PATTERNS OF SUBSPECIES DIVERSITY IN THE GIRAFFE, *GIRAFFA CAMELOPARDALIS* (L. 1758): Comparison of Systematic Methods and their Implications for Conservation Policy.

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"Systematics attempts to organise what evolution has wrought; conservation biology, on the other hand, attempts to ensure that evolution can continue its unpredictable path..."

Buccholz R. and Clemmons J. R., 1997 p. 201.

"Of all the biological information that is needed to manage the world's species, the most fundamental is that provided by the discipline of systematic biology. The four primary components of systematics – discovery and description of species, phylogenetic analysis, classification, and biogeography – provide basic biological information about species, including their name, characterization, relationships to other species, and geographic distribution, thus establishing the foundation for all other biodiversity sciences, such as ecology, population biology, genetics, and behavior. Taken in aggregate, these components support the ultimate aim of systematics to know and understand the taxonomic and phylogenetic diversity of life on Earth."

Wheeler Q. D. and Cracraft J, 1997 (p. 436).

ABSTRACT

This thesis examines the subspecific taxonomic status of the giraffe and considers the role of formal taxonomy in the formulation of conservation policy.

Where species show consistent, geographically structured phenotypic variation such geographic patterns may indicate selective forces (or other population-level effects) acting upon local populations. These consistent geographic patterns may be recognised formally as subspecies and may be of interest in single or multi-species biodiversity or biogeography studies for delimiting areas of conservation priority. Subspecies may also be used in the formulation of management policies and legislation. Subspecies are, by definition, allopatric. This thesis explicitly uses methodology of systematic biology and phylogenetic reconstruction to investigate patterns of variation between geographic groups.

The taxonomic status of the giraffe is apposite for review. The species provides three independent data sets that may be analysed quantitatively for geographic structure; pelage patterns, morphology and genetics. Museum specimens, grouped according to geographic origin, were favoured for study as more than one type of data was often available for an individual.

Population aggregation analysis of forty pelage pattern characters maintained six separate subspecies, while agglomerating some neighbouring populations into a subspecies. A 'traditional' morphometric approach, using multivariate statistical analysis of adult skull measurements, was complemented by a geometric morphometric approach; landmark-restricted eigenshape analysis. Four morphologically distinct groups were recognised by both morphological analyses. Phylogenetic analysis of mitochondrial DNA control region sequences indicates five major clades. Nested clade analysis identifies population fragmentation, range expansion and genetic isolation by distance as contributing to the genetic structure of the giraffe. The results of the analyses show remarkable congruence.

These results are discussed in terms of the formulation of conservation policy and the differing requirements of biological and legal classification systems. The value of a formal taxonomic framework to the recognition, and subsequent conservation, of biodiversity is emphasised.

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Over the last four years or so giraffes have become somewhat of an obsession for me so I should also proffer an apology amongst the thanks to those who have put up with me through this time!

From the outset this project intended to use museum samples to try to derive genetic, morphological and pelage data from the same individual animals as far as possible. Hence, this work would not have been possible without the co-operation of many museum collections in Britain, southern Africa and the United States of America. In all I visited eleven collections and was welcome in each of them. In alphabetical order these collections, along with collection managers and staff who helped me the most, are: The American Museum of Natural History, New York, USA (Bob Randall), British Museum of Natural History, London (Paula Jenkins), Etosha Ecological Institute, Etosha National Park, Namibia (Dr. Nad Brain and Nigel Berriman), Field Museum of Natural History, Chicago, USA (Bill Stanley and Michi Schulenberg), Kruger National Park, RSA (Danie Pienaar), National Museum, Bloemfontein, RSA (Nico Avenant and James Brink), National Museum of Natural History, Bulawayo, Zimbabwe (Woody Cotteril), Powell-Cotton Museum, Birchington, UK (Malcolm Harman), South African Museum, Cape Town, RSA (Denise Drinkrow), Transvaal Museum, Pretoria, RSA (Duncan MacFadyen) and the United States National Museum, Washington DC, USA (Linda Gordon). Other staff at these institutions provided assistance but, again, there are too many to list.

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CHAPTER 1:

SYSTEMATICS, LEGISLATION AND CONSERVATION BIOLOGY: THE RECOGNITION AND CONSERVATION OF BIODIVERSITY

The Context and Aims of this Research Project

The Convention on Biological Diversity (UNEP, 1992) lists its first objective as "the conservation of biological diversity" and goes on to define biological diversity as "the variability among living organisms from all sources including ... diversity *within species*, between species and of ecosystems." (My emphasis added). This study aims to consider the role of the subspecies, as a formal taxonomic grade, in the conservation of biodiversity within species.

The subspecies grade represents a formal taxonomic and nomenclatural recognition of geographically discrete segments within a species that are 'taxonomically distinct' from other such segments. That is, populations that are sufficiently similar to be included within one species may still show locally consistent phenotypic variation as a result of historically different evolutionary environments. Often taxonomists identify consistent characteristics shared by individuals making up a species but miss those features that differ. Such differences indicate contemporary biodiversity within a species.

Subspecific units within species have been named primarily by characterisation of variation in phenotypic characters. In mammals, skeletal proportions, particularly of the cranium, and pelage coloration or pattern have been used to characterise subspecies. Over recent decades methods in the analysis of morphometric data have developed and novel techniques have been introduced. Through a similar time period, introduction of new laboratory techniques, coupled with analytical and conceptual advances, have made it possible to obtain detailed information to infer phylogenetic relationships directly from the genome. Hence, within species diversity may now be characterised by different types and sources of data. Congruence between these independent data sources is clearly desirable. However, the information derived sometimes suggests conflicting interpretation of subspecific units (e.g. Blue wildebeest, *Conochaetes taurinus*: Arctander *et al.*, 1999; Painted hunting dog, *Lycaon pictus*: Girman *et al.*, 1993; Girman *et al*, 2001; Leopard, *Panthera pardus*: Miththapala *et al.*, 1996; Impala, *Aepyceros melampus*: Templeton and Georgiardis, 1996). This project aims to examine the use of independent data sets and different analytical techniques in delineating subspecific units in the giraffe, *Giraffa camelopardalis*, across its recent range in sub-Saharan Africa.

Here, the occurrence of subspecific units is investigated using taxonomic and phylogenetic reconstruction techniques using three independent data sources. Firstly, pelage patterns are analysed using the Population Aggregation Analysis of Davis and Nixon (1992). Secondly, morphological data are analysed using 'traditional morphometrics' (multivariate statistical analysis) on a set of skull dimensions and using eigenshape analysis of skull morphology (Lohmann, 1983; MacLeod, 1999). Thirdly, phylogenetic reconstructions of mitochondrial DNA sequence data (distance, parsimony and likelihood methods) and phylogeny-based techniques (Nested Clade Analysis: Templeton, 1998) are used to infer and interpret cladogenetic events in the population history of the species.

The explicit aims of this project are:

1. to determine patterns of subspecific, geographically structured variation (if any) from morphological, genetic and pelage pattern data and from a variety of analytical techniques;
2. to compare the patterns discovered from the different data sets and to determine the extent of congruence or conflicts between them; and,
3. to discuss the comparisons between the results in terms of the formulation of conservation policy for the protection of giraffe subspecies biodiversity.

The Origin of (Subspecific) Biodiversity

Charles Darwin's realisation that natural selection is an important driving mechanism for evolutionary change is one of the most profound and important biological ideas. The 'struggle for existence' described by Darwin (1859) ensures that unfit individuals do not survive to reproduce, effectively extirpating them from the species and allowing those individuals best adapted to their environment to flourish. The coupling of Darwin's 'descent with modification' with the principles of Mendelian genetic inheritance (the 'modern synthesis' or 'neo-Darwinism': Fisher, 1930; Haldane, 1932; Wright, 1931) provided the mechanism whereby beneficial, fitness-optimising modifications could be inherited. The implication, that selection can cause a species to change over a long period of time, is now generally accepted. Indeed, such is the status of Darwin's theory that it has been suggested that evolution by natural selection "is the only theory that can seriously claim to unify biology" (Ridley, 1993, p. 5).

A logical corollary of Darwinian evolution by natural selection is that species are constantly evolving and separate populations are in all stages of speciation[1]. In Darwin's (1859, p. 107) own words:

> "Certainly no clear line of demarcation has yet been drawn between species and sub-species ... or, again, between sub-species and well-marked varieties, or between lesser varieties and individual differences. These differences blend in to each other in an insensible series; and a series impresses the mind with the idea of an actual passage.
>
> Hence I look at individual differences ... as being the first step towards such slight varieties as are barely thought worth recording in works of natural history. And I look at varieties which are in any degree more distinct and permanent, as steps leading to more strongly marked and more permanent varieties; and at these latter, as leading to sub-species, and to species."

Accordingly, the use of the species as the fundamental currency for measuring biodiversity may effectively disregard a multifarious array of important morphological, behavioural and genetic variability within each species. Most widespread species that have been studied in any detail show some variation across their range. Indeed, it is probable that the observation of geographically structured variation across the range of a single species was what convinced Darwin of the efficacy of his theory (Darwin, 1859; Ridley, 1993). The discovery of contemporary, geographically structured patterns of phenotypic or genotypic variation implies the historical occurrence of differential evolutionary processes across the range of the species. Hence, the discovery (or not) of such patterns is an essential first step in the conservation of subspecific biodiversity. The question then becomes, how should such 'within species' variation be recognised such that it might be conserved?

Units of Biodiversity and Units for Conservation

A central tenet of this thesis is that within species biodiversity is objectively quantifiable and may be defined, at least partly, within the framework of formal taxonomic practice. Types of subspecific variation and their characterisation are discussed below. Before this, a distinction will be made between 'units of biodiversity' and 'units for conservation'. This distinction is instructive when considering the differing requirements of a biological

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[1] 'Speciation' is the process by which species come in to being through evolutionary time. What it actually means will depend upon the species concept used. It could mean the attainment of reproductive isolation (in the sense of the biological species concept; Mayr 1942), the fixation of a certain diagnostic character state (phylogenetic species concept; Cracraft, 1989) or the fulfillment of a particular ecological niche (Van Valen, 1976). The implications of species concepts for subspecific designations are considered later, but a review of the broad and voluminous literature regarding species concepts is beyond the scope of this thesis.

classification and the legislative categories necessary for facilitating and implementing conservation action.

The distinction made here follows, and adds to, that of Dimmick *et al.* (1999), who suggest that units of biodiversity "are the result of evolutionary processes" (p. 659), while units of conservation "may be defined arbitrarily for practical management reasons" (p. 659). I expand and generalise this terminology.

Units of biodiversity are indeed the results of the multitude of evolutionary processes influencing an evolutionary lineage. In this way a taxonomic subspecies (as will be fully discussed and defined below) can be considered a 'unit of biodiversity', but it is not the only unit of biodiversity. Potentially, any morphological, behavioural, ecological or other biological factor could be used to identify unique elements of biodiversity, although not all are deserving of taxonomic recognition (see the discussion characterising subspecific variation, below). However, just because the nomenclatural framework of systematic biology does not provide for formal recognition of such units does not decrease their value.

While a unit of biodiversity is, ideally, an objective reporting of the diversity of the natural world, a unit for conservation is more of a social construction for management purposes. While such a management unit should pay heed to the science of systematic biology, and the resulting classification, it must also take in to account aesthetic, ethical, religious, cultural, social, political and economic values.

Hence, the unit of biodiversity reflects the observations of practising systematic biologists, while the unit for conservation contextualises these observations into the contemporary social and political arena.

Describing Subspecific Diversity

Characterising Subspecific Variability

Genotypic Variation

Genotypic variability within species has, until recently, been the sole realm of population genetics. Genetic distances between conspecific populations have been measured according to allele frequencies in different populations using allozymes or, more recently, microsatellite DNA (Bruford *et al.*, 1996).

Systematic biologists also look at genetic variation within species. The discipline of phylogeography (Avise *et al.*, 1987; Avise, 2000) reconstructs cladogenic events in a

species' history, typically using mitochondrial DNA (mtDNA) sequence variation. Mapping the geographic provenance of each individual onto the mtDNA gene tree allows inferences of the relationships between geographically discrete populations. Coupled with the recent method of nested clade analysis (Templeton and Georgiadis, 1996; Templeton, 1998), which infers the nature of cladogenic events, a detailed population history may be inferred for a given species.

Genetic variation has been used to infer 'Evolutionarily Significant Units' (ESUs) and 'Management Units' (MUs) (Moritz, 1994) that may then be used as units for conservation management (discussed further below). While ESUs have become a popular concept in conservation biology, the term is not synonymous with subspecies and the two are conceptually different. Systematic biology utilises genetic variation for the elucidation of phylogenetic relationships between taxa at all levels and, sometimes, diagnostic molecular motifs for identification. Alpha taxonomy *per se* (taxonomic descriptions) does not use genetic data (although see Smith *et al.*, 1991). Taxonomic descriptions rely on phenotypic, primarily morphological, variation.

Phenotypic Variation

There are many forms of phenotypic variability within 'good' species. All contribute to biodiversity to a greater or lesser extent. Factors such as sexual dimorphism, ontogenetic variation, seasonal variation (e.g. antlers in some male cervids; colour in some orthopterans), social variation (e.g. eusocial insect castes), and differences between generations (e.g. locusts) provide morphological and/or behavioural differences that may be recognised within a species. Such forms have been referred to as 'phena' (Mayr and Ashlock, 1991). Strictly the term phenon (pl. phena) refers to taxonomic units derived from a phenetic analysis. This term may be used here as these within species 'units' are likely to be sufficiently different to come out as separate operational taxonomic units under such analyses. Often the existence of phena is taken for granted and rarely thought of in terms of biodiversity. However, such intraspecific forms may differ in their ecology and must be accounted for in species level taxonomic descriptions.

The distinct phena can be subsumed into the same taxon by biological observations. The minimum number of phena required to produce a self-perpetuating, ecologically functioning unit, that conforms to the typical life history of the species, then makes up a

Least Inclusive Taxon (LIT)[2]. A LIT may consist of a single isolated population or an agglomeration of multiple, local populations. Here I equate the LIT with the subspecies[3], as they contain the same set of individuals, despite having a different ontological basis. The terms are logically and conceptually different and so, strictly, not interchangeable; their use is dependent upon the context. Intraspecific systematics aims to define LITs and infer the relationships between them. The variation that allows a LIT to be defined can occur in any phenon contained within it as certain characters or certain functions may be associated with a particular phenon only (e.g. subspecies of deer described according to variation in the antlers of the male, Geist, 1991). There may still be phenotypic variation within a LIT as the concept is population based. Hence, while phenotypically differentiated, allopatric populations (LITs) represent subspecies, phenotypically distinct sympatric morphs within a population are contained within a given LIT. Such morphs are not of direct interest systematically (although, undeniably, of biological interest).

It should be remembered that a phenon is, typically, a species level phenomenon. That is, individuals of a phenon within a species are more similar to each other (regardless of their subspecific affinities) than they are to individuals of other phena within that species. For example, all adult male giraffe have a heavy skull with well-developed parietal horns. In this character the males within the species as a whole resemble each other more than any individual adult male resembles a female. However, subspecies may still be determined from the geographic structuring of adult male skull variation.

Phenotypic variation within the phena of a species may or may not be geographically structured. Within species diversity in character states may be classified according to the interaction of phenotypic variation and the geographic distribution of this variation. Four types of geographic structure may result from the occurrence of phenotypic variation:

1. No geographic structuring;
2. Smooth clinal variation;
3. Stepped clinal variation; and,
4. Discrete phenotypic classes within isolated ranges.

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[2] This term is introduced here and is different to the Least Inclusive Taxonomic Unit (LITU) concept described by Pleijel and Rouse (2000a) and exemplified by Pleijel and Rouse (2000b) as a replacement for the species in a purely phylogenetic classification system that recognises only monophyletic groups.

[3] The term Least Inclusive Taxon is introduced as a heuristic concept to, at least partly, define my conceptual understanding and usage of the term subspecies. Throughout this thesis the term subspecies will be used for clarity.

The interaction of the incidence of phenotypic variation with geographic structuring is summarised in Table 1.1 along with the terminology used here for each class.

		GEOGRAPHIC VARIATION			
		No Structure	*Gradual Change*	*Stepped Cline*	*Discrete Ranges*
PHENOTYPIC VARIATION	*Yes*	**Morph**	**Cline** (may include **Provisional Subspecies**)	**Definitive Subspecies** (with hybrid zones)	**Definitive Subspecies / Morphotype** (Polytopic Phenotypes)
	No	**Monotypic Species. (Sympatric) Sibling Species**	N/A	N/A	**(Allopatric) Sibling Species**

Table 1.1: *Categories of variation based on the pattern of geographic structuring with respect to the occurrence of phenotypic variability. See text for further descriptions of these terms.*

Those species showing no phenotypic variation can be considered as monotypic species. Where other evidence (e.g. genetic data, breeding behaviour observations) suggests phylogenetic and/or geographic structure to populations within a morphological phenon, where there is no obvious phenotypic diversity, then sibling species may be invoked. The point at which reproductively isolated, allopatric populations become sibling species needs to be considered and justified.

Morphs, clines, subspecies and morphotypes (as defined here) all result from the different patterns of geographical structure in phenotypic variation. Clinal variation is the gradual change of a character across a geographic range such that there is a smooth series of phenotypic variants grading between the extreme phenotypes with no distinct breaks in the distribution. Such variation may be related to environmental factors correlated with latitude, longitude or altitude. Point sampling of a clinally varying species may indicate non-overlapping phenotypic ranges between populations and may result in the description of provisional subspecies (Corbet, 1970). Such subspecies may be common in the literature and are the result of an inadequate sampling regime.

The other categories all show more or less discrete phenotypic variation. Stepped clines show directed variation but are interspersed with narrow regions of rapid phenotypic change between broader areas of gradual change. As such, areas with relatively consistent phenotypes bounded by transition zones may be recognised. Such boundaries allow the description of definitive subspecies (Corbet, 1970).

Morphs show discrete phenotypic variation, but are interspersed together within one population. Morphs may indicate a fixed expression of genetic variation (e.g. the colour morphs of the ship rat, *Rattus rattus*, identified on Lundy Island, UK. Smith *et al.*, 1993) or may change with the social status of the individual (e.g. the silver back of the male gorilla).

Subspecies characterise discrete, phenotypically diagnosable variation in geographically restricted, allopatric populations of a species. The subspecies is the only category used here that is recognised by a formal taxonomic rank. A subspecies is adequately and completely defined by its diagnosis and its geographic range. The diagnosis details how the taxon differs from other similar taxa within the species and includes the phenotypic characters and character states necessary to identify the subspecies. A subspecies may consist of one or more isolated populations. The term morphotype is used here as a notional grade that may include separate subspecies that possess similar phenotypes, but are geographically separated by other physically intervening populations. A morphotype (in my usage) is made up of phenotypically similar subspecies and may be considered as a subspecies group. One of the objections made against the use of subspecific taxonomy is the occurrence of such polytopic populations (Wilson and Brown, 1953). However, where environmental or habitat conditions are similar within the range of a widespread species, convergences between infraspecific populations may be expected.

The recognition of all categories of phenotypic variation is of great importance to understanding and conserving biodiversity. However, this thesis focuses on the recognition of subspecies as a formal taxonomic unit.

'Phenotypic variation' often focuses on morphological variation. Typically systematic biologists use the physical appearance of dead specimens to elucidate characters and character states and to describe species. Mammalian systematists working on subspecies have particularly used features of the skull and variation in pelage colouration. However, much population level variation resides in behavioural characters including interspecific, conspecific and abiotic interactions (Buccholz and Clemmons, 1997). Examples of such behavioural differences include song type variation in oscine passerine birds, vocal dialects in oceanic cetaceans or tool use in chimpanzee troops. Song type, for example, may be a primary prezygotic mating barrier with variants inducing sexual selection in female choice between breeding partners. Tool use and vocal dialects indicate 'cultural inheritance' of information in isolated populations. It has been suggested that behavioural characters may be more environmentally plastic than morphological characters and so of little use in

systematic study. However, studies using ethological characters at the species level indicate that they are no more homoplasious than morphological data sets (de Queiroz and Wimberger, 1993; Wimberger and de Queiroz, 1996). In principle there is no reason why such behavioural characters should not be used to diagnose species and subspecies. In practice this is made more difficult by the requirement of having a type specimen for subsequent referral. Perhaps the future will see the acceptance of audio and/or video recordings as taxonomic evidence.

Inheritance and the Environment

Most biologists consider that any character states used to diagnose subspecies must be genetically based and inherited from the parent. The pervasive assumption of biological determinism (see Rose, 1997) fails to acknowledge a major source of phenotypic variation; that induced by the environment. Clearly the possible range of responses to the environment is determined genetically but most characters will demonstrate some degree of plasticity to the environment experienced during their development. In practical terms it is very difficult, if not impossible, to completely separate the genetic component of geographic variation from that induced by responses to the environment, and to do so would lose an interesting aspect of biogeographical study.

I suggest that the study of geographic variation and subspecific taxonomy should elucidate atemporal patterns of variation irrespective of the process involved. The patterns described then invoke subsequent questions as to their cause. The explication of patterns of subspecific variation and the inference of process are separate activities with the former necessarily preceding the latter. The initial focus on patterns of variation makes no assumptions of the cause of these patterns and provides a more holistic approach to the understanding of biogeographic variation. If environmental effects are producing consistent phenotypic variants that differ from members of the same species in a different area this is of interest in understanding the ecology of the area and the biology of the species. One criticism may be that brief episodic events may affect patterns of variation. Consider an area that suffers from regular droughts (perhaps caused by a cyclical climatic pattern such as El Niño). In such an area there may be regular cohorts of individuals that are smaller, or proportionally underdeveloped in some way, due to a reduction in the quality of food in their early development. A sample of such individuals might lead to the description of a subspecies for such an area. However, this is not a failing of the principle espoused above but a sampling error. Resampling of such an area would identify the more

typical form. Such episodic variation needs to be accounted for in the taxonomic description.

The Taxonomic Subspecies Rank and Other Subspecific Units

The Subspecies – What It Is and What It Is Not

O'Neill (1982, p. 610) states that "The subspecies is not supposed to be an evolutionary unit, but only a taxonomic one." Although he goes on to say "the subspecies concept should be connected to an evolutionary unit if it is to be useful." (p. 611) and that "if subspecies are evolutionary units, then the usefulness of the concept is greatly enhanced." (p. 612). Smith and White (1954, p. 190) go further and suggest that "when the word 'subspecies' is used, it ... [implies that] ... the population has attained such a degree of recognisable differentiation as clearly to demonstrate the potentiality for speciation." Paradoxically, both authors are correct, as their usage of the word subspecies indicates the different connotations that the term may have. O'Neill's (1982) practical assertion, that the subspecies should be taken only as a taxonomic rank and so simply represent a level of organisation of phenotypic diversity below the species level, views the subspecies as a class made up of individual members that may be diagnosed and affiliated to a named subspecies based upon their diagnostic features. Smith and White's (1954) explicit referral to the biological process of speciation and O'Neill's (1982) desire for an evolutionary perspective indicate the notional concept of the subspecies as a 'Darwinian unit' on which evolutionary forces act, in the past, present and into the future. The two approaches show the dichotomy between the practical diagnosis of each subspecies category and its members, and the conceptualisation of the subspecies as the transmutable unit experiencing evolutionary forces.

Mayden (1997) follows Mayr (1957) in advocating the need for two levels of conceptual understanding to describe 'the species'. A similar dual-concept approach is required to describe the subspecies. These reflect the practical, taxonomic approach and the more conceptual, theoretical approach discussed above. The primary concept should clarify the 'idea' behind the subspecies. Meanwhile, the secondary concept must operationalise and apply the defined unit according to the patterns observed in nature.

The primary subspecies concept is as an evolutionary or ecological unit. Individuals experience novel environmental and ecological conditions and varying evolutionary pressures in different geographical regions, leading to phenotypic differentiation of

conspecific populations. Hence, a subspecies represents an isolated population that may be considered as an ecological or evolutionary unit within a species that shows initial signs of evolutionary divergence with the potential for full speciation (but which may or may not reach that point).

The secondary concept involves practical identification and is pattern based. This operational concept is that of the taxonomic rank. The act of naming subspecies indicates discontinuities in the phenotype of the species across its range. This secondary concept is functional and simply reports the patterns observed in nature.

The Subspecies – Defined

Groves (1989) defines subspecies as "geographic segments of a species, which differ morphologically to some degree from other such segments." (p. 6). He goes on to stress that "it is important to grasp the geographic nature of subspecies; they are never, by definition, sympatric." (p. 7). Mayr and Ashlock (1991) consider a subspecies to be " an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species" (p. 43). It may seem tautologous to suggest that taxonomic categories should be classified according to 'taxonomic differences'. However, they clarify their use of language explaining "differing taxonomically" as requiring "sufficient diagnostic morphological characters" (p. 44). Groves (2001. p. 36) has expressed the essence of subspecies more colloquially as "the point along the scale of differentiation at which it becomes worth giving names."

In fact, these 'definitions' should be, more accurately, referred to as diagnoses because they are a prescription as to how to recognise a subspecies. In this way they appeal to the practicality of the secondary subspecies concept discuss above. For this reason, I propose a definition that includes an aspect of the primary subspecies concept indicating the evolutionary and/or ecological nature of the phenotypic differentiation. In accord with the dual nature of the subspecies concepts, and recognising the requirement of geographical and phenotypic patterns, the subspecies is here defined as:

> A phenotypically distinct, geographically restricted sub-unit of a species whose phenotypic variation derives from the effects of differential ecological and/or evolutionary processes.

Population-based versus Individual-based Concepts

An individual based approach to subspecies identification and recognition would see fixed diagnosable differences present in each individual in a subspecies. That is, every individual specimen, possessing the relevant character(s), could be examined and correctly identified to subspecies according to the states of those characters. The occurrence of such unequivocal, fixed character states represents a level of organisation where intraspecific populations have clearly diverged and deserve recognition as subspecies although, in practice, species status will often be conferred upon a taxon with such fixed character states. The subspecies rank attempts to represent a broad spectrum of geographic variation within a species and a less stringent lower boundary is used in practice. Fixed characters state differences are sufficient for the naming of subspecies but they are not necessary.

Most authors (e.g. Rand and Traylor, 1950; Mayr, 1969; Groves 2001) assert that the subspecies is a population level phenomenon. That is, trends in and between populations are compared to ascertain phenotypic differences that allow the description of subspecies. Such an approach makes sense in evolutionary terms as selective forces or simple drift act upon individuals within a population to provide a range of observed phenotypic variants. The geographic distribution of the phenotypes then reflects the evolutionary histories of the populations. However, it is conceivable, indeed it is likely, that the phenotypes observed between two subspecies may overlap in their characters. Under a population-based approach to subspecies designation such overlap is allowable as trends between populations may still be described. As Groves (2001) points out, because of the allowance for overlap of character states, subspecies can not be diagnosed in the strict sense. However, for consistent taxonomic treatment, it remains customary to 'diagnose' subspecies taxa.

Diagnosing Subspecies - How Different is "Different"?

The definitions of the subspecies offered above, including my own, and others in the literature, mention phenotypic differences between populations as being indicative of subspecies. The population-based approach to subspecies designation clearly begs the question how 'different' must a population be to be afforded subspecific status?

Historical Approaches

The typological approach to the description of subspecies and the use of trinomials in the late nineteenth and early twentieth centuries saw the subspecies category as containing

"those individuals that conform to the type of the subspecies" (Mayr, 1963. p. 347). Hence, individuals collected from new localities were compared to specimens from other subspecies type localities. Any mean differences between characters were taken as supportive of subspecific status and a new subspecies was described (Mayr, 1963). Such indiscriminate naming lead to a plethora of names and, while adding to the number of described taxa (particularly in the 'well-known' vertebrate groups), did little to aid the understanding of geographic variation. Such names are still available now and may confuse the understanding of subspecific geographic variation and emphasise the need for subspecific revisions.

The general replacement of the typological, morphological species concept with the biological species concept allowed taxonomists to recognise the extent of geographic variation within biological species. In other words, populations could differ morphologically but still be regarded as the same species. There followed, in the 1920s to 1950s a "veritable orgy of subspecies describing" (Mayr, 1982, p. 594. See also Sabrowsky, 1955 for a quantitative assessment of this phenomenon). No standard for the valid recognition of subspecies (e.g. how much overlap of character values is allowable?) was adopted during this period and authors adopted different conventions. These differed from average character values being different between populations (statistically tested using the Coefficient of Variation, for example) through increasingly stringent separation criteria. Rand and Traylor (1950) discuss the various conventions used through this period and recognise that any arbitrarily set limit may be criticised. They consider a "percentage from all" criterion to have the advantage that it indicates the proportion of a population that may be positively identified to its correct subspecies. Meanwhile, a "percentage from a percentage" approach deals explicitly with populations and allows mutual overlap. Mayr (1969) advocated the use of the '75% rule' (Mayr *et al*, 1953) wherein 75% of population A must differ from 97% of population B to be recognised as a subspecies. This is statistically equivalent to 90% of population A differing from 90% of population B. Of all of the proposed criteria this became the most generally accepted (e.g. Joubert, 1970). Mayr, (1943b; 1969) proposed the 'Coefficient of Difference' (CD), which relates the difference between the means to the standard deviations of the samples. Tabulated values of the CD indicate the separation of the populations. Such calculations were made for each variable separately, and between only two populations at a time. More recent multivariate analyses provide the opportunity to analyse multiple character values for more than two localities. Techniques such as discriminant analysis can also provide classification functions that indicate the proportion of individuals in each sample that can be correctly allocated to their original population.

Biological Criteria rather than Statistical Significance

Any arbitrary rule is open for criticism simply due to its arbitrary nature. The statistical properties of population samples will also vary according to the size of the sample taken and population sampling biases (potentially exacerbated with smaller sample sizes). The adoption of any such arbitrary criterion as an absolute indication of the subspecific status of a population is fallacious. Such a criterion can be indicative and, while a failure to meet the required threshold can be used to deny subspecies status to a population, the attainment of that criterion should not automatically result in the description of subspecies. There are many other factors that a taxonomist must take in to consideration before describing subspecies. Beyond the extent of overlap, other factors include the biological interpretability of the character complexes used to separate the populations, the geographic distributions of the individuals causing the overlap (are the phenotypically overlapping individuals in hybrid zones or showing gradual, clinal variation, or truly homogeneous within each population?) and the demographic structure of the populations compared (is there an age or gender effect between different populations causing bias? Are the phena being analysed comparable?).

Widespread species contain phenotypic variation, some of which is geographically structured. This variation may be thought of as representing different levels in the process of speciation. The decision to describe subspecies must be taken based on the greatest quantity of available information from all sources. No consistent, universally acceptable, arbitrary criteria can be set. Arguably, conventions for what does not constitute a subspecies may be described, but none for what does make a subspecies. By their nature (in representing different stages in speciation), subspecies are not necessarily directly comparable units. It is the species level that represents the fundamental, objectively definable taxonomic unit. Subspecies are hypotheses that summarise observed patterns of phenotypic variation. The description of subspecies comes from the recognition of geographically structured patterns in representative specimens. These patterns can only be recognised *a posteriori* from the empirical results of analysed data. It is not defensible to prescribe, *a priori*, a level of 'difference'. The only valid, defensible criterion that may be asserted before analysis is that the variation discovered must be geographically consistent within an empirically determined tolerance. The differences found should then be translatable to biologically interpretable characters. It is possible (and occurs in these analyses) to have populations that may be separated at some arbitrarily defined, high level

of statistical significance where the differentiating characters have no apparent biological interpretation.

Hence, the answer to the question as to how different a population needs to be to be afforded the rank of subspecies is there is no simple numerical or statistical level that confers this taxonomic status. A subspecies is completely and adequately defined by two factors; the description of geographically structured phenotypic variation within the species and the delineation of discrete geographic ranges demarcating the boundaries of the subspecies. In practice, the valid description of a subspecies (ICZN, 1999) representing consistent, geographically structured variation within a species (as per the subspecies definition offered earlier) based on consideration of the available data and careful analysis, is sufficient to propose the recognition of a subspecies. Acceptance of these observations, and their justification, by the proposer's peers leads to common usage of the new taxon.

Species Concepts – Implications for Subspecies

At the end of nineteenth century Jordan (1896, p. 426) lamented that systematists frequently disagree over "whether a given form of animal or plant is a 'distinct species' or not" because "though using the same term 'species,' the mutual conception of that term is widely different." Over a century later, Hull (1997, p. 357 – 358) noted that "different systematists have different goals for their species concepts, but even those systematists who agree in principle on what a species concept should do frequently prefer different species concepts." That two authors, more than a century apart, should make such similar observations over systematists' perception of the 'species' indicates the complexity of the discussions in the species concept debate, and their lack of resolution. Hull (1997, p. 358) suggests that the reason is that "we have several criteria that we would like an ideal species concept to meet, and these tend to conflict." Hey (2001) is explicit in citing a conflict of interests when defining 'species'. Species, as we refer to them, result from two processes: "(1) the evolutionary processes that have caused biological diversity; and (2) the human mental apparatus that recognizes and gives names to patterns of recurrence." (Hey, 2001, p. 328). The former group alludes to 'real' biological entities while the second invokes the named categories imposed by the recognition of patterns of similarity and difference by a human observer. Hey (2001) suggests that 'the species problem' results from trying to reconcile these two ontologically different concepts. That is, it is difficult to impose categories onto our understanding of evolutionary processes and the natural groups in which they result.

Mayden (1997) lists 24 different proposed species concepts[4] (along with synonyms), with at least one more proposed since his survey (de Queiroz, 1999). It is beyond the scope of this thesis to review species concepts in detail and it is not my intention to do so[5]. However, the conceptualisation of the species taxon has obvious ramifications for the lower level taxonomic rank, the subspecies. Species concepts may be grouped according to the logical derivation of the concept. Ontologically species concepts may be formulated according to biological processes. A more empirical approach sees observed patterns of variation as defining species boundaries. The 'process-based' concepts can be further sub-divided into those based on interpretations of historical processes and those that invoke contemporary processes. These three categories of species concepts are exemplified and briefly considered here to show how they might influence the application of the subspecies rank.

Historical Process-based Concepts – The Evolutionary Species Concept

Simpson (1961, p. 153) defined the Evolutionary Species as "... a lineage (an ancestral-descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies." Wiley (1978) gave a similar definition; "... a single lineage of ancestor-descendent populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate". The laxity of these definitions saw Wiley and Mayden (2000) choose to 'characterise' rather than 'define' the Evolutionary Species Concept (ESC) to avoid "discussions over words rather than concepts" (p. 73). They characterised the evolutionary species as "an entity composed of organisms that maintains its identity from other such entities through time and over space and that has its own independent evolutionary fate and historical tendencies." (p. 73). They amplified their characterisation with nine clarifying points.

Mayden (1997) asserts that the ESC is not an operational concept. That is, it describes the ideal theoretical characteristics of a species rather than providing criteria for their recognition in nature. Despite its theoretical nature (or, perhaps, because of it) the ESC is the only species concept that is generally applicable to all organisms (Mayden, 1997).

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[4] Mayden (1997) actually categorises 22 separate concepts, but one of these (the phylogenetic species concept) is separated into three versions.

[5] The reader is referred to recent discussions of the nature of species, species concepts and speciation and to the references contained therein. Some of the many recent volumes on this topic are: Claridge *et al.*, 1997; Ghiselin, 1997; Howard & Berlocher, 1998; Otte & Endler, 1989; Ridley, 1993; Wheeler and Meier, 2000; Wilson, 1999.

The logical application of a subspecies concept under the ESC is difficult. Wiley and Mayden (2000) preclude the application of the subspecies rank within the ESC by stressing the metaphysical individuality of evolutionary species (see Ghiselin, 1997) and that relationships within ESCs are necessarily tokogenetic and those between ESCs phylogenetic. Hence, the ESC makes no allowance for any substructure within the evolutionary species. The determination appears to be absolute: Evolutionary species or not evolutionary species.

Dimmick *et al.* (1999) assert the importance of systematic biology for conservation biology, and express concern that "the principles of systematic biology ... have not been integrated completely into the practice and principles of conservation" (p. 654). They advocate a systematic approach to determining units for conservation and suggest application of the evolutionary species concept of Wiley (1981). Dimmick *et al.* (1999) suggest that the ontological nature of the evolutionary species concept is advantageous as it does not require recourse to a particular methodology or particular type of data but can accommodate an holistic approach utilising all sources of data. That evolutionary lineages exist is a tacit assumption of evolutionary biology that is generally accepted, all that is then required is evidence to support the evolutionary lineages.

However, the application of the ESC may be equivocal. Many contemporaneously recognised subspecies are well differentiated phenotypically and have, apparently, been following their own 'evolutionary tendency' for some time. These would be considered evolutionary species. But what of a population that has become newly separated from the parent population by a vicariance event of some sort or a founder population that has survived in a new habitat? Are these also evolutionary species? It would appear so as they are independent of other 'entities' (populations) and have embarked upon their own 'evolutionary tendency'. What then happens if secondary contact is established sometime in the future? Wiley and Mayden (2000) suggest that, if tokogenetic relationships are re-established following subsequent sympatry then the separate populations never represented evolutionary species, but we can not know whether secondary contact will happen at some time in the future.

Such problems show the difficulty of applying the ESC to real observations. As stated, the ESC is not an operational concept and should not be thought of as such. It presents a theoretical primary concept (Mayden, 1997) and requires a surrogate concept to allow its application (the secondary concept of Mayden, 1997). While the primary concept (the ESC) may be theoretically sound, the secondary concept provides the practical criteria for

grouping individuals into species. Such concepts include the biological and the phylogenetic species concepts.

Contemporary Process-based Concepts – The Biological Species Concept

The most pervasive of all species concepts and, probably, the one most generally accepted by biologists, either explicitly or implicitly, is the Biological Species Concept (BSC) usually attributed, in its modern form, to Mayr (1940; 1942). Under this concept a species is considered to be an isolated gene pool, the members of which make up a reproductive community separate from all other gene pools. Mayr (1940) defined a species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". Dobzhansky (1950) gave a complementary definition as "the largest and most inclusive ... reproductive community of sexual and cross-fertilising individuals which share a common gene pool." Mayr (in Mayr and Ashlock, 1991) was clear that the origins of the BSC predated his definition and suggests that Jordan (1905) was the first to formulate the concept in some semblance of its modern form.

The shift from a typological concept to a more inclusive biological concept meant that morphological variation could be accommodated within a given species. Polytypic, but reproductively compatible populations, could be subsumed into a single species (Mayr, 1982). These polytypic species contain morphologically diagnosable geographic variants, a number of which had been considered full species under the typological concept. Hence, the BSC clearly allows for, and, arguably, necessitates the description of subspecies to account for such geographically structured variation within the species so defined.

Pattern Based Concepts – The Phylogenetic Species Concept

Cracraft (1983. p. 170) defined a species as "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent." Interpreted from the viewpoint of cladistic analysis such a definition implies that species then represent the terminal taxa that can not be further subdivided on the basis of their character distributions (Cracraft, 1992). This is the essence of the Phylogenetic Species Concept (PSC). It should be noted that there are a number of PSCs that differ slightly in the detail of their conception (Mayden, 1997; See also Wheeler and Meier, 2000).

Vogler, DeSalle and co-workers (Vogler and DeSalle, 1994; Vogler *et al.*, 1993) used an approach based on the phylogenetic species concept to define conservation units. They state that a phylogenetic species is defined as "a cluster of organisms possessing a unique

character or a unique combination of characters" making it "the smallest detectable group of organisms distinguishable by unique attributes." (Vogler and DeSalle, 1994. p. 356). Vogler and DeSalle (1994) operationalise the use of their definition based on the phylogenetic species concept by combining phylogenetic analysis with the 'population aggregation analysis' of Davis and Nixon (1992) where separate populations are aggregated if they contain identical individuals. In this way local populations are grouped into phylogenetic species. In Vogler and DeSalle's (1994) terms this means that "all those clusters of populations that do not overlap in the composition of their members are diagnosably distinct" (p. 357) and hence represent separate conservation units. They note that, in practice, the diagnosability criterion takes precedence over the reconstruction of the phylogeny and that populations may be aggregated and diagnosed without the phylogenetic structure being known. However, this could lead to populations being grouped by homoplasious, rather than synapomorphic, characters.

Cracraft (1992) applied the PSC to the birds-of-paradise, a group formerly classified by application of the BSC. His working hypothesis for the application of his PSC was that "phylogenetic species are those basal[6] [terminal] populations that are 100% diagnosable" (Cracraft, 1992, p. 3). In his revision approximately two-thirds of the subspecies named under the biological species criterion were elevated to full species. Cracraft's (1992) stance on the utility of the subspecies rank is made clear. He states that "adoption of the phylogenetic species concept solves a long-standing source of contention within systematics, namely the taxonomic status of subspecies. Because phylogenetic species are basal (smallest recognisable) differentiated taxonomic units, subspecies could only be applied as arbitrary descriptors of within-species variation. Within that context, therefore, they serve little useful purpose" (p. 4). The supposed lack of significance of the subspecies rank when using the PSC is echoed by McKitrick and Zink (1988) who suggest that subspecies should not be named.

Hence, it would seem then that subspecies could have no biological meaning under a concept that recognises species as its basic phylogenetic unit. However, such a supposition must make clear the nature of the information used and accounted for in its definition of its fundamental units. Indeed, McKitrick and Zink (1988) did recognise that there may still be recognisable geographic variation within identified phylogenetic species (and suggest the

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[6] Cracraft's (1992) use of the term 'basal' when referring to taxa seems to conflict with the generally accepted usage. Cracraft uses it to denote terminal taxa. Typically, the term basal refers to the lower portions of a phylogenetic tree, closer to the root. Cracraft's etymology of the term seems to relate 'basal' to 'basic' or 'fundamental', indicating an irreducible, least inclusive (terminal) taxon. What I consider to be the more typical usage refers 'basal' to 'base' and to the lower branches or the root of the cladogram.

acronym 'PIPS' – Potential Incipient Phylogenetic Species – as a possible rank). Cracraft's own criteria, of complete diagnosability of terminal taxa based on discretely coded character states, leaves room for trends in continuous variation of characters between non-sympatric populations, and, hence, the description of the exact type of variation that the subspecies aims to report.

So, the subspecies can be applied usefully as a nested rank within a phylogenetic species. The PSC is an individual based concept rooted in cladistic thinking. It considers characters as its evidence and uses discrete character states to group individuals together into species, effectively dismissing continuous variation in characters. The phylogenetic species is, then, a population of individuals that may still show phenotypic variation in continuous characters representing informative trends in continuous (non-discrete) geographic variation within a phylogenetic species. In this way, the population based approach of the subspecies rank complements the individual based approach of the PSC.

Does the concept used matter?

The concept used reflects the philosophy of the describing or revising systematist and should be taken account of when interpreting the classification made. Cracraft (1992. p. 1) states that "the entire theoretical and empirical structure of comparative biology depends on how species, and taxa in general, are conceived (Nelson, 1989). Different concepts often have substantially different consequences for the description and explanation of patterns of diversity through space and time." In some sense Cracraft's assertion is correct. However, there remains an implicit and unstated assumption. This is that it is the species that represents the fundamental biological unit on which comparative studies are based, biodiversity is enumerated or conservation policies formulated. The species concept may change the species level classification but, as Cracraft's (1992) own results from a PSC based revision of the birds-of-paradise demonstrated, "the total number of named taxa recognised under the phylogenetic species concept will probably not differ importantly from previous [BSC based] analyses" (Cracraft, 1992, p. 43). In summing up his review Cracraft (1992, p. 43) states that "the goal of systematics ... is to use a concept that will facilitate the naming of all relevant products of evolutionary diversification and our understanding of their history through space and time." I contend that the recognition of subspecies performs this task and provides greater information. Any classification contains two types of complementary relationships within its hierarchical structure, namely similarity and difference. To elevate subspecies correctly designated under a BSC to full species status under a PSC revision will maintain the 'difference' information but may lose

a substantial quantity of 'similarity' information. A specimen with a BSC-based trinomial informs the reader of relationships at two levels; the genus that the specimen belongs to and the species. That is, this form is more closely related to other subspecies within this species than to any other taxon. With elevation to species, and the abandonment of the subspecies rank, one of these sources of information is lost. Without further information, the newly found species may then be related to any of the other species within the genus.

A subspecies concept can, and should, be applied within the BSC or PSC. If the distribution of phenotypic character states between taxa is assumed to indicate phylogenetic relationships (the central tenet of phylogenetic systematics: Hennig, 1950; 1957; 1965; 1966), then the PSC seems the most logical choice of concept. Individuals should be grouped into species where their characters and character states are identical and all individuals may be diagnosed to the correct species. Non-sympatric populations may then be compared to ascertain how the degree of variation within characters is distributed geographically. Significant differences in character state distributions should afford subspecies status to these populations.

The application of different species concepts does not affect the giraffe, as all individuals across the range are fundamentally similar. However, they do show a great deal of phenotypic variation.

The Use, and Abuse, of the Subspecies Rank

The intended purpose of the subspecies rank is to recognise variation within designated species where geographically delimited variation occurs. Hence, it represents the lowest (least inclusive) taxonomic rank and is the only formal taxonomic rank recognised by the ICZN (1999) below the species level.

Hermann Schlegel first used the trinomial combination to represent geographic variation within the range of a species back in 1844 (Sibley, 1954). Since then there has been recurrent and lively debate over the validity of the subspecies rank in taxonomy and its value in recognising and describing intraspecific geographic variation with the ensuing implications for other biological disciplines. Local populations and subspecies are now increasingly recognised in conservation legislation so there is a practical application of the rank of subspecies making the clarification of the status of this rank a necessity.

Discussions of the use of the subspecies rank can be found in the literature going back more than a century (Chamberlain, 1884a; Bogert, 1943; Peters, 1954; Wilson and Brown,

1953; Wiens, 1982[7]). One of the most critical diatribes against the use of the subspecies was that of Wilson and Brown (1953). While they recognised the occurrence of geographic variation by populations gradually accruing morphological changes, they suggested the "eventual abandonment of the subspecies trinomial" (p. 109) and its replacement by the vernacular use of geographical names. However, Wilson (1995, p. 208) later admitted that "Brown and I overstated our case" and that subspecies do offer useful information. Their paper inspired five years of correspondence to the journal *Systematic Zoology* with a split of support and opposition for the subspecies rank. However, even the supporters indicated that, while the subspecies rank was sound conceptually, its application was often at fault.

Many authors point out that some taxonomists saw their role as searching for differences between supposed 'taxa' (Burt, 1954; Wilson and Brown, 1953) and did not relate the variants described to geographical patterns within the species. As Lanyon (1982) pointed out "excesses and abuses in the application of the subspecies concept have led to indiscriminate naming of infraspecific units in the past" (p. 604). He goes on to suggest that "perhaps the deficiencies attributed to the subspecies concept are more to be viewed as deficiencies of the taxonomist rather than of the taxon" (p. 604).

The application of the rank of subspecies is only warranted when the species level taxonomy is established and the species range well known (Blackwelder, 1967 cited by Winston, 1999). In the past subspecies have been named as and when new specimens have been collected from geographically distinct areas (e.g. for giraffes, Lydekker, 1911). On many occasions a single specimen, or a small series of specimens, was available. This may cause individual variation, or the effects of biased sampling, to affect the judgement of the taxonomist. Subsequent sampling from the type locality may confirm the type specimen or type series to represent aberrations of that population or sampling of geographically intermediate sites may indicate a clinal blending with surrounding populations or other subspecies. A survey of subspecific taxonomic practices in entomology (Sabrowsky, 1955) showed that 57.2% of all newly named subspecies were based on specimens from a single

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[7]Discussions in the literature include:
Chamberlain, 1884a; Allen, 1884a; Coues, 1884; Chamberlain, 1884b; Allen, 1884b:
Blair, 1943; Bogert, 1943; Dunn, 1943; Hubbs, 1943; Mayr, 1943a; Hall, 1943; Simpson, 1943:
Peters, 1954; Burt, 1954; Sibley, 1954; Bogert, 1954; Hubbell, 1954; Clench, 1954; Rogers, 1954:
Wilson and Brown, 1953; Edwards, 1954; Mayr, 1954; Moore, 1954; Gosline, 1954; Brown & Wilson, 1954;
Parkes, 1955; Fox, 1955.Sabrowsky, 1955; Durrant, 1955; Fennah, 1955; Edwards, 1956; Gillham, 1956;
Smith and White, 1956; Starrett, 1958:
Wiens, 1982; Parkes, 1982; Gill, 1982; Storer, 1982; Barrowclough, 1982; Lanyon, 1982; Johnson, 1982;
Zusi, 1982; Monroe, 1982; O'Neill, 1982; Phillips, 1982.

location while 18.7% were named based on a single specimen[8]. Similar practices have occurred in mammalogy with subspecies being named from individual specimens. For example, Lydekker (1904) named the south Lado giraffe, *Giraffa camelopardalis cottoni*, in honour of the collector, Major Powell-Cotton from only a single specimen. This subspecies has now been subsumed into the Rothschild's giraffe (*G. c. rothschildi*) (Dagg, 1971).

It is obvious that, where subspecies descriptions derive from single localities the full range of local variation is not taken in to consideration. Similarly, with examination of single specimens, aberrant individual variants may invoke the recognition of a new subspecies. Variation across the entire geographic range of a species and variation within geographically delimited populations must be understood and accounted for when describing subspecies. Corbet (1970) recognised this problem and suggested that a distinction should be made between "definitive subspecies" and "provisional subspecies". Definitive subspecies are considered as those that have been adequately sampled to recognise all spatial and temporal variability and demonstrated to show discrete geographical variation. Meanwhile, "provisional subspecies" are those that are based on too small a sample size or from too few localities to offer confidence in their designation. Zusi (1982) makes an important distinction between "the discovery, analysis, and explanation of geographic variation on the one hand and the describing of subspecies on the other" (p. 607). Indeed, the 'creation' of subspecies should not be the goal of systematic study. Rather the focus should be on the understanding the patterns of geographic variation found in species (Barrowclough, 1982; Storer, 1982). It may then be that the description of new subspecies results from the pattern of variation obtained.

Unfortunately many of the currently recognised subspecies fall in to the "provisional subspecies" category. The problems of subspecies based upon inadequate sampling and insufficient knowledge were recognised long ago (e.g. Sabrowsky, 1955) and have continued to be lamented by taxonomists (Corbet, 1970; O'Neill, 1982). Doubtless this has contributed to the discomfort that many taxonomists feel with this rank and the lack of confidence shown by some conservation biologists in advocating the subspecies as a generalised unit for conservation. It is difficult to chastise past taxonomists too harshly as, when confronted with aberrant material that they could not reconcile to known taxa, they

[8] This study (Sabrowsky, 1955) also categorised the 'variety', an infrasubspecific taxonomic rank no longer recognised by the ICZN. Under current ICZN rules many varieties now hold subspecific status. The survey showed that 64.6% of varieties were from single locations with 26.4% named based on a single specimen.

simply made a new 'pigeon hole' for it. Unfortunately, this practical approach leaves a legacy of problems that stem from the nature of taxonomy and nomenclature itself.

All biological knowledge progresses iteratively and builds upon knowledge gained by others. However, of all disciplines, taxonomy has its foundations strongly rooted in history and in a strict code of rules (e.g. ICZN, 1999). These rules are used to provide stability and consensus in nomenclature. They appeal to precedents to resolve nomenclatural discrepancies often requiring a great deal of detective work from the taxonomist to track down the sequence of valid names of a taxon. The rules also result in 'nomenclatural inertia'. That is, a name remains as the current, accepted name until a more inclusive taxon is reviewed and the new nomenclature becomes accepted. Hence, the names of previously described taxa remain in the literature and in general usage until evidence demonstrating the necessity to change the name comes to light. These names then remain as 'available' names in perpetuity. These historical names describe biological taxa and may, or may not, reflect true biological units.

Recently there has been a change in emphasis from 'traditional' taxonomic revisions utilising phenotypic characters (largely morphological but also ecological and behavioural data where available) to the study of intraspecific phylogeographic patterns (Avise *et al.*, 1987; Avise, 2000) and 'Evolutionarily Significant Units' based on molecular criteria (Moritz, 1994). Some authors are now expressing concern that molecular data is becoming too dominant in conservation management (Crandall *et al.*, 2000) and suggest a return to a more holistic approach where all available data are used to examine the patterns of intraspecific variation (Crandall *et al.*, 2000; Ryder, 1986). These concepts are now discussed.

Evolutionarily Significant Units

The Original Proposition

The term 'Evolutionarily Significant Unit' (ESU) was introduced by Ryder (1986) in response to concerns that described subspecies may not adequately nor accurately represent within species diversity. His discussion arose from concerns of the limited capacity of captive breeding facilities to provide sufficient "captive habitat" (i.e. space) to support multiple populations of each species to represent the different named subspecies. He recognised the goal of captive management programs to be to "preserve gene pools as they exist in nature" (Ryder, 1986, p. 10). The subspecies had been used to represent such infraspecific 'units'. However, if the subspecies taxonomy is ill-founded then much time,

effort and money could be wasted in pursuing captive management programs. Ryder (1986) and Ryder *et al.* (1988) noted the sometimes inconsistent manner in which subspecies have been described in the past. Ryder *et al.* (1988, p. 138) suggested that "criteria for designation of subspecies be refined or that an alternative designation based upon more precise scientific criteria be developed." They suggested that such an alternative may be the ESU.

Ryder (1986, p. 10) proposed that "concordance between sets of data derived by differing techniques be a criterion for identifying ESUs". Such data sets include (p.10) "natural history information, morphometrics, range and distribution data, ... protein electrophoresis cytogenetic analysis and restriction mapping of nuclear and mitochondrial DNA." The very types of data used by systematic biologists when describing new taxa. Hence, it can be argued that Ryder's new term simply represents a reformulation of the subspecies. Apparently, what Ryder (1986) considered to be a problem was not the subspecies concept *per se*, but the application of it.

Although originating in the discussion of captive populations the ESU concept is clearly applicable to the *in situ* conservation of wild populations.

The 'Distinct' Approach

Waples (1991) took up the ESU terminology in an attempt to satisfy the legislative requirements of the US Endangered Species Act. This Act uses the term 'species' very broadly to include "any distinct population segment" of a vertebrate species (in the biological, taxonomic sense). The practical necessity to adequately satisfy the requirements of such a definition and to prove the 'distinctiveness' of each "population segment" requires a consistent, biologically sound approach. Waples (1991) proposed that:

> "A vertebrate population will be considered "distinct" (and hence a "species") for purposes of conservation ... if the population represents an Evolutionarily Significant Unit (ESU) of the biological species" (p. 12).

He qualifies the definition of an ESU, in this context, with:

> "An ESU is a population (or group of populations) that:
>
> 1. Is substantially reproductively isolated from other conspecific population units; and,
> 2. Represents an important component in the evolutionary legacy of the species" (p. 12).

Waples (1991) notes that his criteria reflect the two common uses of the term 'distinct' in requiring the reproductive isolation of the population (i.e. 'distinct' in terms of separation) and the possession of diagnostic characteristics (i.e. distinct in terms of being different or unique).

Waples' (1991) prescription for applying his definition consists of two stages. First, the degree of reproductive isolation must be established. Reproductive isolation can be measured directly using genetic markers or by estimation of migration rates between populations (using tagging studies, for example). Even the identification of physical barriers between populations identifies potential isolated populations, although such habitat information should be augmented with biological evidence. Under Waples' (1991) procedure failure to identify reproductive isolation of a population from conspecifics precludes it from further consideration as an ESU.

Populations shown to be reproductively isolated are then evaluated for evidence of ecological or genetic distinctiveness. Such evaluations must be made within the context of comparable data for the species as a whole, and other appropriate species. The types of data considered important include genetic traits, phenotypic traits (including morphological and meristic characters as well as parasite loads and disease and parasite resistance), life history traits (including breeding behaviour and timing) and habitat characteristics (does the population occupy a unique habitat, compared to its conspecific populations?).

Hence, Waples' (1991) extended the ESU concept into one that could be applied to *in situ* conservation of populations. The guidance offered provided a flexible approach and encouraged consideration of a broad range of data and so maintained the holistic approach advocated by Ryder (1986).

Usurpation by the Geneticists

The treatment of the ESU that has gained the greatest general acceptance is that of Moritz (1994). Moritz (1994) considered the key word that required further definition was 'significant'. He noted that, whilst the ESU concept seeks to identify and preserve diversity into the future, the only information we can glean is about the evolutionary past, by inferring the phylogenetic history. He suggested that historical isolation, rather than current adaptation, indicates a distinct potential in the future. Hence, by Moritz's definition, an ESU is a historically isolated population or set of populations. In an attempt to quantify and formalise the criteria to recognise ESUs, Moritz (1994) used simulations of the progression of mtDNA alleles from two founder populations from polyphyly through

paraphyly to reciprocal monophyly (Neigel and Avise, 1986) to derive an objective definition of an ESU. mtDNA alleles have a relatively low effective population size (due to their clonal nature and exclusively maternal inheritance) and high substitution rate, and so are expected to reach reciprocal monophyly between the populations more rapidly than nuclear alleles. Moritz (1994), therefore, suggested that ESUs should be "reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci." This provides a pattern-based criterion for the recognition of the ESUs dependent upon the phylogenetic structure of the mtDNA alleles and avoids discussions of "how much variation is enough?" Moritz went on to list a series of caveats (e.g. that hybridisation events may affect results) and suggested that his definition should "be applied with common sense" (Moritz, 1994, p. 374). He also states that his criteria for recognising ESUs (and Management Units – MUs - which he synonymised with 'stocks') was not intended to be prescriptive but should promote debate.

Coincident with the use of mtDNA studies in phylogeography (Avise *et al.*, 1987; Avise, 2000) the delineation of ESUs according to genetic evolutionary criteria took over from the more holistic approach of the systematic biologist. In fact Moritz (1994) effectively dismissed the holistic approach by suggesting that current adaptation, as might manifest itself in morphological variation, indicates adaptation to previous conditions and so "may retard the response to natural selection" (p. 373).

The system of Dizon *et al.* (1992) attempted to increase the information content of the classification of ESUs. It provided a theoretical and an operational classification of geographically defined populations according to the level of gene flow between them, taken as reflecting the degree of geographic isolation, and the degree of 'differential selection'. Differential selection "represents differences in characters that are the expression of the locally adapted genome" (Dizon *et al.*, 1992, p. 29) and includes demographic, morphological, allozyme or sequence data that may indicate local selective pressures operating on particular populations. A 2x2 matrix construction of geographic localisation versus differential selection (Table 1.2) provides four categories that may be ranked in terms of their probability of representing an ESU. Category I has the highest probability of being an ESU, while category IV is the least likely. Such a system gets away from the inflexible dichotomy of classifying a population as simply ESU or not ESU. Instead a graded scale of 'importance' (i.e. probability of representing an ESU) is provided based on a range of genotypic and phenotypic data.

Theoretical Classification		PHYLOGEOGRAPHIC TYPES	
Gene Flow	Little or none	III	I
	High	IV	II
		Little	Great
		Differential Selection	

Operational Classification		PHYLOGEOGRAPHIC TYPES	
Geographic Localisation	Great	III	I
	Little or none	IV	II
		Little	Great
		Proxies for Differential Selection	

Table 1.2: *Phylogeographic classification categories based on the methodology of Dizon* et al. *(1992) using genotypic and phenotypic differentiation of geographically defined populations. From Dizon* et al. *(1992) Figure 2. p. 29.*

Dizon *et al.* (1992) recognised that the confidence that can be placed in any classification of populations depends upon the source and quality of the information used. They recommend examination of multiple data sources to establish the status of each population. They categorised the data into four types:

a) distributional data;
b) population response data;
c) phenotypic data;
d) genotypic data.

Their system then allows information for the phylogeographic structure (category) and the supporting data source (criteria) to be presented, in a shorthand notation, for any population.

Recent Critiques of the ESU Concept

Recently, Paetkau (1999) has attempted to revive the debate that Moritz had hoped for by reassessing Moritz's (1994) approach in terms of his original arguments and on subsequent researcher's interpretations of those arguments. Paetkau (1999) points out that many researchers have erroneously referred to Moritz's (1994) criteria for recognising ESUs as the definition of an ESU. Paetkau (1999, p. 1507) goes on to redifine the ESU as "a group of organisms that has been isolated from other conspecific groups for a sufficient period of time to have undergone meaningful genetic divergence from those other groups" and cites Ryder (1986) as his authority for this definition. However, although Ryder (1986) did

discuss ESUs in terms of preserving genetic diversity, he never defined the ESU in genetic terms, instead presenting the holistic approach given above.

Paetkau (1999) points out that the requirement of reciprocal monophyly may be too restrictive and cites the paraphyly of the brown bear (*Ursus arctos*) with respect to the monophyletic polar bear (*U. maritimus*) as an example (Talbot and Shields, 1996). The ancestral polar bear is thought to have diverged from an isolated population of the brown bear in the mid-Pleistocene (Kurtén, 1964). No taxonomist or ecologist would deny the species status of these two taxa, but they do not meet the requirements of reciprocal monophyly suggested by Moritz (1994) as indicative of ESU status. The reason for this was acknowledged by Moritz (1994, p. 375) who cited a similar case for humpback whales, *Megaptera novaeangliae* (Baker *et al.*, 1993). He recognised the progression from polyphyly through paraphyly to reciprocal monophyly. Models of this progression indicate that $4N$ generations (where N is the population size) are required to attain a reasonable probability of reciprocal monophyly (>90% probablility). For mammals with long generation times, where a small daughter population diverges from a large parent population, the time taken to achieve reciprocal monophyly may be longer than the time required to accumulate morphological and ecological adaptations sufficient for recognition of two species. Hence, Paetkau (1999. p. 1508) suggests that "reciprocal monophyly for mtDNA is not a necessary criterion for identifying ESUs, although it may be a sufficient criterion". He goes on to recommend that "ESU definitions should be based on as many sources of information, genetic or otherwise, as are available", effectively returning to Ryder's (1986) original recommendation.

Crandall *et al.* (2000) criticised the emphasis on molecular data encouraged by Moritz's (1994) recommendations noting that "many investigators ... regard these data as essential to conservation management, often to the exclusion of other data." This emphasis focuses on reproductive isolation rather than the discovery of adaptive diversity between populations. Contrary to Moritz's (1994) assertion that maintenance of adaptive diversity may retard future evolutionary potential, Crandall *et al.* (2000) point out that it is the processes that produce this variation that need to be preserved and allowed to continue. The identification of the pattern simply implies that the process is occurring, although does not necessarily identify the process.

Crandall *et al.*'s (2000) solution to the problem of defining 'conservation units' was to examine hypotheses of genetic and ecological exchangeability over recent and historical timescales, with management recommendations based on the rejection or failure to reject these hypotheses. As such they reintroduce ecological criteria (including that inferred by

morphological differentiation) into the decision making process, as in Ryder's (1986) original suggestion. They go further and suggest the abandonment of the ESU terminology as it "hardly seems necessary or appropriate" and has been removed "from its conceptual foundations in evolutionary biology" leading to the "frequent misdiagnosis of conservation units" (all quotes from p. 294). This takes the discussion full circle, as the lack of utility and comparability of described subspecies was the argument used by Ryder (1986) to support the proposed new terminology.

Similarly Cracraft *et al.* (1998) suggest the abandonment of the ESU but argue in favour of using a Phylogenetic Species Concept (PSC) within the framework of a formal taxonomic classification to describe 'units' for conservation. A phylogenetic species (discussed above) is a population of individuals that can not be further subdivided based upon the available character state information. Cracraft *et al.* (1998) suggest that many systematists and conservation biologists view the phylogenetic species as effectively equivalent to the original conception of the ESU (Ryder, 1986). Given the rigorous formality of the taxonomic approach, they commend the adoption of the concept of phylogenetic species in place of ESUs as the basis for biodiversity evaluation.

A practical example where strict application of a solely genetic interpretation of the ESU failed to recognise species and subspecies level diversity is demonstrated in a study of the Cryan's Buckmoth (*Hemileuca* sp.). Legge *et al.* (1996) sought to apply a phylogenetic species approach (after Vogler and DeSalle, 1994) to the elucidation of ESUs in the *Hemileuca maia* species group. Cryan's buckmoth shows marked ecological differences from other members of the species group (including *H. maia*, *H. nevadensis* and *H. lucina*) while being morphologically very similar. Examination of 13 polymorphic allozyme systems showed no resolution between species or populations. Similarly, 160bp of the mitochondrial CO-II gene failed to resolve relationships within the species group with only five polymorphic sites identified within the group. Hence, no diagnostic characters were found that would identify any of the *H. maia* species group as being ESUs. In contrast, controlled host-plant performance experiments and complementary field observations indicated extreme ecological specialisation. Habitat preferences in the wild and larval survivorship on different host plants lead to the conclusion that "in the case of Cryan's *Hemileuca*, direct evidence for ecological separation is available and can be used to recognise evolutionarily significant differentiation." (Legge *et al.*, 1996).

Management Units

Moritz (1994) also described the 'management unit' (MU). He recognised MUs as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles." (p. 374). Such populations, it is suggested, have diverged from their parent population, but sufficiently recently that they have not had a chance to accumulate diagnostic character states. Essentially these populations represent separate breeding units. As such they may possess the potential for independent evolutionary trajectories divergent from the parent population and so may be deserving of conservation protection.

The Subspecies Rank in Taxonomy for Conservation Biology

Conservation Biology, Systematics and Legislation

While systematic biology may offer a key to understanding biodiversity (Wheeler and Cracraft, 1997), legislation is the vehicle by which much large-scale conservation is administered. Patterns of systematic and biogeographic diversity do not recognise arbitrarily imposed anthropocentric national borders making international agreements and treaties a necessary factor in biodiversity conservation. Typically, such conventions are ratified and put in to practice by domestic legislation in the signatory countries.

"The conservation of biological diversity" is a goal of the Convention on Biological Diversity (UNEP, 1992). In this convention, biological diversity is defined as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity *within species*, between species and of ecosystems." (My italics)

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1973), uses the term 'species' throughout its text. However, it extends the definition of 'species' to encompass "any species, *subspecies*, or geographically separate population thereof" (with my italics for emphasis). The implementation of the CITES Convention in the European Union (Council Regulation (EC) No 338/97, 1996) uses the same extension of the definition of species as appears in the text of the convention, but further defines the population as "a biologically or geographically distinct total number of individuals". The US Endangered Species Act uses a similarly broad definition of 'species' to include "any subspecies of fish or wildlife or plants and any other group of fish or wildlife of the same species or smaller taxa in common spatial arrangement that interbreed

when mature". The purpose being the "protection of animals which are in trouble in any significant portion of their range, rather than threatened with worldwide extinction" (U.S. House of Representatives 1973, quoted by Pennock and Dimmick, 1997; see also the Committee on Scientific Issues in the Endangered Species Act, 1995).

The Convention on the Conservation of Migratory Species of Wild Animals (1979) also extends its definition of the species and describes a migratory species as "the entire population or *any geographically separate part of the population* of any species or *lower taxon* of wild animals." (with my italics for emphasis). The only recognised lower taxon is the subspecies.

Hence, there is a *de facto* recognition of diversity below the species level contained within many of the international conventions and ensuing domestic legislation. None of those examined provides an explicit definition of the subspecies but implement their conservation legislation by extending the definition of 'species' or by recognising geographically isolated populations of units potentially requiring conservation. The importance of a formal taxonomic framework supported by good systematic practice has been recognised by a number of authors (Cracraft *et al.*, 1998; Dimmick *et al.*, 1999; Wheeler and Cracraft, 1997). There is an implicit reliance on systematic biologists to provide comprehensive, up to date information regarding the status of taxa. The systematics community needs to recognise as one of its responsibilities the need to review taxa at the species level and lower taxonomic levels to assess their conservation priority.

Requirements and Conflicts of Biological and Legislative Definitions

Systematic biology is largely an observational and relational science. It works by selecting representative samples from populations and comparing characters between these specimens (be they linear or angular dimensions, characterisations of structures or DNA sequence data). Any observational study of such natural systems will entail natural variability in the specimens and a certain amount of sampling error. Statistical and other analytical methodologies are used to examine the relationships between samples and used to infer the properties of the whole population and the relationships between the populations. The population-based approach (discussed above) taken in the recognition of subspecies allows for such overlap in phenotypic variation.

In being based on our knowledge and understanding gleaned from samples of natural populations, the nomenclature, classification and inferred phylogenetic relationships of any group of taxa are hypotheses and, as such, are apposite for review. As a result although

systematists endeavour to maintain the stability of classifications (regulated by the relevant international codes), systematics is a dynamic science in which new discoveries can make significant changes to accepted classifications.

In contrast legislation requires clear, definitive terminology with unambiguous interpretations. For legislation directed towards conserving taxa to work on the level of individual organisms (e.g. the regulation of hunting or trading) an individual-based approach to taxonomic identification is necessary. That is any individual organism must be unequivocally identifiable by features intrinsic to a specimen from any part of the individual. Such a level of differentiation may be troublesome even at the species level. Even lions (*Panthera leo*) and tigers (*P. tigris*), that are readily identifiable by their skins, may be difficult to separate by skull morphology to the untrained eye (Kitchener, 1999). As discussed above, individual recognition is often a feature of species level classification (particularly for a PSC approach), while subspecies classifications typically involve trends in the data with ranges that may overlap between subspecies. This population-based approach to subspecies, coupled with the potentially dynamic nature of systematic biology, may make the application of individual organism oriented legislation problematic.

An alternative legislative scenario is to direct efforts to *in situ* protection of habitat and geographic ranges. In this case sound knowledge of subspecies ranges and biogeography, perhaps including multiple species, can allow designation of areas of land for conservation protection.

The utility of taxonomic ranks in legislation is of great importance. The legislative requirement of unambiguous interpretation may preclude the usefulness of much subspecific taxonomy. Legislation requiring individual identification of specimens is, therefore, perhaps best directed at the species level. Alternatively, good systematic analysis of species across their geographic range may allow the determination of subspecies ranges. Accurate determination of these ranges is compatible with the rigorous requirements of legislation making the subspecies rank useful at this level.

The Giraffe as a Case Study

The giraffe, *Giraffa camelopardalis* (L. 1758), was chosen as a case study for this project for a number of reasons. The species and subspecies level taxonomy of the giraffe is complex and, as a result, replete with names and accompanying type material. The 23 names listed by Grubb (1993) have been subsumed into nine currently recognised subspecies (Dagg, 1971) while some recent authors suggest as few as six subspecies may

be valid (East, 1999). The last major review of giraffe subspecific taxonomy was undertaken at the turn of the 20th century (Lydekker, 1904) with a subsequent reanalysis in 1939 (Krumbeigel, 1939). A greater quantity of specimens and the practical and theoretical development of systematic biology since this time make a revision of giraffe taxonomy timely.

The giraffe was historically widespread in Africa and, although the range is now more restricted and fragmented, it is still widespread over much of sub-Saharan Africa (Dagg, 1962; Happold, 1969; Kingdon, 1979; Sidney, 1965; Skinner and Smithers, 1990; see Chapters 2 and 6). The range of the giraffe reflects the range of many other mammalian species in Africa (see maps in Kingdon, 1997 or Boitani *et al*, 1999) and follows the savannah-type vegetation, missing out the heavily forested and desert regions (Figures 2.3 and 2.4). Hence, useful comparisons of phylogeographic variation may be made with other African taxa. Generalised patterns may allow conclusions regarding historical geological, hydrological or other environmental conditions to be made.

Ex situ captive breeding programs are attempting to maintain separate stocks of giraffe subspecies and need to know which of the currently recognised subspecies are valid (Bingaman-Lackey, pers.comm; Brotzler and Schleussner, 1999).

Finally, and most importantly for the aims of this project is that the giraffe provides three sources of data for the investigation and diagnosis of subspecies:

1. Pelage pattern variation;
2. Morphological variation; and
3. Genetic variation.

Patterns derived from the three sources can be compared and assessed in terms of the implications each would have for the designation of subspecies and hence on proposed conservation policies. The three sources can be combined to provide a comprehensive description of geographic variation in giraffe.

The Scope and Structure of this Thesis

This thesis examines the subspecific taxonomy of the giraffe and considers the application of taxonomic ranks to the practical management of within species biodiversity. Numerous methods are available for the quantification of within-species, population-level variation. This study explicitly draws upon methods used in systematic biology and phylogenetic

reconstruction to elucidate relationships between geographically delimited specimen groups.

The general methodology for gathering data on giraffe subspecies is described in Chapter 3. Before this data was used to investigate the geographic structure of phenotypic and genotypic variation, ontogenetic (Chapter 4) and sexual (Chapter 5) differences are explored.

Hypotheses of similarity and difference between geographically restricted sets of specimens are used to test the occurrence of geographic structure between areas. These sets are derived in Chapter 6.

Currently recognised giraffe subspecies are typically distinguished by their characteristic pelage patterns. Chapter 7 considers the consistency of this variation in pelage pattern for the recognition of subspecies.

Notwithstanding the fact that, in life, giraffe subspecies are recognised by their spot patterns, Lydekker (1904) suggested that all of his subspecies could be determined by differences in skull morphology. This is investigated in two chapters in section 4. A 'traditional' morphometric analyses, using multivariate statistics based on measured skull dimensions (Chapter 9) is complemented by an analysis of general skull shape and of particular skull characters (Chapter 10). Chapter 8 demonstrates the selection of variables for inclusion in the traditional analysis.

The phylogeographic structure of the giraffe in sub-Saharan Africa, as determined from variation in neutral genetic markers, offers indications of the relationships between geographically separate populations (Chapter 11). These relationships are further investigated using nested clade analysis to infer actual events in the population history of the species (Chapter 12).

In the final discussion (Chapter 13) the independent analyses are summarised and the implications for the subspecific taxonomy of the giraffe are presented. The general application of species and subspecies level taxonomy in conservation management and legislation are discussed.

Before this, a discussion of the taxonomic status of the giraffe, with a description of the nine currently recognised subspecies follows in Chapter 2.

CHAPTER 2:

THE TAXONOMIC STATUS OF THE GIRAFFE, *GIRAFFA CAMELOPARDALIS* (L. 1758)

Overview of Current Giraffe Taxonomy

The giraffe is a pecoran ruminant artiodactyl and one of only two extant giraffids remaining from a more speciose group. The classification of the giraffe is summarised in Table 2.1.

Order	Artiodactyla
Suborder	Ruminantia
Infraorder	Pecora
Family	Giraffidae
Subfamily	Giraffinae

Table 2.1: *Classification of the giraffe, Giraffa camelopardalis.*

Grubb (1993) lists 23 specific and subspecific synonyms for the giraffe. Nine subspecies are currently recognised (Dagg, 1971). These are listed, with type localities and synonyms, in Table 2.2. The historical and current range of each subspecies are given in Figures 2.1 and 2.2.

Distribution and Habitat of the Giraffe

The giraffe can be found in savannah, scrub and open wooded habitats throughout most of sub-Saharan Africa, only avoiding deserts and dense wooded areas. Although able to graze the giraffe is typically associated with some degree of tree or bush cover and is rarely far from its preferred browse. The historical distribution of the giraffe (Figure 2.3) coincides with the scrubby savannah, dry bushlands and open woodlands while avoiding the extreme aridity of the deserts and the dense wet forests and moist woodlands (Figure 2.4). A broad band of moist Miombo woodland separates the southern from the northern ranges. A recent analysis based on G.I.S. analysis of environmental data indicates the unsuitability of the uninhabited areas shown on the historical range map (Boitani *et al.*, 1999). The current range is much reduced, and often fragmented, compared with the historic range (Figure 6.1).

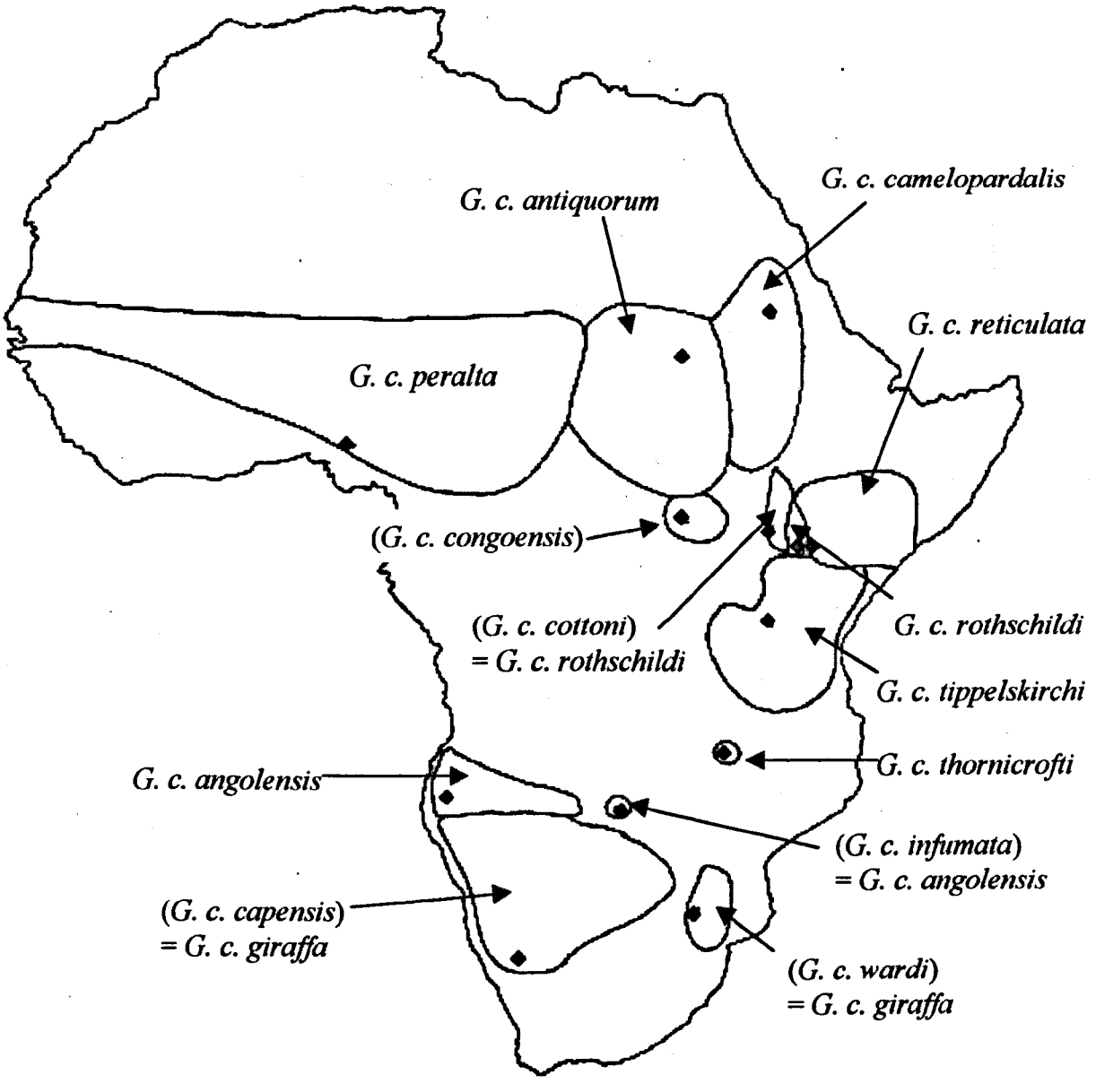

Figure 2.1: *Maximum historical extent of giraffe subspecies ranges. Type localities of currently recognised subspecies are indicated by red diamonds. Type localities for previously recognised subspecies, now subsumed into others, are indicated by blue diamonds. Old subspecies names are given in parenthesis followed by the current name.*
Redrawn from Figure 4-1 of Dagg and Foster (1982).

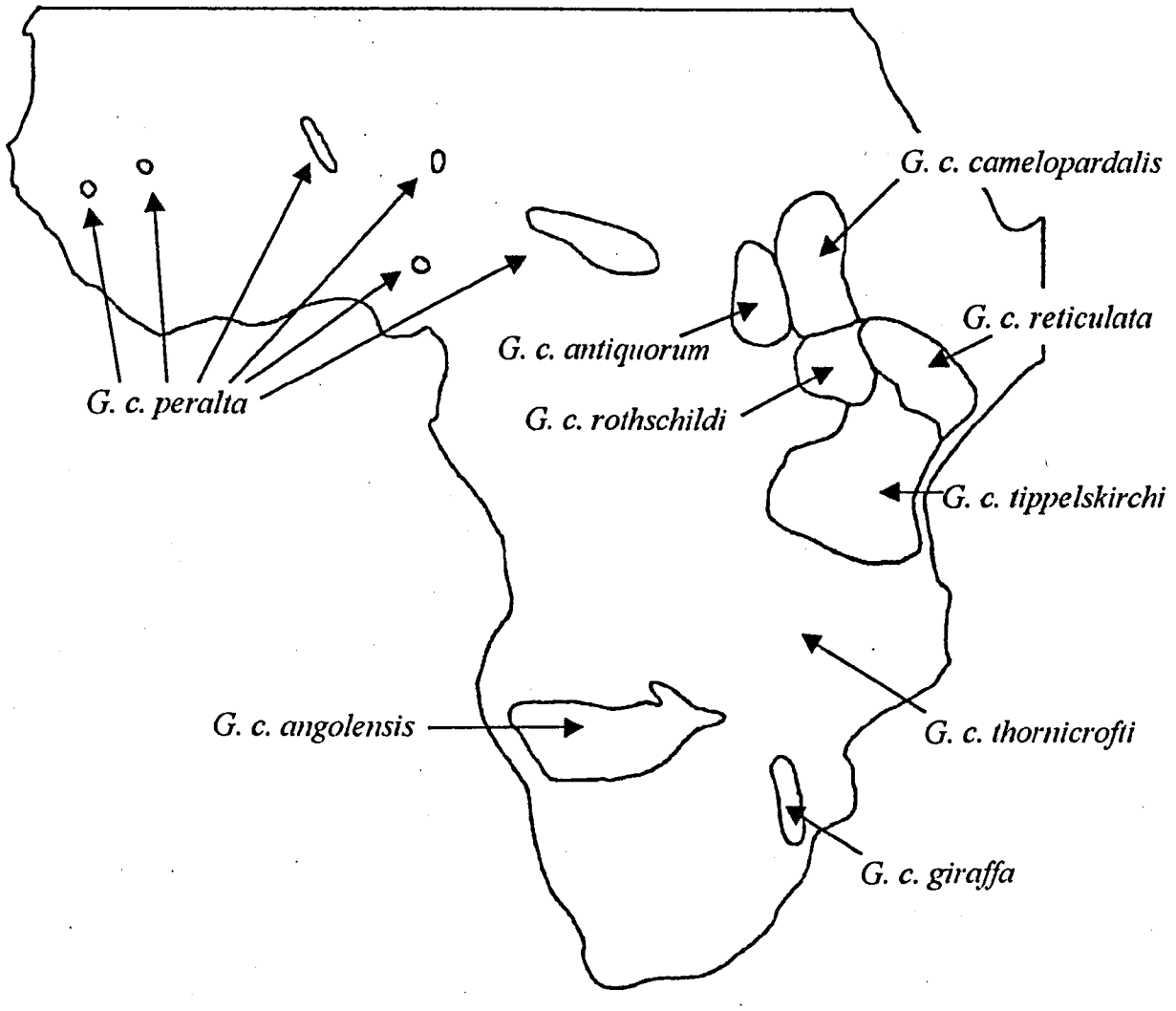

Figure 2.2: *Current giraffe subspecies ranges. Many are restricted in comparison to the historical range (Figure 2.1).*
Redrawn from Figure 3 of Dagg (1971).

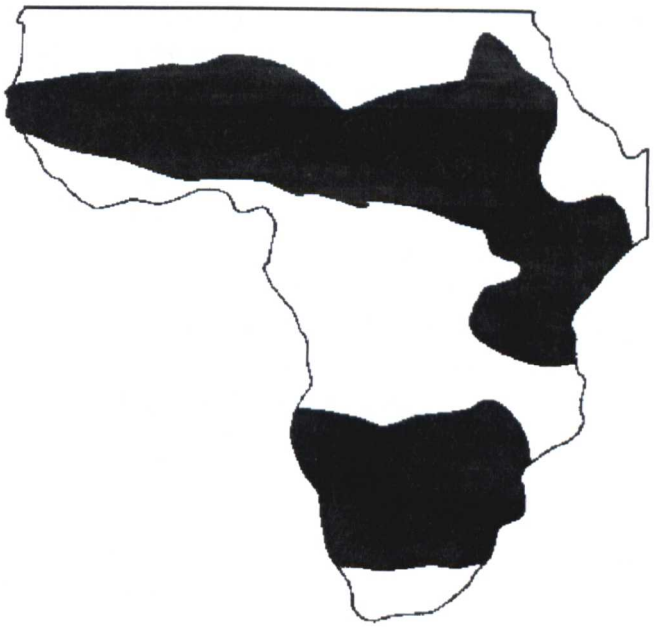

Figure 2.3: *Historical Range of the giraffe. Redrawn from Dagg (1971). The historic range includes evidence from archaeological sites and extends into what is now the Sahara desert, previously less extensive.*

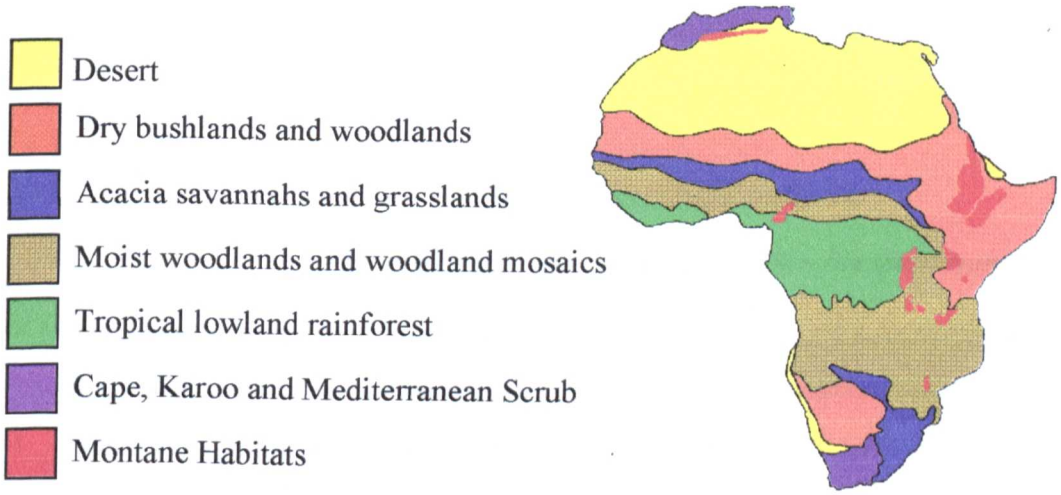

Figure 2.4: *Vegetation Zones of Africa. Adapted from Coe and Skinner (1993) and Kingdon (1997).*

Subspecies	Type Locality	Synonyms
G. c. camelopardalis (Linnaeus, 1758: 66)	"Sennar and Aethiopia"	*biturigum* Duvernoy, *aethiopica* Ogilby, *typica* Bryden, *congoensis* Lydekker (perhaps).
G. c. angolensis Lydekker, 1903: 121.	Cunene River, 240km southwest of Humbe, Angola.	*infumata* Noack.
G. c. antiquorum (Jardine, 1835: 187)	Baggar el Homer, Kordofan (10°N. 28°E) (Harper, 1940).	*senaariensis* Trouessart
G. c. giraffa Schreber, 1784.	Not given. Restricted to Warmbad, South Africa by Dagg (1971) following Brink's (1761) observation of giraffe here.	None.
G. c. peralta Thomas, 1898: 40.	Lokoja, north of the confluence of the Niger and Benue Rivers, Nigeria (Happold, 1969).	None.
G. c. reticulata de Winton, 1899: 212	Loroghi Mountains, Kenya.	*hagenbacki* Knottnerus-Meyer, *nigrescens* Lydekker, *australis* Rhoads (perhaps).
G. c. rothschildi Lydekker, 1903: 122.	West of Lake Baringo (Lydekker, 1908).	*cottoni* Lydekker
G. c. thornicrofti Lydekker, 1911: 484.	Petauke, Zambia.	None.
G. c. tippelskirchi Matschie, 1898: 78	Lake Eyassi, Tanzania.	*schillingsi* Matschie.

Table 2.2: *Subspecies of giraffe, Giraffa camelopardalis, following, and adapted from, the classification of Dagg (1971). The historical extent of subspecies ranges are illustrated in Figure 2.1.*

Higher Level Relationships

The Position of the Giraffidae within the Ruminants

The pecoran ruminant ungulates, or 'higher ruminants', are a monophyletic group diagnosed by a suite of characters of the appendicular skeleton and the dentition with extant taxa possessing a complex four chambered stomach with an omasum (Janis and Scott, 1987). The basal sister taxon of the pecorans is the tragulids (mouse deer or chevrotains).

Within the pecorans the family Giraffidae is characterised by the presence of a bilobed lower canine, the shape of the proximal metatarsus and ossicones that preform in cartilage before calcification to bone (Hamilton, 1978; Janis and Scott, 1987). The Giraffidae is basal to the remaining pecorans with the Bovidae, Moschidae, Antilocapridae and Cervidae sequentially branching above it (Janis and Scott, 1987. See Figure 2.5).

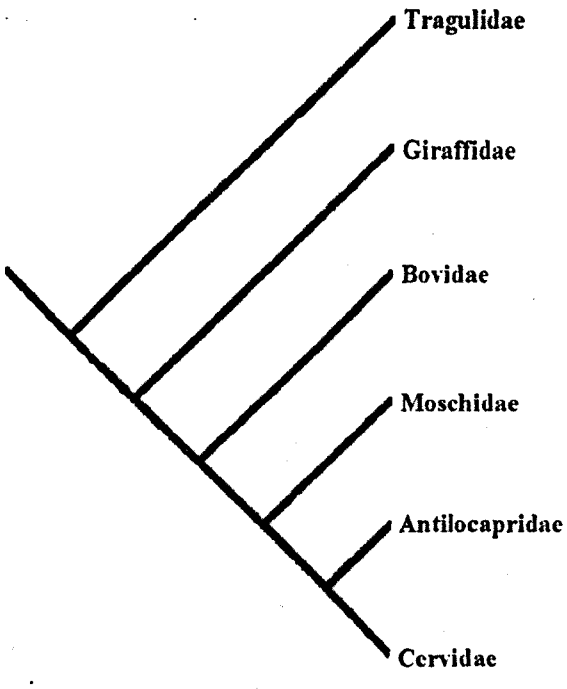

Figure 2.5: *Family level phylogeny of the extant pecoran ruminants (after Janis and Scott 1987).*

The Giraffe and its Relatives

The Family Giraffidae

Only two extant species remain in the formerly more speciose Giraffidae; the giraffe, *Giraffa camelopardalis* and the okapi, *Okapia johnstoni* (Sclater) 1901. The earliest giraffid specimens date from the Miocene and were discovered at Gebel Zeltan, Libya. The smaller specimens, of the genus *Zarafa*, is considered to be related to subsequent forms including *Palaeotragus* and the extant *Okapia*. The second form, the larger *Prolibytherium*, shares a common ancestry with the later, extinct genus *Sivatherium*. The earliest records of the genus *Giraffa* date from the late Miocene and early Pliocene of Kenya (Churcher, 1978).

Churcher (1978) listed nine genera in the family Giraffidae (Table 2.3). Hamilton (1978) provides a phylogeny of the Giraffoidea.

Fossil Relatives

Churcher (1978) describes four extinct members of the genus *Giraffa* besides the extant *G. camelopardalis*; *G. jumae*, *G. stillei*, *G. gracilis* and *G. pygmaea*. *Giraffa jumae* Leakey

1965, is known from localities in east Africa and South Africa and dates from the Late Miocene to Middle Pleistocene. The South African specimens are identified based on isolated teeth and ossicones while the east African material tends to be more substantial. The species is founded on a nearly complete skull and mandible with a substantial proportion of the postcranial skeleton.

Giraffa	Brisson 1756*
Giraffokeryx †	Pilgrim 1910
Helladotherium †	Gaudry 1860
Okapia	Lankester 1901
Palaeotragus †	Gaudry 1861
Prolibytherium †	Arambourg 1961
Samotherium †	Forsyth Major 1888
Sivatherium †	Falconer et Cautley 1832
Zarafa †	Hamilton 1973

Table 2.3: *Genera within the family Giraffidae (after Churcher, 1978). Extinct taxa are marked* †. (* Note that Dagg [1971] considers Brünnich, 1772 as the generic authority for *Giraffa*.)

G. jumae specimens are typically larger than contemporary *G. camelopardalis*. While the dimensions of the skull are greater the teeth are similar in absolute size in comparison between the two species. The ossicones of *G. jumae* originate directly behind the orbital rim, further forward than in *G. camelopardalis*, and extend back parallel to the plane of the skull. The parietal horns end in knobs. No median horn has been identified in specimens assigned to *G. jumae*. No secondary bone deposition is apparent and the occipital ridge is not enlarged. In accord with the larger cranium, the mandible is more substantial than in *G. camelopardalis*. It curves upwards through the diastema and downwards in the incisor region.

Giraffa stellei (Deitrich) 1941, from the Early Pliocene to early Middle Pleistocene of east Africa, was first described as an okapi based on dental characters and forelimb anatomy. Harris (1976) rediagnosed these specimens, according to the dental characters, to "A species of *Giraffa* with teeth of similar morphology but slightly smaller size to those of *G. gracilis*." (Harris, 1976, p. 307, quoted by Churcher, 1978, p. 520). However, Churcher (1978) suggests that the dental characters used to diagnose this taxon are highly individually variable and show greater affinity to another giraffid genus, *Palaeotragus*. It seems that, without additional material, the affinities of the specimens currently assigned to *G. stillei* will remain controversial.

Specimens of *Giraffa gracilis* Arambourg 1947 have been found in east Africa and South Africa from beds dating from the Late Pliocene to Late Pleistocene. The limb bones and neck are of similar absolute length to the contemporary *G. camelopardalis* but are more lightly constructed and show finer proportions in all parts of the skeleton. Dental characters also differ. The bases of the ossicones are oval in cross section, smaller than in *G. camelopardalis* or *G. jumae* and oriented at the same angle as in the modern giraffe. Secondary bone deposition occurs over the ossicones. The area between the orbits is convex (Harris, 1976) and may or may not have a median horn present. Churcher (1978) suggests that, despite generalisations about the relative size of *G. jumae*, *G. camelopardalis* and *G. gracilis*, the skeletal elements overlap in size between these purported species making size alone an unreliable character for species identification.

A fourth *Giraffa* species with ossicones smaller and more delicate than those of *G. gracilis* and flattened on the posterolateral surfaces, occurs in the Early Pleistocene of east Africa. *Giraffa pygmaea* Harris 1976 shows (presumed) sexual dimorphism with secondary bone deposition occurring in the (presumed) males, increasing the proportions of the ossicones, relative to the (presumed) females. Dental characters are typically giraffine but differ from other species by their small size. Churcher (1978) considers this species to be inadequately defined for certain recognition as a separate species.

Extant Relatives

The Okapi, *Okapia johnstoni*

The okapi is the only other extant giraffid and is restricted to north eastern Democratic Republic of Congo where it occurs primarily in riverine forest habitats above 500m. In comparison to the giraffe its smaller body size and relatively shorter legs and neck suit it to its forest habitat. The okapi was originally described as a species of equid and assumed to be related to zebra (Johnston, 1900; Sclater, 1901), as the original specimens were two waist belts made from strips of the black and white striped rump skin. Examination of the hair from these belts suggested a relationship with the giraffe, although did not rule out an allegiance with zebra (Ridewood, 1901). Subsequent examination of skull and skin characters demonstrated the affinities of the okapi to the giraffines (Sclater, 1901; Lankester, 1901a; Lankester, 1901b).

The Specific and Subspecific Taxonomy of the Giraffe

One Species or Two?

The giraffe was included in Linnaeus's (1758) *Systema Naturae* and described based on the work of Belon who had seen a captive giraffe in Cairo some two hundred years earlier. With no specimens to work with, Linnaeus classified the giraffe with the American elk and the red deer into the genus *Cervus*, giving the name *Cervus camelopardalis*. The giraffe was reclassified in 1762 by Brisson and renamed *Giraffa giraffa*. However, Brisson's work was not consistently binomial and so is unavailable under the rules of zoological nomenclature (ICZN, 1999). Brünnich (1772) is the generic authority for *Giraffa*. In 1848 the name was amended to the currently used *Giraffa camelopardalis*. Although now considered a monospecific genus, two species of giraffe have been recognised in the past.

In 1761, about the time Linnaeus published his description of the giraffe, Dutch explorers sent a skin from the Orange River region of South Africa to Leyden University (Dagg and Foster, 1982). The southern giraffe was formally described by Levaillant in his account of his travels in Southern Africa, published in 1790 (Dagg and Foster, 1982). The French anatomist, St. Hilaire, following his study of Levaillant's specimens at the Paris museum and the living northern giraffe in the collection of King Charles X of France (Allin, 1998), decided that the two represented different species. Richard Owen, the British anatomist and zoologist, maintained two species (Owen, 1841) in discussing the features of the Cape and Nubian giraffes separately. Lesson (1842) also classified the northern and southern forms as different species. However, other authors, including Ogilby (1836), Sundevall (1842) and Swainson (1835), considered the two types of giraffes to indicate variation in the same species.

de Winton (1897) reviewed the taxonomic status of the giraffe, considering the paucity of available specimens to be "the reason for the nomenclature of the *two species* being left in a very unsettled state" (p. 274, with my italics added for emphasis), and maintained the separation between northern and southern species. This distinction was further confused by the use of a specimen from Somalia (Thomas, 1894). In the skin the spots were "large, sharply defined, and only separated from each other by narrow pale lines" (Thomas, 1894, p. 135); the pattern now recognised as the reticulated giraffe, *G. c. reticulata*. de Winton (1897) had used this specimen as the basis of his comparison between northern and southern species, rather than material originating from the type locality. de Winton (1899) later recognised his error in using a description of the reticulated giraffe to typify the northern form and sought to "correct a statement ... which may cause confusion if not rectified" (p. 211). He realised that the Somali specimen was, in fact, "very distinct from

the true *Giraffa camelopardalis* from Senaar [the type locality] and the adjacent countries" and was "a strikingly different animal" that was "well worthy of a separate name" (all quotes de Winton, 1899. p. 212). To this end he redefined this taxon as a subspecies of the northern species; *Giraffa camelopardalis reticulata*.

Thomas (1901) argued that the reticulated giraffe deserved specific recognition due to the lack of intermediate forms between it and any neighbouring forms. He further suggests that the northern form (*G. camelopardalis*) grades, through intermediate populations, into the southern form (*G. capensis*) at best making the southern form a subspecies. Hence, while Thomas (1901) also recognised two species these were different from those previously proposed.

In a later review of giraffe taxonomy Lydekker (1904) follows Thomas's arrangement and considers two species of giraffe separated according to the pelage pattern. Lydekker (1904) recognised the 'reticulated' (*Giraffa reticulata*) and the 'blotched' giraffe (*Giraffa camelopardalis*), the latter species containing ten subspecies.

Confusion over the taxonomic status of the giraffe continued with authors differing in their interpretation of giraffe species. Dollman (1929) followed Thomas (1901) and Lydekker (1904) in recognising *G. reticulata* as specifically distinct from all other giraffe populations while Stott (1959) apparently followed de Winton's (1897) classification.

The giraffe is now recognised as a monospecific genus (Dagg, 1971). This is consistent with the biological species concept (Mayr, 1942) as all forms of giraffe may interbreed to produce viable offspring.

Subspecific Variation

Lydekker (1904) carried out the first major review of giraffe subspecific variation that included a reasonable geographic representation of specimens, although his sample sizes were small for many taxa. He considered the reticulated (*G. reticulata*) and blotched (*G. camelopardalis*) giraffe to be separate species with the former monotypic. Hence, he only described subspecific variation in the latter species. Lydekker's subspecies are listed in Table 2.4 and their geographic distributions shown in Figure 2.6. Although Lydekker's (1904) geographic ranges are clearly inadequate by today's standards, and even incorrect in some cases, his classification provides the basis of the contemporary classification. His descriptions of subspecific variation were largely based upon the variation of the pelage

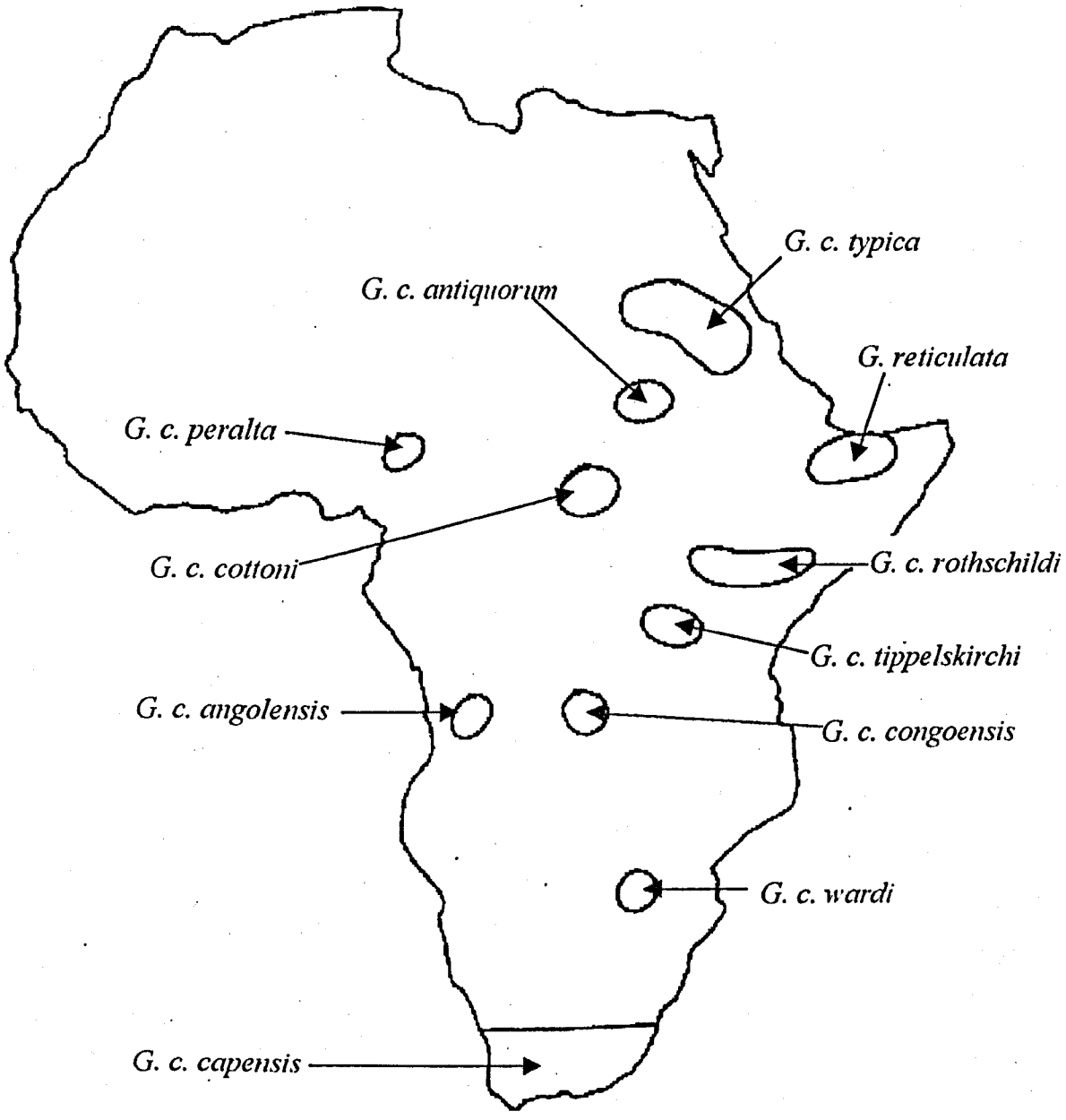

Figure 2.6: *Giraffe subspecies ranges according to Lydekker (1904). Lydekker recognised two species of giraffe;* Giraffa reticulata *and* Giraffa camelopardalis. *Within the latter he recognised ten subspecies. (Redrawn from Lydekker, 1904. Text figure 23).*

patterns. However, he states that "most, if not indeed all, of the subspecies of Giraffe are distinguishable by cranial differences." (Lydekker, 1904, p. 202).

Vernacular Name	Scientific Name
Netted Giraffe	*Giraffa reticulata*
Blotched Giraffe	*G. camelopardalis*
Nubian Giraffe	*G. c. typica*
Kordofan Giraffe	*G. c. antiquorum*
South Lado Giraffe	*G. c. cottoni*
Baringo Giraffe	*G. c. rothschildi*
Kilimanjaro Giraffe	*G. c. tippelskirchi*
Congo Giraffe	*G. c. congoensis*
Angola Giraffe	*G. c. angolensis*
Northern Transvaal Giraffe	*G. c. wardi*
Cape Giraffe	*G. c. capensis*
Nigerian Giraffe	*G. c. peralta*

Table 2.4: *Giraffe species and subspecies described by Lydekker (1904). The geographic ranges delineated by Lydekker are shown in Figure 2.6.*

Lydekker (1911) subsequently named a further blotched giraffe subspecies (*G. c. thornicrofti*) and a subspecies of the netted, or reticulated, giraffe (effectively creating two subspecies within this species, the nominate subspecies and the newly recognised *G. r. nigrescens*).

Krumbeigel's (1939) work followed Lydekker's (1904 and 1911) classification. Rather than reviewing the validity of Lydekker's subspecies Krumbeigel sought to describe them more adequately using larger sample sizes. His classification recognises a single species with two subspecies. Infrasubspecific groups, previously recognised as subspecies, were recognised by a fourth latinised name. As such Krumbeigel's work is not consistent with the requirements of the ICZN (1999). Krumbeigel extends Lydekker's subspecies ranges and presents a more realistic range map that has, apparently, remained the basis for contemporary range maps (see Figure 2.7).

Some of Lydekker's (1904; 1911) subspecies have been subsumed into, and synonymised with, other groups giving the list of nine subspecies currently recognised (Table 2.2). Dagg (1971) is the authority most frequently consulted for the status of giraffe taxonomy. She based her classification on that of Ansell (1968) who provided detailed descriptions of the ranges of each subspecies. Ansell's (1968) classification, in turn, was largely based on Dagg's previous work (Dagg, 1962; 1968) and that of Haltenorth (1962). However, none of these authors examined specimens or undertook an extensive alpha taxonomic systematic analysis in order to rearrange the subspecies and subsume one subspecies into another. Hence, the validity of the content of each subspecies is, apparently, based on

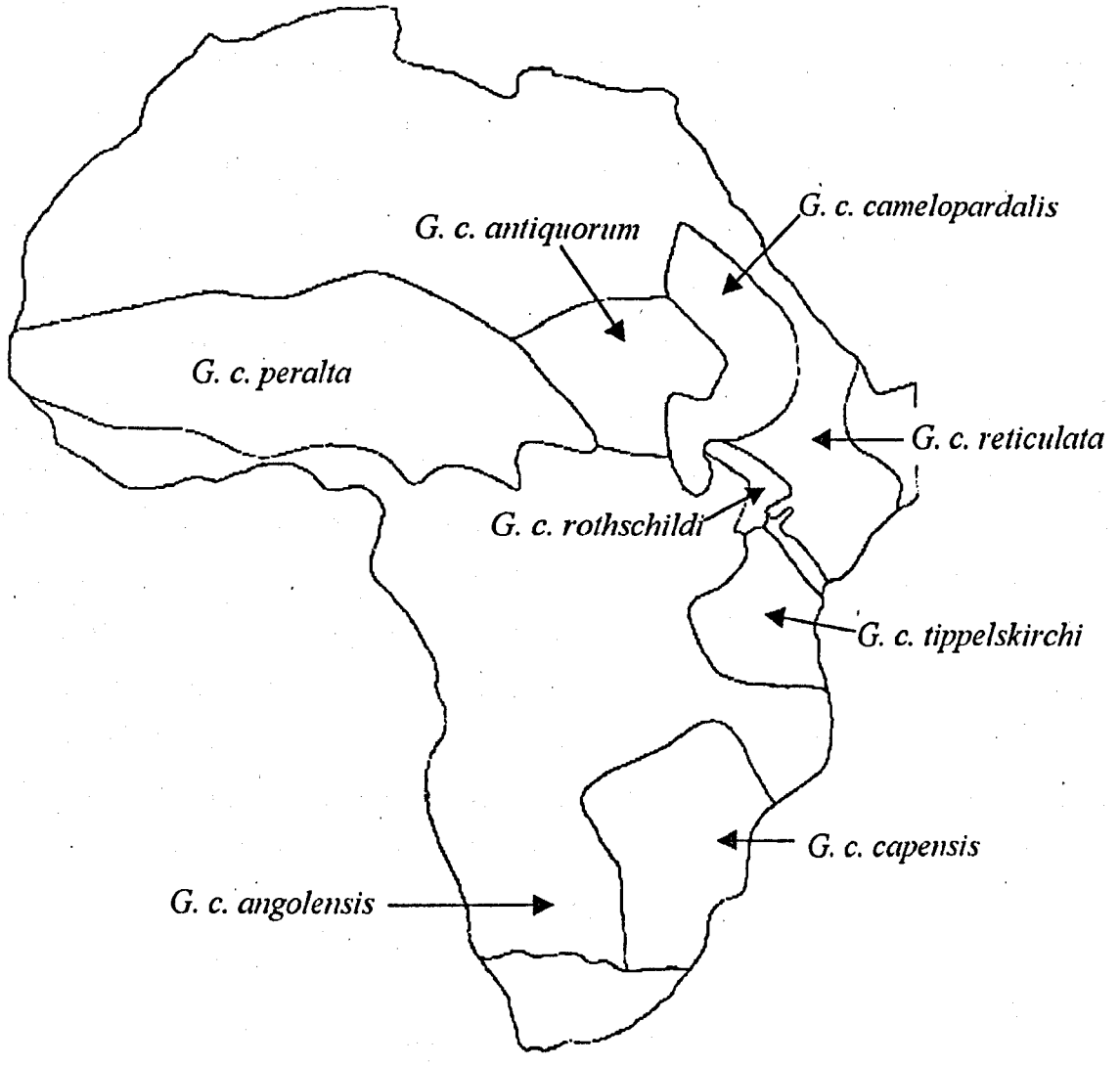

Figure 2.7: *Giraffe subspecies ranges according to Krumbeigel (1939). Krumbeigel reviewed Lydekker's (1904) work and extended his subspecies ranges. Redrawn from Krumbeigel (1939) p. 91, Figure 49. Note that the 'open' ranges (of G. c. reticulata and G. c. angolensis) are reproduced as drawn by Krumbeigel.*

personal preference. Ansell (1971. p. 13, in an updated version of his 1968 paper) stated that his list of subspecies "should be regarded as provisional". Indeed the range described by Ansell (1971) for *G. c. rothschildi* (and, as a result, those of *G. c. reticulata* and *G. c. tippelskirchi*), derived from Dagg's (1968) range map is erroneous and later corrected by Dagg (1971). Hence there is reason to believe that the current classification owes as much to the opinion of previous authors as to rigorous taxonomic appraisal.

Recently East (1999, p. 94) has suggested that "considerable uncertainty surrounds the validity and geographical limits of most of the described subspecies of the giraffe" due to the lack of geographical barriers between supposed subspecies. He suggests instead six "subspecies/subspecies groups" (see Figure 2.8), but states that these groupings are "arbitrary, like other treatments of giraffe subspecies". Hence, a re-evaluation of subspecific variation in the giraffe is clearly warranted.

The uncertainty in giraffe subspecies taxonomy is illustrated by comparing the historical and contemporary ranges of southern African subspecies (Figures 2.1 and 2.2). In the historical range map *G. c. angolensis* is restricted to Angola (except for the inclusion of the former *G. c. infumata* of south-western Zambia) with *G. c. giraffa* occurring in two geographically separate populations. The current range map suggests that *G. c. angolensis* covers the contiguous western populations, while *G. c. giraffa* refers to eastern individuals. Furthermore, Meester *et al.* (1986) agree with the east / west subspecies split but use *G. c. capensis* (Lesson, 1842) in preference to *G. c. giraffa* to designate the eastern subspecies (from northern South Africa, Mozambique and southern Zimbabwe). Their use of this name for this subspecies is incorrect and adds to the confusion.

Subspecies Descriptions

The following briefly summarises the descriptions of the nine currently recognised subspecies. These accounts are based on the descriptions of the type specimens or type series and so may not necessarily accurately represent the range of individual variation seen in each population. Where conflicts occur between the descriptions of different authors these are noted. These descriptions are largely based on those of Dagg and Foster (1982), Krumbeigel (1939) and Lydekker (1904).

Characteristics of the nine currently recognised subspecies (Dagg, 1971), using original descriptions, where available, along with Lydekker's (1904) review, are summarised more extensively in Appendix 1.2.1. Where previously recognised subspecies have been subsumed into current subspecies these are described where they differ.

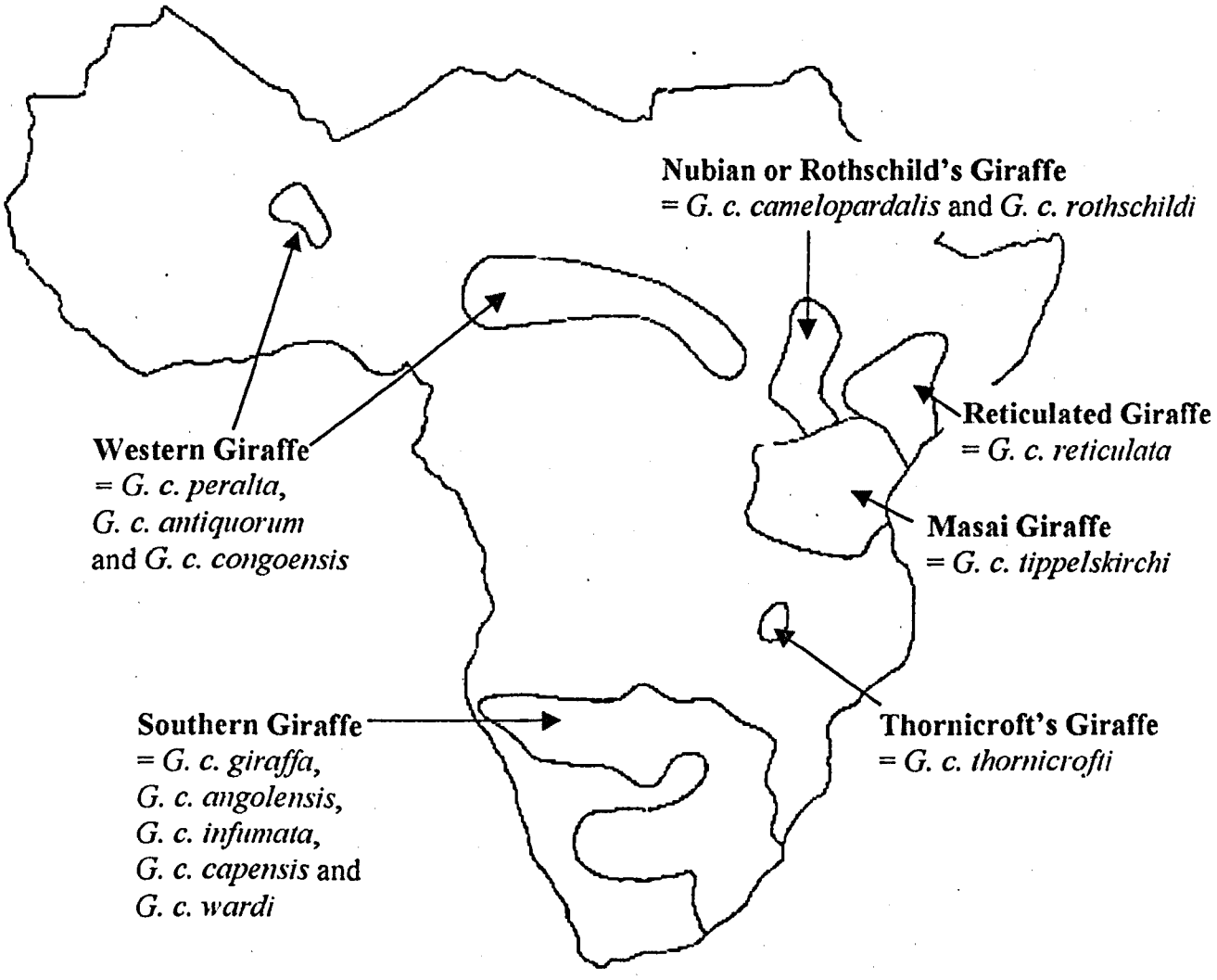

Figure 2.8: *The ranges of the subspecies groups proposed by East (1999). Contiguous ranges are named according to area and subspecies subsumed into these groups. East notes that his six groups are arbitrary, but considers this to be "like other treatments of giraffe subpecies" (p. 94). Redrawn from East (1999) p. 94.*

Giraffa camelopardalis camelopardalis (L.) 1758: 66.

Linnaeus gave the type locality of the species, and hence the nominate subspecies, as "Sennar and Aethiopia". The range of this subspecies is now thought to occupy eastern Sudan and the extreme west of Ethiopia.

The male has a developed median horn. The chestnut coloured body spots are smooth edged and strongly defined, divided by an almost white network of lines. According to Lydekker (1904) the outsides of the legs are spotted below the knee, particularly the hind limb, although Dagg and Foster (1982), Krumbeigel (1939) and Trouessart (1908) all state that the legs are pure white below the hocks. Meanwhile the insides are free of spotting, as is the belly. The sides of the head are spotted, with some spotting on the face.

Giraffa camelopardalis angolensis Lydekker, 1903: 121.

The type specimen of *G. c. angolensis* was collected 240kms south west of Humbe, Angola, close to the Cunene River. The Cunene and Cubango Rivers form a barrier between Angolan and Namibian giraffes effectively separating the ranges of *G. c. angolensis* and *G. c. giraffa*. The range extends eastwards to the Kwando River, although a population to the east of this river in Zambia, previously considered a separate subspecies (*G. c. infumata* Noack 1908) is now considered to be part of *G. c. angolensis*. The range extends northwards into Angola to approximately 13°S.

The male is a two-horned giraffe with large brown body spots, the edges of which are slightly notched (Lydekker, 1904) or with angular projections (Krumbeigel, 1939) on a near-white ground colour. While Lydekker (1904, p. 221) considers the body spots to be "ill-defined", Dagg and Foster (1982) describe them as "well differentiated" (p. 159) and Krumbeigel (1939) suggests that the margins are clear and complete. Spots on the neck and rump break up into much smaller spots than the rest of the body. The legs are fully spotted. Only the lower part of the face is spotted. There is a small white ear patch present.

Giraffa camelopardalis antiquorum (Jardin) 1835: 187.

Jardine (1835) gave a type locality of "Sennar and Darfour". Lydekker (1904) reduced this to "Kordofan", while Harper (1940) suggests a further restriction to Baggar el Homer, Kordofan (approx. 10°N. 28°E). The range of *G. c. antiquorum* includes western and south

western Sudan. Its range borders *G. c. camelopardalis* to the east and, perhaps, *G. c. peralta* to the west.

Males have a median horn. The body, leg and neck spots are somewhat smaller and more irregular than in *G. c. camelopardalis* making the network less regular. The insides of the legs are spotted and spots may sometimes extend below the hocks.

Giraffa camelopardalis giraffa Schreber 1784: pl. 255

This is the southern most subspecies and is considered to contain *G. c. wardi* (Dagg, 1971). Schreber (1784) did not give a type locality, while Boddaert (1785) gave only "Cape of Good Hope" (Dagg, 1971). Dagg (1971) restricts the type locality to Warmbad (24°53'S. 28°18'E), north of the Orange River, South Africa. The range extends from northern Namibia to the west through Botswana to western and southern Zimbabwe, northern South Africa and into south-western Mozambique.

The males do not have a well developed median horn. The dark body spots are more or less round with some fine projections on a tawny ground colour, with no tendency to break up into stars. The legs are fully spotted with dark spots that decrease in size further down the leg.

Although Dagg (1971) synonymises *G. c. wardi* with this subspecies, Lydekker's (1904) description of *G. c. wardi* disagrees with the description given above for *G. c. giraffa* on a number of points (see Appendix 1.2.1).

Giraffa camelopardalis peralta Thomas 1898: 40.

The type specimen was collected at the junction of the Benue and Niger rivers in Nigeria. Thomas (1898) originally reported collection to the south east of the confluence although the contemporary distribution suggests that it was more likely to be to the north (Happold, 1969).

The potential range for the giraffe in west Africa extends from Senegal in the west across to Chad and the Central African Republic where it meets the western extent of *G. c. antiquorum* in western Sudan. The northern range is limited by the southern extent of the Sahara desert, while the southern limit is delineated by the Benue and Niger river systems (Happold, 1969). However, the current range is much reduced (Figure 6.1).

This subspecies was originally described due to its (apparently) great size. The parietal horns diverge and are more erect than in other subspecies. Males have a well developed

median horn and the female type specimen has a bony structure over the frontal bones. The body spots are coarsely divided into large lobes and occur on a reddish ground colour. Spots extend below the hocks.

Giraffa camelopardalis reticulata de Winton 1899: 212.

Perhaps the most strikingly patterned of all giraffe subspecies, the reticulated giraffe was first described in 1894 (Thomas, 1894) in Somalia. de Winton (1899) established the type locality as the Loroghi Mountains in Kenya following subsequent collection of the first fully adult material.

Males have a median horn. The large red-brown spots are well-defined, polygonal and separated by network of narrow white lines. Lydekker (1904) states that the legs are white below the hocks. Meanwhile, Dagg and Foster (1982) assert that "spotting may extend some distance below the hocks" (p. 157). Krumbeigel (1939) considers that spots are generally not found below the hocks, but sometimes may be seen.

Giraffa camelopardalis rothschildi Lydekker, 1903: 122.

The type specimen of this subspecies was reported as taken to the east of Lake Baringo, Kenya, although Lydekker (1908) later adjusted the type locality to the west of the lake (Dagg and Foster, 1982). The range is bounded to the east by the eastern Rift Valley and extends westwards and northwards into Uganda and southern Sudan. The southern extent of the range is approximately 1°S.

Thomas (1901) described this subspecies as the five-horned giraffe due to the development of the occipital ridge into paired 'horns'. Lydekker (1904) concurs but notes that the development of the occipital horns may be individually variable, an opinion echoed by Krumbeigel (1939). The median horn is always well developed in adult males. The large, dark body spots typically have complete margins but are sometimes parted at the edges. The spots show a tendency to break up with paler radiating lines or streaks inside the dark spot. The legs below the hocks are typically unspotted, although where spotting does occur it never extends all the way down to the hooves.

Giraffa camelopardalis thornicrofti Lydekker, 1911: 484.

This subspecies was named after Henry Thornicroft, the collector (Chituta, 1988). The type specimen was taken from Petauke in the Eastern Province of Northern Rhodesia, now Zambia. Thornicroft's giraffe are now restricted to the Luangwa Valley in north eastern Zambia.

The median horn is little developed and unobtrusive in males. The body spots are notched and slightly stellate in their appearance. The neck spots typically appear to be elongated. While Dagg and Foster (1982) suggest that the legs are fully spotted, Lydekker (1911. p. 484) mentions a "uniformly tawny colour of the lower portion of the limbs".

Giraffa camelopardalis tippelskirchi Matschie, 1898: 78.

Lydekker (1904) synonymised Matschie's (1898) *G. tippelskirchi* and *G. schillingsi* into a single subspecies with the type locality on the south east shore of Lake Eyassi (Approximately 3°40'S. 35°15'E). The range extends through southern Kenya into the northern half of Tanzania. The northern limit runs south of Lake Nakuru and Mount Kenya and goes southwards to the Rufiji River and Lake Rukwa in Tanzania. The eastward limit of the range extends to the coast and goes no further west than Lake Rukwa.

The males typically have a median horn, although its presence may be individually variable in this subspecies. The body spots have been described as "jagged" (Lydekker, 1904), splintered (Dagg and Foster, 1982) or "shattered" (Krumbeigel, 1939). They have a sharply lobed, distinctly stellate appearance. The leg spots continue down to the hooves.

Eleven museum and research institution collections were visited for this study to re-evaluate the taxonomic status of the giraffe. The next section gives an overview of the specimens examined and the data obtained from each (Chapter 3). It goes on to investigate the age (Chapter 4) and sex (Chapter 5) of the specimens allowing any possible confounding effects of these variables to be accounted for. A set of specimens for inclusion in subsequent analyses is derived. Finally, specimens are grouped according to their geographical provenance (Chapter 6) to provide primary hypotheses of relatedness in accord with the geographically-based definition of subspecies (p. 11) used in this thesis.

CHAPTER 3:

SPECIMENS EXAMINED, DATA GATHERED AND INFORMATION COLLECTION

Rationale for Data Collection

This study seeks to examine the effects of using different sources of data, and different analytical techniques, on the elucidation of geographically structured variation in giraffe populations across their entire African range. The giraffe was chosen as the study taxon as it allows examination of three independent sources of information; pelage pattern morphological and molecular variation. Museum samples were used so that, where available, all three sources of data could be used to derive information for the same individual animal.

Four types of data were gathered:

1. Pelage patterns information (a series of photographs of each body part);
2. 'Traditional' morphometric data for use in multivariate statistical analyses (point to point measurements of the cranium, mandible and appendicular skeleton);
3. Shape information for eigenshape analysis of aspects of skull shape (a series of photographs of the skull, from different angles);

and,

4. Tissue samples for retrieval of DNA sequence data (a small quantity of muscle tissue was gathered, where available, from the specimens).

This chapter summarises the data gathering protocols followed to assemble the data utilised in the ensuing analyses. Extensive use was made of the collections in the Natural History Museum, London to develop the data collection protocols before visits were made to other institutions. Collections of giraffe specimens were examined in southern Africa, North America and the United Kingdom. Collections in Europe and other parts of Africa were not visited for this study due to time and financial constraints.

Institutions Visited

Eleven Institutions were visited to examine giraffe specimens. These institutions are listed in Table 3.1.

Region	Institution	Location	Abbreviation
UK	Natural History Museum	London	BMNH
	Powell-Cotton Museum	Birchington, Kent	PC
North America	American Museum of Natural History	New York	AMNH
	Field Museum of Natural History	Chicago	FMNH
	United States National Museum	Washington DC	USNM
Southern Africa	Etosha Ecological Institute	Etosha National Park, Namibia	EEI
	Kruger National Park	RSA	KNP
	National Museum	Bloemfontein, RSA	NMB
	National Museum of Natural History	Bulawayo, Zimbabwe	NMZB
	South African Museum	Cape Town, RSA	ZM
	Transvaal Museum	Pretoria, RSA	TM

Table 3.1: *Institutions visited (listed alphabetically by region) to examine giraffe specimens, with locations and institutional abbreviations.*

In addition, tissue samples from four specimens were obtained from Bushmanland, Namibia by Nigel Berrimen.

Material Examined

Museum specimens from 355 individual animals were examined. A complete list of specimens examined in each institution is appended (Appendix 2.3.1). Details of the specimens used in each analysis are provided in the respective sections on pelage, morphology and molecular analysis.

Specimen Information and Condition

All available information from the specimen label and museum records (e.g. card index systems, accessions catalogues) was recorded for each specimen. Detailed notes on the condition of each specimen were made recording any damage to the specimen.

Data Gathered

Pelage Pattern Analysis

Photographs of 72 complete or partial museum skins were taken. Following literature accounts of giraffe pelage variation the patterns were coded for various pelage characteristics and analysed using a modified Population Aggregation Analysis method (Davis and Nixon, 1992).

Age Determination

The age of each skull was determined by examination of the stage of tooth eruption and the extent of tooth wear. Records were made of tooth eruption profiles for all sub-adult animals. The age of each adult specimen was estimated using tooth wear measurements and the equations of Hall-Martin (1975, 1976). All specimens with the full adult dentition in wear were measured and photographed for inclusion in the morphological analyses.

'Traditional' Morphometrics

A set of 59 measurements was used to quantify observed morphological variation. These measurements were used to determine morphological size correlations with age (Chapter 4) and sex-determined dimorphism (Chapter 5) as well as for the multivariate statistical analysis of geographically structured morphological variation in giraffe phenotypes (Chapter 9).

A complete list of the measurements taken, with a full description of each is given in Appendix 2.3.3 (Appendix 2.3.4 gives an alphabetical list of measurements made). Diagrams indicating the measurements taken are presented in Appendix 2.3.6. All measurements taken for each specimen were recorded by hand in purposely designed recording sheets (see Appendix 2.3.2).

Measuring Devices

A spring balance (described in Appendix 2.3.5) was used to weigh the skulls with the skull securely suspended by soft cotton straps.

Three sets of measuring callipers were used to gather linear measurement data while a cloth measuring tape was used to measure curved distances. These are described in Appendix 2.3.5 along with a list indicating which measuring device was used for each

measurement. Generally the smallest set of callipers which could be used for a given measurement was used. As far as possible the same pair of callipers was used consistently for each variable on each specimen.

Geometric Morphometrics

Four views of each adult skull were photographed to characterise skull variation. These included photographs of the left and right skull profiles, a front view of the parietal horns and a plan view of the face and muzzle region from above. A more detailed description of the photographs taken, along with example photographs, and an assessment of the possible associated errors is given in Chapter 10.

Tissue Samples

Each specimen was examined for the presence of dried muscle tissue with small quantities removed from crania or skeletal elements where available. No skin samples were used in this study. The use of tissue samples to derive DNA sequences is detailed in Chapter 11, with the analysis of this genetic data presented in Chapters 11 and 12.

CHAPTER 4:

AGE DETERMINATION OF GIRAFFE SPECIMENS AND CONTROLLING FOR AGE AS A CONFOUNDING VARIABLE IN THE ANALYSIS OF GEOGRAPHIC VARIATION.

Introduction

Ontogenetic variability must be understood and accounted for in any taxonomic study. Typically mammals show deterministic growth whereby growth continues until it closely approaches an asymptotic value. Such a point could be used to define 'morphological maturity' and may be used to mark the transition between sub-adult and adult ontogenetic phases[1]. Hence, it is necessary to have criteria whereby morphologically adult and sub-adult individuals can be distinguished. In male giraffe the problem is complicated further by the continual deposition of secondary bone on the upper surface of the skull throughout the life of each individual (Spinage 1968; Urbain *et al.* 1944). The resulting change in cranial morphology with age could confound any morphological pattern thought to be related to geographical variation if age biases are present in the sampling and not accounted for. Hence, a method to determine either absolute or, at least, relative age is needed.

Here, individuals are considered to be adult when the mandibular canines (C_1) are fully erupted and in wear. Any individuals without C_1 fully erupted and in wear are considered 'sub-adult'[2] and removed from subsequent morphometric analyses. That this approach represents an objective measure of 'morphological maturity' in giraffe is discussed and justified later. Hall-Martin (1975, 1976) states that this criterion separates adult and sub-adult individuals at the age of six years old. Meanwhile, Singer and Boné (1960) give seven years old as the age of full eruption of the mandibular canine. While a captive male giraffe has fathered a calf at the age of two years and eight months (Crandall, 1964), males in the wild reach sexual maturity at three and a half years old (Dagg and Foster, 1982). Hall-Martin *et al.* (1978) report that spermatogenesis begins between three and four years

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[1] An alternative definition of adulthood might be the age of first reproduction representing 'sexual maturity'.

[2] I am using the term 'sub-adult' as a generic designation to avoid confusing or conflicting use of terms. Here it encompasses all descriptive age classes below adulthood used by other authors including foetus, newborn, baby, infant, juvenile, adolescent, immature and sub-adult.

of age with a coincidental rapid rise in testicular weight and diameter of the seminiferous tubule. Testes mass, epididymis mass and bulbo-urethral mass continue to increase with age before reaching an asymptote at approximately 12 years of age (Hall-Martin, 1975). Hall-Martin (1975) and Hall-Martin and Skinner (1978) report age at first conception as 4 years and 8 months for females in the wild (and 3 years and 10 months for captive females). Hence, splitting sub-adults from adults at six years old would represent a conservative estimate of 'physiological adulthood' (i.e. age of sexual maturity) while acting as a useful surrogate for 'morphological adulthood'.

Materials and Methods.

Specimens Examined.

Ages were determined for all skull specimens examined (see Appendices 2.4.1 and 2.4.2). A particular consideration in choosing an age determination method for this study is that all specimens examined came from museum collections or comparative collections of research institutions. Hence a non-destructive method was required.

Age Determination

Tooth Eruption

Tooth eruption was examined in all skulls where teeth were undamaged. Age classes were assigned according to Hall-Martin's (1975, 1976) criteria (See Table 4.1).

A problem arose for those specimens where a mandible was not available or the incisiform teeth were absent or damaged. In these cases eruption of maxillary molariform teeth had to be used as a guide. The second premolar is the last of the permanent molariform teeth to erupt. It shows signs of wear at approximately five and a half years of age and represents age class 11 of Hall-Martin (1975, 1976). At this age the deciduous canine is still present, although all other incisiform teeth are fully erupted and in wear. Hence, in the absence of the incisiform teeth, due to damage to the teeth or a missing mandible, the eruption and wear condition of the second maxillary premolar (PM^2) has been used to separate adults and sub-adults.

The method outlined above leads to the definitive classification of individuals without PM^2 in wear as sub-adults and those with C_1 erupted and in wear as adults. The lack of a mandible for some specimens means that there is no way of determining the state of eruption of the mandibular canine for that individual. This leads to ambiguity in

determining the age of specimens with the PM^2 erupted and in the early stages of wear but, for whom, no mandibular canine is available. For any individual, with the PM^2 well-worn adulthood can be safely assumed. Due to missing mandibles there are a few specimens in between these two definitive points that can not be classified unequivocally as adult or sub-adult on the specified tooth eruption criterion.

Stage	I_1	I_2	I_3	C	PM_2	PM_3	PM_4	M_1	M_2	M_3	Approximate age.
0	(D)	(D)	(D)	((D))	(D)	(D)	(D)	-	-	-	Late foetuses and at birth.
1	D	D	D	(D)	D	D	D	-	-	-	Up to 4 weeks.
2	D	D	D	D	D	D	D	((P))	-	-	Up to 10 months
3	D	D	D	D	D	D	D	(P)	-	-	Up to 12 months
4	D	D	D	D	D	D	D	P	-	-	12 to 15 months
5	D	D	D	D	D	D	D	P	((P))	-	15 to 18 months.
6	D	D	D	D	D	D	D	P	(P)	-	18 to 30 months.
7	D	D	D	D	D	D	D	P	P	-	Up to 3 years.
8	D	D	D	D	D	D	D	P	P	(P)	Up to $3^1/_2$ years
9	P	D	D	D	D	D	D*	P	P	P	Up to 4 years
10	P	D/P	D	D	D/((*P))	D*/(P)	(P)	P	P	P	Up to 5 years.
11	P	P	P	D	P	P	P	P	P	P	Up to $5^1/_2$ years.
12	P	P	P	(P)	P	P	P	P	P	P	Up to 6 years.
13	P	P	P	P	P	P	P	P	P	P	Over 6 years.

Table 4.1: *Giraffe tooth eruption stages with absolute age estimates (adapted from Hall-Martin, 1976).*
*D = deciduous tooth in wear. P = permanent tooth in wear. ( ) = tooth in advanced stage of eruption. (( )) = cusps just emerging above alveolar margin. * = permanents' cusps can be seen between roots of deciduous teeth. D/P = either D or P.*

The use of the second premolar eruption criterion and the acceptance of five to five and a half years of age as the delineating age to separate adults from sub-adults across all specimens would effectively remove this problem (except in those few specimens with bilateral damage to the PM^2s). Could this single criterion be used to separate adults from non-adults?

Although the eruption and coming into wear of the second maxillary premolar may coincide, or come after, the attainment of *sexual* maturity (if not the time of first breeding) the purpose here is to separate adults from sub-adults in terms of *morphological* maturity such that comparable phena can be compared in subsequent morphological analyses. Adult male giraffe deposit secondary bone over the surfaces of the skull lacking muscle attachments, particularly over the median and parietal horns. Females do not. For this reason morphologically sub-adult males resemble adult females in their skull dimensions. Indeed sub-adult male and sub-adult female skulls can not be told apart readily, while sub-adult males can be differentiated from adult females only by examining tooth wear to

indicate age[3]. So, an age where male and female giraffe may be told apart with confidence is required to separate the phena and allow further analysis of the skull morphology of the two sexes separately. This age is taken herein to represent the age of morphological maturity in the ontogeny of the giraffe.

Due to the incidence of secondary bone growth in males but not in females any error is always that male skulls are classified as female. Using the classification function derived from a discriminant analysis of the known adult skulls, both skulls recorded as male at age class 10 (PM^2 not yet in wear) were wrongly classified as female. All skulls in age class 13 (with the full adult dentition in wear) were correctly classified to their gender. Meanwhile of four male skulls in age classes 11 or 12 (with PM^2 in wear but C_1 not fully erupted) one was classified as a female skull.

Although the sample size for the intermediate skulls (classes 11 and 12) is small, combined with the information from other age classes, it may indicate a transition. All skulls in age class 10 or below are unequivocally morphologically immature with males indistinguishable from females. All skulls with a full adult dentition in wear (age class 13) are unequivocally morphologically mature with both sexes correctly classified. While three of the male skulls in age classes 11 and 12 were correctly classified by the classification function derived from adult data, the fourth was not. There is no way of knowing how many of the skulls recorded as or assessed to be female in age classes 11 and 12 ($n = 5$) may, in fact, be misclassified males. Hence, these data may indicate that age classes 11 and 12 represent a transition between morphologically immature and morphologically mature giraffe, as defined above. Accordingly, the age where males and females can be told apart with confidence - the age of morphological maturity - is considered to be the age class 13 (six years old and older). This is coincident with the mandibular canine coming in to wear and is taken as the preferred criterion for assessing the morphological maturity of the skulls and assigning the skull as an adult or sub-adult.

While the problem does remain where mandibles are not available or incisiform teeth are damaged, using the eruption and wear of the mandibular canine as the criterion for separating sub-adults from adults minimises the number of misclassifications which will be included in subsequent analyses.

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[3] A 'feminine' skull with worn teeth must be an old female, but a 'feminine' skull with little wear could be male or female.

Tooth Wear

Hall-Martin (1975, 1976) advises the use of lingual occlusal surface width and lingual cusp height of the first maxillary molar to estimate the age of adult giraffe.

All measurements were taken on specimens with undamaged M^1 using 15cm engineering callipers (see Appendix 2.3.5). Where possible measurements were taken bilaterally and the mean of the two measurements used. Where only one tooth was available measurements were used from the single tooth. Anterior and posterior lingual cusp height was measured directly for all specimens (n = 132).

Assessing the Relationship of Age with Geographical Sampling

Univariate Relationships of Cranial, Mandibular and Skeletal Variables to Estimated Age

Univariate product moment correlation coefficients were calculated to examine the relationship of each variable to estimated age for adult animals of both genders. Male and female data sets were examined separately.

Testing Geographic Sampling Bias of Age Estimates

To test the null hypothesis that there is no difference in age distributions between the geographically defined groups a Kruskal-Wallis test (a non-parametric equivalent to the parametric analysis of variance) was performed on the estimated ages of all grouped specimens (for which such estimates were available). A non-parametric test was used as, for some of the specimens, age estimates were presented in a categorical manner. Those specimens whose wear of the occlusal surface of the first molar was such that the buccal and lingual lophs were no longer distinct can only be classified as greater than a given maximum age. The greatest age estimate for a specimen with distinct lingual cusps was 20.92 years. Specimens with worn flat occlusal surfaces were all assigned an arbitrary age of 25. Tests were carried out for male and female data separately comparing all groups with each other, all groups within regions and the three regions against each other.

Results

Age Determination

Tooth Eruption: Sub-adult skulls

Eruption of the mandibular and maxillary teeth was examined in each specimen. In non-adult animals observations of tooth eruption were compared with Hall-Martin's (1975, 1976) age class data (Table 4.1) and assigned an estimated age accordingly (Appendix 2.4.1). These sub-adult skulls will not be used in the subsequent morphological analyses.

Tooth wear: Adult skulls.

All specimens with full adult dentition are taken to be adult and will be used in the analyses. Ages were estimated for each adult specimen by substituting the measured tooth wear values into Hall-Martin's (1975, 1976) regression equations for lingual height and lingual occlusal surface width (See Appendix 2.4.2 for equations, values used and age estimates). A non-parametric Mann-Whitney U-test was carried out on the sets of age estimates to see whether the rank orders were different between them. This showed a very highly significant difference between the ages estimated by the two equations ($n_1 = 132$, $n_2 = 116$, $U = 2935.00$, $p < 0.001$).

Assessing the Relationship of Age with Geographical Sampling

Univariate Relationships of Cranial, Mandibular and Skeletal Variables to Estimated Age

Product-moment correlation coefficients for each variable are presented in Appendix 2.4.3 for male and female specimens separately. For female giraffe the correlation coefficients of the variable against age estimate are significant for only two variables; the zygomatic width (**ZGW**; $r = 0.440$, $df = 39$, $p<0.01$**) and the width of the mouth at the first maxillary molar (**SWM**; $r = 0.598$, $df = 39$, $p < 0.001$***). Interestingly both of these variables refer generally to the width of the mouth and associated feeding structures. However, it should also be noted that no other width measurements or other measurements of the mouth show a significant relationship with age

In contrast male dimensions were typically associated with age with only twelve variables of the forty six measured not being significant. A summary of the variables significantly associated with age is given in Table 4.2 (see Appendix 2.4.3 for complete results). Re-analysing the data using only males over the age of 12 years reduced the number of

variables significantly associated with age to eight (**PCL, EWB, NOL, MMW, FMP, IPW, NCL, SWM**. See Table 4.2).

p-value	NS. p > 0.05	* 0.05 > p >0.001	** 0.01 > p > 0.001	*** p < 0.001
Variables	**OOL, MMH, LAD, HSP, IWT, PPM, CAW, LMS, ITL, HUL, RUL, MEL.**	**PPHL, MPW, BPH, EWT, EWO, MWO, MXTL, PGW, PACL.**	**MASS, POL, PCL(*), ZGW, APD, TIC, NAC, EWB(*), FMP(**), PGM, TMP, IPW(**), PPL, MAL, MDTL, DIL, WMS.**	**NCL(**), NOL(*), PAOL, MMW(*), EAMW, MOW, LAW, SWM(**).**

Table 4.2: *Summary of association of variables with age for male specimens only. Tabulated p-values summarise the significance levels of the product-moment correlation coefficient of each variable against the estimated age for each individual. The asterisks given in parenthesis after eight of the variables indicate the significance level of that variable's correlation with age for specimens aged over 12 years only (see text for further explanation). Complete results are given in Appendix 2.4.3.*

Testing Geographic Sampling Bias of Age Estimates

The female data showed no significant differences in age distributions between groups or regions in any of the comparisons made (Table 4.3). For the male data there were no significant differences between groups in the southern and western regions. However, statistically significant differences in ages were found between groups within the eastern region, between all groups compared together and between the regions (Table 4.3).

Examination of the data shows that the geographical group EEK from northern Kenya, southern Ethiopia and south western Somalia is represented by a group of relatively young animals. All nine members of this group fall within the first 33 (of 73) specimens when ranked by age. This distribution is by chance as the specimens were taken at different times and for different institutions. Removal of these specimens from the geographic analysis of age distributions gives no significant differences between any of the comparisons made (Table 4.3).

Discussion

Tooth eruption: Sub-adult Animals

Those specimens identified as sub-adult will not be used in subsequent analysis and are not discussed further.

Gender	Scale	n	df	K-W Statistic	p-value
Male	Regional	73	2	6.641	0.036*
Male	All groups	73	12	22.309	0.034*
Male	Eastern groups	32	5	11.155	0.048*
Male	Southern Groups	35	4	5.447	0.244
Male	Western groups	6	1	6.000	0.857
Male	Regional without EEK	64	2	3.427	0.180
Male	All groups without EEK	64	11	11.841	0.376
Male	Eastern groups without EEK	23	4	3.193	0.526
Female	Regional	39	11	14.466	0.208
Female	All groups	39	2	0.991	0.609
Female	Eastern groups	23	6	10.854	0.093
Female	Southern Groups	14	2	0.140	0.932
Female	Western groups				

Table 4.3: *Summary of comparison of sample age distributions to geographic group by sex. A non-parametric analysis of variance (Kruskal-Wallis test) was used to assess sampling bias, with respect to the estimated ages of the specimens, with geographic location. Significant differences between groups and regions were found in the male sample. Removal of the group EEK removed this effect. Female groups showed no differences in age distributions. Note that there was insufficient data to perform a meaningful test between the female western groups.*

Tooth wear: Adult animals

With the two age estimates, calculated from lingual height and lingual occlusal surface width according to Hall-Martin's (1975, 1976) equations, differing significantly, it is not appropriate to estimate the age of each specimen as the mean of the two values, as was recommended by Hall-Martin (1976. p.287).

The purpose of age estimation in this study is to control for any possible error that may be caused by biased sampling of different age groups from different localities. This is particularly important for the male skulls where secondary bone is continually laid down throughout life. Hence, an accurate estimate of absolute age is not necessary. A relative, rank order scale is adequate. If the assumption that relative age may be assessed by using the extent of wear on a given tooth is accepted then can one of the two estimation techniques be used as a useful indicator of relative age?

There are a number of reasons, justified by different criteria, for accepting the lingual width as the better indicator of age. The first is a practical reason. Lingual width measurements are considered to be more accurate than the height measurements because the rugose and discoloured nature of the tooth enamel often made it difficult to locate the cingulum to measure from with confidence. The second, and, perhaps, more compelling, reason is computational. The cut-off point between sub-adult and adult animals, as defined

for this study, is the eruption of the mandibular canine, which is in full wear at 6 years of age. The youngest age estimated from Hall-Martin's equations using the width measurements was 6.2 years and so is as would be expected. In contrast, the youngest animal estimated by the height equation was a mere 1.5 years. Such an estimate is obviously incorrect for a specimen that is certainly adult. Hence, age will be estimated using the lingual occlusal surface data only These estimates were used to assess age bias in the geographic sampling.

Assessing the Relationship of Age with Geographical Sampling

Univariate Relationships of Cranial, Mandibular and Skeletal Variables with Estimated Age in Adult Giraffe

The relationships of the measured variables with estimated age differed between the genders. Females typically show no relationship between the measured cranial, mandibular and skeletal dimensions and the estimate of age. Only two variables in the female data set showed a significant positive relationship with age (**ZGW** and **SWM**). Meanwhile male dimensions were typically related to age with 34 of the 46 variables tested showing a significant association. When only males older than 12 years old were analysed the number of variables giving a positive association with age dropped to eight. All eight of these variables reduced their correlation coefficient with estimated age between the 'all adults' and the 'over 12' analyses.

It should be recalled that the method of determining age used here is based upon patterns in the wear of the molariform teeth (specifically the wear of the lingual cusp of the first maxillary molar, after Hall-Martin, 1975, 1976). Hence the estimated ages should not necessarily be assumed to be accurate reflections of true absolute age as there may be other confounding factors involved (e.g. geographic variation in diets and the coarseness of the food masticated and ingested). As a result, the interpretation of these data is cautious but considered to be robust due to the congruent trends seen in the data.

The pattern of the non-significant male variables in the 'all adults' data set is interesting. It has been reported that secondary bone deposition in male giraffe typically occurs on the upper surfaces of the skull, particularly around the median and parietal horns and also around the occipital ridge at the back of the skull (Spinage 1968; Urbain *et al.*, 1944). Hence, it might be expected that the measurements showing the greatest association with age would be those describing these regions of the skull. However, this is not obviously the

case. The most significant relationships with age are with length and width measurements (**NCL, NOL, PAOL, MMW, EAMW, MOW, LAW, SWM**). Meanwhile, variables that might be expected to increase with the accretion of secondary bone with age, such as the height of the median horn (**MMH**) and the lateral diameter of the parietal horns (**LAD**), are not significantly correlated with age estimates (although other measures of parietal horn size are). The relationship between age and length and breadth measurements that do not describe the area over which secondary bone is thought to be deposited suggests that the increase in size of male giraffe skulls through life may not be due only to the deposition of secondary bone on these surfaces but may involve continuous active growth of the skull. The similar positive associations reflected in the mandibular measurements suggest that growth continues in adult male giraffe. That is the mandible continues to grow to compensate for skull growth. It is beyond the scope of the present study to examine this in any further detail, but this is certainly worthy of further study.

Some of the cranial variables that do not show a significant correlation with age are those that have been suggested as important for the separation of subspecies in the past (e.g. **MMH**) or have been identified during the current analysis (e.g. **OOL**). This indicates that geographic variation can overwhelm the influence of age. It is also noteworthy that none of the three skeletal measurements show a significant relationship with age. This suggests that growth of the limbs is deterministic and stops at maturity. However, the sample size is small making any such conclusion based on these data tentative.

Although wild male giraffe reach sexual maturity at three and a half years old this study uses 'morphological maturity' to separate adult and sub-adult specimens. Giraffes show sexual dimorphism, particularly in the dimensions of the skull, and the age of morphological maturity has been defined in this study as the age at which male and female skulls are sufficiently different that they may be classified unequivocally as male or female in a discriminant analysis. This stage is attained at six years old and coincides with the full eruption and wear of the mandibular canine. However, it is evident from the preceding account that growth continues in male giraffe after this age has been reached. Hall-Martin (1975) reports that the testes, epidydimis and bulbo-urethral gland masses all increase before reaching an asymptote at 12 years of age. Although a captive male giraffe has sired an offspring at the age of two years and eight months (Crandall, 1964) and wild males are sexually mature at the age of three and a half years (Dagg and Foster, 1982) with the onset of spermatogenesis (Hall-Martin *et al.*, 1978) it is unlikely that a wild male would father any offspring until he is much older. Male giraffe undertake ritualised (although sometimes fatal – de Clerck, 1965) fights, known as necking (Coe, 1967) or sparring (Dagg and Foster, 1982). This, coupled with their loose social system with ephemeral associations

rarely lasting more than a few days (Foster, 1966; Foster and Dagg, 1972) means that dominance hierarchies of males can be created and maintained over long periods of time. No published data concerning dominance hierarchies in male giraffe or the proportions of matings obtained by dominant males is available as mating is so rarely observed with pregnancy lasting for fifteen months (Dagg and Foster, 1982). Hence, the majority of male giraffe have to wait beyond both physiological sexual maturity and morphological maturity (as defined for this study) before being sufficiently dominant to obtain regular matings. The continued growth of the sexual organs (testes, epididymis and bulbo-urethral glands. Hall-Martin, 1975) until the age of 12 years suggests that growth in secondary sexual characters may also continue to this age. To examine this a second analysis of product-moment correlation coefficients for each variable against estimated age was performed. In all cases the correlation coefficient decreased and only eight variables remained significantly associated with age (see Table 4.2). Although most of these eight are still length and width measurements this is suggestive that growth in the linear dimensions of the male skull is indeed deterministic but continues until the age of 'social maturity'. In this context social maturity may be defined as the age at which a bull can be expected to reach a sufficient level in the local dominance hierarchy to obtain regular matings.

The strong age associated relationships seen in the adult male giraffe (defined by my morphological maturity criterion as being over six years of age) may introduce problems to the interpretation of the morphological variability seen in the male giraffe skulls. The 'social maturity' criterion, taking male skulls above the age of 12 years, may provide a more reliable assessment of geographic variation by minimising the effect of any age related variation in the data. However, this does reduce the sample size, both overall and for certain geographically restricted groups. Hence, two analyses will be performed on male skull data. Comparisons between the two data sets should indicate whether the age chosen to represent adulthood in the male giraffe affects the interpretation of geographic variation in the skulls.

While every attempt has been made to control for confounding variables and to ensure the statistical validity of all data it is a practical reality that taxonomic revision relies on the available museum samples. The need to account for within region or locality variation (i.e. to maintain an adequate sample size) and to control for all possible factors must be balanced against each other and a compromise struck. Conclusions drawn from the data must be tempered by an understanding of the statistical limitations encountered.

The next chapter considers another, possibly confounding, factor; sexual dimorphism. Differences in skull dimensions between known sex specimens are investigated and used to classify specimens with the sex unrecorded.

CHAPTER 5:

VERIFICATION AND DETERMINATION OF THE SEX OF GIRAFFE SKULL SPECIMENS.

Introduction

Sexual dimorphism is a widespread phenomenon in almost all animal phyla (Shine, 1989). Any difference in size between the two sexes is usually attributed to sexual selection, to intersexual food competition or to reproductive role division (Hedrick and Temeles, 1989; also Shine, 1989). In the mammals males are typically larger than females (Andersson, 1994; see Ralls, 1976 for exceptions). Loison *et al.* (1999) have demonstrated for ungulates that while sexual size dimorphism (the ratio of male to female body mass) increases with increasing female body mass, this effect can be almost completely accounted for by the relationship between polygyny and body weight. Their study indicated that the social structure of the breeding system had the greatest influence on the magnitude of sexual size dimorphism. The ecological variables that they tested (habitat type and preferred diet) were not related to size dimorphism.

In taxonomic studies where sexual dimorphism is known to be present, each specimen must be assigned to its correct sex to control for these differences and avoid introducing unnecessary errors into the analysis.

Sexual dimorphism has been generally recognised between adult giraffe. The mean height of male giraffe is 5.3m, while females attain a mean height of 4.3m (Nowak, 1999). The largest recorded male and female were 5.88m and 5.17m tall respectively (Shortridge, 1934; McSpadden, 1917; both cited by Dagg and Foster, 1982). The heaviest male recorded was 1,930kg with 1,180kg for the largest female. More typical weights are around 1,200kg for males and 800kg for females (Owen-Smith, 1992).

In her study of 55 giraffe skulls, Dagg (1965) recognised sex-related differences in skull mass, parietal horn circumference and skull lengths. The lightest male skull weighed 8.9kg (mean = 9.9kg, n = 3) while the heaviest adult female skull weighed 4.7kg (mean = 3.5kg, n = 7). The reason for the difference in skull size is that males continue to deposit secondary bone onto the upper surfaces of the skull, where there are no muscle attachments, throughout their life. This is thought to be related to the specialised mode of fighting found in male giraffe; the 'necking' behaviour, described by numerous authors (e.g. Spinage, 1959; Coe, 1967; Dagg and Foster, 1982). Necking involves the head being

swung as a club to hit the body and legs of an opponent. Deposition of secondary bone has a two-fold effect; first, along with the extensive bony sinuses, to reinforce the skull from fracturing with the force of the blow, and second to increase the weight of the skull and hence the force of the blow. Consequentially, older males typically have the heavier skulls and an advantage in necking contests. More serious bouts of necking have been termed 'sparring' by some authors (e.g. Innis, 1958; Dagg and Foster, 1982) and can result in such heavy blows as to cause the death of one of the combatants (de Clerk, 1965). Female giraffe rarely engage in necking behaviour.

Whilst the extent of the sexual dimorphism in adult giraffe typically allows easy classification of an adult skull to its sex a more objective classification of the skulls was carried out using discriminant analysis.

Materials and Methods

Specimens Examined

All of the 142 adult skulls measured were included in this analysis. These adult skulls were separately categorised into two simple classes depending upon whether the sex was recorded on the museum label or not. These were termed 'sex recorded' and 'sex not recorded'. The skulls in the sex recorded category were then listed as male or female. Those with the sex not recorded were assigned as male or female by a subjective visual assessment and listed as SNR male and SNR female (with SNR signifying 'Sex Not Recorded'). There were a number of skulls that had a sex recorded on the museum label, but other evidence suggested that the sex had not been determined *in situ* but later according to the assessment of the collector or curator. An example would be a 'pick up' skull found on the ground. In these cases the skull was assigned to the sex not recorded group. In the absence of any evidence suggesting to the contrary it has been assumed that the sex recorded on the label is correct.

Of the 142 adult skulls examined 75 had their sex recorded, with 49 males and 26 females. Of the 67 with no sex recorded 50 were subjectively assessed to be male and 17 female.

Assessing Sexual Dimorphism

Student's t-tests were performed on all measured skull, mandible and post-cranial skeleton parameters measured.

Discriminant Analysis

Canonical Variates Analysis (CVA) describes the relationships between predetermined groups of entities (MacGarigal *et al.*, 2000). The analysis derives linear combinations of the original variables that 'best' discriminate between the predetermined groups. The decision of what is 'best' is made statistically by maximising the F-ratio of the between groups variance to the within groups variance of the newly derived canonical scores. The canonical scores are calculated for each of the sampling entities by multiplying the original variables by the correlation of the variable for the canonical function. This discriminant, or classification, function can be used to classify other entities, not used in the building of the classification function, into one of the groups. Hence CVA can be used to verify the membership of each entity in the predetermined group and to classify previously unclassified entities into one of the groups.

In this analysis a first CVA is used to verify the museum records of the sex of each specimen. The derived classification function is then used to classify all of the sex not recorded skulls into one or the other sex class. A second round of CVA was performed, using all of the sex recorded and the newly assigned specimens, to verify the classification of all specimens using all of the available data.

Results

Sexual Dimorphism

Of the 49 variables cranial, mandibular and post-cranial variables tested for differences between sex recorded male and female specimens using a Student's t-test all but six (**IWT, PPM, IPW, MDTL, CAW** and **ITL**) were significantly different at the 1% level (Table 5.1 and see Appendix 2.5.1 for all results).

One of these (**IWT**) refers to the divergence of the parietal horn tips, two to the internal bony morphology of the throat (**PPM** and **IPW**) and three to the mandibular dentition (**MDTL, CAW** and **ITL**). The latter five, being involved with feeding might be expected not to vary between the sexes. Such a general effect (with most parameters differing with sex) suggests that these differences could be a manifestation of the typically greater size of the males compared to the females and so may represent a simple scaling difference rather than shape differences between the sexes.

Variable	Sex	n	Mean	SD	t	p
IWT	Female	26	101.8	26.88	-1.706	0.094
	Male	49	112.8	25.62		NS
PPM	Female	21	35.26	6.182	-2.181	0.035
	Male	36	38.83	5.570		*
IPW	Female	18	37.00	3.290	-2.113	0.041
	Male	35	43.89	18.72		*
MDTL	Female	27	171.1	5.782	-1.674	0.099
	Male	41	173.8	7.475		NS
CAW	Female	21	25.91	3.057	-2.221	0.033
	Male	18	27.78	2.188		*
ITL	Female	25	41.32	3.537	-0.844	0.403
	Male	37	42.05	3.077		NS

Table 5.1: *Summary t-test statistics for variables that were not significantly different between male and female giraffes, at the 1% level. All other parameters were significantly different when compared between males and females. (See Appendix 2.5.1 for full summary statistics.)*

Examination of the primary data showed that, of the significantly different parameters, most showed some overlap in measured values between sex recorded males and females for each variable. However, eight showed no overlap. These are **MASS**, the four parietal horn dimensions; **APD, LAD, TIC** and **NAC** and the three fore limb measurements; **HUL, RUL** and **MEL**. The five skull variables were used in the CVA (Table 5.2). There were too few limb bones measured to include these variables in further analysis.

	MASS	APD	LAD	TIC	NAC
Male Minimum	3.0kg	35.5mm	26.0mm	93.0mm	93.0mm
Female Maximum	3.0kg	38.5mm	36.0mm	115.5mm	114.5mm

Table 5.2: *Five cranial parameters with non-overlapping values between males and female sex recorded specimens.*

Discriminant Analysis

A classification function was derived using the 49 male and 26 female specimens in the sex recorded data set in a CVA (Table 5.3). As might be expected by using a set of non-overlapping variables, the resulting classification function correctly allocated all sex recorded specimens to the correct sex.

The resulting function was used to classify specimens from the sex not recorded group. All of the assignments made classified the sex not recorded specimens to the sex subjectively assigned to them by a visual assessment.

The second round of CVA using the data from the sex recorded and the newly allocated specimens (i.e. data not included in the first classification function) again saw all specimens correctly allocated to their recorded or assigned sex (Table 5.3). Nineteen skulls

were not assigned to sex in the second CVA due to partial missing data. However, using the classification function on the available data clearly assigns these skulls to one or other of the sexes. Hence they were included in subsequent analyses.

A complete list of the specimens assigned to sex is given in Appendix 2.5.2.

	First Classification Function		Second Classification Function	
	Female	*Male*	*Female*	*Male*
Constant	-16.102	-63.783	-13.266	-49.823
MASS	-2.469	-3.374	-1.581	-1.724
APD	0.595	1.340	-0.015	0.348
LAD	0.001	0.214	-0.657	-0.758
TIC	-0.295	-0.463	0.208	0.306
NAC	0.636	0.960	0.394	0.530

Table 5.3: *Classification functions derived from canonical variates analysis for verifying and determining the sex of giraffe skulls in this study.*

Discussion

CVA was used to separate male from female skulls based on non-overlapping measurements of mass and the dimensions of the parietal horns. One hundred and forty two skulls were unequivocally assigned to one or other sex and will be designated as such in all subsequent analyses.

Six specimens (BMNH671a, BMNH671c, BMNH1901.5.14.1, BMNH1907.7.8.255, NMZB11533 and NMZB60810) had no data available for the five parameters that were used in the classification function to assign sex. The lack of these data is due to missing or damaged parietal horns. Some of these skulls had further damage to other parts of the skull. Due to this damage, and the resulting missing data, the use of these specimens in later analyses would be limited. Hence, they are not included in later analyses.

The specimen NMZB29101 is recorded as being from a known age male animal of 9.5 years. It would be expected that this specimen would be readily classified as an adult male in this analysis. In fact it was categorised as a female. This suggests that either the sex or the age may have been recorded incorrectly for this specimen. In conjunction with the provenance of the animal (It was translocated from Hwange National Park, Zimbabwe to Matopos National Park, Zimbabwe at a young age), these points lend sufficient doubt over the data associated with this specimen for it to be removed from later analyses.

The definitive list of male and female specimens used in future analyses is presented in Appendix 2.5.2.

This chapter and the preceding chapter considered the effect of morphological variation affected by age and sex differences between individuals. The next chapter provides a framework whereby geographically mediated phenotypic and genotypic variation can be investigated. Specimens are grouped into geographically restricted sets determined by physical geography and, to some extent, existing subspecies boundaries. These groups act as hypotheses of geographical relationships for subsequent analyses.

CHAPTER 6:

THE GEOGRAPHICAL RANGE OF THE GIRAFFE, *GIRAFFA CAMELOPARDALIS*, IN AFRICA AND THE ASSIGNMENT OF SPECIMENS TO POPULATION GROUPS.

Introduction

The purpose of this study is to examine the nature of the geographic variation in the giraffe. Giraffe specimens were grouped according to their geographical location using published range maps and considering previously described subspecies ranges and potential geophysical barriers that may prevent movements between regions. These groupings were used in comparisons of pelage, morphological and molecular variation.

Methods

Specimens were grouped at two levels in order to make comparisons between giraffe populations. Firstly, geographically isolated areas populated by giraffe were described by examination of distribution maps (Dagg, 1962; Kingdon, 1979; Sidney, 1965; Skinner and Smithers, 1990. Figure 6.1A-D). Secondly, a distinction was made where described subspecies ranges are contiguous within a continuously occupied geographic area, but the described subspecies boundaries correspond to potential physical barriers between the subspecies.

All specimens examined with reliable provenance information were then assigned to one of these groups. More detailed maps giving ranges within certain countries are given by Sidney (1965) for Cameroon, Central African Republic, Chad, Ethiopia, Kenya, Mozambique, Namibia, Niger, Nigeria, Somalia, South Africa Sudan, Tanzania and Uganda, and by Kingdon (1979) for Kenya, Tanzania and Uganda. Written descriptions of giraffe distributions for each range country, given by Dagg (1962), Dagg and Foster (1982), Sidney (1965) and East (1999), were used to clarify range boundaries and subspecies boundaries within ranges. Regional and national treatise including descriptions of giraffe were consulted, where available.

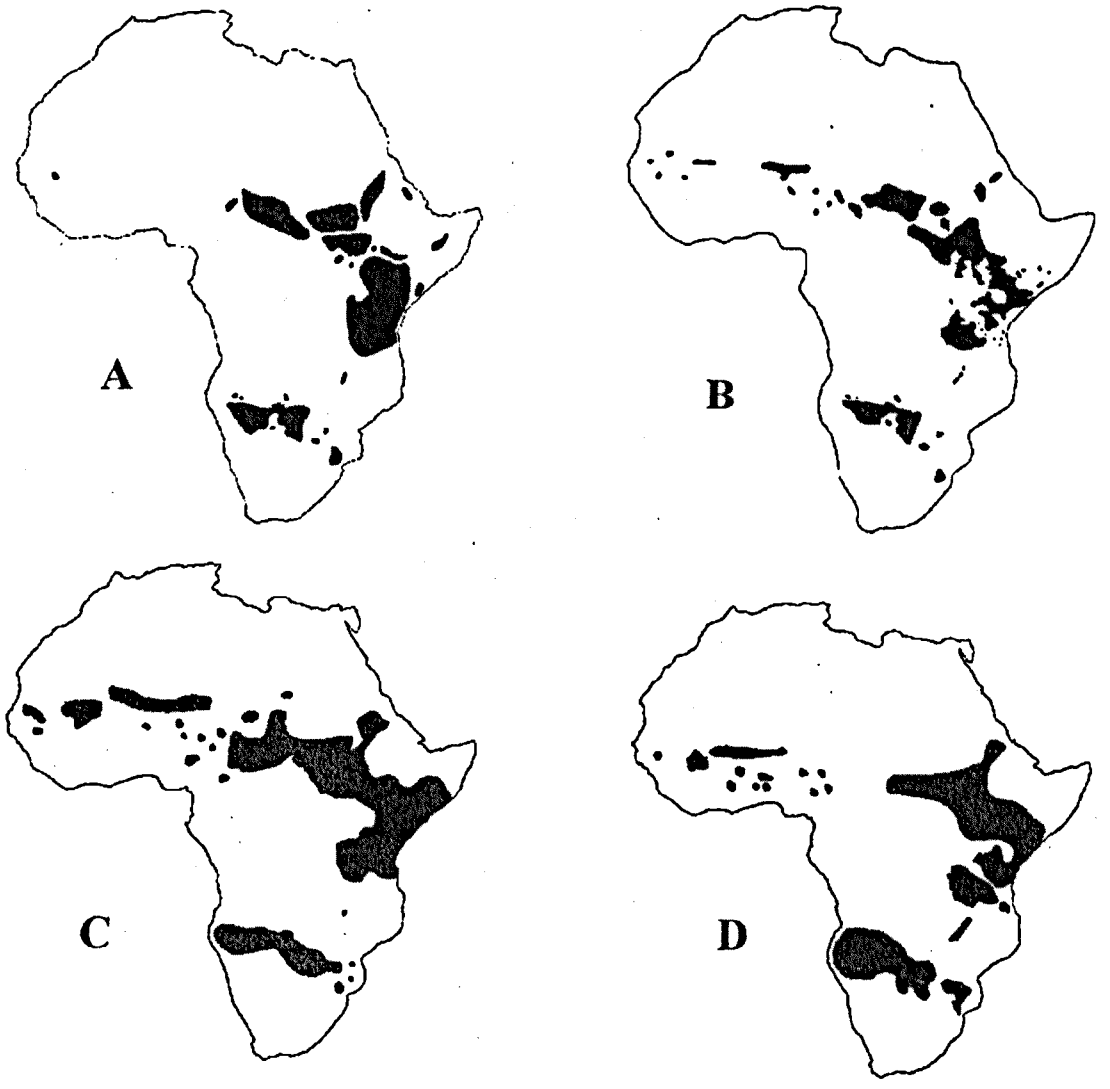

Figure 6.1: Published Range Maps of the Giraffe.

A: *The contemporary distribution of the giraffe, as presented by Dagg (1962). Note that the range in west Africa, with no population between Senegal in the far west and northern Cameroon, is erroneous as presented here. Dagg lists populations from Chad, Cameroon, Niger, Nigeria and Senegal. Redrawn from Dagg (1962) p. 498 Fig.2.*

B: *Distribution of the giraffe (Kingdon, 1979). Note the extensive range in western southern Africa and the fragmented ranges in eastern southern Africa. Also note the fragmentation of the range in east Africa. Redrawn from Kingdon (1979) p. 317.*

C: *Distribution of the giraffe according to Sidney (1965). Note the extensive range in western southern Africa extending in to the eastern part of the region with smaller isolated populations in this western area. The range in east Africa is not as fragmented as in some other range maps. Redrawn from Sidney (1965) p. 140. Map 30.*

D: *Distribution of the giraffe according to Skinner and Smithers (1990). Note the contiguous range in western southern Africa linking populations in South Africa, Mozambique and Zimbabwe that are separated by other authors. The eastern and western ranges separate southern and western Zimbabwean populations. The east African range is similar to that of Sidney (1965. Figure 6.1C) in being unfragmentaed. Redrawn from Skinner and Smithers (1990) p. 606.*

Resolving Conflicts between Range Maps

Generally the pan-African range maps agreed on the distribution of giraffe across the continent. Where differences were apparent these tended to be relatively minor. Any conflicts were resolved by maximising the number of groups recognised. For example, if the ranges of population 1 and population 2 overlapped in different range maps specimens occurring in the region of overlap were assigned to a third population. Hence, the greatest possible resolution between populations, and the greatest information content, was retained for the analysis allowing the affinity of the overlap region to be tested.

The Southern African Range

Sidney (1965) and Skinner and Smithers (1990) indicated different ranges for the giraffe in southern Africa (Figures 6.1C and 6.1D). While each author indicates two major populations in southern Africa, referred to here as the 'western' and 'eastern' populations, they disagree on their separation. Sidney (1965) indicated that the western range extends far across the continent, stretching from southern Angola and northern Namibia eastwards through the south west of Zambia, northern and north-eastern Botswana and south-western and southern Zimbabwe just crossing the border into Mozambique. The eastern range covers a relatively small area in the north east of the Transvaal of South Africa edging in to southern Mozambique. She also maps two small isolated populations in southern Mozambique. Meanwhile Skinner and Smithers (1990) show the western range extending from southern Angola and northern Namibia through southern Zambia and northern and north-eastern Botswana into eastern Zimbabwe. The eastern population starts in the northern Transvaal, as with Sidney, and continues northward up the border with Mozambique into southern Zimbabwe where it extends to central southern Zimbabwe. The essential difference is the separation in Zimbabwe. In Sidney's (1965) arrangement, all of the giraffes of Zimbabwe represent members of a contiguous range with those of the northern Transvaal separate. In contrast, Skinner and Smithers (1990) interpret the giraffe from eastern and from southern Zimbabwe as different historical populations.

Kingdon's (1979) map appears to follow Dagg's (1962) map for the distributions in southern Africa (Figures 6.1B and 6.1A respectively). Both of these authors indicate a western range from Angola and Namibia across Zambia and Botswana into Zimbabwe, similar to that described from Skinner and Smithers (1990). The eastern range then resembles the relatively small range described from the northern Transvaal by Sidney (1965). Dagg (1962) and Kingdon (1979) go on to show two small, isolated populations in

southern Zimbabwe. As such the arrangements of Dagg (1962) and Kingdon (1979) do not conflict with either Sidney (1965) or Skinner and Smithers (1990).

The West African Range

Dagg's (1962) map (Figure 6.1A) is misleading in that it does not show the presence of giraffe in west Africa except for a small range in Senegal. However, this appears to be a simple omission on the map as she lists West African giraffe present in Cameroon, Chad, Niger, Nigeria and Senegal.

Kingdon (1979), Sidney (1965) and Skinner and Smithers (1990) (Figures 6.1B-D) all indicate relatively small, isolated populations throughout West Africa, although they differ slightly in their precise localities.

The East African Range

The range of the giraffe in East Africa is, perhaps, the least controversial in terms of its general shape. However, Dagg (1962. Figure 6.1A) in particular, and to some extent Kingdon (1979. Figure 6.1B), have tended to fragment the range. Sidney (1965. Figure 6.1C) maintained the most coherent range for the giraffe in East Africa extending the range from the east coast throughout the northern two-thirds of Tanzania, most of Kenya and the southern half of Somalia, westwards through northern Uganda and southern Ethiopia into southern Sudan. The range splits northwards following the border between Sudan and Ethiopia and continues west into the Central African Republic and southern Chad. Kingdon (1979) and Skinner and Smithers (1990. Figure 6.1D) both separate central southern Tanzanian populations from populations in the north on the border with Kenya. Kingdon (1979) further separates off the populations in Sudan into a Sudan – Ethiopia border group, a southern central group and a larger western group extending into the Central African Republic.

If the East African range is the least controversial in terms of its general shape (notwithstanding the varying degrees of fragmentation) it is certainly the most complex in terms of the subspecific taxonomy of the populations. Up to seven of Lydekker's (1904) named subspecies are present within this range (Dagg, 1962; Dagg and Foster, 1982), although Lydekker was unsure of the status of the reticulated giraffe and recognised it as a separate species at the time.

Results

Contiguous Regional Populations

This section delimits areas that will be used to group the examined specimens for subsequent comparison and analysis. Each group is defined according to its geographical location and is labelled with an acronym reflecting this classification. The definition and acronym is purely descriptive. In some cases, where two named subspecies have contiguous ranges, a subspecies name is added to the description. The geographical areas defined by each group represent discrete areas described in the literature in the historical range of the giraffe in sub-Saharan Africa. These may not reflect the current distribution of the giraffe. The final set of areas is illustrated in Figure 6.2. The specimens allocated to each described range are presented in Appendix 2.6.1.

The Southern African Range

GROUP SWA

Southern Africa

Western Range

'Angolensis' subspecies.

The giraffe has, historically, been rare in Angola. It has been restricted to the southern part of the country below 15°S. The Cunene and Cubango rivers restrict the southern extent of the range.

GROUP SWC

Southern Africa

Western Range

'Capensis' subspecies

This group extends across northern Namibia, northern and central Botswana, the south west of Zambia and eastern Zimbabwe.

Historically the giraffe occurred Kaokoland in the north west of Namibia, bounded on the western side by the Skeleton Coast National Park. The northern limit borders the SWA group and extends to approximately 20° south as its southern limit. It extends eastwards across the north of Namibia through the Caprivi Strip. The range continues into western Botswana extending slightly further south than the 20°S line of latitude in Ngamiland. The range turns southwards and covers most of the Central Kalahari Game Reserve and westwards across the Makgadikgadi-Nxai Pan National Park to the border with Zimbabwe.

Giraffe occur northwards into the Moremi Game Reserve and the Chobe National Park. The range extends into the south western corner of Zambia no further north than 16°30'S. The westward limit of this group is in western Zimbabwe where it ranges throughout Hwange National Park and slightly beyond its westward and southward borders into the communal lands. To the north of the park its range continues through the Victoria Falls National Park to the Zambezi River (Child and Savory, 1964).

GROUP SCZ

Southern Africa

Central Range

Zimbabwe

Sidney (1965. Figure 6.1C) showed the range of giraffe in southern Zimbabwe to be continuous westwards through western Zimbabwe and across Botswana and into Namibia, grouping with my SWC group. In contrast, Skinner and Smithers (1990. Figure 6.1D) indicate a geographical split between Zimbabwe's southern and western populations. Dagg (1962. Figure 6.1A) and Kingdon (1979. Figure 6.1B) show both the widespread western population (my SWC) and the eastern population (my SEW) as separate from giraffes in southern Zimbabwe.

The arrangement used here reflects that of Dagg (1962) and Kingdon (1979) in recognising the giraffe of southern Zimbabwe as a separate population for inclusion in the analyses. This conservative approach allows the affinities of the southern Zimbabwean giraffe to either the eastern or western group to be tested by empirical analysis rather than making an *a priori* assumption regarding relationships.

GROUP SEW

Southern Africa

Eastern Range

'Wardi' Subspecies

The eastern range of the giraffe in southern Africa extends through southern Zimbabwe, north-eastern South Africa and south western Mozambique. The Lundi River limits the northward and eastward limit of this group in Zimbabwe and extends westwards to the area around West Nicholson (Child and Savory, 1964). The range includes Kruger National Park in South Africa and extends into a limited area south of the Save River in Mozambique (Smithers and Lobão Tello, 1976).

GROUP SZT

Southern Africa

Zambian Range

'Thornicrofti' Subspecies

Giraffe occur in an isolated area in eastern Zambia centred on the Central Luangwa Valley and the Lupande Game Management Area.

Overview and Conflicts:

Five southern African groups have been presented. The SWA and SZT groups represent purported subspecies. The groups SWC, SEW and SCZ represent a conservative characterisation of the range of the giraffe through Namibia, Botswana, Zimbabwe and South Africa. According to Sidney (1965) groups SWA, SWC and SCZ form a contiguous range with SEW separate. Skinner and Smithers (1990) link SEW and SCZ together with SWA and SWC forming a separate contiguous group. Dagg (1962) and Kingdon (1979) link SWA and SWC and have SCZ and SEW as separate populations.

The West African Range

The range in West Africa is the most fragmented and shows the least agreement between authors. Happold (1969) suggested that the potential range of the giraffe extends across the whole of West Africa south of the Sahara and north of the forested areas and the Niger-Benue River systems. Sidney (1965) indicated a contiguous population extending through northern Cameroon, the northern half of the Central African Republic and the south of Chad. The other extensive range occurs through the south of Niger. She gives relatively small populations in northern Chad, Southern Niger and Nigeria. All of the West African giraffes purportedly belong to the same subspecies, *G. c. peralta*. For this analysis the following populations are recognised:

GROUP WNN

Western Africa

Northern Range

Niger population

This group ranges from the Niger River in the west across the southern half of Niger to approximately 12°E. Two isolated populations, close to Niamey in the west and Maradi in the south are included in this group.

GROUP WSN

Western Africa

Southern Range

Nigerian Population

This group conflates the geographically separate populations found in Nigeria. The giraffe is now thought to be extinct in Nigeria, except for infrequent vagrants from northern Cameroon (East, 1999).

GROUP WCP

Western Africa

Central Range

'Peralta' subspecies

This group extends from the north of Cameroon northwards into Chad and eastwards into the Central African Republic. Giraffe occupy northern Central African Republic above approximately 7°N and continue into Chad with a northern limit of Lake Chad in the west across to the Sudan border in the east at about 15°30'E. A population occurred in the Mourdi depression in northern Chad historically but is now extinct.

Two purported subspecies meet in Chad and the Central African Republic. *G. c. peralta* extends eastwards to meet the westward boundary of *G. c. antiquorum*. Therefore animals in the extreme east of Chad and the Central African Republic are not included in this group.

The East African Range

The populations of giraffe in East Africa are complex as they represent numerous, apparently contiguous subspecies whose reported ranges do not fit geopolitical boundaries.

GROUP ENA

Eastern Africa

Northern Range

West Sudan – 'Antiquorum' subspecies

This group ranges from the Nile River in the East across Sudan and into the eastern edge of Chad and the Central African Republic.

GROUP ENE

Eastern Africa

Northern Range

East Sudan, Ethiopia and Eritrea

Sudanese giraffes to the north and east of the Nile River group with giraffes in Western Ethiopia and south eastern Eritrea. There are no giraffe in the mountainous central area of Ethiopia.

GROUP ECU

Eastern Africa

Central Range

Uganda, southern Sudan and north western Kenya.

This group includes giraffes from Sudan to the south and east of the Nile River, into Uganda, partially bounded by Lake Victoria to the south and Lake Albert to the west and into north west Kenya to the west of the Great Rift Valley.

GROUP ECC

Eastern Africa

Central Range

Democratic Republic of Congo

This group includes populations from the north of the Democratic Republic of Congo.

GROUP EEK

Eastern Africa

Eastern Range

Kenya, Southern Ethiopia and Somalia

This group is bounded to the west in Kenya by the Great Rift Valley and on its southern side by the northern slopes of Mount Kenya and the Tana River. It extends northwards into Ethiopia bounded to the north by the Ethiopian Highlands and spreads west into the Ogaden district of Western Ethiopia. The range extends south from Ogaden into Somalia.

GROUP ESK

Eastern Africa

Southern Range

Southern Kenya and northern Tanzania

This group includes giraffe from south of the Tana River and Mount Kenya westwards to Lake Victoria and southwards across the border with Tanzania.

Both Kingdon (1979. Figure 6.1B) and Skinner and Smithers (1990. Figure 6.1D) indicate the northern Tanzanian population to be separated from two populations more southerly in the country. The 'border parks' including Serengeti, Ngorongoro and Tarangire are included in this group.

GROUP EST

Eastern Africa

Southern Range

Tanzania

Kingdon (1979.Figure 6.1B) and Skinner and Smithers (1990. Figure 6.1D) show two populations in central and southern Tanzania. However, the ranges do not correspond so all animals in this region will be grouped together. Lake Tanganyika forms the western boundary of the giraffe while the Rufiji and Ruaha Rivers form the southern boundary with Lake Rukwa. South of this boundary the dominant vegetation changes from *Acacia* woodlands to *Brachystegia*. The northern boundary of this group abuts the southern boundary of group ESK.

Overview and Conflicts:

Kingdon (1979. p. 316) suggests that the region between the Tana and the Athi Rivers is a zone of hybridisation between two "major populations" of giraffe in Kenya (by implication the *G. c. reticulata* subspecies and the *G. c. tippelskirchi* subspecies. My groups EEK and ESK).

Meanwhile Dagg (1962) indicates that the area between these two rivers is occupied by the subspecies *G. c. rothschildi* extending from Uganda and north western Kenya. Dagg's suggestion seems unlikely as the Great Rift Valley offers a physical barrier between group ECU and the Tana – Athi population. Dagg (1971) later withdraws this range of *G. c. rothschildi* allowing the ranges of *G. c. reticulata* and *G. c. tippelskirchi* to abut (see Figure 2.1).

My arrangement has the ESK population extending north to the Tana River, including Kingdon's suggested hybrid zone.

Conclusions

Ranges of Geographic Groups

The geographic ranges of the described population groups are presented in Figure 6.2.

Specimen Assignment to Geographic Groups

Table 6.1 summarises the number of specimens belonging to each of the listed geographic groups. The complete list of grouped specimens is given in Appendix 6.1.

EASTERN AFRICA	
Group	**n**
ENA	6
ENE	1
ECU	30
ECC	8
EEK	23
ESK	21
EST	4
Total	**93**

SOUTHERN AFRICA	
Group	**n**
SWA	5
SWC	58
SCZ	42
SEW	12
SZT	17
Total	**134**

WESTERN AFRICA	
Group	**n**
WNN	2
WSN	5
WCP	8
Total	**15**

Table 6.1: *Summary of geographical grouping of the specimens examined. The sample included 22 specimens that could not be assigned to a group due to the lack of collecting locality information or the ambiguity of the information given.*

The next three sections use these geographically delimited specimen sets to tests hypotheses of morphological similarity and difference (Section 4), genetic relationships (Section 5) and, in the next chapter, geographic distributions of similar pelage patterns (Section 3).

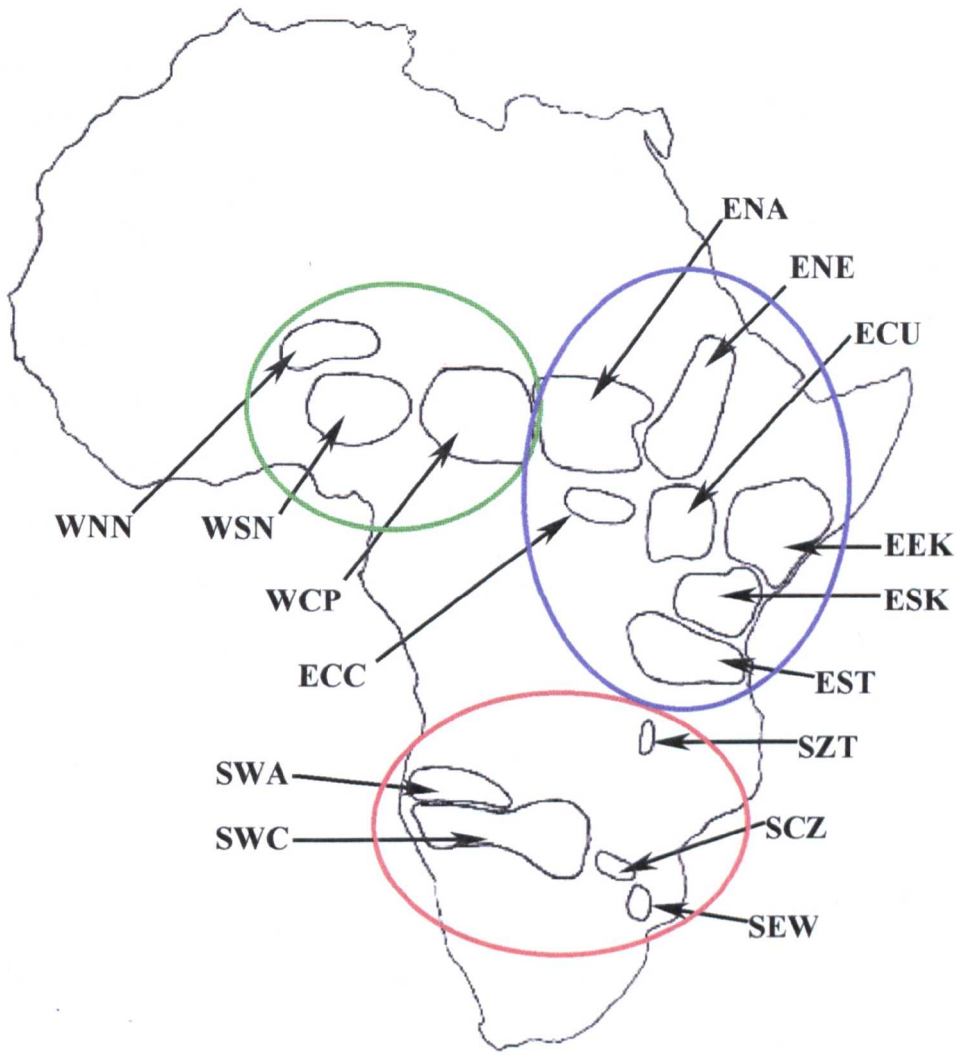

Figure 6.2: *Geographic areas covered by population groups. Each group represents a potentially historically isolated population based on range maps and subspecies ranges. A priori defined regional groupings are indicated by coloured ovals encompassing local population ranges (Western, Eastern and Southern regions). See text for a full explanation.*

CHAPTER 7:

GEOGRAPHICAL STRUCTURE IN GIRAFFE PELAGE PATTERN CHARACTERISTICS

Introduction

The pelage spotting patterns of individual giraffe vary widely across the species' geographic range (e.g. Kingdon, 1979, 1997). Variation in the form, size and arrangement of the spots, as well as the intervening network have been reported as being diagnostic for geographically restricted subspecies (Mitchell, 1905, 1908; de Winton, 1897, 1899; Dollman, 1929; Lydekker, 1904, 1911; Krumbeigel, 1939; Thomas, 1894, 1901). Indeed, despite Lydekker's (1904. p. 202) assertion that "most, if not indeed all, of the subspecies of giraffe are distinguishable by cranial differences", the currently recognised subspecies are diagnosed, primarily, by their pelage pattern (e.g. Dagg and Foster, 1982. See Chapter 2, and subspecies summaries in Appendix 1.2.1).

Dagg (1968. p. 657) observed that "giraffe make a particularly interesting field of study in that individual variations are sometimes traits that have hitherto been thought to differentiate an entire race." In her study of inheritance of pelage pattern characteristics in a captive population with a known genealogy, Dagg (1968. p667-668) concludes that "the coloration... the number, the area and the shape of the spots, and the presence of dark centres in the spots are all inherited features in this species... [and so]... theoretically these could therefore be used as racial characteristics." However, she notes that "spotting which is described as a characteristic of many ... races is often not uniform through a population." (p. 662). Similarly, Dagg and Foster (1982) recognise that while "most of the taxonomic work on the giraffe has been based on coat pattern ... many giraffe have nondescript spots which could belong to any of a number of subspecies."

While it is easy to pick out individual giraffe in each subspecies that conform to the type description (Figure 7.1), the range of individual variation within populations (Dagg, 1962) and the intergradation between populations (Stott, 1959) have been used as arguments against the assignment of individuals to subspecies. Perhaps Grzimek and Grzimek (1960) recognised the underlying problem when they observed that "working on single skins in museums led Europeans to believe at one time that there were several subspecies [of giraffe]" (quoted by Dagg and Foster, 1982). The fact that different authors' descriptions of the pelage characteristics of each subspecies often conflict with one another lends

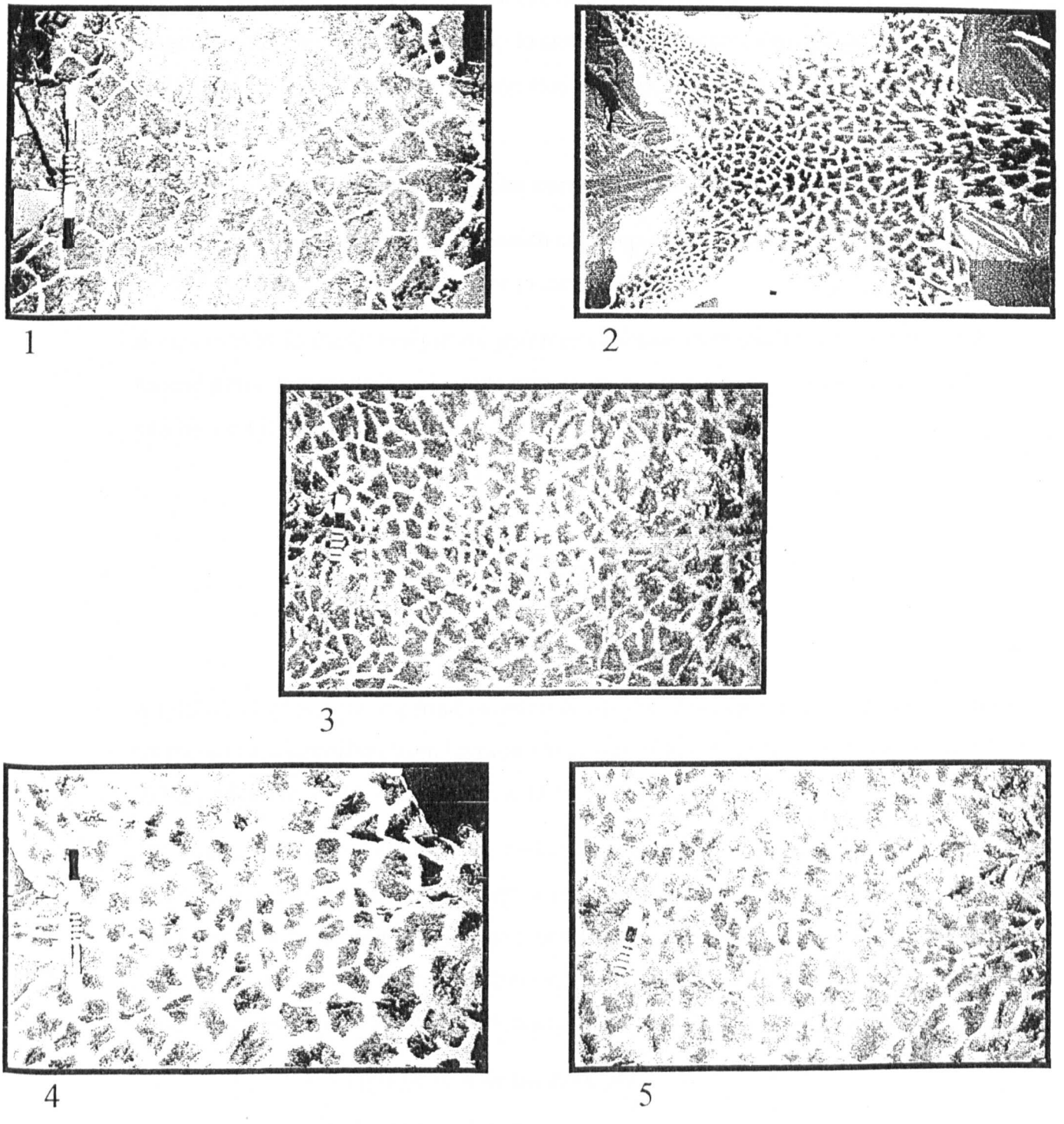

Figure 7.1: *Selected individual skins demonstrating typical giraffe subspecies pelage patterns.*
1 = Giraffa camelopardalis reticulata; 2 = G. c. tippelskirchi; 3 = G. c. thornicrofti; 4 = G. c. peralta; 5 = G. c. giraffa.

another element of doubt to the usefulness of pelage patterns in recognising subspecies (see the subspecies descriptions in Chapter 2).

This chapter considers geographic variation in giraffe pelage patterns. The geographically grouped specimens (Chapter 6) are examined to see whether character states, cited as being diagnostic for particular subspecies, do consistently diagnose that subspecies or whether individual variation obscures the supposed geographic structure to giraffe pelage patterns. This analysis has two, linked aims:

1. To establish which character states are consistent within each population;
2. Of these character states which are diagnostic for geographically restricted subspecies, either singly or in combination.

An attempt to formally analyse the gathered character state information to answer the second point was made based on the population aggregation analysis approach of Davis and Nixon (1992).

Materials and Methods

Specimens used

A total of 72 complete or partial museum skins were examined to analyse pelage pattern characteristics identified from literature accounts of giraffe pelage variation (Krumbeigel, 1939; Lydekker, 1904, 1911; Thomas 1894).

The size and weight of giraffe skins makes them difficult to handle. Skins were laid out flat either on the floor or a large table (protected by a polythene, or similar, cover to protect the skin). Skins were photographed from a consistent height (as consistent as possible given the different conditions in each institute visited). All photographs contained a 20cm linear scale. Black and white 100ASA film was used to take photographs of:

1. The whole body (a view of the back centred on the spine);
2. the fore flanks (right and left);
3. the hind flanks (right and left);
4. the neck (right and left sides);
5. the face (right and left sides)

In addition descriptions of the belly, leg and face and head spotting were taken. A list of skins examined is given in Appendix 3.7.1. Specimens were grouped according to their geographically delimited sets (Figure 6.2, Table 7.1 and Appendix 2.6.1).

Geographic Group	n	Assumed Subspecies	n
SWA	1	*G. c. angolensis*	1
ENA	2	*G. c. antiquorum*	2
SWC	20	*G. c. giraffa*	20
SCZ	3	*G. c. giraffa (wardi)*	6
SEW	3		
ECC	4	*G. c. peralta*	9
WCP	2		
WSN	3		
EEK	8	*G. c. reticulata*	8
ECU	9	*G. c. rothschildi*	9
ESK	11	*G. c. tippelskirchi*	12
EST	1		
SZT	5	*G. c. thornicrofti*	5

Table 7.1: *Number of skins examined and photographed for each geographic group. The assumed current subspecies has been listed with the corresponding sample size. Only one subspecies, the nominate subspecies* G. c. camelopardalis, *is not represented in the sample.*

Selection of Characters and Assignment of Character States

Character states were assigned qualitatively for 40 identified pelage characters extracted from the original taxonomic descriptions of the subspecies (de Winton, 1897; 1899; Lydekker, 1904; 1911; Thomas 1894; 1901. See Appendix 1.2.1 for a précis of subspecies descriptions. Characters and character states are given in Table 7.2). These were compared within and between geographic groups to assess their consistency within local groups.

It was not always possible to give a character state unequivocally for a given character. Some flexibility was exercised in assigning character states to allow for natural variation within an individual that can not be reasonably accounted for by a strictly discrete classification. Parentheses and forward slashes were used to indicate such variation. For example, "General size of fore flank spots: 1/2" is read as "an approximately even distribution of medium and large spots (or transition sizes in between) with the medium spots tending to be more frequent". An entry of "1(2)" for the same character is read as "medium sized spots with some (a few) large spots". In subsequent comparisons the character state in accord with the majority of observations for that character within the geographic group was assumed for the individual showing such variation.

Character.	Character State Code.
Body spots	
1 Size distribution of fore flank spots.	Even 0, Varied 1.
2 General size of fore flank spots.	Small 0, Medium 1, Large 2.
3 Shape of fore flank spots.	Polygonal 0, Crenulate 1, Stellate 2.
4 Width of fore flank lines.	Narrow 0, Wide 1.
5 Size distribution of rump spots.	Even 0, Varied 1.
6 General size of rump spots.	Small 0, Medium 1, Large 2.
7 Shape of rump spots.	Polygonal 0, Crenulate 1, Stellate 2.
8 Width of rump lines.	Narrow 0, Wide 1.
9 Fine projections to the edges of the spots blurring the edges.	Absent 0, Present 1.
10 Spots grouped into rosettes.	Not grouped 0, Grouped 1.
11 Major constrictions in spots.	No more than one 0, More than one 1.
12 Radiating marks within larger spots?	Absent 0, Present 1.
13 Darker centre to spots.	Absent 0, Present 1.
Head Spots	
14 Sides spotted (Below eye to mouth line).	No spots 0, Spots 1.
15 Face spotted (Above eye to mouth line).	No spots 0, Spots 1.
16 Spots between the eye and ear.	No spots 0, Spots 1.
17 Spots between ears and horns.	No spots 0, Spots 1.
18 Spots on top of the head (around parietal horns).	No spots 0, Spots 1.
19 Size distribution of head spots.	Even 0, Varied 1.
20 General size of head spots.	Small 0, Medium 1, Large 2.
21 White ear patch present or absent.	Absent 0, Present 1.
Limb Spots	
22 Fore limb spots below the hocks.	No spots 0, Partly spotted 1, All spotted 2.
23 Hind limb spots below the hocks.	No spots 0, Partly spotted 1, All spotted 2.
24 Fore limb spots between the legs.	Absent 0, Present 1.
25 Hind limb spots between the legs.	Absent 0, Present 1.
26 Size distribution of hind limb spots.	Even 0, Varied 1.
27 General size of hind limb spots.	Small 0, Medium 1, Large 2.
28 Shape of hind limb spots.	Polygonal 0, Crenulate 1, Stellate 2.
29 Size distribution of fore limb spots.	Even 0, Varied 1.
30 General size of fore limb spots.	Small 0, Medium 1, Large 2.
31 Shape of fore limb spots.	Polygonal 0, Crenulate 1, Stellate 2.
32 Change from larger body spots to small limb spots.	Abrupt 0, Gradual 1.
Belly Spots	
33 Belly spots present or absent.	Absent 0, Present, chest only 1, Present, chest and belly 2.
Neck Spots	
34 Continuous transverse bands.	Absent 0, Present 1.
35 Radiating lines within spots.	Absent 0, Present 1.
36 Size distribution of neck spots.	Even 0, Varied 1.
37 General size of neck spots.	Small 0, Medium 1, Large 2.
38 Shape of neck spots.	Polygonal 0, Crenulate 1, Stellate 2.
39 Neck spots are elongated (K).	"Round" 0, Elongate 1.
40 Width of neck lines.	Narrow 0, Wide 1.

Table 7.2: Pelage characters (with corresponding codes) classified according to body region. Characters used were extracted from the literature describing giraffe subspecies (see Appendix 1.2.1 for a summary of subspecies descriptions.) These character codes are used in an adapted population aggregation analysis (Davis and Nixon, 1992) to assess geographic variation in pelage patterns.

Consistency of Character States within Geographically Delimited Groups

Only phylogenetically informative character states are useful in assessing relationships between groups. The characters selected for analysis here have been derived from literature descriptions of the pelage patterns of giraffe subspecies. Different authors offer a variety of opinions over the occurrence and consistency of certain characters in different subspecies and hence the efficacy of these characters as diagnostic for giraffe subspecies is compromised (see the introduction to this chapter). For this reason, pelage characters that showed either excessive individual variation within geographic specimen sets or that were consistent between all geographic groups were removed from the wider analysis. Any character was deemed to show excessive individual variation if:

1. For a multistate character, more than two character states are represented in any geographically delimited population;
2. For a binary or multistate character represented in the data matrix by two character states:
   - Either, (with $n \geq 10$) the most frequent character state is represented by less than 90% of individuals;
   - Or, (where $4 \leq n < 10$) the most frequent character state is represented by fewer than n-1 individuals; i.e. more than one aberrant individual is present in the locality;
   - Or, (where $n < 4$) at least one aberrant individual is present.

These cut-off points have been decided arbitrarily, but do allow a certain amount of variation to be present in a population for given characters while taking account of the sample size of each population. If dealt with in a way cognisant of the nature of the variation, variability of characters or character states within populations may be allowed. It is the consistency of the variation between these populations that is of importance in determining subspecific units.

The characters corresponding to the first requirement were removed from further analysis. Characters corresponding to the second requirement were used in a modification of population aggregation analysis.

Population Aggregation Analysis

A formal analysis using the assigned character states for each specimen was attempted following the Population Aggregation Analysis approach advocated by Davis and Nixon (1992) for the delineation of phylogenetic species. Davis and Nixon (1992) summarise their method with two principles:

1. All individuals of a local population are regarded as belonging to the same [phylogenetic] species;
2. If identical individuals can be drawn from two local populations (i.e., if no character distinguishes the two populations), the two populations belong to the same [phylogenetic] species.

In this way, the character state distributions of successive local populations are compared and either aggregated or maintained as separate groups.

Following assessment of the consistency of character state distributions between and within the geographically defined specimen groups, an amended 'hierarchical' approach to population aggregation analysis was used. This effectively weights each character according to its level of between group consistency. The first level of analysis includes all characters where all groups are internally consistent. Any agglomerations of geographic groups are then compared, using the set of characters that are internally consistent within each distinct group remaining in a cluster after the first round of analysis. At each sequential step, characters that are consistent within each geographical set in an agglomerated group are used. Rounds of analysis continue until specimen sets are separated or until agglomerated units (consisting of more than one population) remain that can not be differentiated further.

Population Aggregation Analysis versus the Parsimony Approach

Population aggregation analysis (Davis and Nixon, 1992) differs from a cladistic (parsimony based) approach (e.g. Kitching *et al*, 1998) in the nature and the level of the hypotheses of homology erected during the analysis.

The cladistic approach invokes hypotheses of homology at the level of the character state. Identically coded character states are hypothesised as homologous by an assumption of primary homology (de Pinna, 1991; Brower and Schawaroch, 1996). Corroboration of primary homology hypotheses, following cladistic analysis, leads to secondary hypotheses of homology. The 'optimal' branching diagram indicating sister group relationships is then

the topology that maximises the proportion of secondary homologues; that is, the most parsimonious tree.

In contrast, population aggregation analysis makes its comparisons (its hypotheses of homology) at the level of the whole organism, or, at least, the set of character state data available for each individual. The whole spectrum of character state information is compared between individuals and between populations. Any difference between the individuals or populations compared creates a new population set.

Hence, the parsimony approach allows for homoplasious character states (non-corroborated hypotheses of primary homology) when analysing sister group relationships between individuals and groups. Population aggregation analysis does not and deals with any differences between individuals by invoking a new population set.

Results

Full results, with characters scored for all individuals, are given in Appendix 3.7.2. The consensus scores, representing each population, are given in Table 7.3.

Assessment of Character State Variation

Invariant Characters

Two characters were found to be completely non-varying throughout the entire sample. Four further characters were shown to vary in only a small fraction of individuals with this variation distributed in a non-structured way throughout the geographic range. These characters (summarised in Table 7.4) were removed from further analysis.

Individually Varying Characters

Some characters showed variation that was not geographically structured. This varied within each geographically delimited population and was assumed to be individually variable. Multistate characters giving more than two character states within a geographic set were considered to be excessively individually variable and removed from the analysis (Table 7.5).

	Population Group	ECC	West	ECU	EEK	ENA	ESK	EST	SCZ	SEW	SWA	SWC	SZT
	Body Spots												
1	Size distribution of fore flank spots.	1	1	0	0	-	-	1	1	-	1	1	1
2	General size of fore flank spots.	2	1	2	2	-	-	1	1	-	2	1	1
3	Shape of fore flank spots.	1	1	1	0	-	2	2	1	-	1	1	1
4	Width of fore flank lines.	1	-	-	0	-	-	0	-	-	2	-	-
5	Size distribution of rump spots.	1	1	-	-	-	1	1	1	-	1	1	1
6	General size of rump spots.	1	1	1	2	-	1	0	1	-	1	1	1
7	Shape of rump spots.	1	1	1	0	-	2	2	1	-	1	1	1
8	Width of rump lines.	0	-	0	0	-	0	0	-	-	1	-	-
9	Fine projections blurring spot edges.	1	0	1	0	-	1	1	1	-	1	1	0
10	Spots grouped into rosettes.	0	-	0	0	-	0	0	0	-	0	-	0
11	Major constrictions in spots.	0	1	-	0	-	2	2	1	-	1	1	1
	Head Spots												
14	Sides spotted (Below eye-mouth line).	1	-	1	1	1	1	1	0	-	-	-	1
15	Face spotted (Below eye-mouth line).	1	0	-	1	0	-	1	0	0	-	0	0
16	Spots between the eye and ear.	1	0	1	1	-	1	1	0	-	-	0	1
17	Spots between ear and horns.	1	0	1	-	-	1	1	0	-	-	0	1
18	Spots around parietals.	0	0	0	-	0	-	1	0	0	-	0	-
19	Size distribution of head spots.	0	1	-	1	-	1	1	-	-	-	-	1
20	General size of head spots.	1	0	-	2	1	1	1	-	-	-	-	0
21	White ear patch.	0	-	0	0	-	0	1	-	0	-	-	0
	Limb Spots												
22	Fore limb spots below the hocks.	1	0	0	-	-	-	-	-	-	1	-	0
23	Hind limb spots below the hocks.	1	0	-	1	-	-	-	-	-	1	-	-
26	Size distribution of hind limb spots.	0	1	0	-	-	-	0	1	-	1	-	1
27	General size of hind limb spots.	1	0	1	-	-	0	0	-	-	1	0	0
28	Shape of hind limb spots.	1	1	1	-	-	2	2	1	-	1	1	1
29	Size distribution of fore limb spots.	1	-	1	-	-	1	1	1	-	1	1	1
30	General size of fore limb spots.	1	-	-	-	-	1	0	-	-	1	-	0
31	Shape of fore limb spots.	1	-	1	-	-	2	2	1	-	1	1	1
	Belly Spots												
33	Belly spots present or absent.	1	-	-	-	-	-	1	1	-	1	-	-
	Neck Spots												
36	Size distribution of neck spots.	1	1	-	-	-	1	1	-	-	-	1	1
37	General size of neck spots.	-	1	-	2	1	1	1	1	-	-	1	1
38	Shape of neck spots.	1	1	-	0	0/1	2	2	1	-	-	1	1
40	Width of neck lines.	2	2	-	0	2	-	2	-	-	-	-	2

Table 7.3: *Consensus scores for pelage pattern characteristics for each population group.*

Character	Common Character State	Varying Individuals (n)	Non-varying Individuals (%)
32. Change from body spots to limb spots.	Gradual.	0	100%
34. Continuous transverse bands around the neck.	None.	0	100%
35. Radiating lines within the spots of the neck.	None.	1	98%
12. Radiating marks within the larger body spots.	None.	2	96%
13. Darker centre to spots.	None.	2	96%
39. Neck spots are elongated.	None.	6	90%

Table 7.4: *Summary of non-varying pelage characters. Only two characters were found to be completely non-varying through all specimens, while a further four varied in only a few individuals, regardless of geographic origin.*

Character	Reason for Rejection
8. Width of rump lines.	>2 character states per group.
22. Fore limb spots below the hocks.	>2 character states per group.
23. Hind limb spots below the hocks.	>2 character states per group.
33. Belly spots.	>2 character states per group.
40. Width of neck lines.	>2 character states per group.

Table 7.5: *Multistate characters showing more than two character states within a geographically delineated group. These characters are considered to be excessively individually variable and were removed from the population aggregation analysis.*

Certain characters were observed to be consistent (by the above criteria) within certain geographically delimited specimen sets, but excessively individually variable in others. Table 7.6 summarises the proportion of groups in which each character showed individual variation.

Consistent in All Sets	Variable in Two Sets
3. Shape of fore flank spots.	5. Size distribution of rump spots.
6. General size of rump spots.	10. Spots grouped into rosettes.
7. Shape of rump spots.	15. Face spotted (above eye - mouth line).
9. Fine projections blurring spot edges.	16. Spots between the eye and ear.
28. Shape of hind limb spots.	19. Size distribution of head spots.
29. Size distribution of fore limb spots.	21. White ear patch.
31. Shape of fore limb spots.	26. Size distribution of hind limb spots.
	30. General size of fore limb spots.
Variable in One Set	37. General size of neck spots.
1. Size distribution of fore flank spots.	
2. General size of fore flank spots.	**Variable in Three Sets**
11. Major constrictions in spots.	14. Face sides spotted (below eye - mouth line).
20. General size of head spots.	17. Spots between ear and parietal horns.
27. General size of hind limb spots.	18. Spots around parietal horns.
38. Shape of neck spots.	
	Variable in Four Sets
	36. Size distribution of neck spots.
	Variable in Six Sets
	4. Width of fore flank lines.

Table 7.6: *Summary of individual variation of characters within geographically delimited specimen sets. The table indicates in how many groups the listed character was deemed to be excessively individually variable. Excessive individual variation was determined according to the criteria presented in the text. A limited amount of individual variation was allowed by these criteria.*

	Population Group	*ECC*	*ECU*	*SCZ*	*SWC*	*SWA*	*SZT*	*West*	*ESK*	*EST*	*EEK*
	Consistent in All Groups										
3	Shape of fore flank spots.	1	1	1	1	1	1	1	2	2	0
6	General size of rump spots.	1	1	1	1	1	1	1	1	1	2
7	Shape of rump spots.	1	1	1	1	1	1	1	2	2	0
9	Fine projections blurring spot edges.	1	1	1	1	1	0	0	1	1	0
28	Shape of hind limb spots.	1	1	1	1	1	1	1	2	2	-
29	Size distribution of fore limb spots.	1	1	1	1	1	1	-	1	1	-
31	Shape of fore limb spots.	1	1	1	1	1	1	-	2	2	-

	Population Group	*ECC*	*ECU*	*SCZ*	*SWC*	*SWA*
	Consistent in Level 1 East/South Groups					
1	Size distribution of fore flank spots.	1	0	1	1	1
2	General size of fore flank spots.	2	2	1	1	2
16	Spots between the eye and ear.	1	1	0	0	-
17	Spots between ear and horns.	1	1	0	0	-
18	Spots around parietals.	0	0	0	0	-

	Population Group	*SCZ*	*SWC*
	Consistent in Level 2 SCZ/SWC Groups		
5	Size distribution of rump spots.	1	1
11	Major constrictions in spots.	1	1
15	Face spotted (Below eye-mouth line).	0	0
19	Size distribution of head spots.	-	-
20	General size of head spots.	-	-
37	General size of neck spots.	1	1
38	Shape of neck spots.	1	1

	Population Group	*ESK*	*EST*
	Consistent in Level 1 ESK / EST Group		
5	Size distribution of rump spots.	1	1
10	Spots grouped into rosettes.	0	0
11	Major constrictions in spots.	2	2
14	Sides spotted (Below eye-mouth line).	1	1
16	Spots between the eye and ear.	1	1
17	Spots between ear and horns.	1	1
19	Size distribution of head spots.	1	1
20	General size of head spots.	1	1
21	White ear patch.	0	1
27	General size of hind limb spots.	0	0
30	General size of fore limb spots.	1	1
36	Size distribution of neck spots.	1	1
37	General size of neck spots.	1	1
38	Shape of neck spots.	2	2

Table 7.7: *Complete results of Hierarchical Population Aggregation Analysis of coded spot pattern information. Population groups are agglomerated or separated according to similarities or differences between character state codes. A double border demarcates separate groups. According to this analysis the only agglomerated groups are SCZ with SWC and ESK with EST. All other groups show unique (potentially diagnostic) character state combinations.*

Population Aggregation Analysis

Ten geographically defined groups, representing eight of the nine currently recognised subspecies were used in this analysis (Table 7.1). Two of these localities (EST and SWA) were represented by a single specimen. Two further groups (ENA and SEW) were represented by two head and neck mounts and by three mounted heads respectively providing a limited range of characters that did not allow their group affinities to be adequately assessed. These two groups were removed from the analysis.

The first round of aggregation used only those characters that were locally consistent for all groups. This set of characters allowed the unequivocal separation and isolation of only one of the geographic groups (EEK) while maintaining three agglomerated sets, one containing five groups (ECC, ECU, SCZ, SWA and SWC), the others with two sets each (SZT / West and ESK / EST).

The second round compared three independent character combinations, within each of the agglomerated groups. These reflected the non-varying characters in each of these groups. The results of the hierarchical population aggregation analysis are presented in full in Table 7.7 and summarised in Table 7.8.

Discussion

Population Aggregation Analysis

The result of hierarchical population aggregation analysis showed eight discrete populations, reduced from ten geographically delimited specimen sets. Only two pairs of sets were found to be identical at all consistently scored characters, while the remaining six sets possessed unique, apparently diagnostic character state combinations.

Interpretation of Resulting Population Groups

Character Distributions and Geographic Origins

The addition of knowledge regarding the geographic origin of the specimen sets, to the character distributions, allows interpretation of the derived groups. It should be remembered that subspecies are, by definition, geographically delimited and must show differences from neighbouring populations. The occurrence of a similar phenotype in a geographically distant location does not preclude the recognition of a subspecies and may indicate ecophenotypic adaptation to a similar environment, or coincidental convergence.

No	Round of Analysis and included Specimen Sets	Characters Used	Agglomerated Groups and Distinct Populations
1	**Round 1:** ECC, ECU, EEK, ESK, EST#, SCZ, SWA#, SWC, SZT, West*.	3, 6, 7, 9, 28, 29, 31.	Group A: (ECC, ECU, SCZ, SWA, SWC) Group B: (SZT, West) Group C: (ESK, EST) *Distinct Population:* *EEK.*
2	**Round 2:** Group A. ECC, ECU, SCZ, SWA, SWC.	1, 2, 16, 17, 18.	Group D: ECC, SWA. Group E: SCZ, SWC. *Distinct Population:* *ECU.*
3	**Round 2:** Group B. SZT, West.	1, 2, 5, 11, 15, 16, 17, 19, 20, 21, 26, 27, 30, 36, 37, 38.	*Distinct Populations:* *SZT, West.*
4	**Round 2:** Group C.	5, 10, 11, 14, 16, 17, 19, 20, 21, 27, 30, 36, 37, 38.	*Agglomerated Populations:* *ESK / EST.*
5	**Round 3:** Group D. ECC, SWA.	4, 5, 10, 11, 14, 15, 19, 20, 21, 26, 27, 30, 36, 37, 38,	*Distinct Populations:* *ECC, SWA.*
6	**Round 3:** Group E. SCZ, SWC.	5, 11, 15, 19, 20, 37, 38.	*Agglomerated Population:* *SCZ / SWC.*

Table 7.8: *Summary of hierarchical population aggregation analysis. Character state distributions of geographically delimited specimen sets were compared and agglomerated or separated depending upon the sharing of consistent character states. The characters used were consistent within the specimen sets, but differed between them (see text for a full explanation). Specimen sets are determined as either 'distinct populations', where local sets are distinct from other groups, or as 'agglomerated populations' where two or more local groups are identical in the character comparisons made and so can not be separated on the basis of these characters. These 'final groups' are italicised in the table. (* = the group 'West' consists of individuals from the WCP and WSN geographically defined sets. # = Sets EST and SWA are each represented by data from a single individual.)*

The first round of analysis saw the immediate separation of the EEK set and the ESK/EST combined sets (the ESK/EST group could not be separated with comparison of all available characters). These two sets correspond to the reticulated (*Giraffa camelopardalis reticulata*) and Masaai (*G. c. tippelskirchi*) giraffes respectively. Both of these subspecies have distinctive pelage patterns that are readily differentiated from all other pelage variations (Dagg, 1968; Dagg and Foster, 1982; Lydekker, 1904). The body, neck and upper limb spots of the reticulated giraffe are large, regular, straight edged polygons with sharp edges contrasting the dark spot with the light background colour. There are few, if any, constrictions into the spot margin. The Masaai giraffe represents an opposite extreme with irregular, jagged spots, described as "star-like" (Lydekker, 1904) or "shattered"

(Krumbeigel, 1939). The irregular spots have multiple, deep involutions into the spot margin producing the stellate spot pattern typical of this subspecies.

The first round of analysis also separated off two other groups. A pair of local sets (from Zambia - SZT - and west Africa) were differentiated from a larger group of southern and eastern central African local sets (ECC, ECU, SCZ, SWA, SWC) by the lack of fine projections around the spot margin blurring the edge of the body spots. These two groupings of specimen sets do not connect geographically neighbouring populations.

Although superficially similar in many of their character states, the SZT and West African specimens are clearly separated geographically and are recognisably different qualitatively, primarily by the broader interspot lines in the western giraffe. Of the coded characters, they both share consistently sharp, smooth edge spots that differ from their geographically closest neighbours. This consistent difference allows this character to be accepted as diagnostic of these geographically separate populations.

In the other group, the southern (SWA, SCZ and SWC) sets can be distinct from the northern sets (ECC and ECU) due to their geographical separation.

Accounting for Within Group Individual Variation

The ECC and ECU specimens are differentiated according to the pattern of body spots, particularly on the fore flanks. While both possess large body spots, these spots are consistently large in the ECU individuals but may be more variable in size in the ECC set. However, inspection of the character data for individual animals indicates that this pattern may vary and that some ECU individuals may demonstrate the ECC character distribution pattern.

In the south, the two widespread southern populations could not be differentiated following comparison of all internally consistent characters. These two specimen sets either derive from a single subspecies (*G. c. giraffa*) or represent two subspecies (SCZ = *G. c. wardi*; SWC = *G. c. giraffa*).

The Angolan giraffe (*G. c. angolensis* - SWA) differs from the other southern populations by the larger size of the fore flank spots. However, the SWA group is represented by a single specimen. Also, almost half (9 of 22) of the combined SCZ / SWC individuals showed a proportion of large flank spots. Hence, on closer inspection of the character state distributions SWA group can not be separated from the remaining southern giraffe specimens.

Data Quality and the Geographic Groups

Pelage characters were selected from the literature descriptions of giraffe subspecies pelage pattern variation (de Winton, 1897; 1899; Lydekker, 1904; 1911; Thomas 1894; 1901). The characters selected reflect pertinent aspects of the pelage and, taken as a whole, provide a useful general description of the pelage. However, in attempting to categorise the pelage pattern into discrete statements, of characters and character states, information is unavoidably lost, perhaps resulting in the disparate geographical groupings observed.

Apparent character state convergence may be the result of the character state coding system. In retrospect, it is no accident that the two populations that were recognised as distinct in the first round of analysis were the two with the most distinctive body spot shape. Four of the seven characters used in the first round related to the shape of the spots. The character states recognised 'polygonal' and 'stellate' as describing two extreme spot morphologies, but then allowed all of the spot shapes in between to be classified into the intermediate 'irregular' character state. In this way, extremes of character states were recognised and adequately coded, while a large proportion of specimens were lumped into a single category that encompassed a number of different spot shapes. Similarly, size characters adequately characterised extremes of 'large' and 'small', but grouped together a range of 'medium' spots. Therefore, many of the character states coded represent a broad range of variation that is not adequately accounted for in a discrete coding system. Boundaries within the broad intermediate categories were not easily described, although variation was observed and noted. This lack of sensitivity in the character coding system may have resulted in some of the 'unnatural' (i.e. geographically disparate) higher order groupings in the earlier rounds of analysis.

Locally Consistent Characters Previously Considered Diagnostic for Subspecies

Lydekker's (1904) revision of giraffe subspecies remains the basis of the current classification, with some modification (Dagg, 1971). Lydekker described for each subspecies the spot patterns of the body, head and limbs in some detail. It is primarily these characters that have been used as diagnostic for the giraffe subspecies. More recent authors have maintained the use of body and limb spotting patterns (e.g. Dagg and Foster, 1982), but head spotting was deemed to be individually variable by Krumbeigel (1939).

Lydekker (1904) had a limited quantity of giraffe material available to him at the time of his revision. As Dagg and Foster (1982. p. 50) point out "at best it was hoped that no

important racial characters were attributed to individual idiosyncrasies". This section considers some of Lydekker's 'diagnostic' characters and attempts to identify valid differences between subspecies and where further data are needed.

Body and Upper Limb Spots

Thirteen body spot characters were scored for all specimens. Four of these (3, 6, 7 and 9) were shown to be consistent within all geographical sets while differing between some. Three (1, 2 and 11) and two (5 and 10) characters showed individual variation in one and two sets respectively. The tenth (4) showed variation in six sets. Three characters were excluded from analysis either for being consistent between all individuals (12 and 13) or for being excessively individually variable (8) (Table 7.6).

Body spot shapes, and the shapes of the upper limb spots, were consistent in all geographically delimited groups. This consistency may accurately represent the true pattern, or may be an artefact of the coding system. Two 'extreme' pelage patterns were immediately identified; the regular polygons of the reticulated giraffe (*G. c. reticulata*) and the 'shattered', stellate patterns of the Masaai giraffe (*G. c. tippelskirchi*). All other patterns were classified as 'irregular' (see the preceding discussion). Potentially such lumping may artificially enhance the consistency within the specimen set scores for these characters. However, interpretation of the results of the broader analysis, along with geographic provenance information, showed that other characters separate out these specimen sets at subsequent levels in the analysis hierarchy providing the resolution needed by the population aggregation analysis to separate or agglomerate groups.

The occurrence of projections that blurred the edges of the spots was consistent in all groups. This character grouped together the Zambian population of Thornicroft's giraffe (SZT - *G. c. thornicrofti*) with the west African giraffe (West – WCP and WSN – *G. c. peralta*) to the exclusion of other southern and central-eastern African sets that were otherwise similar in the first round of analysis. The disjunct geographic distribution of these grouped specimen sets suggests that this is a convergent character. The presence of these sharp edged spots may be an important diagnostic feature of the isolated Thornicroft's giraffe.

The width of the interspot lines is the most variable feature of the body spot pattern with the width of the rump lines (8) being excluded from analysis as being excessively individually variable while the width of the fore flank lines (4) was identified as individually variable in six geographical sets (Table 7.6). Hence, while spot shape and

distinctiveness are of great importance, the spaces between spots have little taxonomic value.

Limb Spots – Below the Hocks

Lydekker (1904) described the occurrence of spots and the background colour of the legs below the hocks for most of his subspecies. These features have, since then, been regarded as diagnostic for subspecies and are reported in recent descriptions of subspecies patterns (e.g. Dagg and Foster, 1982). However, according to the spot patterns observed in the sample the occurrence of spotting below the hocks is individually variable within geographic specimen sets, making this feature useless as a taxonomic character.

Head Spots

Krumbeigel (1939) considered the head and face spots to vary individually and did not use them in his analysis of spot patterns (Dagg and Foster, 1982). The presence or absence of head and face spots (characters 14, 15, 16, 17 and 18) was individually variable in only two (15 and 16) or three (14, 17 and 18) geographical areas. The results suggest a trend for the presence of head and face spots in northern populations with a lack of head spotting in the southern groups. The presence or absence of head spots is then a valid character to be used in combination with other characters.

Conclusions from Pelage Pattern Analysis

Analytical Aspects

The modified 'hierarchical' population aggregation analysis approach presented above separates six geographically restricted specimen sets while agglomerating two pairs of sets, resulting in eight differentiated populations.

The character coding protocol used often effectively recognised extremes of variation (e.g. in spot shape or size) while clumping together 'in between' patterns into a broad, central character state category. Spot patterns require greater quantification to provide the resolution to more adequately define the observed variation. Image analysis techniques such as erosion cycling and erosion-dilation cycling (e.g. Ehrlich *et al.*, 1984) may provide an opportunity to objectively quantify the observed variation in spot pattern to more adequately resolve the differences seen.

Geographical Differentiation and Subspecies Recognised

The analysis presented here indicates that combinations of character states that are consistent within local geographically delimited specimen groups can be used to differentiate between currently recognised subspecies. Also, populations from within a subspecies range are sufficiently similar to be agglomerated together into one group. This suggests that subspecies, recognised by their pelage patterns are valid. However, sample sizes are small and the validity of the characters and character states selected require further validation with greater numbers of samples.

This analysis supports six of the currently recognised subspecies. *G. c. reticulata* (EEK), *G. c. tippelskirchi* (ESK and EST), *G. c. rothschildi* (ECU - including *G. c. congoensis*, ECC), *G. c. peralta* (West – WCP and WSN), *G. c. thornicrofti* (SZT) and *G. c. giraffa* (SCZ and SWC, perhaps including SWA) are all identified according to unique character state combinations, interpreted alongside geographical provenance information.

Another subspecies (*G. c. angolensis* - SWA) is represented by a single specimen and could not be separated from the other southern subspecies. A greater sample is needed to clarify the relationship. A lack of adequate characters from two of the recognised areas (ENA and SEW) and the omission of any specimens from a third (ENE) means that the status of these populations, with respect to their pelage patterns, can not be established at this time. Two of these populations represent separate currently recognised subspecies with ENA representing *G. c. antiquorum* and ENE representing *G. c. camelopardalis*. Meanwhile, the SEW population is considered as part of *G. c. giraffa* by many authors (e.g. Dagg and Foster, 1982) while others consider it a separate subspecies (*G. c. wardi*. e.g. Lydekker, 1904). If considered a separate subspecies the range of *G. c. wardi* would include the SCZ population, here agglomerated with SWC into *G. c. giraffa*. The SEW population is represented only by three mounted heads, the spot patterns of which are congruent with SEW being agglomerated with the other two populations. On this basis it is suggested that SCZ, SEW SWC and SWA make up a single southern subspecies, *G. c. giraffa*.

These results must be interpreted and applied with some care. The hierarchical analysis used here is one-way. That is, it allows separation of the local specimen sets but the results obtained can not then be generalised and used to classify an unknown specimen as a member of a particular subspecies. The selective nature of the hierarchical analysis presented and the occurrence of individual variation in some groups for certain characters means that the results obtained are locally optimal and particular to the data set in hand.

Generalisations can not be made with confidence. This same truth is applicable to the original descriptions of the subspecies, due to the limited sample sizes available to these authors (de Winton, 1897; 1899; Lydekker, 1904; 1911; Thomas 1894; 1901). While some of the results derived from this analysis appear robust (such as the recognition of the EEK and ESK/EST groups as separate subspecies and the conjoining of the SCZ and SWC groups into a single subspecies), and the character states diagnostic of the subspecies inferred, other separations appear more subjective and are deserving of further investigation.

The same geographically restricted specimen sets examined for diagnostic differences in pelage patterns are investigated in the next section for structured morphological variation.

CHAPTER 8:

SELECTION OF CRANIAL AND SKELETAL DIMENSIONS FOR INCLUSION IN TRADITIONAL MORPHOMETRIC ANALYSIS OF GEOGRAPHIC VARIATION IN GIRAFFE

Introduction

'Traditional' morphometrics applies multivariate statistical procedures to sets of measured variables (Marcus, 1990; Rohlf and Marcus, 1993). The variables are typically linear, inter-landmark distances which quantify the lengths, heights or widths of given structures, although sometimes angles or ratios may be used (Rohlf and Marcus, 1993).

Selection of Variables

Selection of variables can have a profound effect on the results of multivariate analyses and, therefore, on their interpretation. Results of multivariate analyses will be 'locally' optimal, but may not be 'globally' optimal. That is they reflect the relationships for the variables and cases entered into that analysis, but these relationships may change when additional data, a different subset of data from the same set of specimens or data from additional specimens are entered into the analysis. Such ambiguity is not unique to multivariate techniques and is a feature of all analytical procedures where a 'local' sample is taken from a 'global' population. The assumption common to all sampling techniques is that the sample is a representative selection from the population and that the chosen variables somehow summarise the 'relevant' variation. Although this assumption is implicit in most studies it is rarely addressed.

Poor selection of variables can a provide information that is difficult to interpret. The data may contain insufficient information, or it may contain redundant information. Obviously, if insufficient or irrelevant information is used the relationships between sampling entities may not reflect the 'true' relationships. Meanwhile, redundant information (where two or more variables are highly correlated) can add 'noise' to the analysis interfering with the signal. Hence, the selection of variables is of great importance[1].

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[1] Of course, a poor selection of specimens that are not representative of the underlying populations may also cause similar problems. Therefore, the sampling regime is of equal importance. Within the confines of the current study every attempt was made to maximise the number of specimens included in each population. The assumption that the sampled specimens are representative of their populations is implicit in all of the

In previous studies a number of approaches to variable selection have been taken. Some authors have selected a limited number of variables, apparently arbitrarily. Others have used as many variables as practically possible, following the Numerical Taxonomy paradigm (Sneath and Sokal, 1973) of attempting to summarise the 'total' shape variation. A third, more rigorous approach, considers the organism as a unified integration of a set of functional units and seeks to remove redundancy and represent variation by identifying and quantifying these functional units (e.g. Taylor and Meester, 1993; Chimimba and Dipenaar, 1995; Chimimba *et al.*, 1999). This approach is based on the concept of morphological integration proposed by Olsen and Miller (1958, see below).

Morphological Integration

Organisms and parts of individual organisms show concerted development with functional units undertaking particular tasks within the larger structure. This is the basis for the concept of morphological integration. The concept was introduced and developed in 1950s, particularly by Olson and Miller (1958) and in earlier works by Miller (1950), Miller and Weller (1952) and Olson and Miller (1951). Morphological integration considers each individual organism as a complex whole made up of functionally autonomous parts, each of which is 'responsible' for a different function (although, in reality, many structures fulfil, or are involved in, multiple functions or combine to fulfil a single function). For example, different parts of an organism may be responsible for locomotion, for food acquisition and/or processing, for receiving sensory information, etc. All of these functions come together to make up a functional whole; the organism. Olson and Miller (1958, p. v) describe morphological integration as referring to "the summation of characters which, in their interdependency of form, produce an organism." They go on to suggest that "[i]t would seem logical that the degree of interdependency of any two or more morphological components in development and function would bear a direct relationship to the extent of their particular morphological integration." (p. v). That is, highly correlated variables may indicate a nexus of measurements all describing aspects of one of the functional parts of the integrated whole that is the organism. As a result of their studies, Olson and Miller (1958) developed a method whereby functional groups, or F-groups, are determined for an organism from *a priori* knowledge of the organism's behaviour and ecology. Correlation coefficients between the measured variables are then examined to find groups of closely correlated variables, identifying basic pairs (pairs of the most closely related variables) and

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following analyses. As noted earlier, taxonomic entities represent hypotheses of relatedness that may be updated with additional data.

larger phenotypic, or ρ-groups. The members of the intersections between the F- and ρ-groups (termed ρF-groups) are interpreted to identify the integrated units that go to make up the whole organism.

The ensuing analysis utilises the concept of morphological integration, and implements a rigorous set of selection criteria. These criteria aim to reduce the effect of redundant information and missing data and to maximise the sample size used in the subsequent multivariate statistical analyses by identifying phenotypic – functional group intersects and selecting representative variables from these sets.

Materials and Methods

Sexual Dimorphism

Giraffe skulls show sexual dimorphism and were, therefore, analysed separately. The determination of sex is described in Chapter 5.

Measurement of Variables

The data acquisition protocol used in this study (described in Chapter 3) sought to optimise the efficiency of initial information gathering by maximising the quantity of good quality information gathered in a reasonable time, and then to subsequently remove redundant information. The approach used has four major advantages:

1. It avoids the possible, unknown biases that may be introduced by arbitrary selection of a relatively small number of variables;
2. It removes highly correlated variables from further analysis;
3. It ensures that each functional unit is represented in the analysis; and,
4. It ensures that variables included in the ultimate analysis are derived from a repeatable empirical assessment of the data.

Measurements were chosen following the literature where giraffe subspecies were described or other variability in giraffe morphology had been discussed. When an author has drawn attention to a particular feature of the skull or post-cranial skeleton these features were closely examined in a selection of specimens from the collection of the Natural History Museum, London and a series of measurements devised to quantify the relevant variability. Features were examined if highlighted in a description by any author

even if a subsequent author refuted their usefulness[2]. Some of the measurements that extend across more than one structure in the skull were subdivided into two or more separate measurements in order to try to acquire more information on the detailed variability of the skull. In addition to the variables selected from the literature descriptions, a series of measurements was made to acquire information on the 'gross morphology' of the skull. Where there was an obvious gap additional measurements were taken simply to 'fill in' these areas. Hence, an attempt was made to characterise the entire skull by a series of linear point to point, inter-landmark measurements.

Measurements taken

In total 59 different measurements were taken from each specimen; 38 of these were from the cranium (including mass and 9 measurements directly related to the parietal horns), 8 from the mandible (including one of the canine tooth), 10 from the teeth, and 3 from the post-cranial skeleton. Of these 19 of the 38 cranial measurements and 6 of the 8 mandibular measures were taken bilaterally. All of the tooth and post-cranial measurements were taken bilaterally making a total of 97 separate measurements for each specimen.

A list of the measurements taken, with a full description and illustration of each is given in Appendices 2.3.3, 2.3.4 and illustrated in Appendix 2.3.6.

Dealing With Missing Data in the Raw Data Matrix: Implications for the Analysis

In any taxonomic or morphological study missing data might occur in the raw data matrix due to a specimen lacking a particular part (i.e. the character was present in life but the part was not collected or has been subsequently damaged or lost in storage). Often specimens show common patterns of breakage due to weakness in a particular structure making it liable to breakage (e.g. loss of premaxillae at the maxillary / premaxillary suture) or specimens are damaged in handling (e.g. damage to occipital condyles when removing the head from the body). As a result, missing values are often concentrated in certain variables and not randomly distributed through the data matrix. A method that will remove the effect of these variables from the analysis while minimising the loss of information is needed.

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[2] For example, Lydekker (1904) described differences between subspecies in the shape of the parietal horns. Later Dagg (1968) and Singer and Boné (1960) disputed the usefulness of this character in recognising subspecies.

Missing data are difficult to deal with for statistical analyses that require complete data sets. A number of different solutions to the problem of missing data have been proposed, but all introduce new assumptions and potential biases into the data. Typically statistical computer packages deal with the problem of missing data in one of two ways; either by pairwise deletion or by listwise deletion of variables. In deriving correlation coefficient matrices (as used in morphological integration analysis) pair-wise deletion disregards a specimen when one of the two variables being compared is missing. This, potentially, leads to a data matrix with different sample sizes for each correlation coefficient in the matrix giving different levels of confidence around each value. Case-wise deletion removes any specimen from the analysis that has any missing value. Although an apparently Draconian approach, this does maintain consistency in sample sizes throughout the matrix.

In the morphological integration analysis performed here case-wise deletion of specimens has been used for all analyses. This has some serious implications for the data available for analysis. For the 143 adult specimens included in this study 111 (77.6%) of them had a mandible present, while only 24 (16.8%) had limb bones available. Use of casewise deletion while including cranial, mandibular and appendicular skeletal elements would leave no male specimens and only one female specimen with all values available. Inclusion of cranial and mandibular data only gives nine males and seven females. Clearly these are not useful sample sizes. Cranial data is complete for 34 male and 13 female specimens. Hence, only cranial variables were used in this analysis.

It must now be assessed whether the sub-sample of skulls with a complete data set available (hereafter referred to as 'complete skulls') is representative of the entire sample. Student's *t*-tests and a variance ratio test (*F*-test) comparing the complete against the incomplete specimens were performed for each variable.

Data Quality

Statistical Assumption Testing

Canonical Variates Analysis (CVA), a multivariate discriminant analysis technique that will be used to analyse the final data set, makes a number of statistical assumptions regarding the sampling, distribution and quality of the input data. These include multivariate normality, random sampling without outliers and linearity of variable relationships to the underlying variables (McGarigal *et al.*, 2000).

There is no way to explicitly test the multivariate normality of data. However, the univariate normality of standardised variable scores was assessed by comparison to the

expected distribution of scores according to a normal distribution using a Kolmogorov-Smirnoff test, as implemented in Systat (Version 8). Tests of skewness (shifting of the median scores from the centre of the distribution) and kurtosis (flattening or heightening of the bell-shaped curve) were also performed on the standardised data.

Outliers were assessed by eye by examining Normal Probability plots of the standardised data against values expected if the data conform to a Normal Distribution. All outliers deviating by more than 2.5 standard deviations from the mean were removed from further analysis.

Replication and Measurement Error

Fourteen male skulls in the collection of the Natural History Museum, London were measured on two separate occasions for all variables (bilaterally, if relevant). Each pair of measurements was then tested using a paired t-test to examine consistency between the two sets of measurements.

Five of these male skulls were measured multiple times to assess measurement error. Each skull was measured three times for all measurements in a single session. Each was subsequently measured for all variables on two more occasions over the course of two months. The handling time for each skull, determined by their large size and the number of measurements to be taken, precluded the measurement of a larger set of skulls or a greater number of replicates.

Due to the number of replicates measured the coefficient of variation is not an appropriate statistic to express measurement error in this case. A similar statistic, introduced here and termed the 'standard maximum difference' or SMD was calculated for each variable and makes use of the maximum difference found between each pair of variables. The SMD took the maximum difference between any pair of measurements and divided by the mean of the three values. The resulting fraction was expressed as a percentage. Any variable within a specimen giving an absolute maximum difference greater than 2mm over the three measurements was highlighted and the SMD value examined. If the SMD percentage was then greater than 2 per cent, a potential for measuring error in these variables was inferred.

Replication and measurement error was only assessed on a sub-set of male skulls.

Morphological Integration, F-sets and ρ-sets.

Olson and Miller (1958)-style F-sets were created subjectively by consideration of the suite of cranial variables measured. Cheverud (1982) recognised two major components of the skull; the neurocranial and the orofacial components. He further sub-divided the neurocranial component into three partially independent sub-units; the frontal, parietal and occipital sub-units. The orofacial component was sub-divided into the orbital, nasal, oral and masticatory units. Similar terminology, with some modification relevant to giraffe skulls, has been followed in the present study. Basic pairs and ρ-sets were determined empirically from the correlation matrix (Pearson product-moment correlation coefficients). Pairs of variables with population correlation coefficients (ρ) greater than 0.85 were linked together to create ρ-sets. The intersection of the F- and ρ-sets were examined and the integrated functional – phenotypic groups determined.

The final set of variables to be included in the PCA were chosen such that:

1. Three 'gross morphology' parameters were included;
2. At least one member of each basic pair was included;
3. At least one member of each ρ-group was included;
4. At least one member of each F-group was included;
5. The eight variables not assigned to a ρ-group were treated as a separate group and variables only removed if they had a large proportion of missing values.

Decisions to remove particular variables considered the following criteria:

1. Variables that were significantly different between within-session replicate measurements were removed;
2. Variables with a high measurement error (between-sessions) were removed;
3. Where two parameters from different F-groups occur in the same ρ-group, the pair with the lower correlation coefficient was used (e.g. in choosing whether to include **FMP** or **HSP** with **NOL**);
4. Combinations of variables that define particular relationships discussed in the literature (e.g. in the parietal horn F-group **EWB** and **EWT** describe parietal horn divergence);
5. At all stages, priority was given to removing variables with a large proportion of missing data, hence maximising the sample size for inclusion

in the subsequent analysis. All variables with a frequency less than 80% were excluded.

6. The variables **FMO** and **FMS** have been excluded from all analyses due to practical concerns over the consistency of the measurement of these variables. These measurements might not be consistently homologous between skulls as the upper landmark was effectively positioned at an extreme of curvature that manifested itself in a different position, relative to other structures, for each skull.

Results

Measured Variables

Summary statistics of all measured variables for each specimen are listed in Appendix 4.8.2. Raw data are available from the author. The frequency of recording of each variable is given in Appendix 4.8.1.

Dealing With Missing Data in the Raw Data Matrix: Implications for the analysis.

Complete results of Student's *t*-tests and variance ratio tests are given in Appendix 4.8.3 for male and female specimens separately.

Male Data

Univariate Student's *t*-tests comparing mean values of the complete and incomplete male skull variables show no significant differences between the two data sets. Variance ratio tests (*F*-tests) indicate that four of the variables show significantly different variances (**NCL**, **OOL**, **ZGW** and **PGW**).

Female Data

Twenty-one of the 35 variables showed significant differences between their means between excluded and included female skulls when tested with a Student's *t*-test. Meanwhile four variables have significantly different variances (**ZGW**, **MMW**, **MXTL** and **IPW**).

The results of the Student's t-test indicate that, while the complete male data set is representative of the univariate structure of the entire sample, the complete female data set is not. Hence, use of case-wise deletion in the analysis of the female raw data matrix can not be justified. For this reason the selection of cranial variables to be used in multivariate statistical analysis of both male and female data will be based upon the analysis of male data only.

Data Quality

Statistical Assumptions Tests

The results of tests for normality, skewness and kurtosis are presented in Appendix 4.8.4.

None of the standardised variables departed significantly from a normal distribution. Similarly, none were kurtotic, while only **MMH** (the height of the median horn from the maxillary tooth row) was significantly positively skewed. However, outliers were noted for each variable. Typically, no individual specimen was seen consistently as an outlier over multiple variables indicating that none was distinctly larger or smaller than the others were. Hence, no specimens were excluded from the analysis at this stage.

Replication and Measurement Error

Of the 53 cranial replicate measurements tested by a paired *t*-test (including 18 bilateral measurements) only two were significantly different between sampling sessions (all results are given in Appendix 4.8.5). The measurement from the foramen magnum to the tip of the parietal horn (**FMP**) on the left side is significantly different ($n = 14$, $t = 2.543$, $p = 0.025$). However, the mean difference is 1.5mm representing less than 0.5 per cent of the mean value for this measurement. In ten of the fourteen cases the difference between measurements is less than 1mm. The reason for the significant difference is that, by coincidence, the first of the replicates is consistently the higher value for all specimens. Hence, it is justified to continue using this variable in future analysis.

The second variable giving a significant difference between sampling sessions is the external width of the occipital ridge (**EWO**: $n = 14$, $t = -2.709$, $p = 0.018$). In this case the mean difference is 2.64mm. While eight of the 14 replicated measurements were within 1mm of each other, the remaining six had differences of 3, 5, 6, 7, 7, and 10mm

respectively. This suggests a problem with the consistent location of landmark points for the measurement of this variable. Hence, **EWO** was excluded from future analyses.

The standard maximum difference (SMD) was calculated for each variable from two sets of three replicate measurements. The first set was measured within one session ("Within session SMD"). The second set was measured separately in three distinct sessions ("Between sessions SMD"). Full results are presented in Appendix 4.8.6. The variables identified as being potential sources of error are listed in Table 8.1. **EWO** occurs on five occasions with high absolute maximum difference values and relative SMD scores. Hence, the exclusion of this variable is justified again. Of the other variables only **TIC** (three times) occurred more than twice. Only **PGW** had an SMD value greater than 5 per cent. Both of these variables were removed from the analysis.

Within Sessions			
Specimen	*Variable*	*Maximum Difference*	*SMD*
BMNH1898.7.2.5	**OOL**	5.5mm	2.04%
	EWO	12.0mm	8.07%
BMNH1938.7.8.22	**TIC**	6.0mm	3.72%
BMNH1962.220	**TIC**	4.0mm	2.33%
BMNH1986.1604	**MMH**	8.5mm	3.06%
Between Sessions			
Specimen	*Variable*	*Maximum Difference*	*SMD*
BMNH1898.7.2.5	**TIC**	8.0mm	4.09%
	EWO	18.0mm	12.65%
BMNH1899.7.8.5	**EWO**	6.0mm	4.32%
	SWM	3.0mm	2.21%
BMNH1938.7.8.22	**LAD**	2.5mm	4.49%
	EWO	5.0mm	3.65%
	MWO	3.0mm	2.13%
	PGW	6.0mm	7.79%
BMNH1962.220	**NOL**	5.0mm	2.04%
	IWT	6.0mm	3.90%
BMNH1986.1604	**MMH**	6.0mm	2.14%
	EWO	8.0mm	4.82%

Table 8.1: *Variables identified as being potential sources of error according to within and between session replicate measurements. See text for full explanation.*

Morphological Integration, F-sets and ρ-sets.

The measured variables were subjectively classified into functional sets and are listed in Table 8.2.

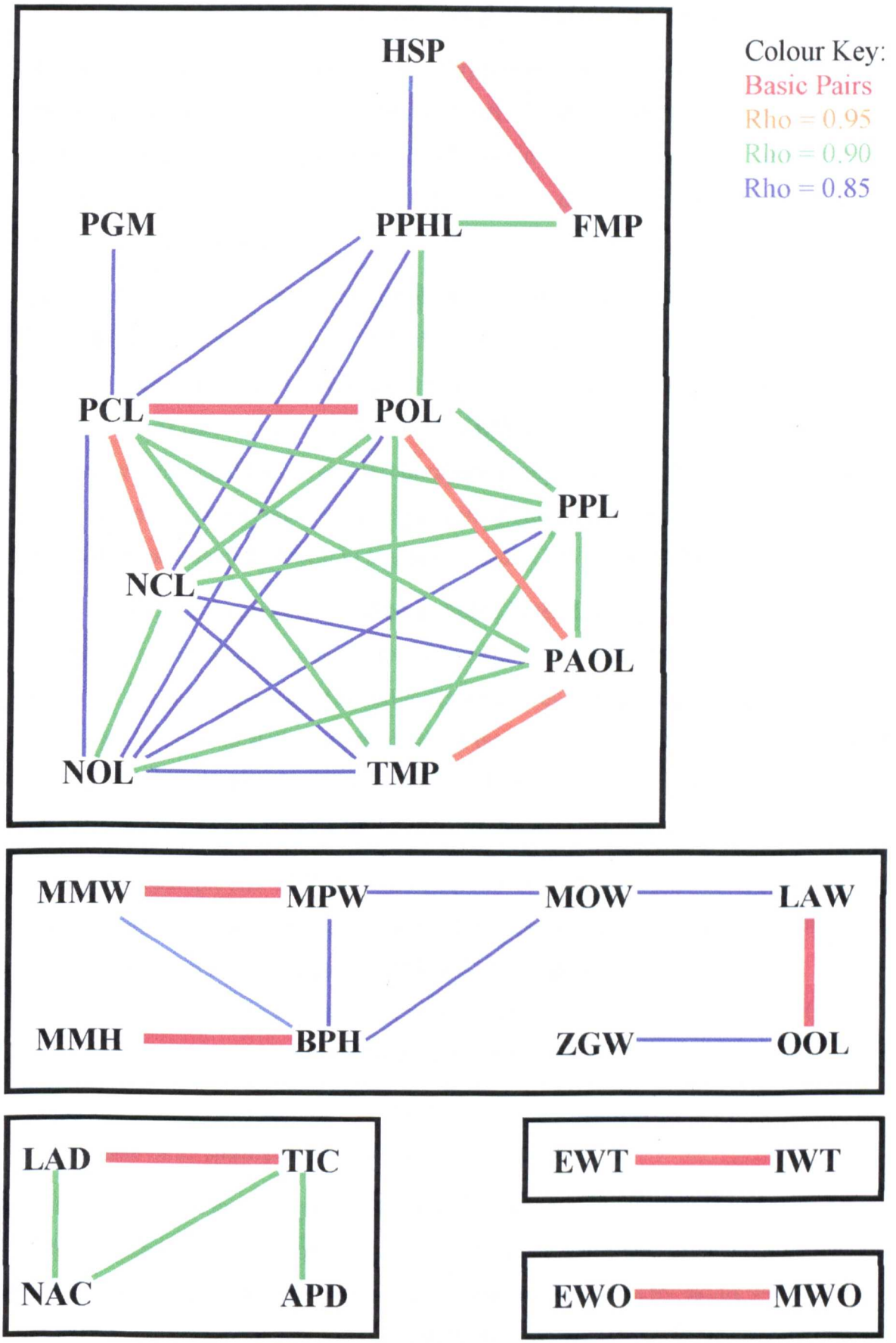
Functional Set	Sub-unit	Variables
Facial		**PAOL, MMW, MPW, MOW, LAW.**
Oral – Masticatory		**MXTL, TMP, SWM, PPL.**
Oral – Pharyngeal		**PPM, PGM, IPW.**
Neurocranial	*Lengths* *Widths* *Height*	**OOL, PACL,** **EWO, EAMW, PGW, EWB,** **BPH.**
Parietal Horns	*Girth* *Height* *Divergence*	**APD, LAD, TIC, NAC,** **PPHL, HSP, FMP,** **EWB, EWT, IWT.**
Median Horn		**MMH.**
Occipital 'Horns'.		**EWO, MWO.**
Gross Morphology	*Lengths* *Widths* *Heights*	**PPHL, POL, PCL, NCL, NOL,** **ZGW, MOW, EAMW, EWB,** **MMH, BPH.**

Table 8.2: *Functional sets, with sub-units, for the 35 cranial variables. Note that some of the variables occur in more than one functional set.*

Empirical ρ-sets were derived from Pearson product-moment correlation coefficients for male and female data separately (correlation coefficients are given in Appendix 4.8.7). The correlation coefficient ρ, as used in the morphological integration model, represents the population correlation coefficient. From the empirical analysis of correlations between measured variables a sample correlation coefficient, r, is obtained. In order to derive ρ-sets from r-values the level of r corresponding to a given level of ρ must be estimated with a certain confidence. Calculating the 95 per cent confidence limits around r and taking the lower bound gives the critical value of ρ to be used (calculation of confidence limits for r follows Zar, 1984. p. 311).

Network diagrams linking parameters at different levels of ρ were constructed. The critical value of ρ chosen for this study is 0.85 as this value represents a level where coherent, discrete ρ-groups are formed with no intersections between groups. The resulting network diagram is presented in Figure 8.1[3]. A ρ value of 0.85 is equivalent to an r-value of 0.718, with 95 per cent confidence.

[3] A 'relaxation' of the correlation coefficient criteria to ρ = 0.80 linked the two major groups with a single linkage (between **POL** and **MMW**) and added two variables (**EWB** and **PPM**) into the network not included at ρ = 0.85.



Not classified into ρ-sets: **EAMW**, **EWB**, **IPW**, **MXTL**, **PACL**, **PGW**, **PPM** and **SWM**.

Figure 8.1: ρ - *sets derived from Pearson product moment correlation coefficients of skull measurements of giraffe.*

Selection of Characters for Multivariate Analysis

In this study the purpose of considering morphological integration in the giraffe skull is primarily to derive a set of representative, relatively uncorrelated variables to be used in the multivariate analysis of geographic variation in skull shape an objective way. A secondary aim was to minimise the effect of missing data and to maximise the sample size included in the analysis.

All variables were included in the morphological integration analysis. Variables were selected to represent the *a priori* derived F-sets and the empirically derived ρ-sets. Variables were removed if they showed significant differences between replicate measurements taken on 14 male skulls (only **EWO** removed by this criterion). Similarly, they were removed if they gave a standard maximum difference (a measurement error statistic defined above) greater than 2 per cent in more than one skull (**TIC** and **EWO**) or an SMD greater than 5 per cent in any single skull (**EWO** and **PGW**).

The final set of thirteen variables to be included in the CVA are listed in Table 8.3 and illustrated in Figure 8.2. Tables 8.4 to 8.8 give descriptive statistics for each of these variables (Table 8.4), results of *t*- and *F*-tests between 'complete' and 'missing' skulls (Table 8.5), results of statistical assumptions tests (Table 8.6) and results of measurement replication efficacy tests (Tables 8.7 and 8.8). Complete results for each of these tests for all variables are given in Appendix 4.8.2 to 4.8.6.

This gives a sample size of 77 specimens for the male analysis. All of the ρ-groups are represented. All of the F-groups except for the oral-pharyngeal are represented. This group contained the variables **PPM, PGM** and **IPW,** all of which were frequently unmeasured due to damage and so were excluded from the analysis (each was measured in 72%, 77% and 72% of specimens respectively).

Using the same set of parameters to examine variation in female skulls gives a sample size of 29 specimens for inclusion in the analysis.

Data and statistics pertaining to the 13 selected variables are presented in Tables 8.4 to 8.8. Table 8.4 gives summary statistics for the 13 parameters. Table 8.5 presents the results of Student's *t*-tests between the means of the values for skulls with a complete complement of data and those with some data missing. Table 8.6 provides results of tests of the univariate normality of each of the variables. The accuracy of replication for the 13 parameters is presented in Table 8.7. Finally, measurement error (assessed as the 'Standard Maximum Difference') is indicated in Table 8.8. Corresponding data for all measured variables can be found in Appendices 4.8.2 to 4.8.6.

BPH	*Bottom of brain case to base of Parietal horns* - Minimum height of the skull from the base of the braincase to the base of the parietal horns.
EWB	*External width of bases* - The maximum width of the base of the parietal horns, including secondary bone deposition.
EWT	*External width of tips* - The maximum width measured to the outside surface of the tips of the parietal horns.
HSP	*Height (from sagittal point)* - The height of the parietal horns measured to the top of the skull. A straight edge was rested across the tops of both horns and the height to the closest point of the skull was measured from the centre point of the straight edge.
LAD	*Lateral diameter of tip* - Measured bilaterally, as the maximum diameter of the tip of the parietal horns in the lateral orientation, typically at right angles to the antero-posterior plane.
MMH	*Posterior M^2 to tip of Median horn* - Maximum height of the skull from the most ventral edge of the maxillary bone immediately posterior to the third maxillary molar to the highest point on the mid line of the median horn.
MMW	*Minimum Maxillary width* - The minimum width across the maxillary bones anterior to the molariform teeth.
MOW	*Maximum Orbital width* - Maximum width across the frontal bones forming the dorsal margin of the orbit.
MWO	*Maximum width of the occipital ridge* - Measured to the maximum width of the secondary bone growth on the occipital ridge. If no secondary bone growth was present the value for **EWO** was used here.
MXTL	*Toothrow length (maxillary)* - The length of the maxillary toothrow from the anterior margin of the PM^2 alveolus to the posterior margin of the M^3 alveolus. The measurement was made to the edges of the alveoli, rather than to the teeth themselves so that the same measurement could be taken on skulls with missing teeth.
NOL	*Nasal Notch to anterior Orbit* - Measured from the most posterior angle of the nasal notch to the most anterior margin of the orbit.
OOL	*Posterior Orbit to Occipital ridge* - Measured bilaterally from the most posterior margin of the orbit to the furthest point of the occipital ridge.
SWM.	*Snout width at M^1* - Minimum distance across the palate measured between the M^1 alveoli.

Table 8.3: *List of variables selected for inclusion in the 'traditional' multivariate statistical analysis of morphological variation in the giraffe skull. The parameters were selected from a suite of 59 measured variables using the principles of morphological integration (Olsen and Miller, 1958). See the text for further details. These measurements are illustrated in Figure 8.2.*

Discussion

This chapter has presented a method that selects dimensions (characters) for inclusion in a traditional, multivariate statistical analysis of morphometric variation. Using the principles of morphological integration (Olson and Miller, 1958) 51 of the original 59 variables were grouped into five correlated 'phenotypic sets'. Comparison with eight *a priori* defined 'functional sets' allowed selection of the final 13 variables. The purpose of this selection procedure is to quantify the relevant variation between specimens, reduce the inclusion of

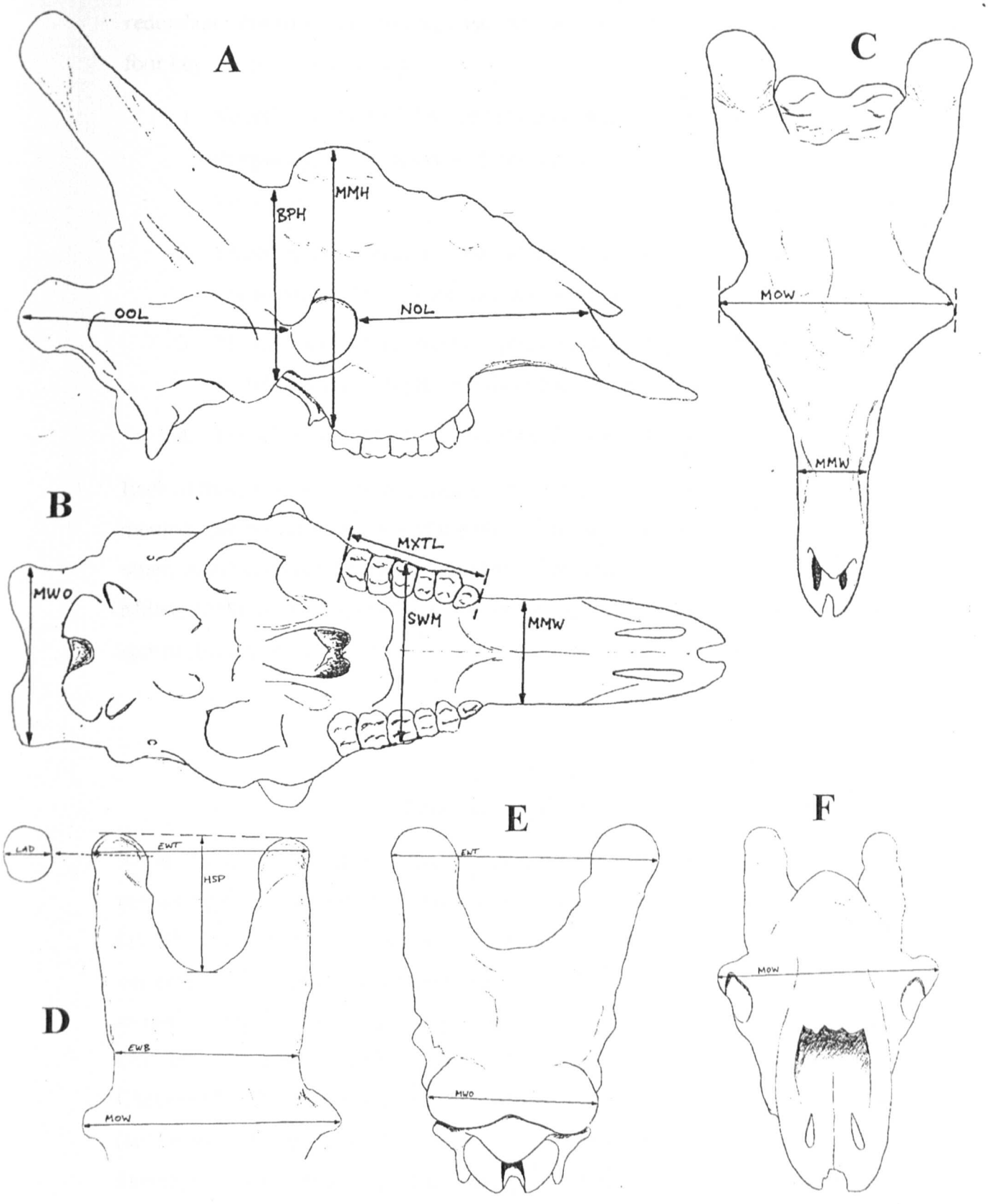


Figure 8.2: *Measurements selected to be included in the traditional morphometric analysis. Thirteen parameters were selected from 59 cranial measurements taken using the principals of morphological integration (Olsen and Miller, 1958). The selected varaibles are described in Table 8.3. The full set of 59 measurements taken are illustrated in Appendix 2.3.6. Diagrams based on skull BMNH1986.1604.*

A - *Side view;* **B** - *Bottom of skull;* **C** - *Top view of skull;* **D** - *Front view of parietal horns;* **E** - *Back of skull;* **F** - *Front view of skull.*

redundant information and to minimise the loss of data due to missing values. It identifies four key stages in the procedure:

1. Selection of the initial set of variables based on literature discussions of the morphology of the species of interest and direct observation of the specimens;
2. Screening of the acquired data to meet the assumptions of the proposed statistical tests and for consistency in measuring;
3. The derivation of functional – phenotypic sets, based on the principles of Morphological Integration (Olson and Miller, 1958);
4. The selection of the final data set for subsequent analysis.

Each of these points has been discussed at the relevant points in this chapter. However, the method used for the derivation of the phenotypic sets does not follow the approach of other, recent authors (although it does follow the original recommendations of Olson and Miller, 1958). A discussion of the alternative approaches, with a justification of the approach used here is warranted.

Derivation of Phenotypic Sets

Recent authors using a morphological integration approach to the selection of morphological dimensions for multivariate statistical analysis include Taylor and Meester (1993), Chimimba and Dipenaar (1995) and Chimimba *et al.* (1999). They differ from the current study, and from the original method of Olson and Miller (1958), by using cluster analysis techniques to derive their phenotypic groups. Taylor and Meester (1993) and Chimimba and Dipenaar (1995) justify their use of cluster analysis with reference to Cheverud (1982). However, there are potential problems with cluster analysis techniques (see below). The current study returns to Olson and Miller's (1958) original method in directly employing Pearson product – moment correlation coefficients to assess the relationships between variables.

Cluster analysis is a generic term for a family of techniques that attempt to organise sampling entities into discrete classes or groups using particular criteria to minimise within group variation and maximise between group variation (McGarigal *et al.*, 2000). A problem arises when different clustering algorithms provide different structures to the data set. That is, to some extent, the structure apparent in the derived clusters, perhaps

represented as a branching dendrogram, represents an artefact of the similarity / dissimilarity index and the clustering algorithm chosen for the analysis, rather than an 'analysis neutral' summary of the true relationships among the data.

Cluster analysis is typically an iterative procedure with groupings of entities made at each step according to a predefined set of criteria. At the first step individual entities are linked together according to a similarity value of some sort (e.g. Euclidean distance). It is usually easy to see which values are the closest and so are linked together into groups. At the next step these groups are linked together, and this is where the clustering algorithm used exerts its effect. How should groups be linked together? Different algorithms use different approaches and may have an effect on the structure of the hierarchy (McGarigal *et al*, 2000). There is rarely a biologically valid reason for choosing between hierarchical clustering techniques. In the present study the use of different clustering algorithms was investigated. The result was a lack of consistency between the techniques, which worsened higher in the hierarchy. The widely differing placement of entities within groups indicating that the clustering algorithm used may have a greater influence on the results than the signal in the data.

Olson and Miller (1958), when developing the principals of morphological integration and its analysis, considered this very problem. They state that during their work "various types of multivariate analysis were considered as possible means for studying the association of measures. All were rejected as unsatisfactory for our purposes, generally because they tended to obscure the networks of measures which were the very things that we wished to examine." (p. vi). They returned to the simple, non-hierarchical division of entities according to their degree of relatedness indicated by their Pearson product – moment correlation coefficients. This approach is justified as the method makes the fewest assumptions, does not impose an unjustifiable model on the data and is readily understandable. This approach has been used in this study.

The 13 representative variables selected are used in the next chapter to investigate variation in skull morphology using 'traditional' multivariate statistical techniques.

Male Data

Variable	Gender	n	Min	Max	Mean	SD
BPH	Male	95	160.0	250.0	196.6	16.38
EWB	Male	98	170.0	275.0	222.5	19.73
EWT	Male	98	169.0	296.0	222.6	27.82
HSP	Male	99	109.0	229.0	157.6	21.04
LAD	Male	99	36.0	78.0	54.25	9.636
MMH	Male	98	212.0	402.5	288.0	36.96
MMW	Male	93	76.0	111.0	94.41	8.040
MOW	Male	98	249.0	337.0	291.6	20.03
MWO	Male	99	113.0	180.0	146.0	11.25
MXTL	Male	98	130.5	163.0	147.3	6.149
NOL	Male	87	218.0	290.0	250.1	15.53
OOL	Male	96	227.5	302.5	263.0	15.60
SWM	Male	96	137.0	169.0	152.7	7.267

Female Data

Variable	Gender	n	Min	Max	Mean	SD
BPH	Female	41	137.0	190.0	160.85	12.56
EWB	Female	43	140.0	202.0	170.1	15.16
EWT	Female	42	97.0	227.0	138.7	24.63
HSP	Female	43	66.0	153.0	94.19	17.53
LAD	Female	41	14.0	36.5	20.14	3.719
MMH	Female	43	193.5	288.0	221.9	18.67
MMW	Female	35	60.0	163.0	79.74	15.62
MOW	Female	43	227.0	290.0	258.4	14.97
MWO	Female	43	103.0	135.0	120.7	8.142
MXTL	Female	43	131.5	154.0	142.7	4.862
NOL	Female	38	195.0	244.0	220.5	9.867
OOL	Female	43	207.0	258.5	231.3	10.24
SWM	Female	43	131.0	160.0	147.7	6.937

Table 8.4: *Summary statistics for male and female data for the thirteen variables selected for inclusion in the multivariate statistical analysis of morphometric variation in giraffe skulls. See text for further details.*

Male Data

Variable	Group	n	t	p	F	p
NOL	Complete	34	0.685	0.495	1.097	>0.05
	Missing	53				NS
OOL	Complete	34	-0.429*	0.669	1.873	0.026
	Missing	62				*
MMW	Complete	34	-0.237	0.813	1.477	>0.05
	Missing	59				NS
MOW	Complete	34	0.174	0.862	1.202	>0.05
	Missing	64				NS
MMH	Complete	34	1.837	0.069	1.073	>0.05
	Missing	64				NS
BPH	Complete	34	0.633	0.528	1.428	>0.05
	Missing	61				NS
LAD	Complete	34	0.140	0.889	1.535	>0.05
	Missing	65				NS
HSP	Complete	34	-0.854	0.395	1.150	>0.05
	Missing	65				NS
EWB	Complete	34	-0.132	0.895	1.667	>0.05
	Missing	64				NS
EWT	Complete	34	-0.195	0.846	1.548	>0.05
	Missing	64				NS
MWO	Complete	34	-0.606	0.546	1.518	>0.05
	Missing	65				NS
MXTL	Complete	34	-0.046	0.963	1.448	>0.05
	Missing	64				NS
SWM	Complete	34	-0.140	0.889	1.218	>0.05
	Missing	62				NS

Female Data

Variable	Group	n	t	p	F	p
NOL	Complete	13	1.763	0.086	1.161	>0.05
	Missing	25				NS
OOL	Complete	13	4.305	<0.001	1.196	>0.05
	Missing	30		***		NS
MMW	Complete	13	-0.213*	0.833	13.134	<0.001
	Missing	22				***
MOW	Complete	13	3.333	0.002	1.790	>0.05
	Missing	30		**		NS
MMH	Complete	13	2.190	0.034	1.609	>0.05
	Missing	30		*		NS
BPH	Complete	13	1.721	0.093	1.230	>0.05
	Missing	28				NS
LAD	Complete	13	-1.209	0.234	2.048	>0.05
	Missing	28				NS
HSP	Complete	13	2.481	0.017	1.275	>0.05
	Missing	30		*		NS
EWB	Complete	13	3.008	0.004	1.586	>0.05
	Missing	30		**		NS
EWT	Complete	13	2.202	0.033	1.620	>0.05
	Missing	29		*		NS
MWO	Complete	13	3.689	0.001	1.317	>0.05
	Missing	30		***		NS
MXTL	Complete	13	1.428*	0.161	4.123	0.006
	Missing	30				**
SWM	Complete	13	2.720	0.010	1.188	>0.05
	Missing	30		**		NS

Table 8.5: *Results of statistical tests of differences between data derived from skulls with a complete data set and those skulls with some values missing from the data set for the thirteen variables selected for inclusion in the analysis of morphometric variation in giraffe skulls. All t values are pooled variance t-values except for *, which have significantly different variances (as identified by the F-values).*

Male Data

Variable	n	Normality p	Skewness G1	G1/SES	Kurtosis G2	G2/SEK
NOL	86	0.204	0.304	1.171	-0.263	-0.512
OOL	96	0.325	-0.069	-0.280	-0.220	-0.451
MMW	93	0.773	-0.072	-0.287	-0.570	-1.15
MOW	98	0.806	-0.206	-0.846	-0.651	-1.348
MMH	98	0.069	0.835	3.427	0.604	1.251
BPH	95	0.550	0.530	2.143	0.451	0.921
LAD	99	0.553	0.225	0.928	-0.752	-1.564
HSP	99	0.855	0.304	1.251	0.381	0.794
EWB	98	0994	0.009	0.037	-0.156	-0.323
EWT	98	0.799	0.406	1.665	-0.497	-1.030
MWO	99	0.940	0.277	1.142	0.193	0.403
MXTL	98	0.757	-0.086	-0.354	0.232	0.480
SWM	97	0.982	-0.117	-0.478	-0.530	-1.091

Female Data

Variable	n	Normality p	Skewness G1	G1/SES	Kurtosis G2	G2/SEK
NOL	36	0.674	-0.394	-1.004	1.802	2.347
OOL	41	0.798	-0.301	-0.815	0.499	0.689
MMW	34	0.696	-0.435	-1.08	1.489	1.890
MOW	41	0.928	-0.205	-0.554	0.003	0.004
MMH	40	0.965	0.344	0.919	-0.444	-0.606
BPH	39	0.930	0.394	1.042	0.383	0.517
LAD	39	0.962	0.191	0.504	-0.086	-0.116
HSP	41	0.993	0.382	1.035	0.441	0.608
EWB	41	0.536	0.220	0.596	-0.709	-0.979
EWT	40	0.451	-0.265	-0.708	-1.099	-1.501
MWO	41	0.876	-0.135	-0.366	-0.460	-0.635
MXTL	41	0.860	-0.196	-0.530	0.270	0.372
SWM	41	0.678	-0.517	-1.398	0.112	-0.154

Table 8.6: *Results of univariate statistical assumptions tests for the thirteen variables selected for inclusion in the multivariate morphometric analysis of giraffe skulls for male and female data. See the text for details of the selection procedure. All tests were performed on standardised data (mean = 0, SD = 1). Probability values testing for Normality were derived from a Kolmogorov – Smirnov one sample goodness of fit test using a Normal distribution with mean of 0 and SD of 1 as implemented by Systat (version 8). No male or female variable departed significantly from the Normal distribution. Skewness (G1) and Kurtosis (G2) statistics are given. Skewness and Kurtosis values are considered significant when the ratio of these values to their standard errors (SES and SEK respectively) is greater than 2. Significant values are highlighted. For the male data* **MMH** *and* **BPH** *are slightly positively skewed (shifted to the right). For the female data* **NOL** *is slightly positively kurtotic (more 'peaked' than a Normal distribution).*

Variable	Side	n	t	p	Variable	Side	n	t	p
NOL	Left	14	1.325	0.208	**LAD**	Left	14	-0.763	0.459
	Right	13	-0.267	0.794		Right	14	-0.249	0.807
OOL	Left	13	-1.148	0.273	**HSP**		14	1.102	0.291
	Right	14	0.000	1.000	**EWB**		14	-0.812	0.431
MMW		14	-1.472	0.165	**EWT**		13	-1.389	0.190
MOW		14	1.295	0.218	**MXTL**	Left	14	0.000	1.000
MMH	Left	14	1.727	0.108		Right	13	-0.365	0.721
	Right	14	1.685	0.116	**SWM**		14	-0.154	0.880
BPH		14	0.000	1.000					

Table 8.7: *Paired t-test results for comparison of replicated cranial measurements for 14 male skull specimens for the thirteen parameters selected for inclusion in the multivariate morphometric analysis of giraffe skulls. Note that no test was performed on the* **MWO** *parameter.*

Specimen	BMNH 1898.7.2.5		BMNH 1899.7.8.5		BMNH 1938.7.8.22		BMNH 1962.220		BMNH 1986.1604		Mean Values	
	MD	*SMD %*	*MD*	*SMD %*	*MD*	*SMD %*	*MD*	*SMD %*	*MD*	*SMD %*	*MD*	*SMD %*
NOL	1.5	0.57%	1.0	0.42%	0.5	0.21%	5.0	2.04%	0.5	0.19%	1.7	0.62%
OOL	1.0	0.37%	0.5	0.21%	0.0	0.00%	1.0	0.37%	1.0	0.35%	0.7	0.27%
MMW	0.0	0.00%	0.0	0.00%	1.0	1.08%	0.0	0.00%	0.0	0.00%	0.2	0.21%
MOW	0.0	0.00%	1.0	0.35%	1.0	0.35%	0.0	0.00%	2.0	0.63%	0.8	0.29%
MMH	1.0	0.34%	0.0	0.00%	1.0	0.60%	2.0	1.02%	6.0	2.14%	2.4	0.86%
BPH	2.0	1.01%	1.0	0.56%	3.0	0.97%	2.0	0.63%	1.0	0.52%	1.6	0.94%
LAD	0.0	0.00%	0.5	1.04%	1.0	0.53%	3.0	1.49%	0.5	0.72%	0.8	0.96%
HSP	2.0	1.37%	1.0	0.80%	2.5	4.49%	0.5	0.93%	2.0	1.23%	1.2	0.74%
EWB	0.0	0.00%	4.0	1.97%	0.0	0.00%	1.0	0.72%	3.0	1.17%	2.2	0.94%
EWT	1.0	0.47%	1.0	0.53%	2.0	0.96%	2.0	0.85%	0.0	0.00%	0.4	0.19%
MWO					0.0	0.00%	0.0	0.00%	0.0	0.00%	1.5	0.97%
MXTL	1.5	1.01%	0.0	0.00%	3.0	2.13%			1.0	0.70%	0.8	0.62%
SWM	1.0	0.64%	3.0	2.21%	0.0	0.00%	1.0	0.60%	1.0	0.60%	1.2	0.62%

Table 8.8: *Measurement Error assessed by calculating Standard Maximum Difference for the thirteen variable selected for inclusion in the multivariate morphometric analysis of giraffe skulls.*

CHAPTER 9:

GEOGRAPHICALLY STRUCTURED MORPHOLOGICAL VARIATION IN THE GIRAFFE SKULL USING A TRADITIONAL MORPHOMETRIC APPROACH

Introduction

'Traditional' morphometrics utilises multivariate statistical techniques to summarise and describe the variation found in biological structures. The application of multivariate statistics to traditional morphometrics can involve three generalised types of analysis. Firstly, to identify trends in the data. Second, to characterise related groups where individual affinities may be known or inferred beforehand. Thirdly, to classify specimens of unknown provenance to one of the defined groups. The first of these analysis types uses ordination methods. The second is explanatory, or descriptive, discriminant analysis and uses *a priori* defined groups of entities, or can be combined with clustering algorithms where no *a priori* groups can be defined. The third is predictive discriminant analysis and uses classification functions derived from the analysis (MacGarigal *et al.*, 2000). These two uses of discriminant analysis derive their information from the same analytical procedure, but differ in their focus.

The traditional morphometric approach taken here uses descriptive discriminant analysis to investigate geographical structure in morphological variation of giraffe skulls. This is accomplished by examination of selected skull dimensions between geographically restricted specimen sets to ascertain whether trends in skull dimensions can be identified in geographically delimited specimen groups. The occurrence of consistent, biologically interpretable, geographically structured morphological variation may confer the recognition of subspecies.

In this context discriminant analysis is used to 'explain' differences between pre-specified, geographically defined groups based on a set of cranial variables. Weighting each variable according to its ability to discriminate between the groups produces linear combinations of the original variables (canonical functions). The optimal linear combination of variables is achieved by maximising between group variance while minimising within group variance (i.e. maximising the ratio of the two). These canonical functions represent the dominant, underlying trends in the multivariate data set between the specimen groups and can be interpreted in terms of the inclusion and the weighting of the original variables. Each

specimen has a unique score along these linear axes, derived by summing the products of each variable with the corresponding weight. The mean value for each group is calculated. These are the centroid scores and indicate the 'typical' (mean) location, in multivariate space, of a group. The proximity of each sample point in multivariate space to the group centroids can then assess the efficacy of the discrimination with each specimen assigned to the group whose centroid it is closest to. The proportion of correctly allocated specimens indicates the quality of the discrimination obtained by the discriminant function.

Materials and Methods

Specimens Examined

This study is based on an original sample 142 adult giraffe skulls held in museums and research institutions in Africa, North America and the United Kingdom. Missing data were dealt with by casewise deletion (a specimen with any missing data was removed from the analysis).

Sex Determination of Specimens

Giraffe are sexually dimorphic so male and female specimens were treated separately in multivariate analyses. Approximately half (67 of 142, 47.2%) of the specimens examined did not have a sex recorded in the associated museum records. Predictive Discriminant Analysis was used to derive a classification function from those specimens of known sex in order to classify those of unknown sex (Chapter 5). A complete list of male and female skulls used in the analysis is given in Appendix 4.9.1.

Age Determination of Specimens

Adult skulls were distinguished from sub-adult skulls by the presence of the full permanent dentition (Hall-Martin 1975, 1976). The mandibular canine is the last tooth to erupt and come in to wear at the age of six years. This age is taken to represent 'morphological maturity' and is the age where the development of sexual dimorphism allows male and female skulls to be identified unequivocally (Chapter 4).

Ages of adult skulls are estimated according to tooth wear criteria. Hall-Martin (1975, 1976) presented linear regression equations linking lingual occlusal surface width of the first maxillary molar (M^1) to an estimate of absolute age. The methodology for age

determination of giraffe skulls is given in Chapter 4. Age estimates for adult specimens are given in Appendix 2.4.2.

Selection of Skull Characters for Analysis

A total of 59 cranial, mandibular and appendicular skeletal measurements were taken. Only cranial measurements were used in the analysis as many specimens did not have either mandible or limb elements available. The 38 cranial variables were reduced to 13 variables (Chapter 8) for the final analysis using the principals of morphological integration (Olson and Miller, 1958) to identify highly correlated 'phenotypic-functional groups' among the measurements. The final data set includes representatives of the functional and phenotypic groups identified. The selection of the final set of variables also allowed the maximisation of sample sizes by minimising the quantity of missing data.

Statistical Analysis of Cranial Variation

Discriminant Analysis – Justification of the Approach Taken

Discriminant Analysis was carried out on the standardised linear measurements of each specimen. This method requires that sampling entities be grouped according to an *a priori* determined scheme and comparisons made between these groups. Subspecies recognition and description relies on the discovery of consistent, geographically structured, morphological trends within a species. Hence, geographically delimited specimen groups derived from historical range information were used to group the specimens for this analysis. For brevity, the term 'group' and the descriptor 'local' are used for the range delimited geographic groups identified previously, while 'region' and 'regional' are used to refer to larger scale, agglomerated specimen sets used in other analyses. The generic term 'set' is used when referring to either a group or a region.

The nested nature of the defined groups allows analyses at different scales (specimens are derived from individual localities that are agglomerated into local groups that in turn are linked into regional comparisons). This raises the issue of 'local' versus 'global' optimality in the analyses. A global analysis is henceforth taken to mean inclusion of all available data for all specimens across all groups. Therefore, the global discriminant functions optimise group distinctions across the entire sampled data set. In contrast, local analyses can be used to explore distinctions between subgroups. The locally derived discriminant functions optimise only within the scope of variation of the groups included.

Often the patterns of discriminating variation identified at one geographical scale are not those that dominate the discriminant functions at a different scale. Hence, in practice, the results of local analyses do not necessarily reflect the same major sources of discrimination as does the global analysis, and should not be expected to. The use of local analyses 'nested' within the global analysis is consistent with the definition of subspecies used in this thesis. According to the definition used here (see Page 11) subspecies are phenotypically distinct local populations that are distinguishable from neighbouring or nearby conspecific populations. By this criterion pairs (or groups) of subspecies separated by intervening subspecies may be similar morphologically. Here I have used the term morphotype to describe such infraspecific agglomeration of taxonomic groupings. Hence, local comparisons of geographically neighbouring populations are a necessary aspect of taxonomic investigations below the species level.

It is the nature of discriminant analysis that successive discriminant functions use different variables to separate different groups. Consequently, it might be expected that examination of sequential discriminant functions and discriminant scores would ultimately allow each group to be distinguished. Such an expectation may be met where all data conforms to the theoretical assumptions required by the method. In practice small departures from these theoretical assumptions can be made with minimal effect on the robustness of the results (see MacGarigal *et al.*, 2000 for assumptions and a discussion of departures from the assumptions, as well as references therein). These departures from the theoretical ideal may 'smear' residual variation into subsequent discriminant functions. Hence, attempting to differentiate between many groups using the lower order discriminant functions can obscure the relationships between the groups causing a lack of clarity in the expressed relationships. The global regional comparison is, therefore, complemented by smaller scale within region and pair-wise comparisons of geographic groups to examine the detail of the morphological trends in the giraffe skull.

The analysis started with comparisons made between multiple sets at the regional scale (Eastern versus Western versus Southern regions) and at a local scale within each region. Results of these comparisons then prescribed the local, typically pair-wise, analyses. All groups included in an analysis had at least two members. Where a local group included only one specimen the group was removed from the local analysis, but the specimen data was retained in the regional analysis. (See Appendix 4.9.2 for a list of comparisons made and Figure 6.2 for group locations, ranges and composition.)

Structure of the Analysis

All discriminant analyses were carried out using the Systat (version 8.0) statistical analysis package. The multigroup analyses utilised an automatic stepwise approach to extract the variables that contribute most to the differentiation of the defined groups and to examine the patterns of classification and misclassification of specimens. A tolerance test and partial *F*-tests controlled the inclusion of variables in the automatic stepwise analysis model. These were adjusted to provide the greatest differentiation between sets and the maximum number of correctly assigned specimens.

The tolerance represents the percentage of variance in that parameter not already accounted for by the other parameters already included in the model. The tolerance was set at 0.05 ensuring that each variable included in the model accounted for at least 5 per cent of remaining variation. A small tolerance value allows inclusion of variables that offer little additional information to the analysis and so can enter highly redundant information into the model that may offer more noise than signal and may obscure the relationships between groups by increasing the number of misclassifications. Small tolerance values can cause computational inaccuracies in the eigen analysis due to the accumulation of rounding errors and may cause the matrix to be singular (MacGarigal *et al.*, 2000). The partial *F*-tests include an *F*-to-enter step and an *F*-to-remove step. The *F*-to-enter value evaluates the added discrimination provided when a variable is added to the model. It indicates whether the addition of the variable significantly improves the discriminatory power of the model. The *F*-to-remove is the converse whereby the significance of the decrease in discriminatory power after the removal of a variable is tested. The *F*-to-enter and the *F*-to-remove values were adjusted to provide the variable combination that offered the greatest resolution between the *a priori* identified geographic groups.

For the pairwise analyses between neighbouring groups, a 'manual stepwise' approach was used to derive the variable combination that provided the greatest proportion of correctly classified specimens (i.e. classified into their *a priori* defined groups). Under this protocol all 13 variables were included in the analysis. The reported '*F*-to-remove' statistic was used to order the variables in terms of their unique discriminating power (MacGarigal *et al.*, 2000). The variable offering the least explanatory power was then removed and the analysis repeated with the remaining 12 variables. Rounds of analysis were then undertaken until only a pair of variables remained in the analysis. Using the proportion of specimens identified into the correct group as the optimality criterion, the combination of variables that offered the highest proportion of correctly assigned specimens was selected and interpreted in terms of the morphological variation described between the predefined sets. Alternative, slightly less optimal combinations, were assessed in some cases.

The manual stepwise approach offered a methodology to discover the optimal combination of variables for maximum separation of pairs of sets. In contrast, a similar manual stepwise approach did not always discover the optimal combination when examining relationships between more than two groups. The manual procedure, as described, is a simple, linear sequence of decisions. At each step the variable to be removed from the subsequent analysis was determined based on analysis of data included in that step of the analysis. Hence, the sequential removal of variables is, in a sense, predetermined and results in the testing of only one series of combinations of the variables in order to find the combination that best separates the groups.

This is another example of a difference between local and global optimisation. Thirteen variables provide in excess of 3.1×10^9 possible sequences of removal. In the pairwise analyses, where the relationships between the two groups are relatively simple, the manual stepwise approach finds the optimum combination each time. That is, in this data set, no amount of manipulation in an automatic stepwise model can better the optimum combinations derived from the manual approach. In contrast, the manual procedure does not always find the optimal combination in the multiple group analyses. The more complex relationships between the multiple groups affect the importance of each variable at each stage. The amount of discriminating power provided at each step in the multiple group situation changes as relationships between different sets are emphasised with different combinations of variables. The simpler pairwise comparisons do not suffer from such an effect. The two methods are utilised for the different circumstances.

Interpretation of Discriminant Analysis Results

The approach taken in this study has been to search for the combination of variables that maximises the discrimination between groups. That is, the chosen optimisation criterion is to maximise the proportion of specimens allocated to the correct *a priori* defined group. However, unless groups are completely homogenous, the progressive inclusion of variables in the model will eventually find a combination of variables that will separate the defined groups to a reasonably high level of efficiency, particularly at low sample size.

The ability of the mathematical model to successfully separate the defined groups must be interpreted in a manner that is biologically meaningful. For a systematic study this means that the results should be interpretable in terms of taxonomic character states or character trends that allow diagnosis and description of taxa, in this case subspecies. Simply because an algorithm is able to differentiate between the *a priori* groups does not mean it provides useful biological evidence of subspecies. In this way discriminant analysis is used as an

exploratory procedure to investigate the nature of the differences between the groups. The biological importance and relevance of these differences may then be assessed.

A number of factors affect the interpretation of the discriminant analyses performed and so affect the conclusions drawn. One factor is the simplicity or complexity of the relationships that allow groups to be discriminated from each other. The most obvious reason for groups being classified separately is that they are indeed distinct from each other in some measured morphological character, or suite of characters. However, other factors (e.g. the structure of the model, the number of groups defined, the sample size) have a bearing on the interpretation of the results. The interpretation may be further confounded as these factors may not be independent of one another.

Arguably, sample size should be the easiest to deal with as certain 'rules' for minimum sample sizes required in analyses have been proposed (Table 9.1). The minimum number of samples that must be used in discriminant analysis is equal to the number of variables used plus two with at least two sampling entities in each group (MacGarigal *et al.*, 2000).

Rule A	$N \geq 20 + 3P$	Johnson, 1981.
Rule B	If $P \leq 4$, $N \geq 25G$ If $P > 4$, $N \geq [25 + 12(P-4)]G$	Wahl and Kronmal 1977.
Rule C	Each group, $N \geq 3P$.	Williams and Titus 1988.

Table 9.1: *Suggested 'rules' determining the recommended sample size for Discriminant Analysis. N = sample size, P = number of variables, G = number of groups. Reproduced from MacGarigal* et al. *(2000).*

In the current analysis the availability of specimens and the occurrence of damage limit sample size (as DA does not accommodate missing data). Hence, the sample sizes used have a fixed maximum. The reduction in the number of variables included in a model resulting from the stepwise approach effectively raises the sample size relative to the number of variables used. Whether these suggested rules have any true value is not clear and there are instances where my analyses are based on very small sample sizes. The effect of a small sample size is to introduce artefactual biases to the model. Discriminant analysis seeks differences between groups. There is an obvious problem that a small sample might not be representative of the population as a whole. Any artefactual bias exposed due to the limited sampling will be exploited by the analysis and presented in the discriminant function. Care must be taken in interpreting any analysis using a low number of samples.

The number of *a priori* groups that the total sample is split into has a bearing on the sample size in each group, but there is a further effect of the number of groups included in an

analysis. The more groups included in an analysis the greater the number of distinctions that have to be made between the groups. This leads to increasingly complex relationships between a greater number of variables in the classification function. The inclusion of one set of discriminating variables can affect the separation of groups at subsequent levels in the analysis. This effect is apparent in the regional analysis undertaken here where the separation of the eastern, western and southern groups combine the variables that discriminate the separate northern against southern and eastern against western analyses. The agglomeration of eastern and western sets into a single northern set to compare with the southern set allows efficient classification of the two groups with only two variables. Inclusion of the three regions provides a more complex relationship involving six variables. The relationships are still evident from the canonical scores plots and can be derived from the standardised canonical functions and group mean values but the pairwise comparison provides a more straightforward simple interpretation. Therefore, the number of groups and the scale of analysis can be a factor in the interpretation of results.

The complexity of the classification function is also a factor in interpretation of the results. Traditional morphometrics uses multivariate statistical analysis to investigate and identify character states and trends in characters that may define taxa. Hence, the mathematical relationships derived from the analyses must be biologically interpretable to be useful. Typically the fewer the number of variables included in the classification function, the simpler the model and the easier it is to interpret. Without a biological explanation the classification becomes a mathematical representation lacking a useful interpretation. If differences can be explained with a few variables, a practical classification can be produced. If a large number of variables are used to define the model it may indicate that the model is seeking finer levels of refinement to enforce the defined groups and is exploiting the biases in the distributions of individual values in each group for each variable. Such biases may be increased due to the relatively small sample sizes of some of the groups in the foregoing analyses. Sometimes complex, multi-variable models discriminate between groups very efficiently (a high proportion of specimens are correctly classified). However, such complex models may not be readily interpretable as biological features that may be observed on the specimen making them useless in biological terms. There is no reason, in principle, why a classification function should not be complex, with a relatively large number of variables, as long as the model can be interpreted in a biologically meaningful way.

Model Validation and Classification Efficacy

Interpretation of Classification Matrices and Jack-knifed Classification Matrices

The two types of classification matrix indicate the proportion of specimens correctly classified by the classification function under two different circumstances. In both cases the classification function works by calculating the position of each specimen in multivariate space and then assesses each specimen's Euclidean distance to the centroid of the *a priori* defined groups. The specimen is then assigned to the group whose centroid it is closest to.

The classification matrix shows how well the classification function separates the groups of measured specimens. It addresses the question "how distinctive are the *a priori* defined sets of specimens in the analysis?" The distinctiveness indicated by the classification matrix is used here primarily following the multi-set analyses to see which of the groups clearly separate and which overlap by looking at the pattern of misclassifications. The jack-knifed classification matrix results from a resampling procedure where individual specimens are removed from the analysis, the classification function is calculated and the removed specimen is then classified according to the derived function (MacGarigal *et al*, 2000). Examination of the jack-knifed classification matrix indicates how robust the classification function is to the influence of individual specimens.

Any large discrepancy between the classification matrix and the jack-knifed classification matrix suggests that individual specimens may be having disproportionate influence over the model (i.e. the removal of a specimen alters the structure of the model) indicating an unstable model. Biologically the misclassification of specimens in the jack-knifed classification matrix would suggest that the members of the group are not, in fact, sufficiently morphologically similar and are relatively widely dispersed in the morphological multivariate shape space. Thus, the removal of a specimen significantly shifts the group centroid of those remaining such that the removed specimen is subsequently classified into a different group (The classification criterion being the proximity of the nearest group centroid).

A preferred validation procedure is to use a split sample approach where a portion of the data acts as a 'training set' to derive the model. The remaining specimens are then classified according to the derived classification function. The success of the model can then be inferred from the proportion of correctly classified specimens in the second set. Small sample sizes in the current data set prevented the use of this split-sample approach.

The foregoing discussion shows that the results of the discriminant analysis must be viewed with a critical eye. Just because a linear discriminant model can be produced that effectively discriminates between the *a priori* defined groups it does not mean these groups should be recognised taxonomically. The results must be interpreted to decide whether the separation seen reflects biologically relevant differences, the confounding effect of biases due to sample sizes or a pattern of variation that has a mathematical definition but no biological interpretation. The interpretations made from these analyses must, additionally, be assessed with reference back to the original specimens.

Support Statistics

Three statistics are reported to provide an indication of the quality of each classification. These are Wilks' lambda (λ) statistic, the proportional chance value (C_{pro}) and Cohen's Kappa (κ) Statistic (MacGarigal *et al.*, 2000).

Wilks' λ is a likelihood-ratio statistic that assesses the separation between and the cohesiveness within the groups. It tests the null hypothesis that there is no difference between the group means in the population as a whole. A value approaching zero indicates greater separation. The Wilks' λ statistic is calculated from the classification matrix.

The proportional chance criterion is presented here only for pairwise comparisons. It shows the proportion of correct assignments that would be expected by random assignment of specimens into groups and takes account of differing sample sizes in the sampling groups. It is calculated according to:

$$C_{pro} = p^2 + (1-p)^2 \qquad \text{... Equation 9.1}$$

Where p is the proportion of the specimens in the first set and, by implication, $(1-p)$ is the proportion of specimens in the second set.

Cohen's κ is a chance-corrected indicator of how well the classification function performs as compared to random allocation of samples. It assumes prior probability of classification to a group is proportional to the relative group sample sizes and is a combination of these prior probabilities of correct classification and the final classification proportions. It is defined as:

$$\kappa = \frac{p_0 - \sum_{i=1}^{G} p_i q_i}{1 - \sum_{i=1}^{G} p_i q_i} \quad \text{... Equation 9.2}$$

Where G is the number of groups, p_0 is the proportion of all samples correctly classified, p_i is the proportion of samples in the i^{th} group and q_i is the proportion of samples classified into the i^{th} group. A κ value of 1.0 indicates perfect assignment of specimens to groups. A κ value of 0.0 shows no better than chance assignment of specimens to groups. A negative value of κ indicates a classification that is worse than would be expected by chance alone. In the current study κ values are always calculated on the jack-knifed classification matrix.

It must be remembered when interpreting classification accuracy using C_{pro} and the κ-statistic that they are only unbiased when calculated on data which has not been used to create the model. Bias will enter the statistics when they are calculated using the data that has been used to create the model. Hence, the assessment of classification function quality offered is positively enhanced by the use of data used in the creation of the model so the interpretation of these statistics must be tempered by caution and used only as relative indicators of model quality.

Accounting for Potential Confounding Errors in the Discriminant Analysis

Despite selecting only 'morphologically mature', adult specimens it was discovered that age may act as a potentially confounding factor in these data. Adult specimens were diagnosed as those at the stage of morphological maturity at which development was such that male and female specimens could be told apart unequivocally. These were defined as individuals with the mandibular canine fully erupted and in wear corresponding to an age at morphological maturity of approximately six years old (see Chapter 4 for all age related analyses).

Pearson product-moment correlation coefficients showed that, for the female data used in the discriminant analysis, the only variable significantly correlated with estimated age was mouth width (SWM) (Chapter 4). A Kruskal-Wallis test showed that there was no geographically structured age bias in the distribution of ages between geographically defined groups. Hence, the complete female data set was used in the analysis.

In contrast, nine of the thirteen variables were significantly correlated with estimated age in male specimens. This dropped to only four (all with weaker associations with increasing age) in specimens estimated to be over twelve years old. Also, one of the geographically defined groups (EEK from northern Kenya, southern Ethiopia and south eastern Somalia) was significantly younger than other groups. Hence, there may be a confounding age effect in the male data.

These problems are dealt with empirically by undertaking three separate analyses and interpreting the effect of each differing treatment on the results. The first analysis contains data from all available male specimens. The second analysis seeks to minimise the effect of the size correlation with age by examining male skulls estimated to be over 12 years old (the age when growth of the male reproductive organs stops, Hall-Martin *et al.*, 1978). The third analysis removes the EEK group from all analyses.

Results

Discriminant Analysis

An overview of the results of the analyses is presented. Some discussion of the interpretation of the discriminant analysis results in terms of the original variables and specimens is included here. Detailed results for each analysis undertaken are provided in Appendix 4.9.2. General descriptions of the important variables are given here as the variables used in this analysis represent the broader functional-phenotypic sets identified by morphological integration analysis.

Interpreting the 'Alternative Analyses'

Male Data

Three sets of analyses were undertaken for the male data set due to observations pertaining to the estimated ages of the specimens. The results of the three analyses, including the efficiency of the classification function as reported in the jack-knifed classification matrix and the important discriminating variables, were compared subjectively. The results of all three alternative analyses are presented in Appendix 4.9.2. In the discussions that follow the complete analysis is discussed unless it is indicated otherwise.

Female Data

Fewer female skulls were sampled than male skulls. Also, due to the relatively more delicate nature of the female skull (lacking secondary bone deposition) damage occurred more often giving missing values in the data set. Two analyses were carried out. First, only skulls with a complete complement of measured skull variables were used in the manual stepwise discriminant analysis. The most efficient discriminant function was then used on all skulls, testing the efficacy of the discriminant function on a larger sample size.

Geographical Structure in the Cranial Morphology of Giraffes

Regional Comparisons (Male Data)

The comparison of all three regions together shows 87 per cent of skulls to be correctly allocated to their group by the jack-knifed classification matrix with only nine specimens misclassified (Table 9.2 and Figure 9.1). The jack-knifed classification efficiency for the 'old males' and 'no EEK' analyses were 93 per cent and 86 per cent respectively. The northern (combined east and west) group compared to the southern sees only three misclassifications in the jack-knifed classification matrix, differentiating 96 per cent of all specimens (Table 9.3) (98% and 86% for the 'old male' and 'no EEK' comparisons). Finally, east and west split with 85 per cent efficiency with all western specimens correctly classified in the jack-knifed classification and five eastern specimens misclassifed as being from the west (Table 9.4).

Classification Matrix				
	East	South	West	% Correct
East	23	3	2	*82*
South	1	35	0	*97*
West	0	0	6	*100*
Total	*24*	*38*	*8*	*91*

Jack-knifed Classification Matrix				
	East	South	West	% Correct
East	21	3	4	*75*
South	1	35	0	*97*
West	1	0	5	*83*
Total	*22*	*38*	*9*	*87*

Table 9.2: *Classification matrix and jack-knifed classification matrix for all male data from the three regions (East, West and South). See Figure 9.1.*

Classification Matrix			
	North	South	% Correct
North	32	2	*94*
South	1	35	*97*
Total	*33*	*37*	*96*

Jack-knifed Classification Matrix			
	North	South	% Correct
North	32	2	*94*
South	1	35	*97*
Total	*33*	*37*	*96*

Table 9.3: *Classification matrix and jack-knifed classification matrix for all male data from the Northern (agglomerated East and West) and Southern regions.*

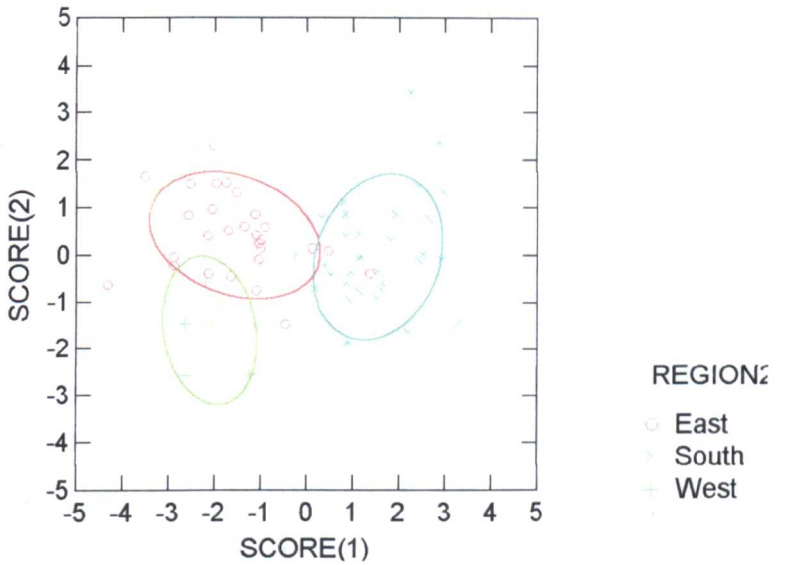


Figure 9.1: *Canonical scores plot indicating the separation between the three regional sets for all male data.*

Classification Matrix			
	East	West	% Correct
East	23	5	82
West	0	6	100
Total	23	11	85

Jack-knifed Classification Matrix			
	East	West	% Correct
East	23	5	82
West	0	6	100
Total	23	11	85

Table 9.4: *Classification matrix and jack-knifed classification matrix for all male data from the Eastern and Western regions.*

Northern and southern forms can be readily separated using only two variables. Comparison of median horn height (**MMH**) and orbital width (**MOW**) show that giraffes with a median horn height (measured from the rear of the third maxillary molar to the tip of the median horn) greater than the width across the orbital ridges originate in the northern region, while those with the width greater than the height come from the south. (In fact the differentiation between north and south occurs at a median horn height to orbital width ratio of 1:0.96.) A plot of median horn height versus orbital width demonstrates this relationship (Figure 9.2). The absolute values of these two parameters overlap extensively between the two regions requiring the expression of the relationship as a ratio of the height and width.

It must be demonstrated that the proposed 'diagnostic ratio' is not confounded by the age or size of the specimens. That is, is the relationship consistent regardless of the age or size of the specimen? Three measures of age or size were tested against the two 'diagnostic parameters', and the ratio of these two values. The measures were the estimated age of each specimen (derived from tooth wear data, Chapter 4), the mass of the skull (as male

giraffe continue to lay down secondary bone on the upper surfaces of the skull throughout adulthood) and the nasal-occipital length of the skull (**NOL**; used as a general indicator of skull size). While the univariate values for the two parameters separately are correlated with the age and size measures, the ratio values are not correlated with either the estimated age or skull length (**NOL**). The ratio values were significantly correlated to the mass of the skull (Table 9.5). **MMH** and **MOW**, and the ratio of these two parameters are plotted against the three age/size indicators in Figure 9.3. Regression analysis indicates that the slope of the regression line in the plots of the ratio value against age and skull length do not differ significantly from zero (Age analysis: Northern data: $F = 0.002$, $p = 0.962$, NS. Southern data: $F = 0.063$, $p = 0.803$, NS. Skull length analysis: Northern data: $F = 3.369$, $p = 0.075$, NS. Southern data: $F = 2.895$, $p = 0.096$, NS). Inspection of the plots (Figure 9.3) shows that skull mass is correlated to the ratio for the northern specimens. This is confirmed by the regression analysis, which also shows that the slope of the ratio versus the mass best fit line does significantly differ from zero for the northern specimens (Mass analysis: Northern data: $F = 26.677$, $p < 0.001$, ***. Southern data: $F = 0.000$, $p = 0.996$, NS). The strong correlation of the northern skulls against the mass of the skull is to be expected. In the northern form, secondary bone is laid down to make up a discrete, conical or cylindrical median horn. In the southern specimens secondary bone is laid down more generally over the upper surface of the skull resulting in a lower dome-like structure and no relationship between skull mass and median horn height. Extrapolation of the regression lines (not shown in Figure 9.3) for the northern and southern specimen sets in the plots of the ratio against the mass of the skull show that they intersect at a point well before morphological adulthood[1] is reached. Hence, there should be no confusion when comparing adult skulls.

Notwithstanding the relationship between mass and median horn height in northern specimens the ratio does stand up to reasonable scrutiny. Inspection of the plots indicates that, with a few overlapping points, the two regions are effectively separated where the width of the skull approximately equals the height of the median horn (as measured here from the back of the maxillary toothrow). Even a comparison of old southern skulls against young northern skulls is robust to this comparison. Therefore, it is suggested that, the proposed ratio is reliable for any age adult male skulls.

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[1] Morphological adulthood has been defined here (Chapter 4) as the point at which male and female skulls can be told apart unequivocally, due to the ontogeny of sexual dimorphism. This is concurrent with the full eruption and coming in to wear of the permanent mandibular canine.

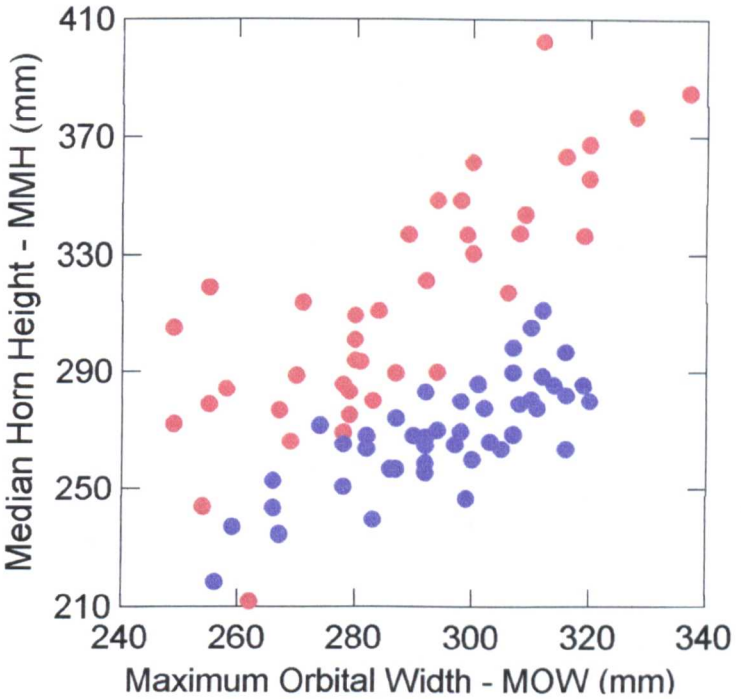

Figure 9.2: *Plot of median horn height versus orbital width for male giraffe skull specimens demonstrating the distinction between northern and southern specimens. Northern specimens are shown in red (●); southern specimens are shown in blue (●).*

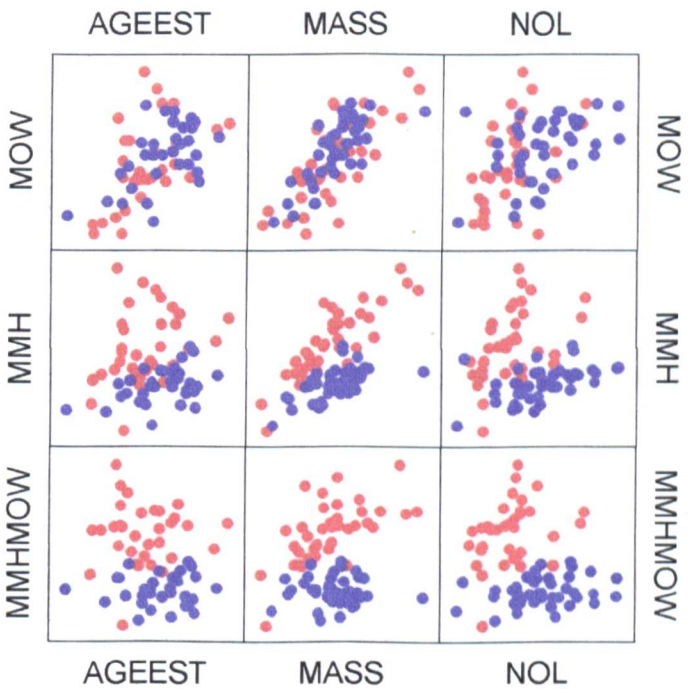

Figure 9.3: *Plots of* **MMH** *and* **MOW** *and the ratio of the two against three age/size indicators. The univariate plots show extensive overlap between the northern (red; ●) and southern (blue: ●) specimens. The dimensions within the regional groups are correlated with each of the age/size indicators. However, the ratio is independent of (i.e. not correlated with) age and skull length, while only the ratio in the northern specimens is correlated to skull mass. The ratio of* **MMH** *to* **MOW** *provides a robust indicator of the geographic provenance of an adult male specimen. Note that the northern specimen that provides the lowest ratio value is a specimen originating from the variable EST area and was ommitted in calculating correlation coefficients and regression analysis.*

Age / Size Estimator	MOW			MMH			MMH/MOW		
Northern Specimens	r	n	p	r	n	p	r	n	p
Age	0.587	33	***	0.354	33	*	0.009	33	NS
Mass	0.727	37	***	0.866	37	***	0.658	37	***
NOL	0.450	35	**	0.461	35	**	0.304	35	NS
Southern Specimens	r	n	p	r	n	p	r	n	p
Age	0.575	33	***	0.433	34	*	0.045	33	NS
Mass	0.804	42	***	0.665	43	***	-0.001	42	NS
NOL	0.419	43	**	0.496	44	***	0.257	43	NS
All Specimens	r	n	p	r	n	p	r	n	p
Age	0.534	75	***	0.175	76	NS	-0.115	75	NS
Mass	0.756	89	***	0.556	90	***	0.185	89	NS
NOL	0.462	86	***	0.082	87	NS	-0.192	86	NS

Table 9.5: *Correlation coefficients of the two 'diagnostic variables' used to differentiate between northern and southern specimens. It is proposed that specimens with well-developed median horns, such that the skull is taller (***MMH***) than it is wide (***MOW***) originate in the north. Meanwhile, those with less well-developed median horns come from the south. Three measures of age or size are tested against the two parameters; the estimated age, the mass of the skull and the nasal-occipital length. The univariate correlations indicate that the measures are associated with each of the age/size indicators. However, the ratio values are independent of these age/size indicators (except for the* **MMH** *versus Mass correlation). See the text for further discussion and Figure 9.3.*

The occurrence of a median horn was used as a diagnostic character between northern and southern forms by Lydekker (1904) and de Winton (1897), although Dagg and Foster (1982) considered this character too labile to be diagnostic. However, the current analyses indicate that the proposed ratio is consistently different between northern and southern specimen sets.

An examination of the specimens indicates an obvious difference in the form of the median horn, even in those specimens that are close to the borderline separating the two regions. Figure 9.4 shows the extremes of form for the two regions as well as the specimen that most closely approximates the mean score for all of the correctly classified skulls. Specimen NMZB29100 is the southern skull with the highest **MMH**:**MOW** ratio (0.949:1) while BMNH1899.7.8.5 has the lowest ratio (0.989:1) of any northern skull. Despite their proximity, according to this ratio, the difference in the conformation of the median horn is clear. Northern specimens have a distinct conical, cylindrical or domed median horn. The corresponding structure in the southern specimens is a broader based more diffuse bony structure that spreads across the top of the skull.

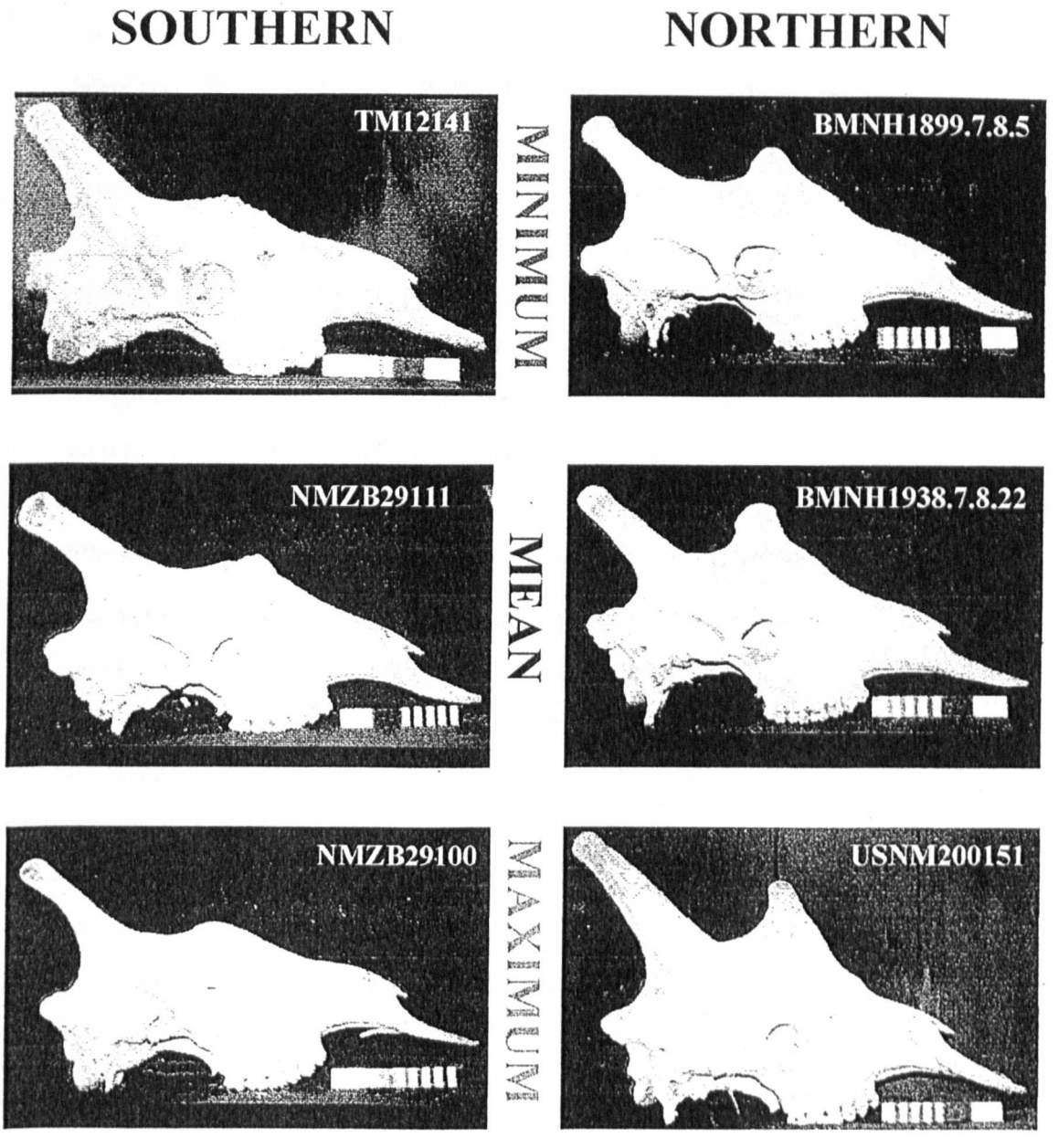

Figure 9.4: *Comparison of the form of the median horn of northern and southern male skulls. Discriminant analysis shows that males from northern and southern regions of Africa can be discriminated according to the ratio of the height of the median horn (measured from the rear of the maxillary toothrow) to the maximum orbital width. If the skull is taller than it is wide it is from the north, if wider than tall it originates in the south. This effectively equates to the development of the median horn. In the northern form the median horn forms a discrete conical or cylindrical horn. In the southern specimens secondary bone deposition is more diffuse giving a low boss. This figure shows three skulls from each region that have the minimum, maximum and closest to the mean discriminant scores. Note that all of the northern skulls have the discrete median horn formation.*

There are three misidentified skulls (Figure 9.5); two that originated in the north and classified as southern (BMNH1898.7.2.5 and USNM251799) and one from the south assigned to the northern region (NMZB26180). BMNH1898.7.2.5 and NMZB26180 are very close to the 'dividing line'. Examination of the specimen photographs suggests that NMZB26180 is clearly a southern form and owes its misclassification to its slightly narrower than might be expected orbital width. BMNH1898.7.2.5 is a northern skull but is less readily classified as such. It does show a domed structure on the forehead, but this is not a simple discrete structure and there is further deposition of secondary bone more generally across the upper skull surface. Prior knowledge of its origin leads to its acceptance as a northern skull, without this knowledge this skull would be difficult to assign with confidence. The third skull, USNM251799, is from the north but is classified into the middle of the southern specimens. This specimen was collected in Tanzania and, for a northern specimen, has an unusually low median horn. Unfortunately no photograph was taken showing the profile of this specimen (due to damage to other structures).

The efficiency of the discriminant analysis classification, the ease of translation of the results into taxonomic characters and the corroboration of the classification by examination of specimens clearly demonstrates that the northern and southern forms are distinct in their skull morphology. The two misidentified northern skulls originated in southern Kenya or Tanzania. Lydekker (1904) recognised that the presence of a median horn in giraffes from this area may be variable (See Appendix 1.2.1). Examination of the other specimens from this region indicates that all possess the typical northern discrete median horn. A comparison of these specimens to the nearest southern population (ESK/EST versus SZT) indicates that same two variables to separate the two sample sets with only one misclassification in either group.

In the northern versus southern region analysis male specimens from the east and west are freely mixed within the northern group. However, an analysis contrasting eastern and western sets shows that the western specimens can be unequivocally assigned to the correct group, while 82 per cent of eastern samples are correctly identified (Table 9.4). The defining classification function is somewhat more complex than the north versus south split and relies on the relationship between the size of the brain case (length – **OOL** – and depth – **BPH**) and the size of the mouth (**MXTL**). The length of the parietal horns (**HSP**) increases the efficiency of the model slightly (one additional correct allocation). The standardised canonical discriminant functions contrast **MXTL** and **BPH** against **OOL** and **HSP**. That is, as **MXTL** and **BPH** increase **OOL** and **HSP** show relative decreases (i.e. the rate of increase in size is lower). In three of the four variables included in the

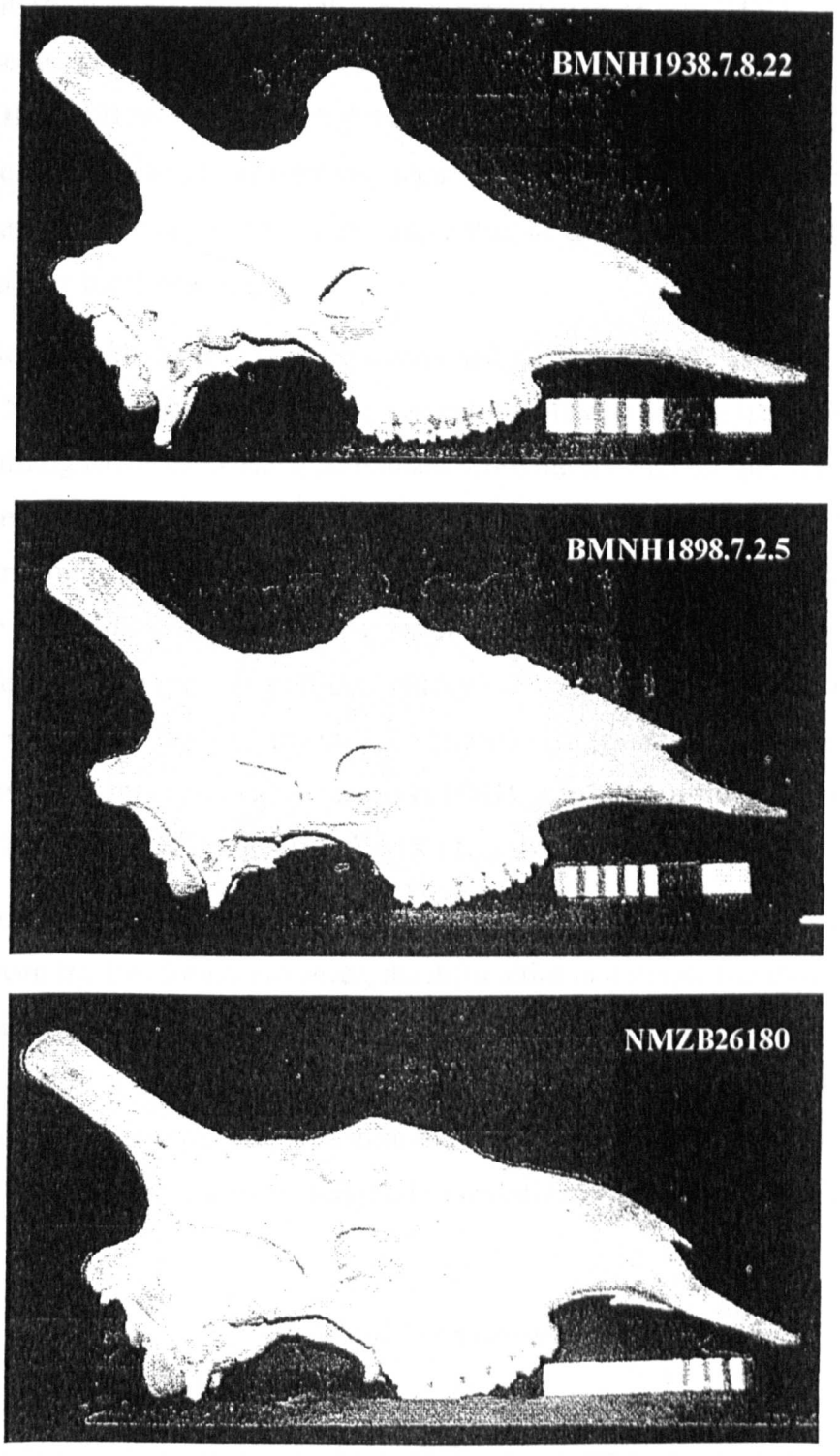

Figure 9.5: *Misidentified Male Skulls. Skull BMNH1938.7.8.22 is a 'typical' northern male skull (with the discriminant score closest to the mean for the northern region). Northern and southern skulls can be discriminated according to the ratio of the median horn height versus the maximum orbital width. Skull BMNH1898.7.25 is an adult male skull from northern Africa classified by discriminant analysis as a southern skull. The skull shows a discrete domed structure but secondary bone is diffused across the upper surface of the skull. Skull NMZB26180 is a southern skull classified as being from the north. The misclassification is due to a smaller than expected orbital width making the skull taller than it is wide. This skull is clearly a southern skull.*

classification function the eastern group has the largest dimensions (**OOL, BPH** and **HSP**), while the western group has the longer maxillary tooth row (**MXTL**). Interpretation of these factors suggests that a western giraffe tends to have a relatively small braincase (**OOL** and **BPH**), which is slightly shorter in comparison to the eastern individuals. The parietal horns tend to be relatively shorter in the west too. In contrast the western giraffe has a relatively larger masticatory apparatus, as exemplified by the relatively greater maxillary tooth row length.

Although both the classification matrix and the jack-knifed classification matrix indicate that 5 of the 26 eastern specimens are misclassified, when the specimens are ordered according to the canonical discriminant score the six western specimens have the lowest scores with only one eastern specimen in their midst. This, perhaps, suggests a greater separation of the western forms than indicated by the classification matrices. However, none of the six western specimens show congruent patterns in the four variables used in the model (i.e. consistent proportional changes reflected by all specimens). Moreover, of the four variables utilised by the model, none are significantly different between the eastern and western sets in univariate analyses (**OOL**, $t = 1.910$, $p = 0.065$; **BPH**, $t = 0.805$, $p = 0.427$; **HSP**, $t = 1.514$, $p = 0.140$; **MXTL**, $t = -1.703$, $p = 0.291$; $df = 32$ for each analysis).

The comparison of eastern and western sets is suggestive of morphological differentiation between the two forms. However, the difficulties in a simple translation of the classification function into observable character differences and the lack of consistent trends in the data suggest that the derived classification function may be artefactual. This lack of clarity probably derives from the low sample size available for the western region. More western specimens are required to investigate this relationship further.

Regional Comparisons (Female Data)

The female analysis does not conflict with the male conclusions, but provides little evidence of a difference in skull morphology between north and south (Table 9.6). By itself the female analysis offers no simple interpretation that provides evidence of character state differences between the regions. The 86 per cent classification efficiency of the jack-knifed classification matrix relies primarily on a contrast between the depth of the braincase (**BPH**) and the width of the skull at the base of the parietal horns (**EWB**) (the diameter of the parietal horn tips [**LAD**] is included in the classification function). The results presented do not justify the separation of female specimens from the two regions on morphological grounds.

Classification Matrix			
	North	South	% Correct
North	12 (20)	1 (3)	*92 (87)*
South	1 (1)	10 (13)	*91 (93)*
Total	*13 (21)*	*11 (16)*	*92 (89)*

Jack-knifed Classification Matrix			
	North	South	% Correct
North	11 (20)	2 (3)	*85 (87)*
South	1 (2)	10 (12)	*91 (86)*
Total	*12 (22)*	*12 (15)*	*88 (86)*

Table 9.6: *Classification matrix and jack-knifed classification matrix for female data from the Northern and Southern regions. Two analyses of female date were performed to compensate for the relatively small sample size of skulls with the complete data set available. The first analysis used data from all complete skulls to obtain the classification and jack-knifed classification matrices. The second (presented in parentheses in this table) uses the classification functions derived from the first analysis to classify all available skulls. The number of parameters used by the classification function to optimally separate the northern and southern groups effectively allows an increase in the sample size in this second analysis.*

Local Comparisons

Southern Region

Southern groups include two distinctly separate geographic ranges. The first population inhabits a small area isolated in Zambia's Luangwa Valley (SZT). The second shows a very large, and potentially contiguous distribution from southern Angola into northern Namibia and westwards across Botswana into south western Zambia, western and south western Zimbabwe into Northern South Africa and southern Mozambique. Different authors separate the range within this area in different ways (See Figure 6.1). Hence, there are two distinct questions posed in the analysis of variation in southern specimens. Is the isolated population (SZT) morphologically unique? Are specimens from the large range characterised by discrete, geographically structured variation that may indicate historical isolation?

The Luangwa Valley group is represented by only four adult male specimens. Comparison of these against the other southern specimens correctly classifies all four of the Zambian specimens. However, three (of 30) of the remaining southern specimens are misclassified as SZT specimens (An overall jack-knifed classification efficiency of 91%. Table 9.7).

Classification Matrix			
	SCZ/ SWC	SZT	% Correct
SCZ/ SWC	27	3	*90*
SZT	0	4	*100*
Total	*27*	*7*	*91*

Jack-knifed Classification Matrix			
	SCZ/ SWC	SZT	% Correct
SCZ/ SWC	27	3	*90*
SZT	0	4	*100*
Total	*27*	*7*	*91*

Table 9.7: *Classification matrix and jack-knifed classification matrix for comparison of local groups of male data within the Southern region. The SZT population is a population geographically isolated from the other southern African populations. See the text for further discussion.*

The SZT group is primarily separated by median horn height (**MMH**) and parietal horn length (**HSP**) with maxillary tooth row length (**MXTL**) and maxillary width (**MMW**) included in the classification function. The original values for these variables indicate that the SZT specimens are robust skulls with wider snouts and greater median horn development (although not a discrete horn as in the north) and a relatively long maxillary tooth row. In contrast, the parietal horns are typically shorter.

Examination of the SZT specimens does indeed show them to be robust specimens with ample secondary bone deposition and relatively short parietal horns. However, all of the variables fall within the range of the southern group in general and no biologically meaningful interpretation of the variables is immediately apparent. The differentiation of SZT from the southern group generally, based on skull dimensions, is suggestive and is deserving of further attention but here must be interpreted cautiously as a potential artefact due to the small sample size for this population.

Of the remaining four geographically delimited groups SWA and SEW were represented by only one skull each, precluding these groups from further analysis. Comparison of SCZ and SWC groups (Table 9.8) derives a complex classification function that suggests that the SCZ skulls are relatively longer (with respect to width – **MMW** and **EWB** – and height – **MMH**) compared to the SCZ, while the SCZ skulls have thicker (**LAD**) and more divergent (**EWB** and **EWT**) parietal horns. However, attempts to derive meaningful relationships that can be expounded in terms of characters to define morphological disparity between the two groups failed to provide a diagnostic set.

Classification Matrix			
	SCZ	SWC	% Correct
SCZ	13	2	*87*
SWC	3	12	*80*
Total	*16*	*14*	*83*

Jack-knifed Classification Matrix			
	SCZ	SWC	% Correct
SCZ	12	3	*80*
SWC	4	11	*73*
Total	*16*	*14*	*77*

Table 9.8: *Classification matrix and jack-knifed classification matrix for comparison of local groups of male data within the Southern region. SCZ and SWC are two groups that may represent historically contiguous groups, or historically or recently isolated groups, depending upon the author consulted.*

Western Region

Only six specimens were available within the western region, separated into two geographical groups. Despite the derivation of a classification function that successfully assigned all six specimens to their assigned groups the jack-knifed classification matrix showed that the classification was not robust (Table 9.9). No univariate differences could be found to separate the two groups. While acknowledging the inadequacy of the sample size this suggests that there are no differences between these groups.

Classification Matrix			
	WCP	WSN	% Correct
WCP	4	0	*100*
WSN	0	2	*100*
Total	*4*	*2*	*100*

Jack-knifed Classification Matrix			
	WCP	WSN	% Correct
WCP	2	2	*50*
WSN	2	0	*0*
Total	*4*	*2*	*33*

Table 9.9: *Classification matrix and jack-knifed classification matrix for comparison of local groups of male data within the Western region. While the classification matrix indicates perfect separation of the two groups, the jack-knifed classification matrix clearly demonstrates the lack of differentiating characters in the data set.*

Eastern Region

The situation in the eastern region is more complex with four groups separated by a classification function containing six variables with 85 per cent jack-knife classification efficiency (Table 9.10 and Figure 9.6). The majority of misclassifications occurred between ECU and EEK so these two groups were compared more closely.

It took ten variables to separate the nineteen specimens (ECU n = 7; EEK n = 12. Table 9.11). The ECU group was larger in all variables. Recalling that the EEK group was significantly younger than all other groups and many, but not all, of the variables were correlated with the estimated age of the male specimens this is, perhaps, not surprising. This result notwithstanding, the standardised canonical discriminant functions demonstrate

that this is not a case of isometric scaling and that there is shape variation in the dimensions of the skull. However, it is difficult to discern any trend in this variation. Arguably, as the length measurements increase (**NOL**, **MXTL**) the width measurements (**MMW**, **EWB**, **SWM**) effectively decrease in relative terms (i.e. increase at a slower rate), but height shows opposing trends (**MMH** and **BPH**). Other variables, particularly those of the parietal horns (**LAD**, **HSP**, **EWT**) add confusion to any attempted interpretation. No biologically meaningful characters can be discerned from the complexity of the classification function.

Classification Matrix					
	ECU	**EEK**	**ESK**	**EST**	**%Correct**
ECU	6	1	0	0	*86*
EEK	1	11	0	0	*92*
ESK	0	0	6	0	*100*
EST	0	0	0	2	*100*
Total	*7*	*12*	*6*	*2*	*93*
Jack-knifed Classification Matrix					
	ECU	**EEK**	**ESK**	**EST**	**%Correct**
ECU	5	1	1	0	*71*
EEK	2	10	0	0	*83*
ESK	0	0	6	0	*100*
EST	0	0	0	2	*100*
Total	*7*	*11*	*7*	*2*	*85*

Table 9.10: *Classification matrix and jack-knifed classification matrix for comparison of four locally defined groups of male giraffe specimens within the Eastern region.*

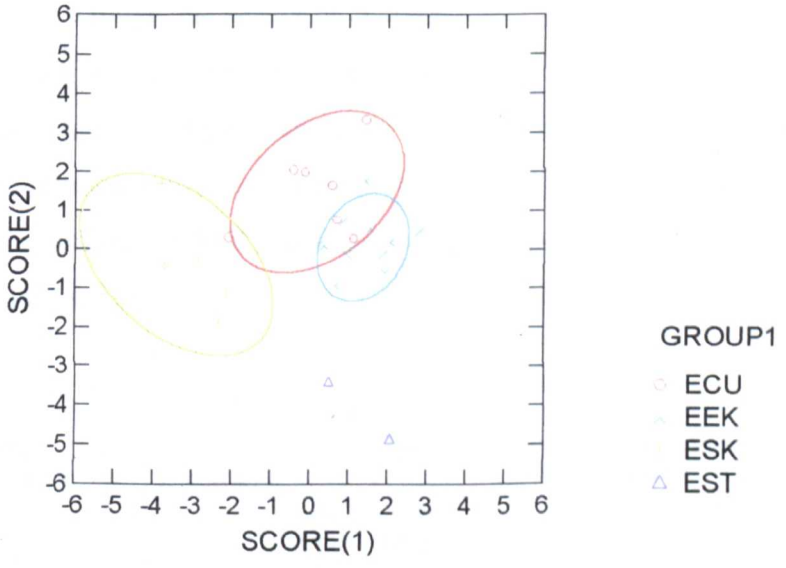

Figure 9.6: *Canonical scores plot indicating the separation between the four locally defined specimen sets in the eastern region for all male data.*

Classification Matrix			
	ECU	EEK	% Correct
ECU	7	0	*100*
EEK	0	12	*100*
Total	*7*	*12*	*100*

Jack-knifed Classification Matrix			
	ECU	EEK	% Correct
ECU	6	1	*86*
EEK	1	11	*92*
Total	*7*	*12*	*89*

Table 9.11: *Classification matrix and jack-knifed classification matrix for comparison of the ECU and EEK locally defined groups of male giraffe specimens within the Eastern region. These two groups provided most of the misclassifications in the original, four group analysis and so were compared.*

The comparison of ECU and EEK using the 'old male' data set reduces the efficiency of the jack-knifed classification matrix to 67 per cent. Of the six variables in the classification function only three are shared with the 'all male' classification function.

As no meaningful differences could be found between ECU and EEK and between ESK and EST (Table 9.12), a comparison was made between the combined groups (ECU with EEK versus ESK with EST. Table 9.13). Five variables were included in the classification function to separate the two groups, with only one specimen misclassified in the jack-knifed classification matrix. Two variables (**MMH** and **SWM**) dominate the classification function and a plot reveals the relationship between the two (Figure 9.7). The two groups can be effectively separated by taking the ratio between the median horn height (**MMH**) and the width of the mouth at the first maxillary molar (**SWM**). For the specimens in this data set, when the median horn height is approximately double the width of the mouth (in fact approximately 1.9 times) the specimen is from northern Kenya, Ethiopia, Somalia or Uganda. Meanwhile if it is less than this ratio it is likely to come from southern Kenya or northern Tanzania. This simplified relationship generally holds, but is slightly imperfect. However with the removal of the outlier from the ECU / EEK group, the model need only be tempered by the addition of **NOL** (skull length, perhaps controlling for absolute size) to make the distinction perfect.

Classification Matrix			
	ESK	EST	% Correct
ESK	6	0	*100*
EST	0	2	*100*
Total	*6*	*2*	*100*

Jack-knifed Classification Matrix			
	ESK	EST	% Correct
ESK	2	4	*33*
EST	0	2	*100*
Total	*2*	*6*	*50*

Table 9.12: *Classification matrix and jack-knifed classification matrix for comparison of the ESK and EST locally defined groups of male giraffe specimens within the Eastern region.*

Classification Matrix			
	ECU/ EEK	ESK/ EST	% Correct
ECU/ EEK	19	0	*100*
ESK/ EST	0	8	*100*
Total	*19*	*8*	*100*

Jack-knifed Classification Matrix			
	ECU/ EEK	ESK/ EST	% Correct
ECU/ EEK	18	1	*95*
ESK/ EST	0	8	*100*
Total	*18*	*9*	*96*

Table 9.13: *Classification matrix and jack-knifed classification matrix for comparison of the agglomerated ECU and EEK groups versus the ESK and EST groups of male giraffe specimens within the Eastern region.*

A similar analysis to that carried out to investigate the relationship between northern and southern skull parameters suggests that this ratio (between **SWM** and **MMH**) to discern between ECU/EEK and ESK/EST specimens is not as robust. The ratio of these two measurements are correlated with the mass and length indicators (Table 9.14). Similarly, regression analyses indicate that the slope of the regression line between the ratio and the size indicators differs significantly from zero for both area sets (Mass analysis: ECU/EEK $F = 33.440$, $p < 0.001$, ***. ESK/EST $F = 14.606$, $p = 0.005$, **. Length analysis: ECU/EEK $F = 28.006$, $p < 0.001$, ***. ESK/EST $F = 6.784$, $p = 0.031$, *). There is a significant association between the ratio and estimated age for the ESK/EST group (Age analysis: ECU/EEK $F = 2.162$, $p = 0.162$, NS. ESK/EST $F = 7.475$, $p = 0.026$, **). Examination of the univariate plots (Figure 9.8) shows extensive overlap of the two geographic groups for each variable. The ratio plots do show some structure that suggests separation may be possible. However, the shape of these graphs, coupled with the significant correlations and regression analysis results suggest that there may be an age or size related effect.

It should be recalled that the two measurements highlighted are part of a group of five measurements that make up the discriminant function that is able to separate these two groups. Consequently, perhaps it should not be expected that they can provide a general rule to separate these groups. Also the sample size presented here is relatively small. It seems that there is some overlap in the ratio values between older ESK/EST individuals and younger ECU/EEK specimens. The purpose here is to present an interpretation of the multivariate data in a relatively simple format. As such the ratio may still provide an approximate rule of thumb to distinguish between these east African geographic groups, if an indication of age or overall size is considered.

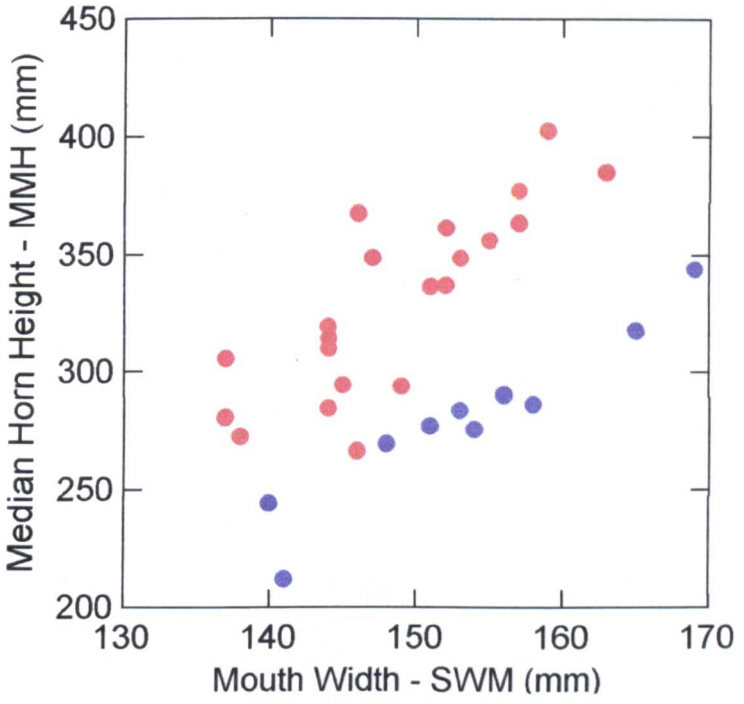

Figure 9.7: *Plot of median horn height versus mouth width showing the strong relationship between the two in differentiating the two pairs combined groups. Groups EEK & ECU are shown in red (●); groups ESK & EST are shown in blue (●).*

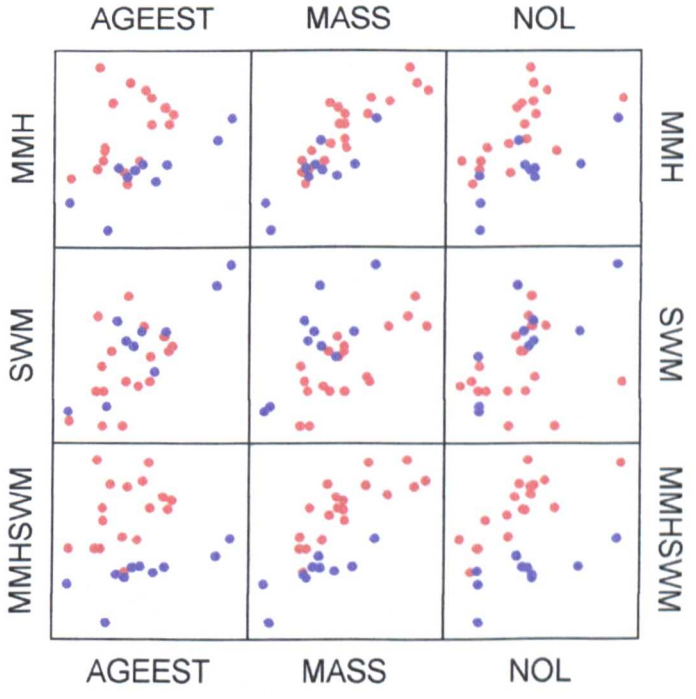

Figure 9.8: *Plots of* **MMH** *and* **SWM** *and the ratio of the two against three age/size indicators. The univariate plots show extensive overlap between the ECU/EEK (red; ●) and ESK/EST (blue; ●) specimens. The ratio of* **MMH** *to* **SMW** *provides a general indicator of the geographic provenance of an adult male specimen. See the text for further discussion.*

Age / Size Estimator	MMH			SWM			MMH/MOW		
ECU & EEK Specimens	r	n	p	r	n	p	r	n	p
Age	0.428	17	NS	0.443	17	NS	0.355	17	NS
Mass	0.885	20	***	0.736	20	***	0.806	20	***
NOL	0.649	18	**	0.157	18	NS	0.798	18	***
ESK & EST Specimens	r	n	p	r	n	p	r	n	p
Age	0.799	10	**	0.856	10	**	0.695	10	*
Mass	0.850	10	**	0.835	10	**	0.804	10	**
NOL	0.746	10	*	0.768	10	**	0.677	10	*
All Specimens	r	n	p	r	n	p	r	n	p
Age	0.383	27	*	0.664	27	***	0.158	27	NS
Mass	0.883	30	***	0.559	30	**	0.774	30	***
NOL	0.518	28	**	0.431	28	*	0.409	28	*

Table 9.14: *Correlation coefficients of the two 'diagnostic variables' used to differentiate between ECU/EEK and ESK/EST specimens. An approximate association between the ratio of these two measures and the geographical provenance of east African adult male skulls is proposed. This association is acknowledge as being imperfect. Three measures of age or size are tested against the two parameters and their ratio; the estimated age, the mass of the skull and the nasal-occipital length. The univariate correlations indicate that the measures are associated with each of the age/size indicators for the ESK/EST group, but may or may not be for the ECU/EEK specimen set. However, the ratio values are significantly correlated with both mass and length measures. See the text for further discussion and Figure 9.8.*

Within the eastern region the female data shows that the EEK group is consistently narrower (**MOW**) than all of the other specimens (except one EST individual) and is definitely smaller than all of the neighbouring ECU females (Figure 9.9). No other character was found to consistently differentiate other groups.

Discussion

Morphological Variability in Giraffe Skulls as Revealed by a Traditional Morphometric Approach using Multivarite Statistics.

The foregoing analysis used a traditional morphometric analysis approach with point to point measurements of skull dimensions analysed using multivariate statistical techniques. A subset of 13 skull measurements, representing functional – phenotypic units within the cranium, were derived from a suite of cranial dimensions and compared between geographically delimited specimen sets. The geographic boundaries were defined according to historical range and physical barriers in the landscape. Adult specimens of the

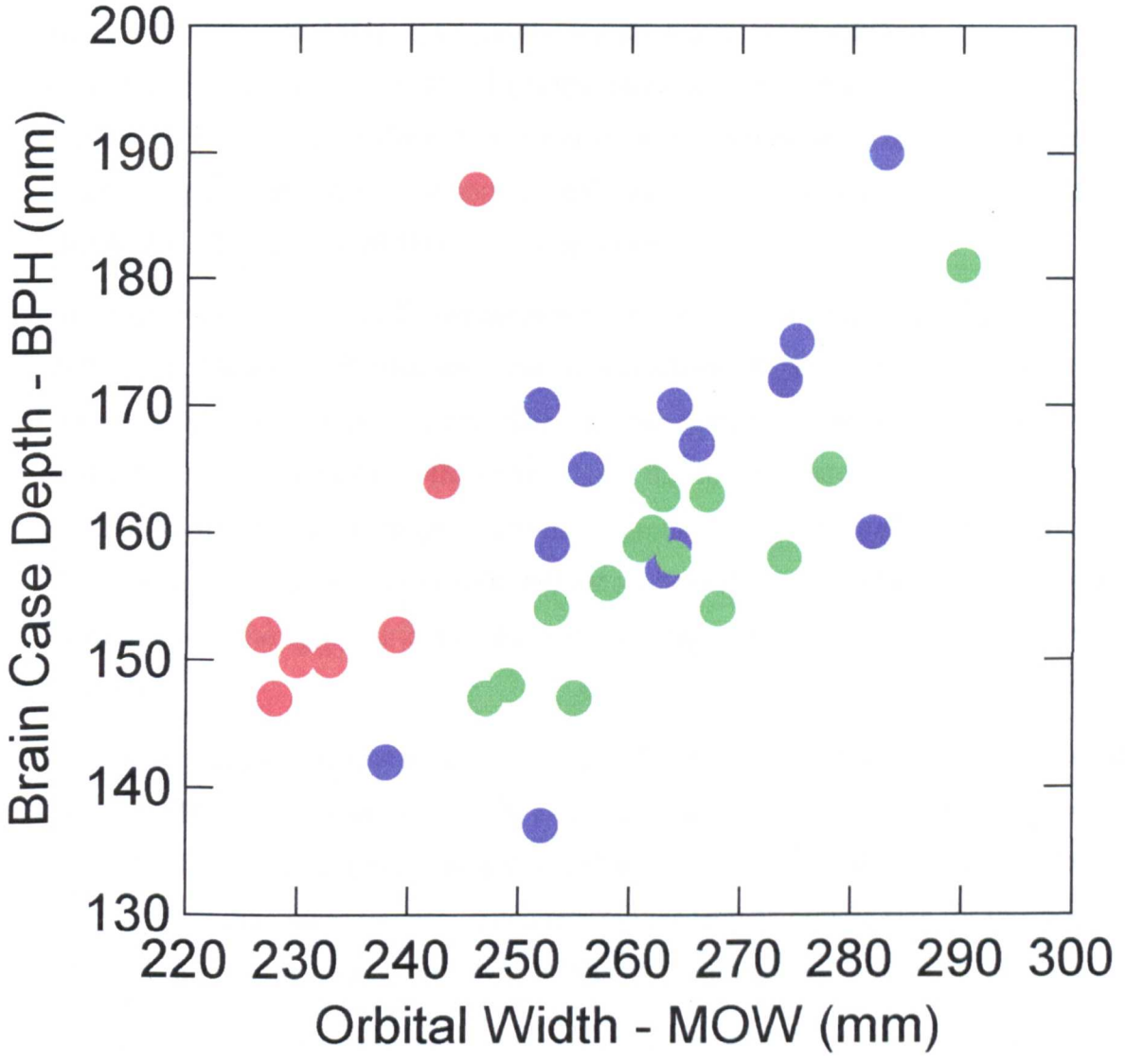

Figure 9.9: *Plot of orbital width (against brain case depth) for female skulls demonstrating the typically narrower nature of skulls from northern Kenya, southern Ethiopia and south western Somalia (EEK). Group EEK are shown in red (●); all other eastern groups are shown in blue (●); all southern specimens are shown in green (●).*

two genders were analysed separately to control for the effects of age and sexual dimorphism.

Generally female skulls show little difference between areas with only the EEK group, from northern Kenya, southern Ethiopia and south western Somalia showing a consistent narrow orbital width (**MOW**) suggesting morphological differentiation. There is a complementary tendency for male EEK specimens to be narrower than other male specimens but this is insufficient for definitive separation of this group from the other males. Female specimens from northern and southern regions may differ according to the depth of the brain case (**BPH**) but this is inconsistent.

Male specimens can be attributed to the northern or southern range according to the ratio between the height of the median horn (measured from the rear of the tooth row) versus the orbital width. Any skulls taller than they are wide come from the north, those of greater width than height originate in the south. This is due to the greater development of the median horn in the northern specimens into a prominent conical or dome-topped cylindrical structure. In the southern specimens the secondary bone deposition is more diffuse across the upper surface of the skull, creating a lower, irregular boss on the forehead.

No differences between localities were discovered in the widespread southern distribution, although the isolated population in the Luangwa Valley in Zambia may be morphologically distinct. The Luangwa specimens appear to have more robust skulls with less parietal horn development than other southern specimens but inadequate sample size for this population makes it impossible to categorically confirm any morphological disparity.

Sample sizes in the western region were inadequate to assess geographic variation. However, based on the limited sample available, there appears to be no differentiation in this region.

In the east male ECU and EEK could not be differentiated. Neither could ESK and EST specimen sets. These groups combined and compared with each other show different morphologies with the more northerly groups (ECU and EEK) showing larger skulls with greater median horn development, with relatively smaller mouths.

Inter-regional comparison of groups shows that southern Kenyan (ESK) and Tanzanian (EST) specimens can be separated from Zambian (SZT) specimens according to the same criteria used to separate the northern and southern regions indicating that this represents a robust character state demarcating a true difference. A contrast between western specimens and their nearest group, ECU, shows that the two can not be adequately separated.

In summary, four different 'morphotypes' can be discerned from the traditional analysis. Firstly, northern and southern male specimens can be separated according to the development of the median horn, as related to the width of the skull. In the eastern region the southern Kenyan and northern Tanzanian male giraffes can be differentiated from those further north. Meanwhile, female giraffes from northern Kenya separate from western Kenyan and Ugandan giraffes. In the south the widespread, perhaps contiguous southern population is undifferentiated. Arguably, the isolated Zambian population may be separated morphologically.

The recognised groupings (in terms of my geographic groups. See Chapter 6) are:

1. West Africa eastwards to the White Nile and the Great Rift Valley (WCP, WSN, ECU). Typified by males with large skulls and well developed median horn;
2. Northern Kenya, southern Ethiopia and south-western Somalia (EEK). Males possess a well-developed median horn. Females skulls are consistently narrower, while male skulls show a tendency to be narrower than other specimens;
3. Southern Kenya and northern Tanzania (ESK and EST). With smaller mouths and less well developed median horns than other male eastern specimens;
4. Southern Africa (SWC, SCZ, arguably SZT). Males show no development of a conical median horn, instead having a low irregular boss of secondary bone depositions. SZT may be more robust skulls with shorter parietal horns.

The geographic groups SWA, SEW, ENA, ENE, ECC and WNN were inadequately represented, or not represented at all, and could not be included in this analysis.

The traditional morphological analysis presented in this chapter uses linear measurements of specimens to represent variation in the dimensions of the skull. However, useful information may be contained in the shape of the skull that can not be represented by simple point-to-point measurements. The next chapter uses landmark restricted eigenshape analysis to investigate variation in the shape of the cranium.

CHAPTER 10:

GEOGRAPHIC VARIATION IN GIRAFFE SKULL MORPHOLOGY: AN APPROACH USING LANDMARK-RESTRICTED EIGENSHAPE ANALYSIS

Introduction

A fundamental objective of taxonomic work is the description of taxa to portray the salient features of each taxon to allow for their future recognition and comparison. This requires the careful use of language to depict morphological features accurately and unambiguously. The descriptive language used often invokes relational comparisons between taxa or between taxa and commonly known shapes and forms. Such descriptive language can be illustrated from Lydekker's (1904) description of the southern African giraffe subspecies, the Transvaal giraffe, *Giraffa camelopardalis wardi*. He states that the skull of the male Transvaal giraffe is characterised by an "aborted frontal [median] horn ... [that] forms an irregular nodular boss, measuring 6 inches in antero-posterior length" (p. 224). The occipital horns are "enormously developed" (p. 221) and "very much larger than in the Baringo giraffe [*G. c. rothschildi*]" and "present a marked contrast to both the Lado [*G. c. cottoni*] and the Cape [*G. c. capensis*] forms" (p. 222-3). The length of the parietal horns "is 7 inches, against $5^1/_4$ inches in a male skull of the Baringo giraffe", although a specimen from Sudan has parietal horns that are "nearly as long ... but are much more slender" (both quotes from p. 223).

In this one description Lydekker exemplifies four types of descriptive language. He uses abstract descriptive language, invoking an unrelated shape ("an irregular nodular boss"). He proffers comparative descriptive language (in comparing the Transvaal giraffe with specimens of the Baringo, Lado, Cape and Sudanese giraffes). He uses relative comparative descriptions requiring some knowledge of the other forms of giraffe skulls ("enormously developed" occipital horns). Finally, he presents measurements as definite indicators of size.

The paired objectives of all taxonomic descriptions are (1) to communicate the shape of the specimen in question and (2) to indicate similarities and differences in relation to other forms. The first of these aims is best done using the accepted anatomical vocabulary with descriptive language and measured dimensions. The second aim may be augmented by the use of geometric morphometrics and shape analysis.

Shape analysis is one of an array of techniques in the field of study known as morphometrics. As Bookstein (1991) states, "The objects of morphometric study are not the forms themselves, but rather their associations, causes, and effects." Such shape analysis does not analyse or describe shape *per se*, but facilitates the elucidation of differences and similarities between comparable objects. Morphometric analysis goes on to interpret covariations between shape and functional, developmental, phylogenetic or other information relating to the individuals and taxa involved.

The particular technique discussed here is extended eigenshape analysis (MacLeod, 1999) and examines the shapes of comparable outlines or other continuous linear forms. Extended eigenshape analysis is used here as a tool to allow quantitative comparisons between different aspects of variation in giraffe skulls. The information provided allows the variability of characters to be quantified and the distribution of character states to be established. This variation is related to the provenance of specimens to examine trends in the geographic structure of the observed character states. Consistent trends in character variation may be used to diagnose subspecies taxa.

Standard and General Eigenshape Analysis

Standard eigenshape analysis was introduced by Lohmann (1983) and illustrated with a study of latitudinal ecophenotypic shape variation in a planktonic foraminiferan, *Globorotalia truncatulinoides* (d'Orbigny). Lohmann's (1983) method is restricted to the analysis of closed curves and has two unnecessary parts. First, it detrends the shape function by removing a constant vector of angular deviation (= a circle) from each shape function. Second, it standardises the shape function so that each shape has a constant angularity. Rohlf (1986) questioned the necessity of the standardisation. MacLeod and Rose (1993) developed a variant of eigenshape analysis that did not require either standardisation or detrending. MacLeod (1999) reviewed the details of the MacLeod and Rose (1993) method and showed how additional modifications could allow eigenshape analysis to (1) analyse open curves and (2) analyse 3-dimensional curves.

The shape of curves must be quantified and described in some way to allow comparisons and can be represented in a number of formats. The most generally used format is

Cartesian (x, y) co-ordinates. An alternative is polar co-ordinates[1]. Lohmann (1983) and Lohmann and Schweitzer (1990) argue against the use of polar co-ordinates, as might be commonly used in Fourier analysis, for two reasons. First the decision of where to locate the centre point (from which measurements are taken) can have a profound effect on the results as any variation in the positioning of the centre point becomes an additional source of variation in the analysis. If the centre point was calculated as a mathematical function and, as a result, this 'measurement hub' resided on a different, non-homologous biological structure for each outline, a biological interpretation of the results would be problematic, if not impossible. Choosing a biologically comparable point may give the practical problem of identifying exactly which point is to be selected as being comparable between objects and may make interpretation of the results more complex. The second reason is that the polar shape function is not necessarily a single valued function of the angle (θ)[2], leading to potential ambiguities in the data and problems in computation. The Zahn and Roskies' (1972) shape function was originally developed for use with Fourier analysis to solve the problem of locating the centroid and is preferred for eigenshape analysis as it consistently provides a simple, complete and accurate description of any curve, within a given tolerance.

Under the Zahn and Roskies' protocol a starting point on the outline is chosen and the desired outline traced. The line is divided into equal length segments by interpolating a series of equally spaced points. The number of points used should describe the curve in sufficient detail so as to retain the useful information contained the original outline. The angular deviation from a standard baseline between sequential points is recorded. The constant step length combines with the angular deviations to provide a complete, unambiguous description of the shape of any curve (within a certain tolerance). This is an efficient way to summarise the data describing a curve as the Zahn and Roskies' (1972) shape function requires n+1 data items for a curve defined by n points; that is n angular deviation values plus the standard step length. In contrast both Cartesian and polar co-ordinates require $2n$ data items; an x and a y element for Cartesian co-ordinates; a distance and an angle for polar co-ordinates. A further advantage of the Zahn and Roskies' (1972) shape function is that it describes shape (i.e. form and angularity) separately from size (= step length) (Lohmann, 1983; MacLeod, 1999). Hence, eigenshape analysis may be made

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[1] Polar shape functions are defined according to a centre point, an angular deviation and a distance measure. The choice of centre point may be arbitrary, it could be the geometric centre or it could be located on a (geometrically) comparable point.

[2] From the centre point a straight line is drawn to the outline and the distance measured. This is repeated at a set angular increment. The polar shape function is reported in the form (r, θ); that is (distance from the centre

size-independent by sequestering the step size information from the analysis (Lohmann, 1983; MacLeod, 1999).

In standard eigenshape analysis the raw angular deviations (the "ϕ" form of the Zahn and Roskies' shape function) at each step are converted to normalised net angular deviations ("$\phi^*(l)$") by subtracting the constant angular deviation value expected on a circle of equivalent size from the observed angular deviation of the curve (Lohmann, 1983). In this way, the $\phi^*(l)$ function is restricted to only the consideration of closed curves. MacLeod's insight (Macleod and Rose, 1993; Macleod, 1999) was to use the ϕ function directly in the analysis, without normalising to a circle, thus generalising the technique to include open, as well as closed, forms.

Both standard and generalised eigenshape analysis use singular value decomposition (Jöreskog *et al.*, 1976) to summarise the patterns of shape variation represented in the data matrix of the shape functions of the relevant objects. This summarises the greatest proportion of observed shape variation in the fewest number of independent axes. The analysis results in a set of eigenvalues that represent the proportion of the total variance explained by each of the associated eigenvectors, equations for the eigenvectors (= eigenshape axes) and covariance or correlation-based scores of the original specimens with the latent shape-deformation trends that are represented on each eigenvector. Plotting the covariance or correlation scores allows the degree of similarity (and difference) between objects to be assessed. Lohmann (1983) specifies the use of a correlation, rather than covariance, matrix as the basis for the singular value decomposition calculation. However, this is equivalent to normalizing each object's angularity and so removes a valuable component of between species variation from the analysis and imposes an artificial weighting (Rohlf, 1986). MacLeod and Rose (1993. See also MacLeod, 1999) pointed out that, unless the weighting can be justified, it is best to carry out analyses without weights and so suggest following Rohlf's (1986) recommendation to use the interobject co-variance matrix.

The production of equations representing the principal axes (latent shape trends representing the major modes of shape variation between objects) allows models of shape variation along axes to be made. Multiplying through by a constant term scales the latent shape trend, effectively altering the angularity without changing the form.

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point to the outline followed by the angle). For complex shapes that curve back on themselves a line drawn at a given angle may cut the outline at more than one point and so have more than one value.

Hence, eigenshape analysis allows the comparison of similarities and differences between a set of objects in three ways:

1. By summarising trends in shape variation as a series of independent latent shape trend axes,
2. By comparison of the covariation scores of the original objects against the shape trends represented by the axes,
3. By the creation of models of variation that allow variation along the axes to be visualised.

Two Problems with Standard and Landmark Restricted Eigenshape Analysis

There are two problems associated with both standard and generalised eigenshape analysis. The first is to do with the number of points used and the second relates to the comparability ("homology") of the equivalent points between objects.

In Lohmann's (1983) original description of standard eigenshape analysis the number of points to be used was a subjective decision made by the investigator. He suggests that "usually, a few hundred segments [bounded by points] are sufficient to smoothly and precisely represent the details of a typical shape outline." (p. 663). MacLeod (1999) states that "in standard eigenshape analysis, the digital resolution employed by the investigation is determined without reference to the range of shape complexities present within the data set." This is true to the extent that there is no formal consideration of the shape complexity with reference to the number of points used. However, it is to be hoped that any researcher would use his judgement and digitise a smooth outline with less resolution than a highly variable one. This said, most people will still be attracted to the 'round' numbers, choosing multiples of a hundred rather than any figure in between.

To counter any charge of subjectivity, MacLeod (1999) recommended a pre-processing step whereby the number of co-ordinate points in the outline is determined algorithmically and referenced to a specific quality, fidelity or 'tolerance' criterion. For example, each curve may be subjected to an iterative search for the minimum number of boundary co-ordinates required to represent the length of the original digitised curve. That is, the number of points can be set at the minimum level whereby, for the most complex individual curve, the sum of the lengths of the line segments is greater than, for example, 95 per cent of the length of the original curve. For a given tolerance level, more points are required for more complex curves.

The fundamental requirement in digitising the original curve is simply that the digitisation should be at sufficiently high resolution to closely approximate the true length of the curve. Increasing the number of points on a curve causes rapid convergence of the summed length of the interpolated line segments with the true length of the curve (Figure 10.1). Hence, as long as the original number of points is a close approximation of the length of the curve then over-estimation of the resolution required will not cause computational problems and will result in a similar number of representative points following the pre-processing procedure. Judgement must be used when deciding on the tolerance level to be used. Here the level of detail in the outline influences the decision with more intricate curves requiring greater tolerances to capture subtle variation. A precedent may be borrowed from statistical analyses where a 95 per cent confidence level is considered a reasonable minimum level to work at.

The second problem is one of correspondence. In any comparison like must be compared with like in order to draw any valid conclusions. As expressed by Lohmann and Schweitzer (1990), "meaningful comparisons of form depend critically on the correspondence of features, point for point." (p. 153). In standard eigenshape analysis Lohmann (1983) used a single, consistently identifiable reference point to bring the outline points of the foraminifera studied into correspondence[3]. The correspondence introduced here is a geometrical correspondence and not, necessarily, biological. Relying on a single reference point to register the correspondence of all outlines can introduce artefactual 'shape variation' that can obscure the true shape trends. Where the compared structures vary slightly in their form relative to one another (that is parts of the curve vary in their proportions) the correspondence between points can be lost. For complex outlines that may incorporate more than one biological structure this effect may cause the points contained in one structure to 'smear' into another. This effect is illustrated in Figure 10.2 which shows three male giraffe skulls, digitised to the same number of outline co-ordinates with numerically corresponding points highlighted around the outline.

MacLeod (1999) suggested that, in order to bring the outline points back in to closer correspondence, additional internal landmarks should be added where biologically or geometrically comparable points are present in the complex outline. These landmarks then mark the limits of the curves representing homologous structures within the whole outline

[3] Lohman (1983) offered an alternative, superposition approach for bringing his outlines into correspondence. The series of outline points could be rotated so as to maximise the correlation between the individual outline and a reference shape (either a selected individual or a mean shape). However, as MacLeod (1999) pointed out, this alternative has not been widely used.

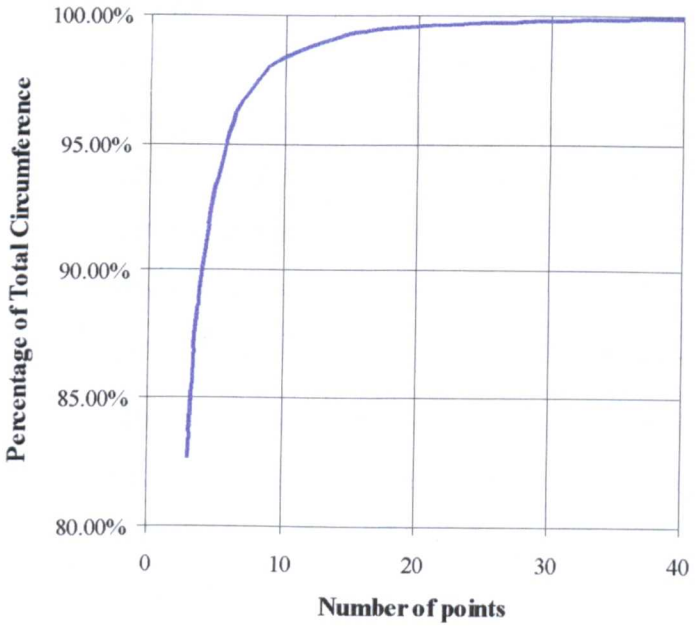


Figure 10.1: *Convergence of true circumference length and summed inter-point line segment length for a circle. As the number of line segments, as defined by the number of points, increases the concordance between the true and estimated circumference rapidly converges. With forty points on the circle the summed length of the straight line segments is 99.90% of the true circumference. Note that a circle is a simple two-dimensional object. More complex objects, particularly with sharp angles, will require a greater number of points to converge on the perimeter length.*

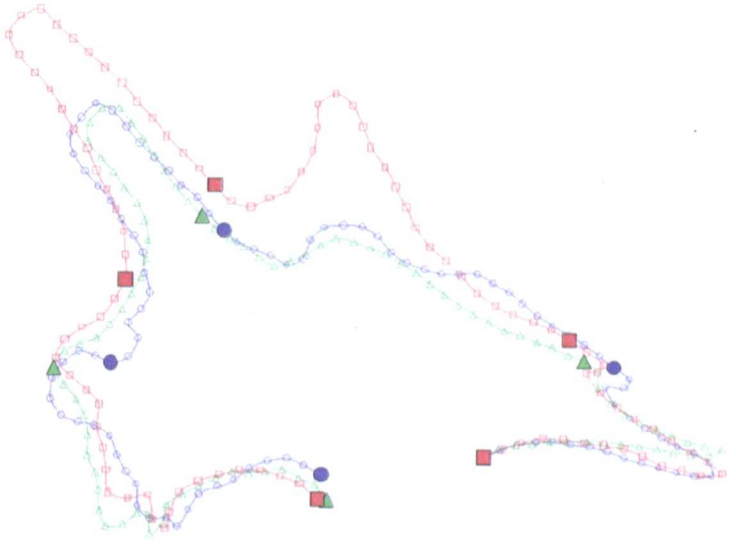


Figure 10.2: *Outlines of three male giraffe skulls digitised with 100 points illustrating the effect of 'smearing'. The 25th, 50th and 75th points are highlighted as filled shapes. The numerically corresponding points are not in geometric correspondence and occur on different structures of the skull. The correspondence is particularly poor at the back of the skull. Note that the skulls are shown with 100 points for clarity, the original outlines were digitised to 400 points. The skulls are scaled to have the same premaxillary – occipital ridge length.*
● *= BMNH1986.1604;*
■ *= USNM162017;*
▲ *= USNM200151.*

and bring the sequence of outline points back in to closer correspondence at these points. This new variant is known as extended or landmark-registered eigenshape analysis. The use of landmarks along the outline in this manner does not remove the mismatching effect completely, but it does correct it to the extent possible given accepted hypotheses of correspondence (geometric and/or biological) between landmarks. This method also has the useful feature of facilitating a clear demarcation between different structural units along the outline. Comparing standard and extended eigenshape analysis results MacLeod (1999) demonstrated that, for a relatively simple data set consisting of six mammalian distal phalanges, the artefactual smearing effect seen in the standard analysis accounted for over 15 per cent of the variance in 'shape' between these six objects.

The use of landmark points makes good operational sense beyond the practical benefit of reducing any smearing effect as these defined line segments identify biological structures that can be examined in isolation allowing particular structures (characters) to be investigated more fully.

Linking Landmark Registration with Tolerance Criteria

In presenting landmark registration with tolerance criteria as an answer to the problems of subjective selection of point sampling density and outline point smearing MacLeod (1999) reinforces standard eigenshape analysis and provides a robust generalised method for examining shape variation in complex outlines, such as skull profiles.

The inclusion of these two techniques allows differences in complexity between line segments to be accommodated in the analysis. Each line segment demarcated by landmark points can be subjected to the tolerance criterion independently. In this way the information present in relatively simple curves can be captured by a lower density of outline points while the variation present in more complex curves requires representation with a greater density of points to describe the segment to the same fidelity. A result of the independent application of the tolerance criterion to landmark-restricted line segments is that the more complex segments enjoy greater weighting in the ensuing multivariate analysis. This effect, termed complexity weighting (MacLeod, 1999), is not a problem in itself, but should be understood by the investigator and assessed with regard to the problem in hand.

The aims of this chapter are to investigate the variation in giraffe skull morphology by application of landmark-restricted eigenshape analysis. The results of the analysis will

allow the major trends in the variation of skull morphology to be identified and any geographic structure to this variation to be determined.

Materials and Methods

Specimens Examined

This study was based on 142 adult skulls examined during visits to museums and research institutions in Africa, North America and the United Kingdom. The analysis uses photographs of giraffe skulls to document trends in morphological variation of the skull. Due to damage to individual specimens in different parts of the skull, the sample composition and sample sizes differ for each analysis. A list of all specimens included in each analysis is given in Appendix 4.10.1. Giraffe skulls are sexually dimorphic so male and female skulls are analysed separately (Chapter 5). Only adult skulls were included in this analysis. Adults are defined as those skulls with the full adult (permanent) dentition in wear, corresponding to an age of approximately six years (Hall-Martin, 1975, 1976. Chapter 4).

Data Acquisition

Photographing for Geometric Morphometrics

Four views of each adult skull were photographed to characterise skull variation. Photographs of the left and right skull profiles were taken parallel to the mid-sagittal plane of the skull with the camera positioned centrally relative to the horizontal and vertical axes of the skull (Figure 10.3A and 10.3B). The rear parietal horn was covered with a black cloth to exclude it from view. A front view of the parietal horns, parallel to the central long axes of the horns was taken (Figure 10.4). The lower boundary of the image included the orbital ridges. The occipital ridge was covered with a black cloth to exclude it from the image and prevent it from obscuring the bottom of the curvature between the parietal horns. A plan view of the face and muzzle region from above, parallel to the basifacial plane was taken (Figure 10.5). The extent of the photograph included the orbital ridges. Where necessary the maxillary teeth were covered with black cloth to prevent them obscuring the outline of the muzzle. All photographs had either a 20cm bar or a 5cm disc to provide scale. All photographs were taken against a black background. At least two, but typically three replicates of each view were taken for each specimen.

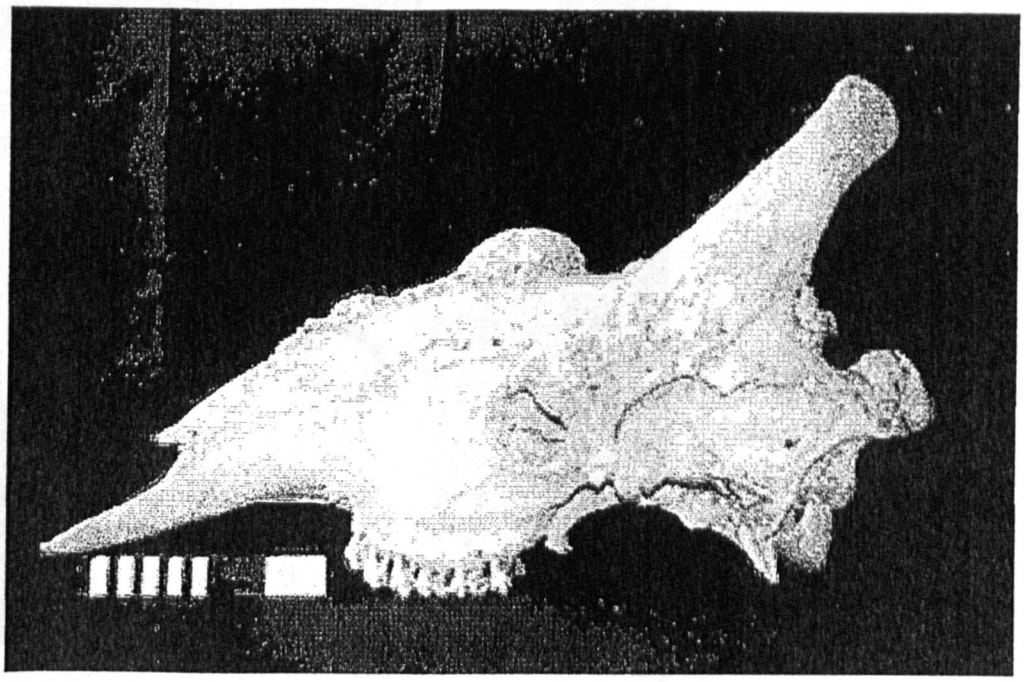


Figure 10.3A: *Example photograph of the left side of the skull of specimen BMNH1986.1604.*

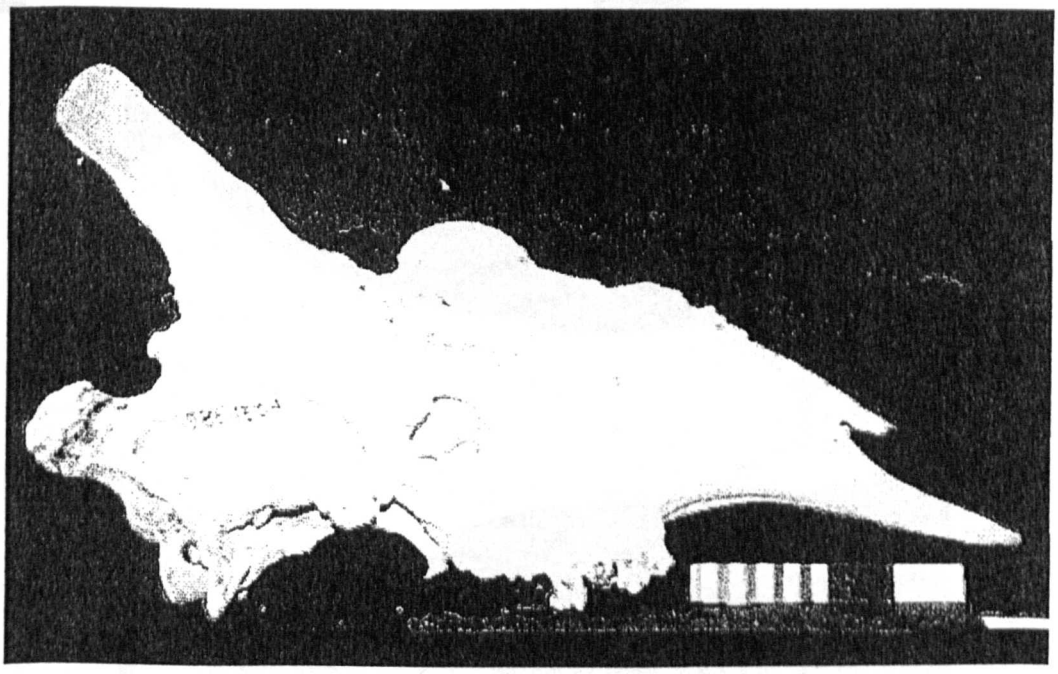


Figure 10.3B: *Example photograph of the right side of the skull of specimen BMNH1986.1604. Photographs were taken with the objective lens of the camera placed centrally on both horizontal and vertical axes of the skull. Skull outlines were digitised from the front of the maxillary toothrow to the rear of the maxillary tooth row, excluding the teeth from analysis. Note that the rear parietal horn has been masked in each case to prevent it obscuring the outline. A 20cm scale bar was included in all photographs.*

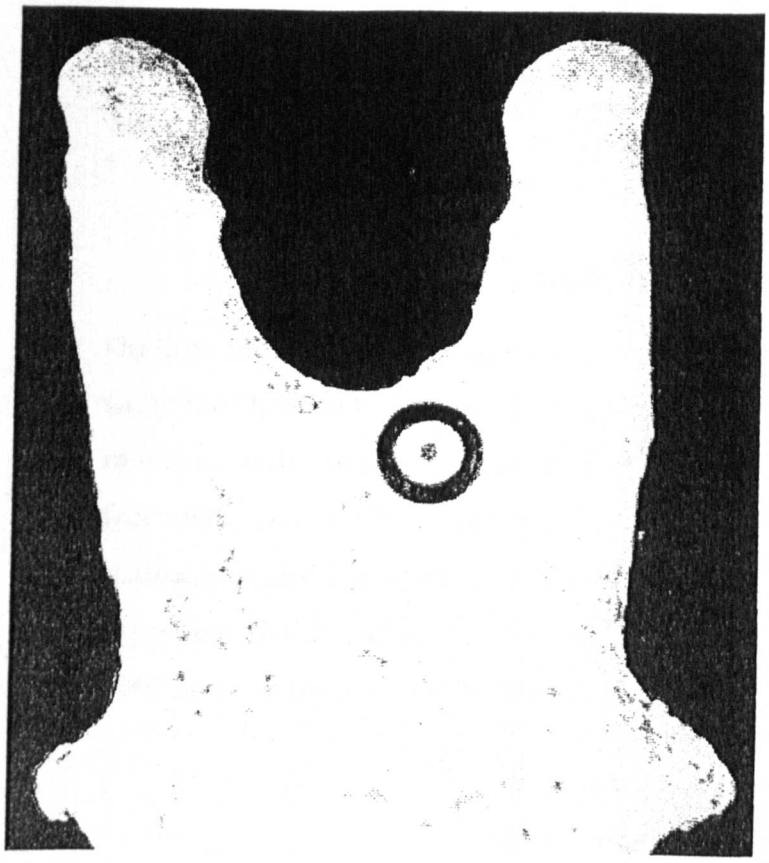


Figure 10.4: *Example photograph of the parietal horns of specimen number BMNH1986.1604 taken parallel to the central, long axis of the horns. The occipital ridge was masked to prevent it obscuring the bottom of the curvature between the horns. Note the orbital rims at the bottom of the picture. These are included to act as the corresponding start and end points for outline digitisation. A 5cm scale disc was included in all photographs.*

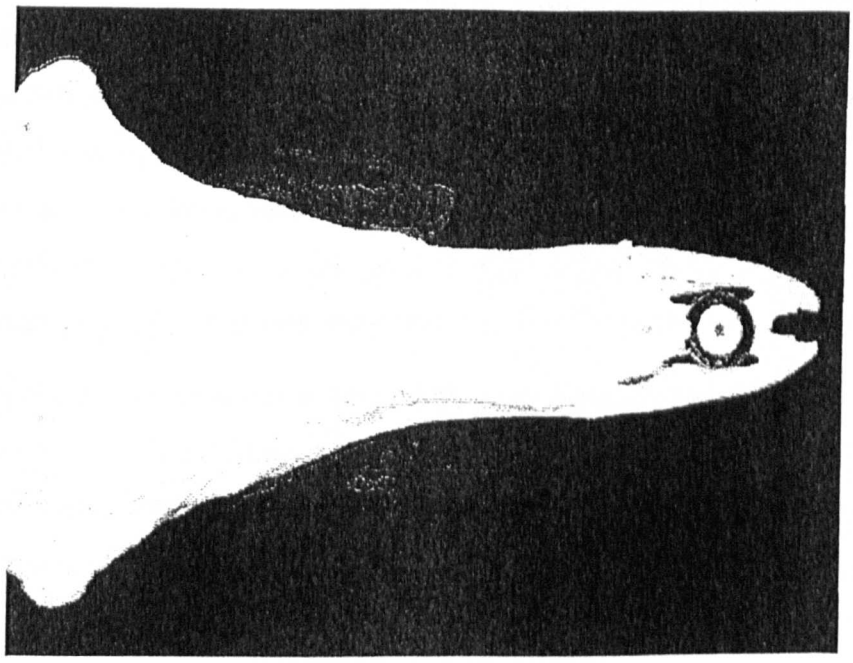


Figure 10.5: *Example photograph of the face and muzzle region of specimen number BMNH1986.1604. Plan view taken with the camera parallel to the basifacial plane. Maxillary teeth, that may obscure the outline, were masked. Note the orbital rims to the left of the picture. These are included to act as the corresponding start and end points for outline digitisation. A 5cm scale disc was included in all photographs.*

All photographs were taken using black and white 100ASA film. The developed negative images were subsequently scanned and processed using Adobe Photo Shop to produce a positive image. Contrast was adjusted to maximise the contrast between the skull and the black background.

Digitising of Skull Outlines

Outlines of the scanned images were traced and acquired as Cartesian co-ordinate data files using the Optimas 6.0 Image Analysis Software (Optimas Corporation, 1997). A resolution of 400 co-ordinate pairs per profile outline and 200 co-ordinate pairs for parietal horns and face and muzzle outlines was used to record the outline shapes (Figure 10.6). The profile outline included the whole profile except for the maxillary teeth. For the premaxillae and the parietal horns outlines the rim of the orbit (at the widest point) was chosen as the start (left) and end (right) point for the digitising process (Figure 10.6).

Positioning of Landmark Points

All of the outlines analysed in this study are open curves and so are delimited by two terminal landmark points. Additionally, internal landmark points are located on each outline defining the structural elements of the skull outline. The internal points provided a source of common reference between shapes, improving the correspondence between outlines and allowing complexity weighting to be carried out on the line segments independently. A secondary, and useful, effect of internal landmarks is that it allows examination of the structures defined by the segments in isolation, where relevant.

Positioning of the landmark points for the parietal horn and face and muzzle outlines was straightforward. These images are bilaterally symmetrical and the single internal landmark point was placed centrally to maintain this symmetry. For both types of image the point was positioned on the sagittal plane, where the plane of bilateral symmetry intersected the outline (Figure 10.6).

The profile outlines were divided into seven segments using six internal landmark points (plus the start and end points). These internal landmark points were (see Figure 10.6):

1. The tip of the premaxillae,
2. The angle between the premaxillae and the nasal bones,
3. The tip of the nasal bones,
4. The base of the parietal horns on the upper surface of the skull,

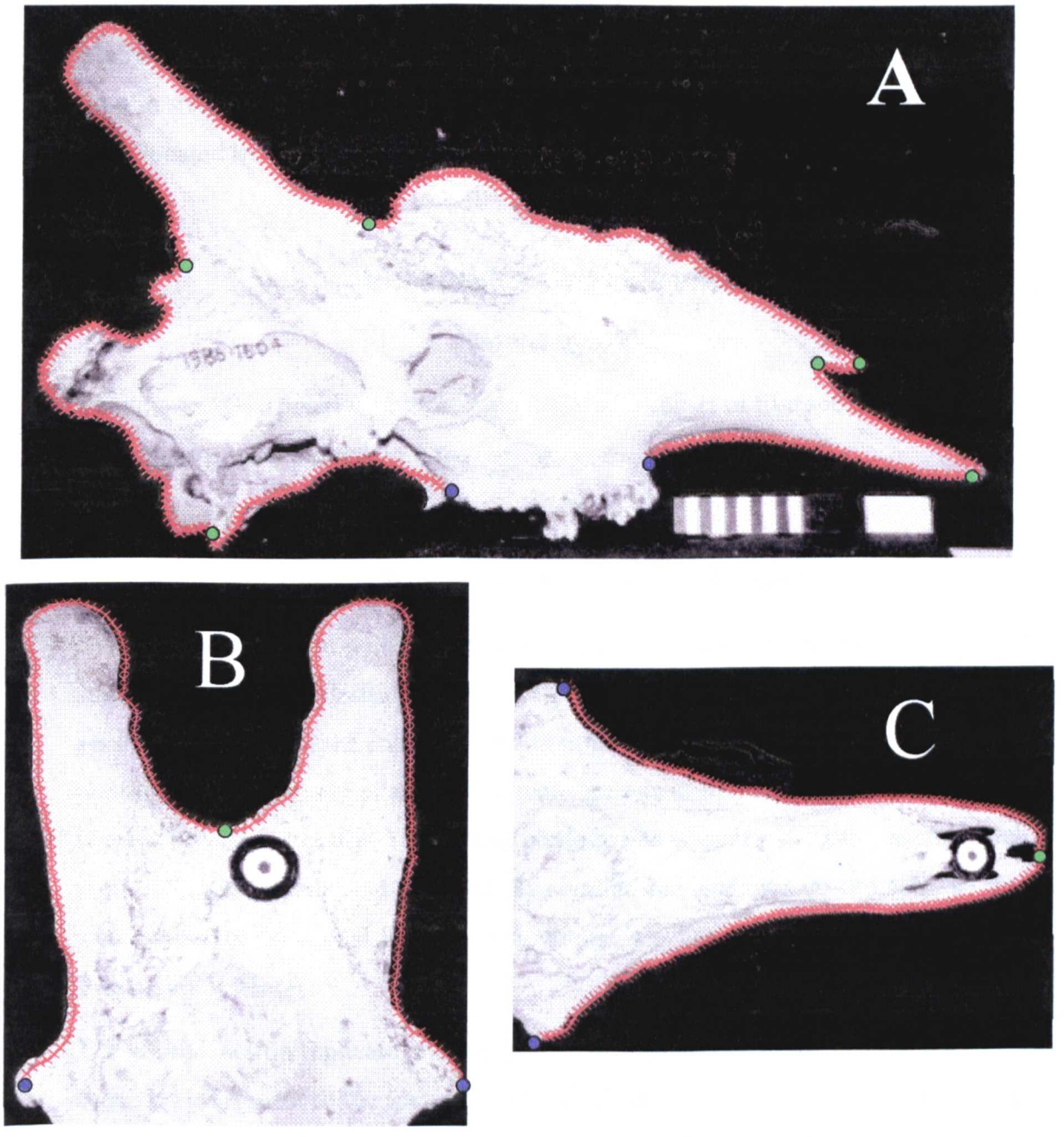


Figure 10.6: *Digitisation of Outlines. Photographs of specimen number BMNH1986.1604 demostrating the digitisation of A) profile, B) parietal horns and C) face and muzzle outlines used in the analyses. Profile outlines were digitised to 400 points (red crosses ×), while parietal horns and face and muzzle were digitised to 200 points (red crosses ×). Note the start and end points of each digitisation (shown with blue dots •): The profile started at the anterior end of the maxillary toothrow and ended at the posterior end; parietal horn and face and muzzle outlines start and end at the outermost point of the orbital rims.*

5. The base of the parietal horns on the back of the skull,
6. The angle between the occipital condyle and the post-glenoid process.

Many of the points were readily locatable as they occurred at obvious points either at the tip of a structure (1 and 3) or at the junction of two structures (2 and 6). Two (4 and 5) were located by eye to demarcate the start and end of the outline of the parietal horn.

Accounting for Potential Sources of Error

Positioning of Landmarks

Landmarks were positioned three times each on a subset of five specimens. The resulting co-ordinates were plotted onto the outlines and assessed.

Between and Within Sessions Variation

Variation between and within sessions was assessed to ensure that the setting up of the skull and the camera position was consistent such that the photographs taken in different sessions and through the course of an individual session are comparable. Replicate photographs were made of a subset of male specimens in the collection of the Natural History Museum, London. Up to three photographs of each view were taken in each of three different sessions and digitised. The outline and landmark co-ordinates so derived were plotted and assessed for any variation that may introduce artefactual variation into subsequent analyses.

The within-session replicate photographs focus attention on to the positioning of the camera with respect to the specimen. The skull was set up once for each view and multiple photographs taken. Between-sessions replicates test the consistency with which the specimen and camera were set up through time and at different locations where specimen photographs for analysis were taken. Between sessions replicates were taken at gaps of between seven and fourteen months with sessions in October 1998, May 1999 and December 1999.

Comparison of Left and Right Profiles

Variation in profile shape was examined using the right profile of each specimen. Giraffe skulls are (to a reasonable tolerance) bilaterally symmetrical. Therefore, in specimens with damage to the right side only, it should be reasonable to substitute a reflected image of the left side. Deriving outline and landmark data from left and right profiles of the same

specimen tested this assertion. The outlines and landmark points from both the left side and the right side were superimposed and assessed by eye for any variation.

Data Processing and Analysis

Male and female skulls were analysed separately for each of the three skull views. The landmark-restricted eigenshape analysis was undertaken using a suite of programs written by Dr. Norman MacLeod of the Palaeontology Department of the Natural History Museum, London[4].

The initial outline data, in the form of Cartesian co-ordinates segmented by the inclusion of internal landmark points, were converted to ϕ shape functions at 99 per cent tolerance for the parietal horns and premaxillae photographs and 95 per cent for the profile outlines.

Eigenshape analysis was performed on each data set. The size element was sequestered from these analyses, removing the influence of scale and making the procedure purely a comparison of shape.

Modelling and Interpreting Major Trends in Shape Variation

Following eigenshape analysis and the resulting derivation of the principal latent shape functions, the trends described by these functions were modelled. It is important to understand the distinction between the two components of shape - form and angularity – in order to understand the procedure behind modelling the principal modes of shape variation. Form refers to the position of any deformations to the outline. That is, form is determined by the position on an outline where bulges (convex curves) or hollows (concave curves) occur. Angularity is the magnitude of these deformations. These terms are explained further and illustrated in Figure 10.7.

Eigenshape analysis provides equations (= the eigenvectors or eigenshape axes) that describe the principal shape trends between the objects included in the study. Each latent shape function can then be represented graphically by plotting the series of ϕ-values. A useful heuristic procedure is to plot these values as a line graph to visualise the shape function (Figure 10.8). The position of the troughs and peaks in the function's trace represents variation in the form of the shape. Meanwhile, the amplitude of the trace represents the angularity of the particular shape model. These equations may be scaled by a constant to alter the amplitude of the plot. Hence, by equating the constant value with

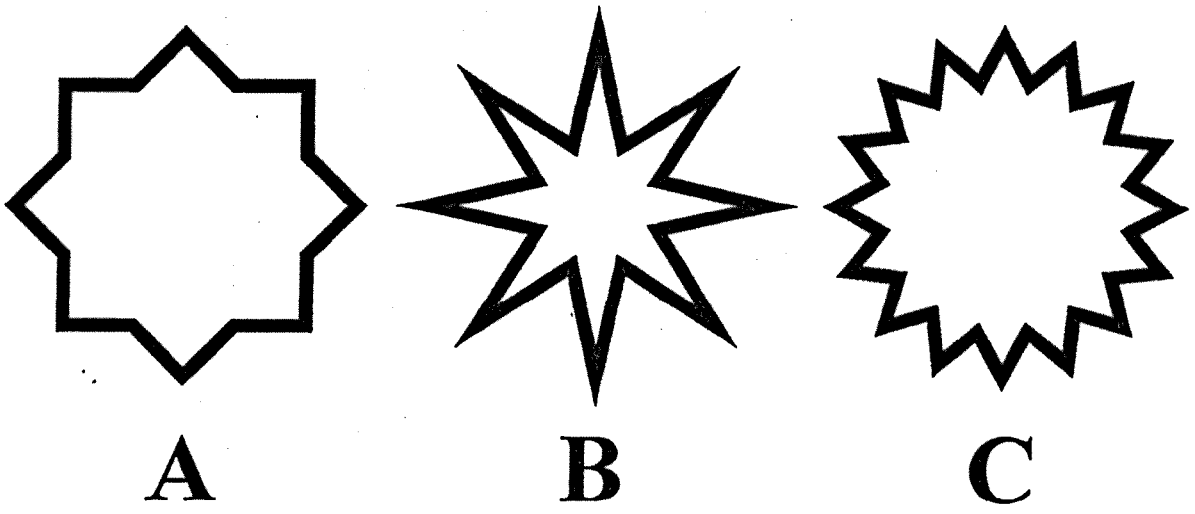


Figure 10.7: *Shape, Form and Angularity.*

Shape is made up of two factors, form and angularity. Form describes the positions of deformations in the outline (2-D) or surface (3-D) of an object, whereas angularity indicates the magnitude of these deformations.

The three stars above illustrate the point. Stars A and B are both 8-pointed stars, and hence, they have the same form. On each one the points originate from the same relative positions at regular angles. Meanwhile star C has 16-points and so has a different form. The points protrude from different positions. The difference between stars A and B is angularity. That is the angles made by the points are much sharper in star B.

Note that, in this context, angularity is only a useful concept when referring to shapes of the same form.

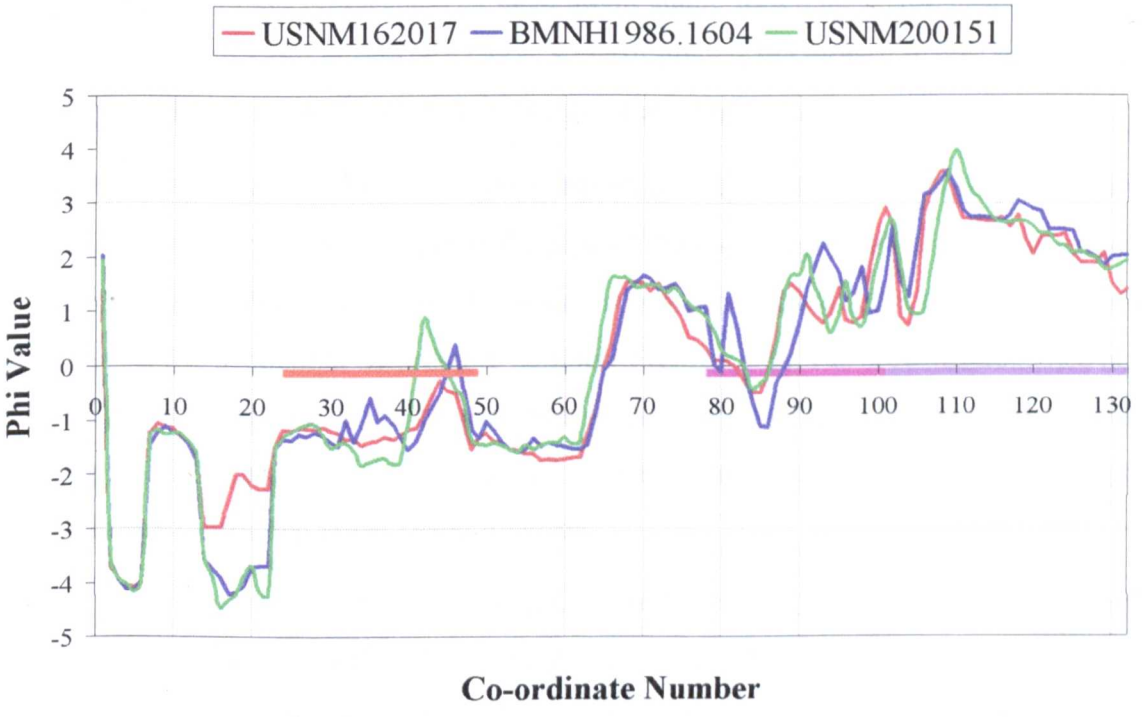


Figure 10.8: *Phi shape functions for three skulls. The phi shape function represents the same information as the outline data in a different form. Phi functions from the interpolated outlines of three skulls (USNM162017, BMNH1986.1604 and USNM200151) are presented. Skull outlines were interpolated from 400 points to 132 total points with 5, 7, 9, 25, 31, 24 and 31 points in each segment (as defined by internal landmark points). Shape differences at corresponding co-ordinates can be easily compared using phi plots. For example the variation in the median horn apparent in the photographs is indicated by the region of the orange bar. The concave approach and high peak of the median horn in USNM200151 contrasts with the lower profile of USNM162017. The pink and mauve bars show the variation present in the back and base of the skull outlines.*

covariance values along each axis the shape at any point along the axis can be modelled. By modelling the shape variation at the maximum and minimum covariances and using three equally spaced values in between, five modelled shapes were calculated for each axis. Plotting these models onto common axes indicates the shape variation, due to changing angularity, seen along each axis (See Appendix 4.10.4 for modelled shapes).

Three sources of information are available to aid in interpretation of latent shape trends and resulting shape variation models. The most useful are the modelled shapes themselves. Plotting the shapes allows the modelled shapes to be described directly and hence indicate the shape changes represented by each latent shape function. Subtle changes may be difficult to pick out in separate plots. Superimposing the models makes the comparisons of shape differences between models obvious (see Appendix 4.10.4).

A second source of information for interpreting amplitude-mediated shape variation in the principal latent shape functions is the plot of the modelled ϕ-values as a line graph. This provides direct correspondence between each point on each curve (as the x-axis of such a graph is the sequential co-ordinate number. Figure 10.8). Close correspondence between the plotted lines indicates little variation in that region of the outline in that modelled shape. Differences between the plotted lines indicate the regions of the outline primarily associated with that latent shape function.

The third source of information is recourse to the original specimens via the eigenshape scores calculated for each axis. These are the covariance coefficients of each of the original shapes with the latent shape function. The scores therefore indicate how closely the original outline corresponds to the shape function. Hence, the interpretation of the shape function can be aided and should be confirmed by returning to the original specimens. However, when examining the original specimens, it should be remembered that the shape functions represent latent shape variation and are independent (orthogonal) functions that model the variation remaining after previous shape trends have been described. Any two of the original specimens with the same, or similar, covariance values for a particular axis need not look very similar in close comparison. Each of the original specimens are described completely only by a linear combination of all of the latent shape functions. Although similar for one of the shape trends, they may be different for the others, and so look different. The greater the eigen-value associated with the eigenvector the greater the

[4] Available from: http://www.nhm.ac.uk/hosted_sites/paleonet/ftp/ftp.html.

proportion of the total variance associated with it and the stronger the shape trend identified.

Assessing Regional and Local Geographically Structured Variation

Comparisons were made between geographically delimited sets of specimens. Inter-regional comparisons were followed by within region (local) comparisons. Following the eigenshape analysis the shape variations described by the principal axes were modelled and interpreted accordingly. Plots of covariance values between objects used to constrain the eigenvectors and the eigenvectors themselves were examined to ascertain the covariation of the shape trends identified with the geographical provenance of the specimens. Statistical tests, using the eigenscores of geographically grouped specimens, were conducted to test the null hypothesis of no geographical structuring to the identified shape variation (See Appendix 4.10.2).

Results

Potential Sources of Error

Positioning of Landmarks

Visual assessment of the positioning of landmark points showed this to be consistent and precise for each of the views used (Figure 10.9). As the same criteria were used to place each point on each specimen, it is reasonable to assume that the chosen landmarks are consistently recognisable and so may be considered as being geometrically corresponding locations. These points show high fidelity in placement within and between specimens, that adequately demarcates the limits of the biologically corresponding structures represented within the complex outlines. As such, the placing of landmark points within the outlines increases the correspondence of the compared outline points and reduces artefactual 'shape variability' attributable to smearing. The slight error that exists in placing the landmark points is negligible compared to the improvement in correspondence of the points resulting from their inclusion.

Between and Within Sessions Variation

Visual assessment of outlines derived from replicate photographs for each view, both within and between photographing sessions, showed close, accurate replication. Typically

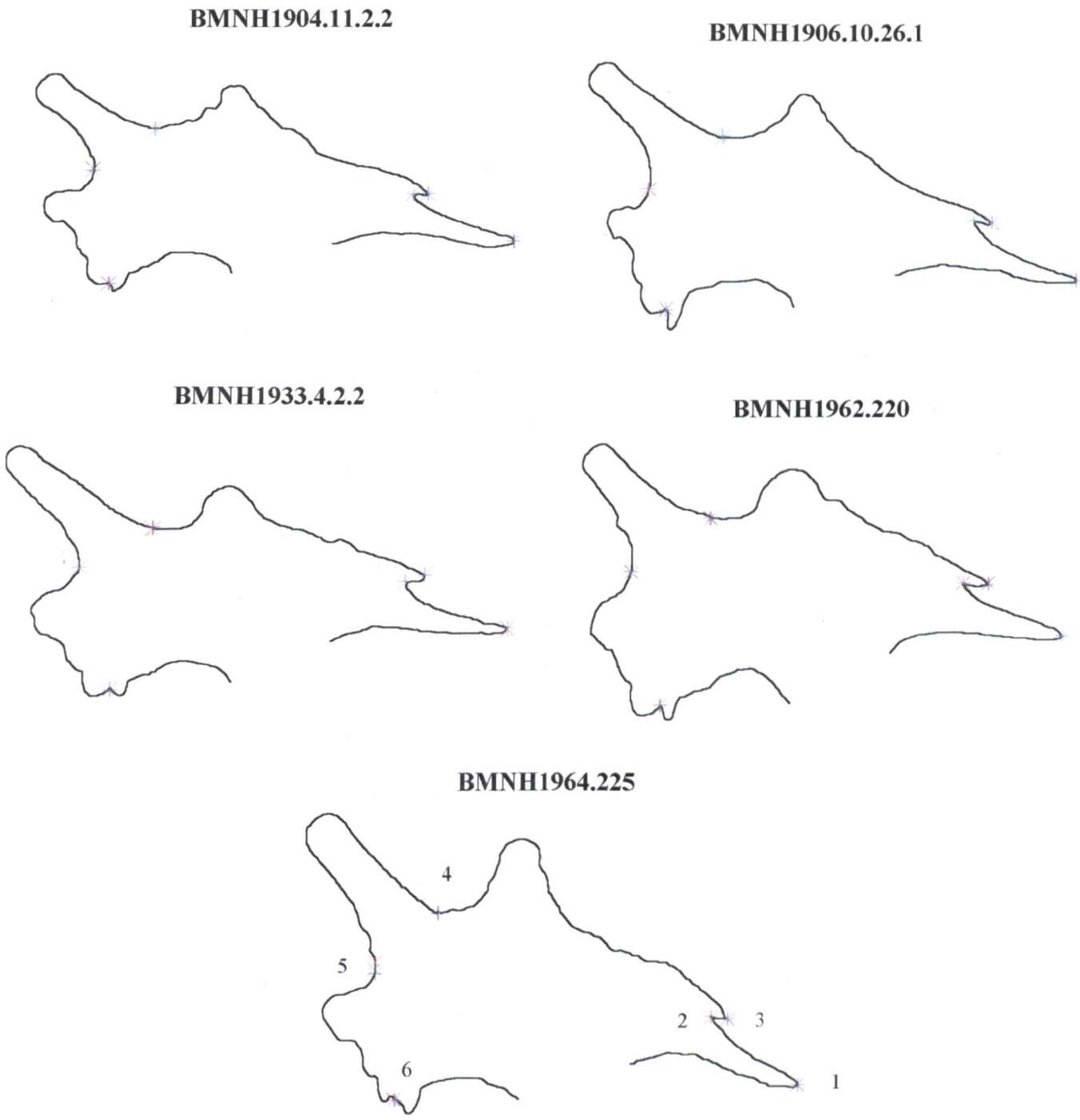


Figure 10.9: *Replication of the placing of landmarks on profile outlines. Landmark points were located on digitised outlines on three ocassions to assess observer error in the placing of landmark points. Internal landmark points 1 and 3 were placed at the tip of structures (the premaxillae and nasal bones respectively) while landmarks 2 and 6 were positioned at the abuttal of two structures (between the premaxillae and nasal bones and the occipital condyle and post-glenoid process). Landmarks 4 and 5 were expected to be more problematic, being placed at extremes of curvature demarcating the base of the parietal horns. With one exception (slight variation in point 5 in BMNH1964.225) points were placed with great consistency suggesting comparability between all specimens.*

the variation was slightly greater between sessions than within, as might be predicted, although this was not necessarily so. In either comparison, the difference between replicate outlines was considered negligible with respect to possible between subspecies variation (Figures 10.10 and 10.11). The outlines derived from the between sessions photographs provide consistent results, suggesting that the same standards were used in all photographing sessions through time and in each institution visited.

Left and Right Profile Comparison

The right side profile was arbitrarily chosen for the analysis of shape variation in the profile. The comparison between the right profile and the reflected left side profile of the same specimen shows little difference. Only specimens where an asymmetry was apparent from the specimen itself gave significantly different profiles (Figure 10.12).

The reflected left side profile was included in the analysis of profile variation where a right side image was not available to enhance sample sizes and to attempt to classify the greatest proportion of the examined specimens as possible. It was typically used under two circumstances; where either the right premaxilla or the right parietal horn was damaged or missing. In the former case the symmetry of the parietal horns could be examined. In any case where it was known that the parietal horns were distinctly asymmetrical the left profile was not used. Where one of the parietal horns was damaged, no such comparison could be made and it was assumed that the skull was bilaterally symmetrical and the reflected left profile was included for analysis with the right profile outlines.

Giraffe Skull Shape Variation

Each data set has been analysed and modelled. Due to sexual dimorphism in giraffe skulls male and female data were analysed separately. In each gender all data, for each data set, were analysed together. Subsequently, data were separated into northern (incorporating eastern and western) and southern regions and analysed again to examine more localised trends in variation. The major trends of shape variation identified from the analyses are described. Detailed results are presented in Appendix 4.10.2, eigenshape score plots in Appendix 4.10.3 with modelled shapes given in Appendix 4.10.4.

The first axis indicates the major shape trend within the data and so, in most instances, represents an axis of shape similarity (MacLeod, 1999), so a high eigenvalue is expected for the first axis when the shapes compared are fundamentally similar. The shape variation

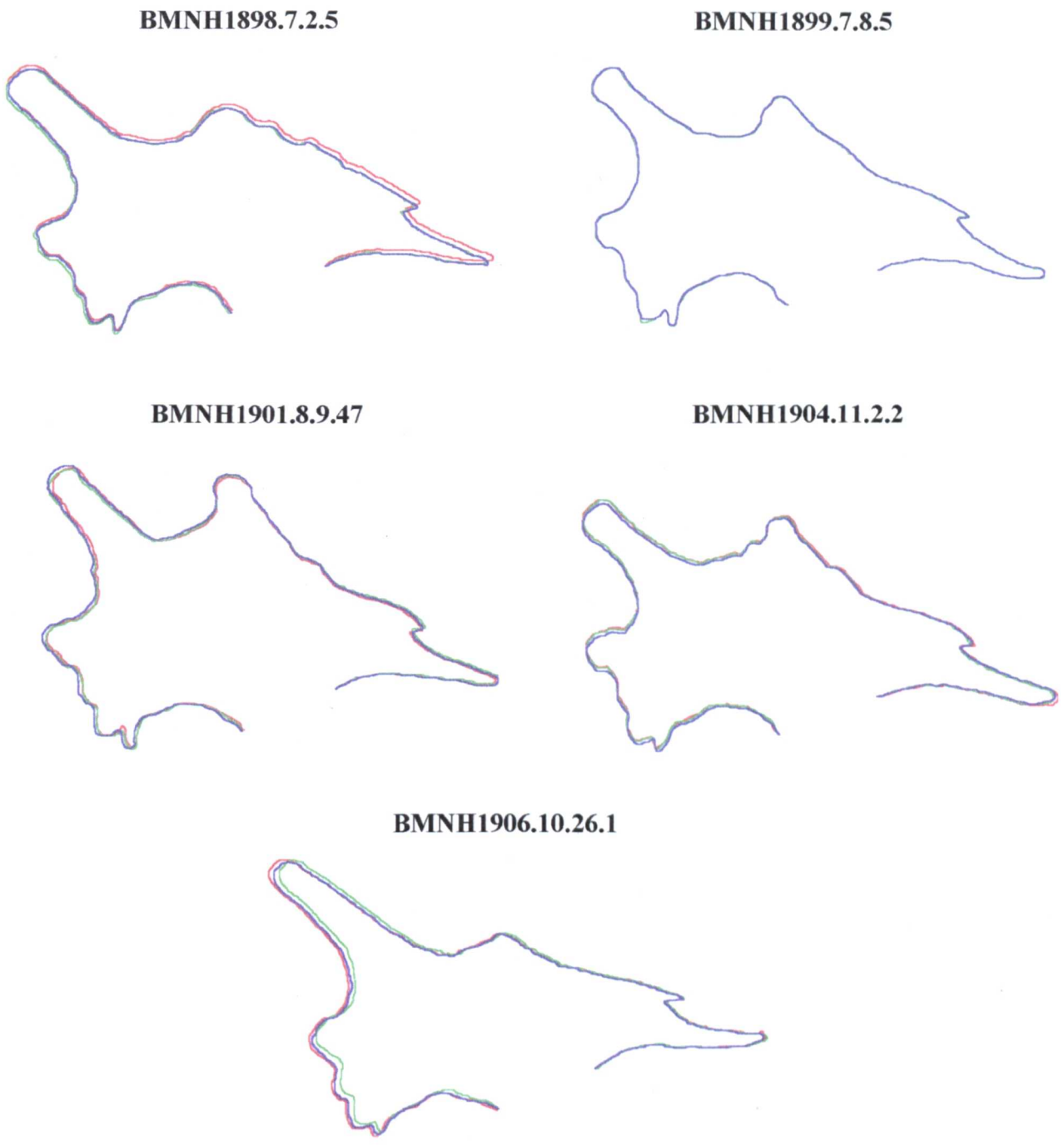


Figure 10.10: *Within sessions variation in outlines. Outlines derived from three photographs, taken in the same session, of each of five skulls are presented. Red, green and blue outlines show close congruence, indicating that the positioning of the camera relative to the skull had a minimal influence on the outline obtained. Note that Skull BMNH1899.7.8.5 shows only two outlines. Both parietal horns and face and muzzle outlines were assessed in the same way.*

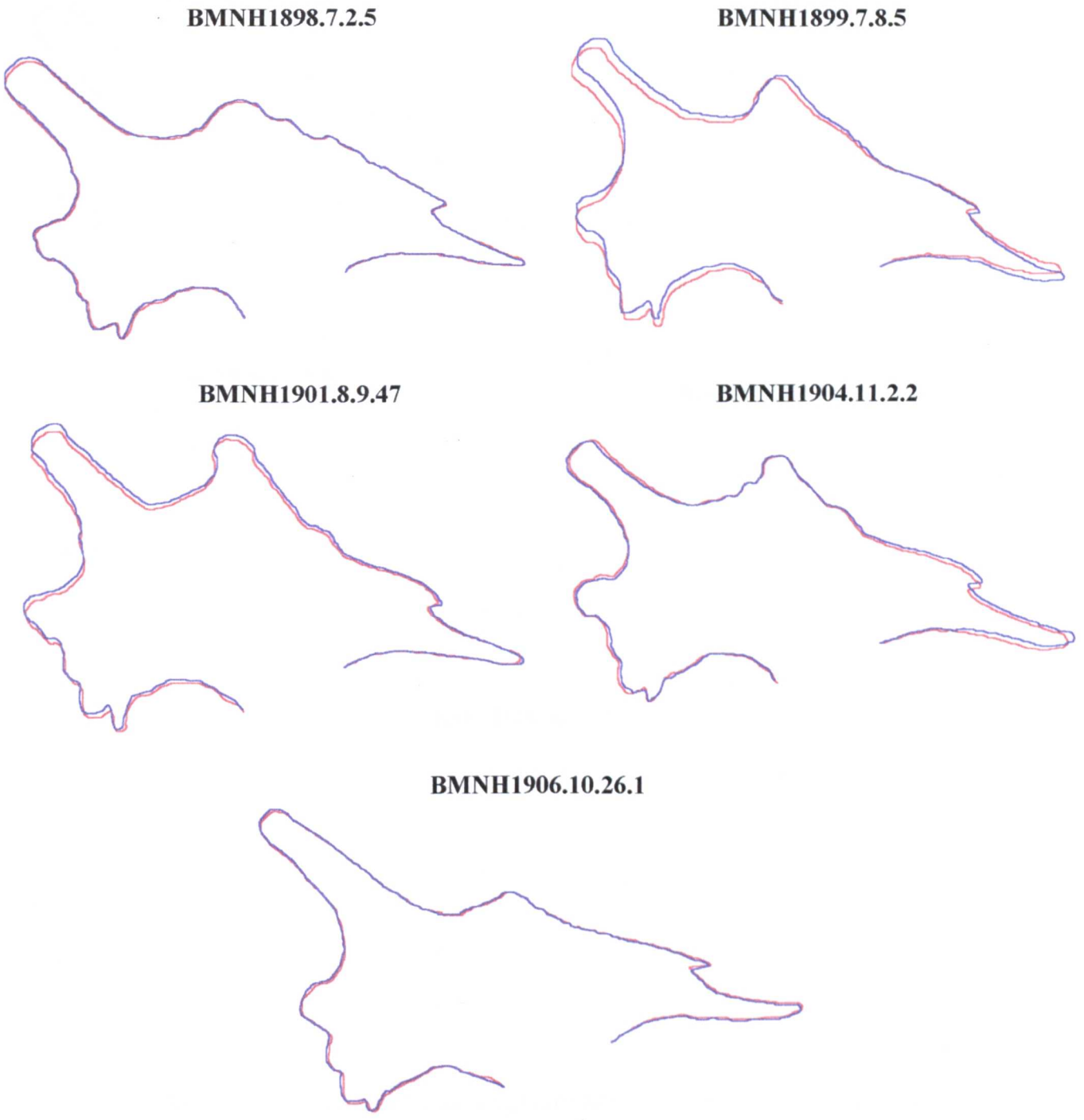


Figure 10.11: *Between sessions variation in outlines. Outlines derived from two photographs, taken in different sessions, of each of five skulls are presented. Red and blue outlines show close congruence, indicating that the positioning of the camera relative to the skull had a minimal influence on the outline obtained and was consistent over time. Skull BMNH1899.785 shows the greatest difference. This results from a slight angle to the camera. But shape is characterised independently of orientation. The shape is still fundamentally similar. Both parietal horn and face and muzzle outlines were assessed in the same way.*

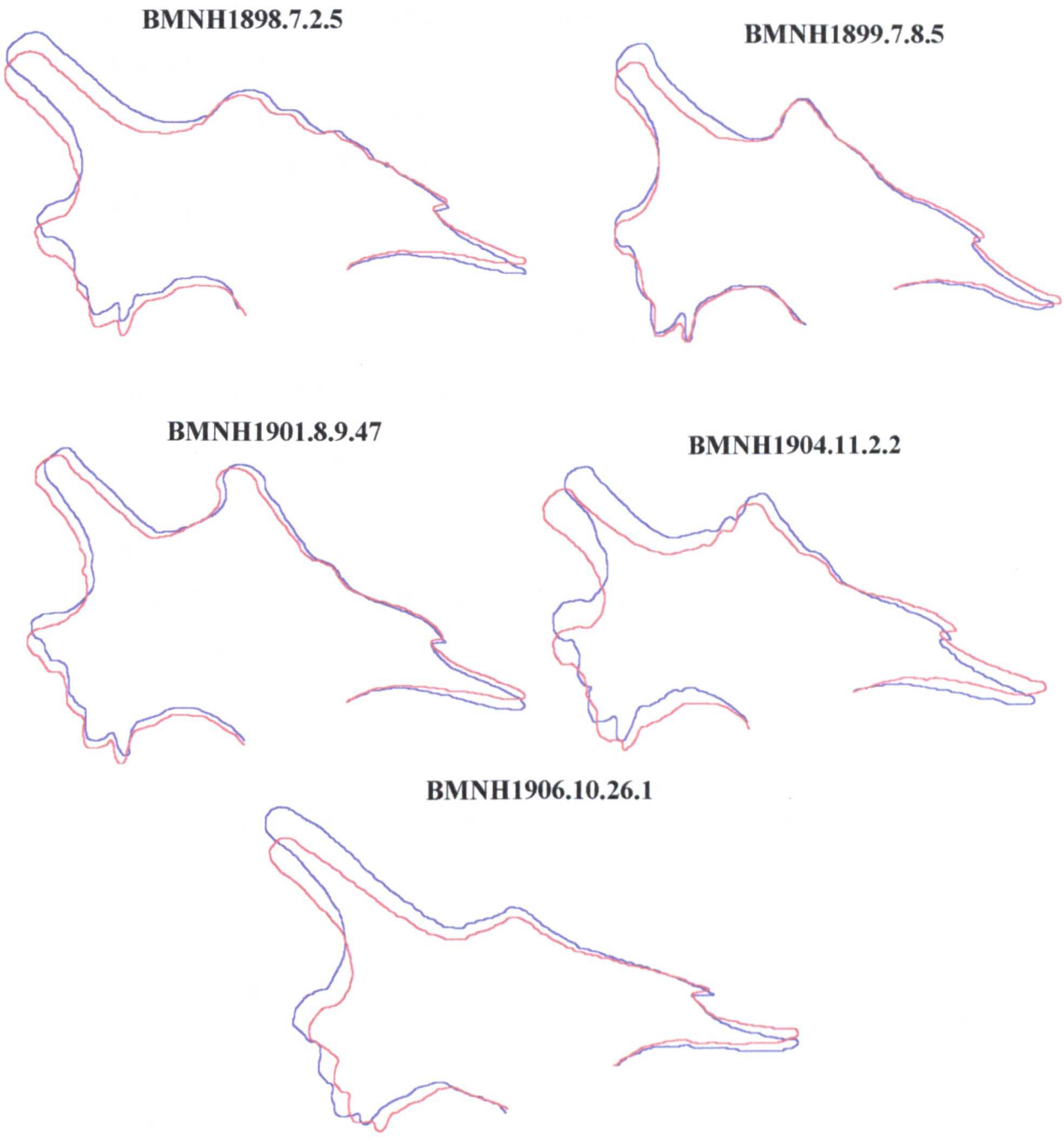


Figure 10.12: *Comparison of left and right profiles of five male skulls. Left (blue) and right (red) profiles are seen to be fundamentally similar. Rotational differences are due to the way that the skull rests on a flat surface. Shapes of the paired skull outlines closely correspond. Skull shape is characterised independently of orientation. Slight differences in the size of the parietal horns occur, particularly in BMNH1899.7.8.5 and BMNH1906.10.26.1. However, no systematic variation favouring either side was observed. For a few specimens where a right profile photograph was not available (due to damage to the right premaxillae or parietal horn) a reflected left profile photograph was included in the analysis to maximise the sample size (see text for full explanation).*

described by the first axis is typically general in nature and is spread throughout the outline. Each sequential axis indicates the residual, or latent, shape trend remaining once the foregoing shape trends have been accounted for. Each axis accounts for the greatest proportion of the remaining variation. Hence, the eigenvalues (which represent the proportion of total variance accounted for by that axis) reduce in magnitude with each sequential axis. The trends indicated by these higher level axes tend to be more localised in their variation.

Male Data

Skull Profile and Median Horn Profile

The modelled shapes for all male skulls show the major trends in shape variation (accounting for 98.5% of identified variation. Table 10.1) to be the flexion of the skull; that is the angle of the basicranial to basifacial planes (axis 1) and the conformation of the median horn (axis 2). The thickness of the parietal horns and the relative depth of the brain case also vary (axis 1). The modelled shapes for the first three axes are shown in Figure 10.13.

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	275.061	98.140	98.140
2	1.076	0.384	98.524
3	0.591	0.211	98.735
4	0.462	0.165	98.900
5	0.372	0.133	99.033

Table 10.1: *Eigenvalues for the first five eigenvector axes for all of the male skull profile outlines. The percentage of total variance and the cumulative variance are presented.*

Isolation of the median horn segment shows that the 'angle of approach', the height and form of the median horn and its position on the nasal bones account for much (99.3%) of the identified shape variation (See Table 10.2 and Figure 10.14).

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	142.898	97.391	97.391
2	1.947	1.327	98.718
3	0.866	0.590	99.308
4	0.193	0.131	99.440
5	0.181	0.123	99.563

Table 10.2: *Eigenvalues for the first five eigenvector axes for all of the male skull median horn profile outlines. The percentage of total variance and the cumulative variance are presented.*

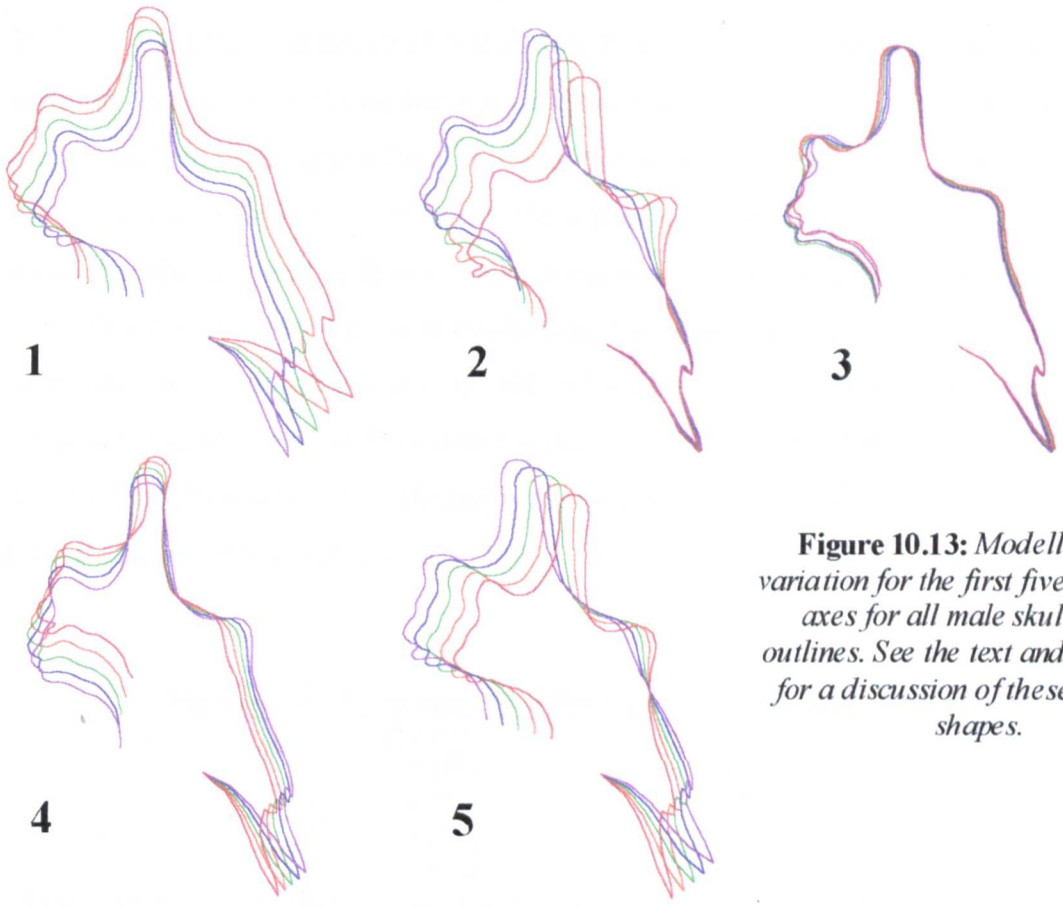


Figure 10.13: *Modelled shape variation for the first five eigenshape axes for all male skull profile outlines. See the text and Table 10.1 for a discussion of these modelled shapes.*

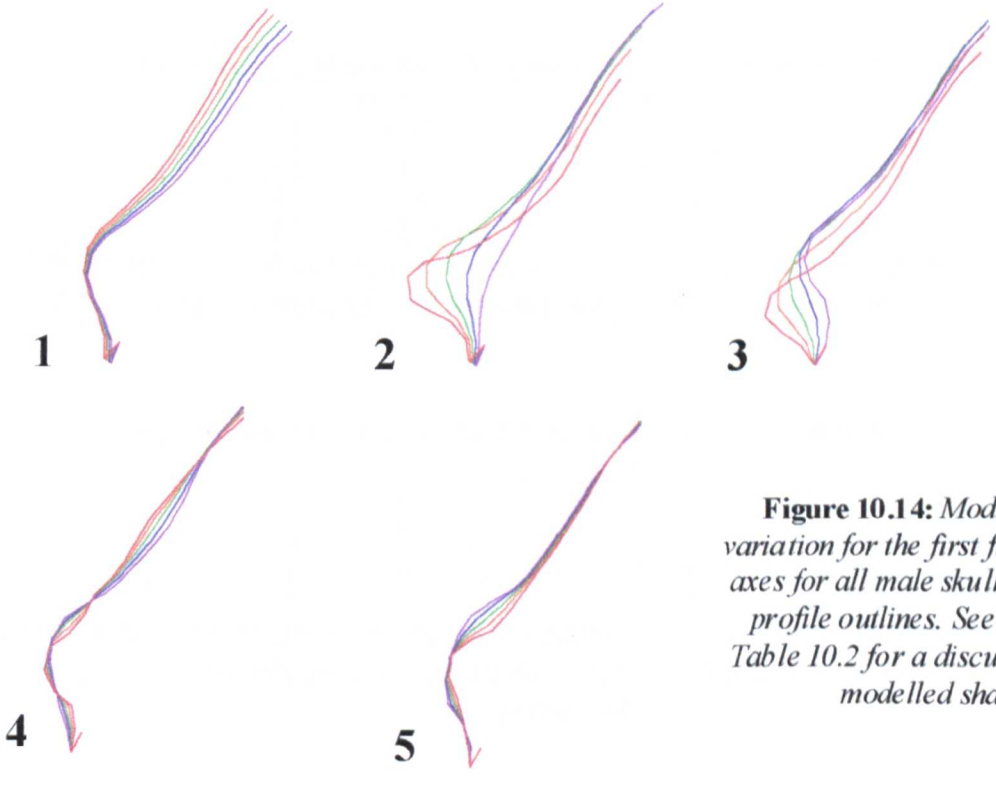


Figure 10.14: *Modelled shape variation for the first five eigenshape axes for all male skull median horn profile outlines. See the text and Table 10.2 for a discussion of these modelled shapes.*

The major trend (axis 1) in the two regional comparisons indicate similar shape variation as the general analysis with skull flexion and parietal horn thickness predominating (Tables 10.3 and 10.4 and Figures 10.15 and 10.16). The northern data analysis then indicates the position and height of the median horn and the relative length of the parietal horns (axis 2) and the curvature of the profile of the nasal bones (the position of the point of inflexion in the curvature. Axis 3) to be important shape trends. Isolation of the median horn profile show that the 'angle of approach' (axis 1) and the position (axis 2) and height of the horn (axis 3) account for 99.4 per cent of observed variation (Table 10.5 and Figure 10.17). Similarly, in the southern skulls the median horn also varies in position (axis 2) and height (axis 3) but does not show the extreme conical shape of the northern specimens (Figure 10.18). Variation in height in the southern specimens accounts for only 0.18 per cent of total shape variation (Table 10.6).

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	117.190	98.260	98.260
2	0.443	0.372	98.631
3	0.285	0.239	98.870
4	0.201	0.168	99.038
5	0.150	0.126	99.164

Table 10.3: *Eigenvalues for the first five eigenvector axes for northern male skull profile outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	155.010	98.554	98.554
2	0.447	0.284	98.838
3	0.427	0.271	99.109
4	0.180	0.114	99.224
5	0.166	0.106	99.329

Table 10.4: *Eigenvalues for the first five eigenvector axes for southern male skull profile outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	65.389	97.815	97.815
2	0.636	0.952	98.767
3	0.400	0.598	99.365
4	0.126	0.188	99.553
5	0.076	0.114	99.667

Table 10.5: *Eigenvalues for the first five eigenvector axes for northern male median horn profile outlines. The percentage of total variance and the cumulative variance are presented.*

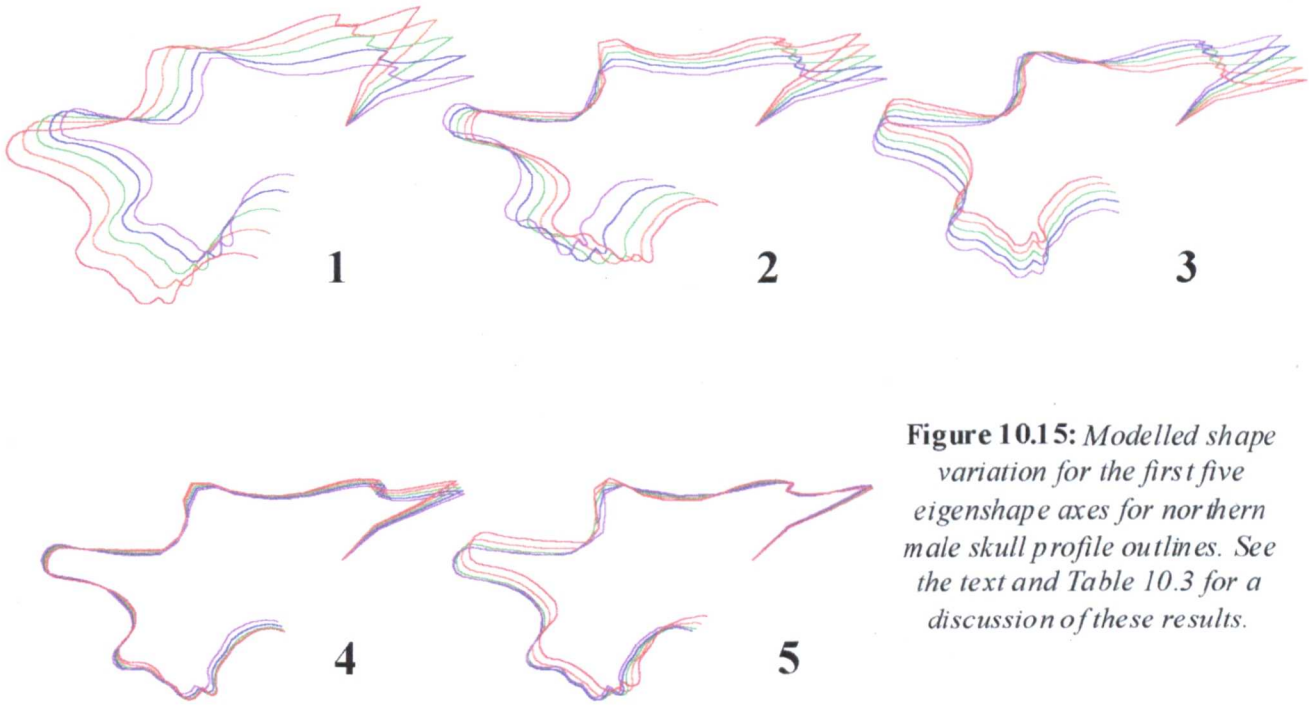


Figure 10.15: *Modelled shape variation for the first five eigenshape axes for northern male skull profile outlines. See the text and Table 10.3 for a discussion of these results.*

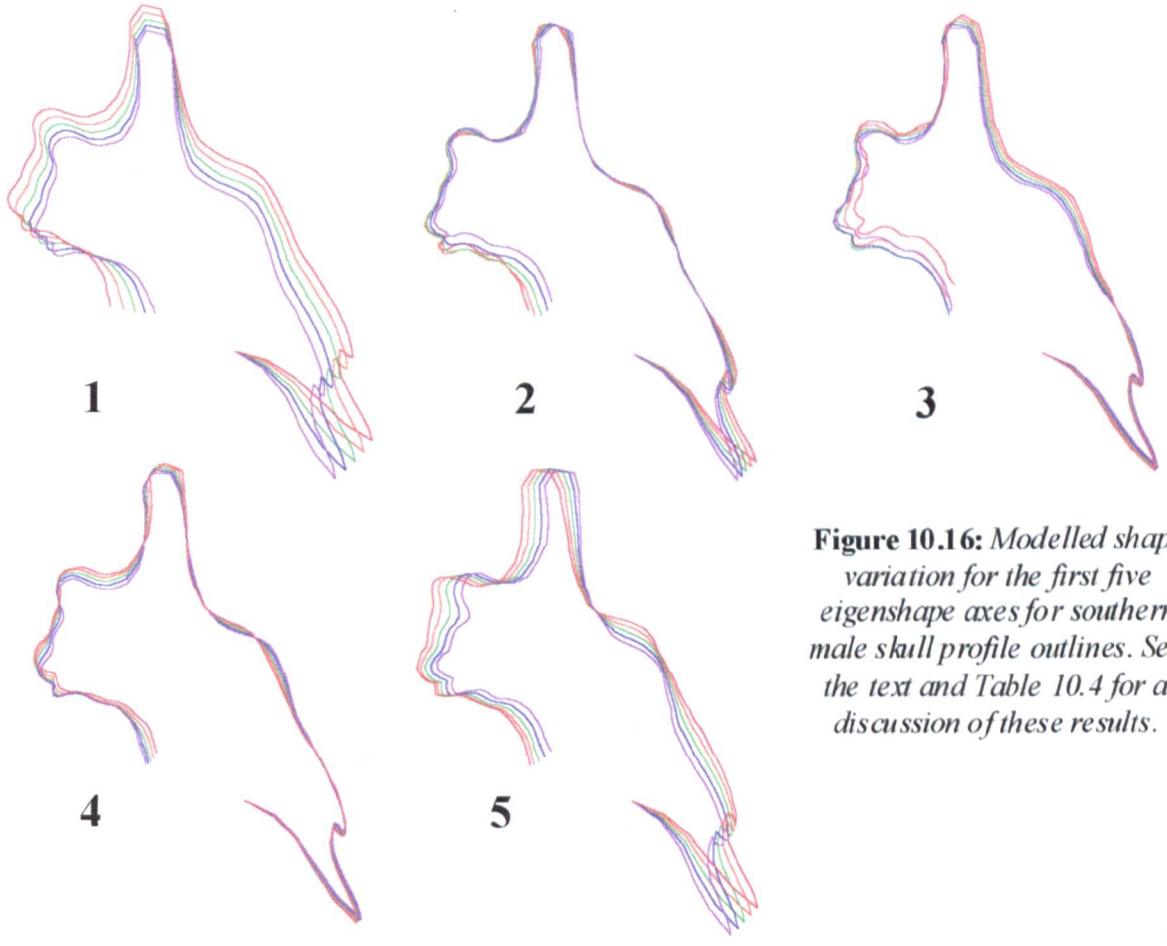


Figure 10.16: *Modelled shape variation for the first five eigenshape axes for southern male skull profile outlines. See the text and Table 10.4 for a discussion of these results.*

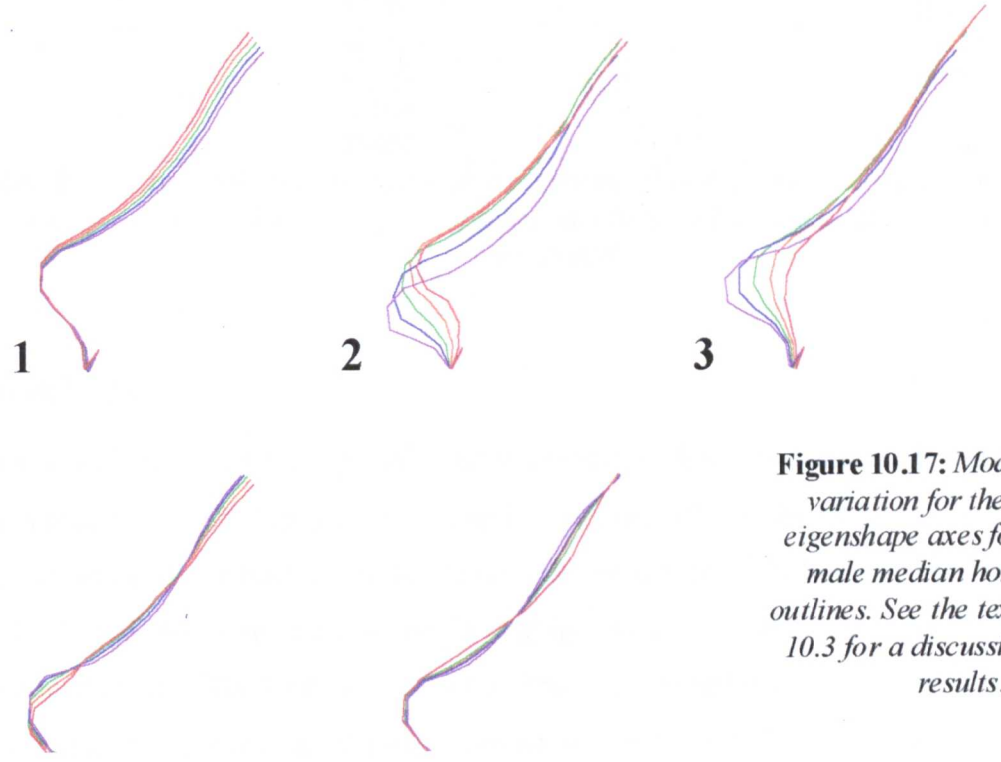


Figure 10.17: *Modelled shape variation for the first five eigenshape axes for northern male median horn profile outlines. See the text and Table 10.3 for a discussion of these results.*

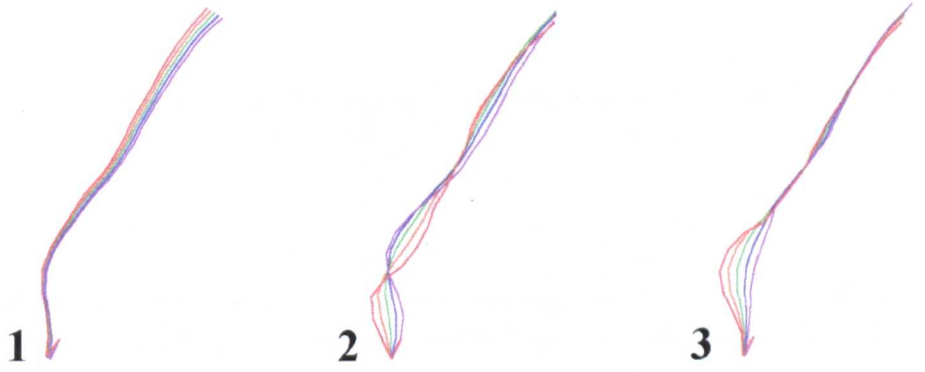
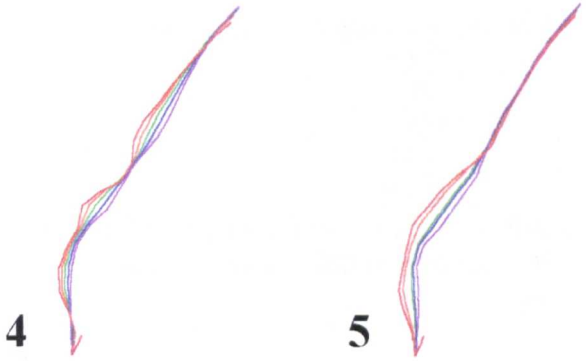


Figure 10.18: *Modelled shape variation for the first five eigenshape axes for southern male median horn profile outlines. See the text and Table 10.6 for a discussion of these results.*



Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	70.279	98.813	98.813
2	0.283	0.397	99.210
3	0.125	0.176	99.385
4	0.104	0.146	99.531
5	0.082	0.115	99.646

Table 10.6: *Eigenvalues for the first five eigenvector axes for southern male median horn profile outlines. The percentage of total variance and the cumulative variance are presented.*

Parietal Horns

The northern and southern regional and the combined data sets demonstrate similar shape trends (Figure 10.19). The basal width and divergence of the horns accounts for the major shape trend (axis 1 in each case), while the relative length of the horns and the breadth of the 'U-shaped' segment between the parietal horns vary on the second eigenshape axis. The constriction of the horn (to produce a 'knob' at the tip) is also represented in the second axis. The percentage of total variation accounted for by each axis for each analysis is given in Tables 10.7, 10.8 and 10.9 for all specimens, northern and southern analyses respectively.

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	144.261	98.613	98.613
2	0.752	0.514	99.127
3	0.224	0.153	99.280
4	0.186	0.127	99.407
5	0.168	0.115	99.522

Table 10.7: *Eigenvalues for the first five eigenvector axes for all male specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	58.847	97.742	97.742
2	0.380	0.632	98.374
3	0.184	0.306	98.680
4	0.097	0.161	98.841
5	0.091	0.151	98.992

Table 10.8: *Eigenvalues for the first five eigenvector axes for northern male specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

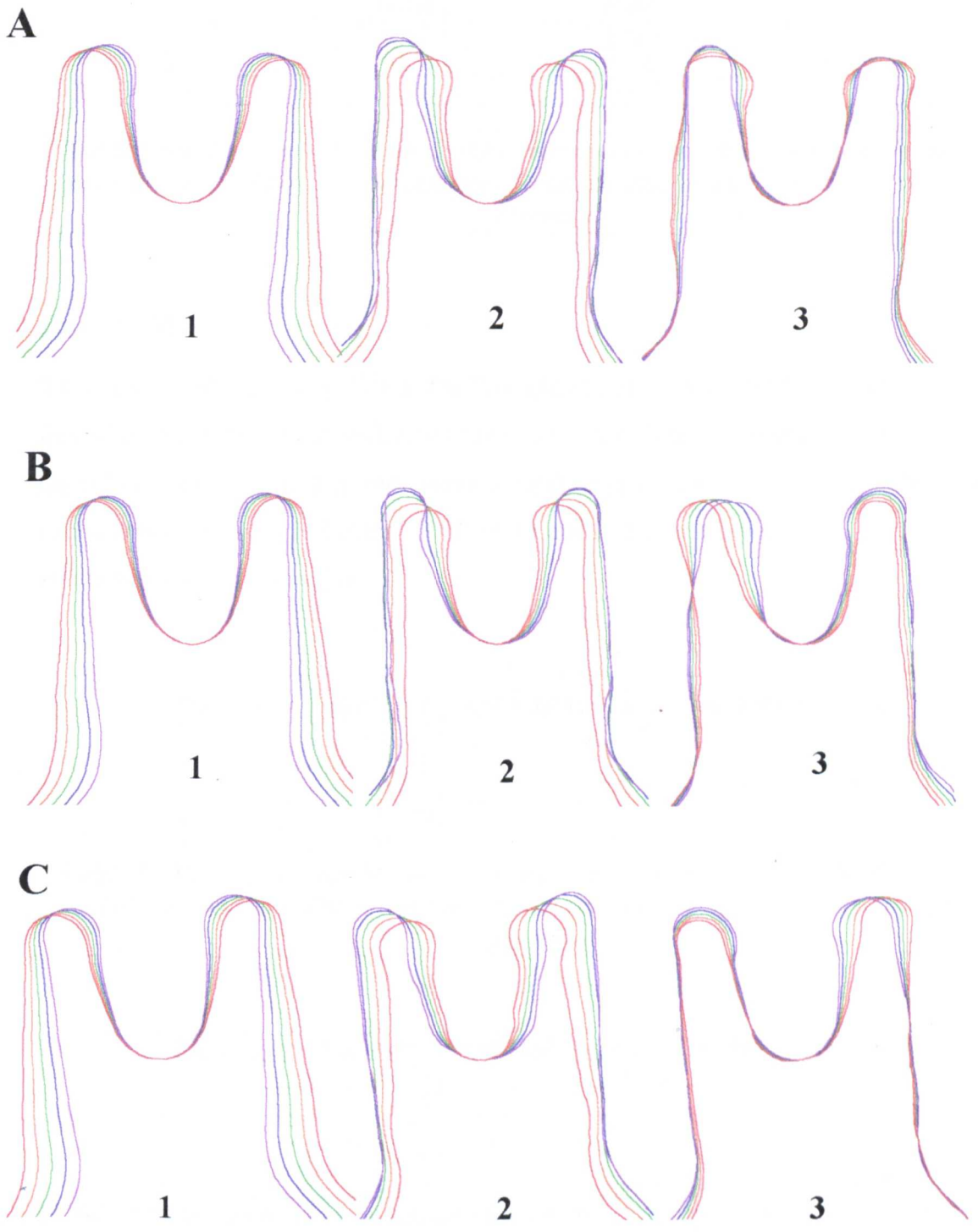


Figure 10.19: *Modelled shape variation for the first three eigenshape axes for all male parietal horn outlines (A), for northern male parietal horn outlines (B) and for southern male parietal horn outlines (C). The three data sets indicate similar shape trends. See the text for discussion.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	144.658	97.891	97.891
2	0.827	0.560	98.451
3	0.287	0.195	98.646
4	0.214	0.145	98.790
5	0.199	0.135	98.925

Table 10.9: *Eigenvalues for the first five eigenvector axes for southern male specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

Face and Muzzle

The majority of shape variation in the face and muzzle profile for all three data sets describes the width of the skull (axis 1) and how rapidly the shape tapers in to the muzzle (axis 2) (Figure 10.20). The percentage of total variation accounted for by each axis for each analysis is given in Tables 10.10, 10.11 and 10.12 for all specimens, northern and southern analyses respectively.

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	99.677	99.357	99.357
2	0.110	0.112	99.469
3	0.073	0.074	99.543
4	0.040	0.041	99.584
5	0.036	0.036	99.620

Table 10.10: *Eigenvalues for the first five eigenvector axes for all male specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	36.790	99.536	99.536
2	0.045	0.122	99.659
3	0.023	0.063	99.722
4	0.016	0.044	99.766
5	0.015	0.040	99.805

Table 10.11: *Eigenvalues for the first five eigenvector axes for northern male specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	56.199	99.348	99.348
2	0.076	0.134	99.482
3	0.038	0.067	99.549
4	0.030	0.054	99.603
5	0.024	0.043	99.646

Table 10.12: *Eigenvalues for the first five eigenvector axes for southern male specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

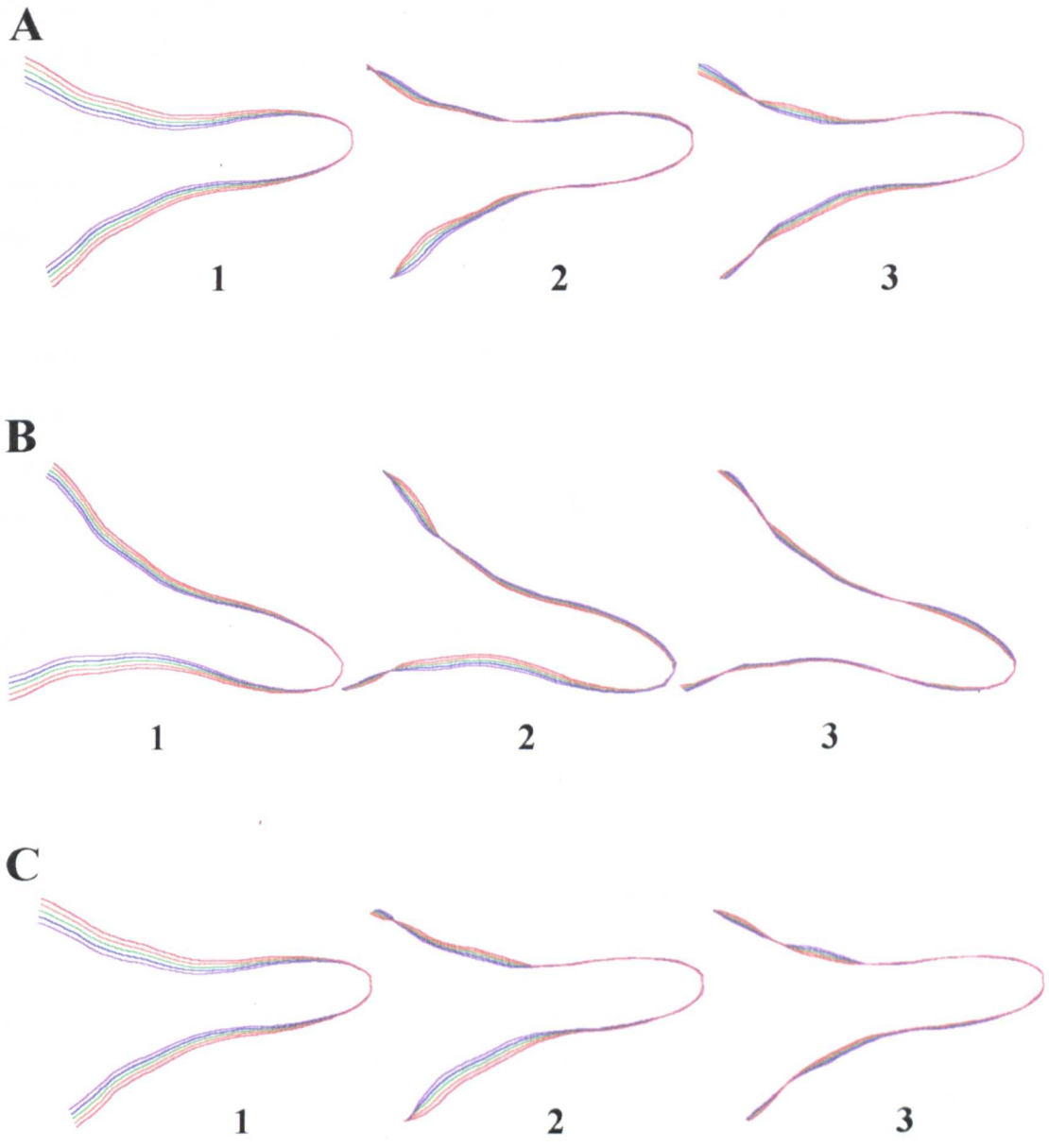


Figure 10.20: *Modelled shape variation for the first three eigenshape axes for all male face and muzzle outlines (A), for northern male face and muzzle outlines (B) and for southern male face and muzzle outlines (C). The three data sets indicate similar shape trends. See the text for discussion.*

Female Data

Skull Profile

The major shape trends identified in the first axis largely reflect those shown by the male skulls. Skull flexion dominates each model with some variation in the width of the parietal horns and the depth of the brain case (Figure 10.21).

For all data the second shape trend largely reflects variation in the parietal horn. The position, thickness (axis 2), angle (axis 4) and the steepness of the approach to this horn (axis 3) varies (Figure 10.21). The second axis also shows some variation in the angle of the premaxillae.

The regional models are also dominated by skull flexion. The northern data show differences in the depth of the brain case and the position of the parietal horns (axis 2) and in the angle and length of the parietal horns (axis 3) (Figure 10.22). Beside skull flexion the angle of the parietal horns (axis 2) varies in the southern data set (Figure 10.23).

The percentage of total variation accounted for by each axis for each analysis is given in Tables 10.13, 10.14 and 10.15 for all specimens, northern and southern analyses respectively.

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	109.523	93.008	93.008
2	5.898	5.009	98.017
3	1.280	1.087	99.104
4	0.280	0.238	99.342
5	0.193	0.164	99.506

Table 10.13: *Eigenvalues for the first five eigenvector axes for all female specimens skull profiles. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	68.632	98.607	98.607
2	0.308	0.443	99.049
3	0.184	0.265	99.314
4	0.124	0.178	99.492
5	0.110	0.157	99.649

Table 10.14: *Eigenvalues for the first five eigenvector axes for northern female specimens skull profiles. The percentage of total variance and the cumulative variance are presented.*

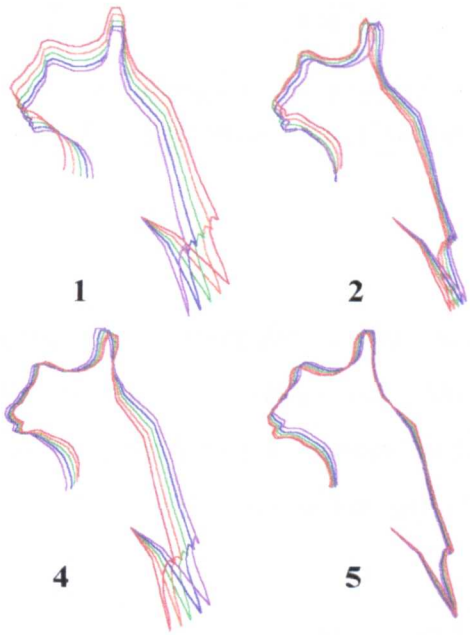


Figure 10.21: *Modelled shape variation for the first five eigenshape axes for all female skull profile outlines. See text for discussion.*

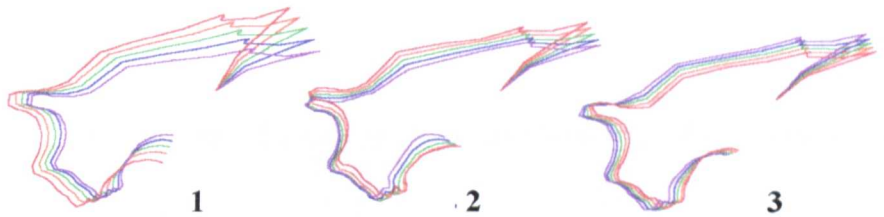


Figure 10.22: *Modelled shape variation for the first three eigenshape axes for northern female skull profile outlines. See text for discussion.*

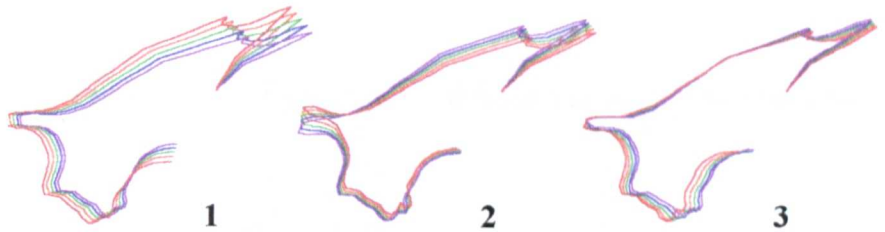


Figure 10.23: *Modelled shape variation for the first three eigenshape axes for southern female skull profile outlines. See text for discussion.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	46.400	99.199	99.199
2	0.114	0.244	99.443
3	0.079	0.170	99.612
4	0.048	0.103	99.715
5	0.042	0.089	99.805

Table 10.15: *Eigenvalues for the first five eigenvector axes for southern female specimens skull profiles. The percentage of total variance and the cumulative variance are presented.*

Parietal Horns

The shape trends in all three data sets are largely concordant. The width of the skull at the orbital rim (where outlines are bounded. Axis 1), the relative length of the parietal horns and the breadth of the U-shape between the horns (axis 2) and the internal curvature of the horns (axis 3) account for over 99 per cent of the identified variation in each case (Figure 10.24).

The percentage of total variation accounted for by each axis for each analysis is given in Tables 10.16, 10.17 and 10.18 for all specimens, northern and southern analyses respectively.

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	55.380	97.427	97.427
2	0.807	1.420	98.846
3	0.139	0.244	99.091
4	0.115	0.202	99.293
5	0.084	0.147	99.440

Table 10.16 *Eigenvalues for the first five eigenvector axes for all female specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	32.803	97.490	97.490
2	0.483	1.437	98.926
3	0.101	0.301	99.227
4	0.068	0.201	99.428
5	0.063	0.186	99.615

Table 10.17 *Eigenvalues for the first five eigenvector axes for northern female specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

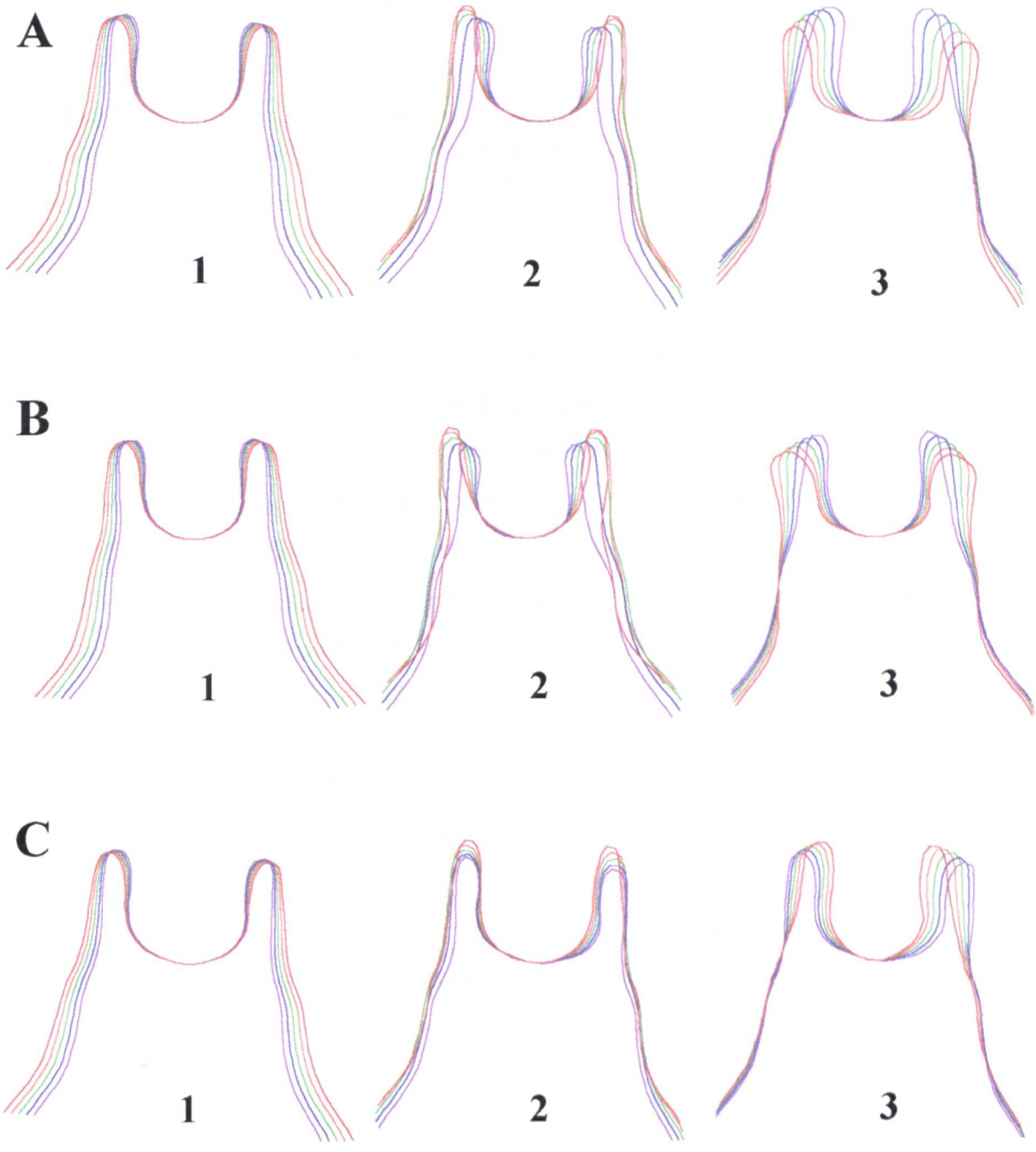


Figure 10.24: *Modelled shape variation for the first three eigenshape axes for all female parietal horn outlines (A), for northern female parietal horn outlines (B) and for southern female parietal horn outlines (C). The three data sets indicate similar shape trends. See the text for discussion.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	21.332	98.508	98.508
2	0.141	0.650	99.158
3	0.045	0.208	99.366
4	0.040	0.187	99.553
5	0.031	0.145	99.698

Table 10.18 *Eigenvalues for the first five eigenvector axes for southern female specimens parietal horn outlines. The percentage of total variance and the cumulative variance are presented.*

Face and Muzzle

The major shape trend in the face and muzzle, for all, northern and southern female specimen data sets, is the width of the skull reflecting the commonality with the parietal horn data set of the start and end points of the outlines (Figure 10.25). In each case the first axis accounts for nearly, or more than, 99.5 per cent of modelled shape variation (Tables 10.19, 10.20 and 10.21 give the percentage of total variance values for all, northern and southern female data sets respectively). Variation in subsequent axes represents the concavity of the facial profile as it narrows into the muzzle (Figure 10.25).

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	39.591	99.422	99.442
2	0.053	0.133	99.575
3	0.031	0.077	99.652
4	0.022	0.055	99.707
5	0.018	0.046	99.753

Table 10.19: *Eigenvalues for the first five eigenvector axes for all female specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	19.880	99.544	99.544
2	0.030	0.151	99.695
3	0.019	0.096	99.792
4	0.009	0.045	99.837
5	0.008	0.040	99.876

Table 10.20: *Eigenvalues for the first five eigenvector axes for northern female specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

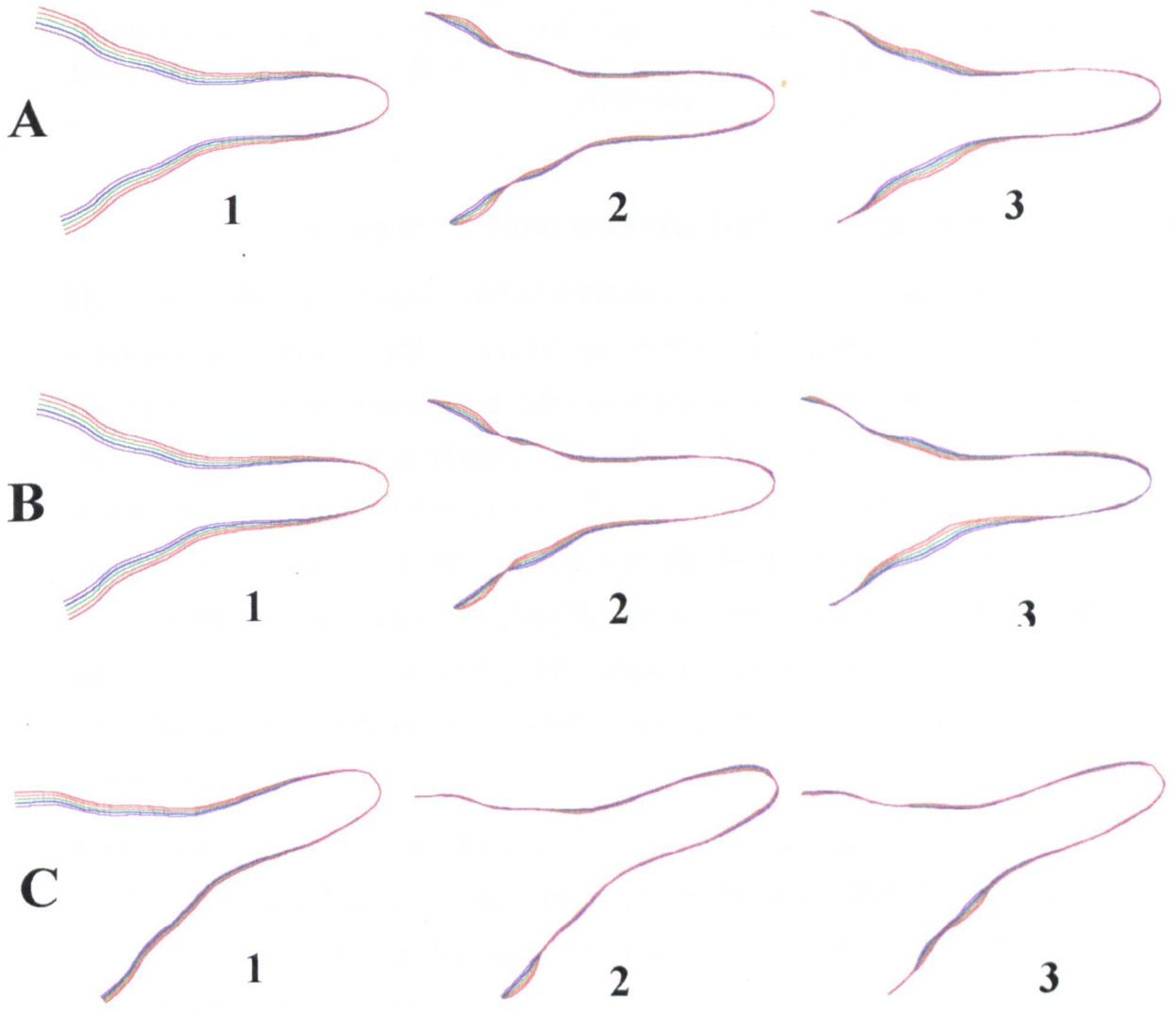


Figure 10.25: *Modelled shape variation for the first three eigenshape axes for all female face and muzzle outlines (A), for northern female face and muzzle outlines (B) and for southern female face and muzzle outlines (C). The three data sets indicate similar shape trends. See the text for discussion.*

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	15.202	99.553	99.533
2	0.022	0.141	99.694
3	0.012	0.079	99.773
4	0.009	0.058	99.831
5	0.008	0.054	99.885

Table 10.21: *Eigenvalues for the first five eigenvector axes for southern female specimens face and muzzle outlines. The percentage of total variance and the cumulative variance are presented.*

Geographic Structure to Giraffe Skull Shape Variation

Hypotheses relating the identified shape trends to geographic structure were tested using eigenscore covariation coefficients and *a priori* defined geographic groups (see Chapter 6). Null hypotheses assuming no geographic structure to the identified shape trends were tested using non-parametric Mann-Whitney U-tests for paired comparisons and Kruskall-Wallis tests for multigroup comparisons. Non-parametric tests were used due to the small sample sizes in some of the geographic groups. Parallel parametric tests were performed on groups with sufficiently large samples (Student's t-tests for paired comparisons and one-way analysis of variance for multigroup comparisons) and gave equivalent results in all cases. Results of the non-parametric tests for all comparisons are given for consistency (Table 10.22).

Pairs of the eigenscores were plotted as two-dimensional Cartesian plots and examined for the first five latent shape trends resulting from each analysis. Where rejection of the null hypotheses implied statistically significant geographic structure to the eigenscores the relevant plots were examined

Although differing, hierarchically structured, shape trends were described for each analysis, statistical analysis and examination of the eigenscore covariance plots suggests that most of the shape trends demonstrate variation that is dispersed across individuals and not geographically structured. The following section highlights only those relationships, in both the between and within regional analyses, that show some geographic structure.

Male Skull Profile Data

Between Regions Variation

Statistically significant geographical structuring was evident in ES2 and ES5 covariation coefficients (Figure 10.26). Examination of the graphs show that male skull profiles were strongly structured according to axis 2, relating to the development of the median horn,

Comparison	Eigenshape	Test	Statistic	df	p
Male All Data – Complete Skull Profile					
North v South	ES2	Mann-Whitney	52.500	1	<0.001
North v South	ES5	Mann-Whitney	272.500	1	0.002
Male Northern Region – Complete Skull Profile					
Eastern Groups	ES2	Kruskal-Wallis	13.838	4	0.008
Male, All Data – Median Horn Profile					
North v South	ES1	Mann-Whitney	1010.00	1	<0.001
North v South	ES2	Mann-Whitney	26.500	1	<0.001
Male Northern Region – Median Horn Profile					
Northern Groups	ES1	Kruskal-Wallis	17.821	6	0.007
Northern Groups	ES3	Kruskal-Wallis	19.257	6	0.004
Northern Groups	ES4	Kruskal-Wallis	13.350	6	0.038
Eastern Groups	ES1	Kruskal-Wallis	14.732	4	0.005
Eastern Groups	ES3	Kruskal-Wallis	15.479	4	0.004
Eastern Groups	ES4	Kruskal-Wallis	10.318	4	0.035
Male Southern Region – Median Horn Profile					
Southern Groups	ES3	Kruskal-Wallis	11.092	4	0.026
Male Northern Region – Parietal Horns Outline					
Northern Groups	ES5	Kruskal-Wallis	13.048	6	0.042
Eastern Groups	ES5	Kruskal-Wallis	9.807	4	0.044
Male Southern Region – Parietal Horns Outline					
Southern Groups	ES1	Kruskal-Wallis	16.058	4	0.003
Southern Groups	ES2	Kruskal-Wallis	14.170	4	0.007
Male All Date – Face and Muzzle Outline					
North v South	ES3	Mann-Whitney	638.000	1	0.001
Male Northern Region – Face and Muzzle Outlines					
East v West	ES1	Mann-Whitney	8.000	1	0.040
Northern Groups	ES1	Kruskal-Wallis	15.759	6	0.015
Northern Groups	ES3	Kruskal-Wallis	16.838	6	0.010
Northern Groups	ES5	Kruskal-Wallis	13.660	6	0.034
Eastern Groups	ES1	Kruskal-Wallis	12.443	4	0.014
Eastern Groups	ES3	Kruskal_Wallis	13.378	4	0.010
Eastern Groups	ES5	Kruskal-Wallis	12.633	4	0.013
Male Southern Region – Face and Muzzle Outlines					
Southern Groups	ES3	Kruskal-Wallis	11.317	4	0.023
Female Northern Region – Complete Skull Profile					
East v West	ES2	Mann-Whitney	26.000	1	0.027
Female All Data – Parietal Horns Outline					
North v South	ES2	Mann-Whitney	257.000	1	0.001
North v South	ES4	Mann-Whitney	92.000	1	0.044
Female All Data – Face and Muzzle Outlines					
North v South	ES3	Mann-Whitney	94.00	1	0.025
North v South	ES4	Mann-Whitney	20.500	1	0.009

Table 10.22: *Summary of statistical tests of geographical structure to giraffe skull shape variation. Non-parametric tests (Mann-Whitney U-test for paired comparisons; Kruskal-Wallis test for multigroup comparisons) were used to test between group eigenscores (covariance coefficients with sequential eigenshape axes). Only statistically significant test results are shown. See the text for further discussion.*

with northern (including eastern and western regions) and southern regions generally separated, with some overlap (Figure 10.13). Separation on axis 5 was less clear with a tendency to separate but extensive mixing. Interpretation of axis 5 largely reflects residual variation in the median horn (Figure 10.13).

A separate analysis isolating the median horn shows very strong separation with both eigenshapes 1 and 2 (Figure 10.27). These shape trends relate to the curvature of the nasal region approaching the median horn and to the height of the median horn respectively (Figure 10.14). The position of the median horn (accounted for by eigenshape 3) showed no significant geographic structure (Figure10.14).

Within Regions Variation – North

Significant statistical comparisons indicate a trend with the second eigenshape separating western specimens (including geographical groups WCP, WSN, ECU) from eastern groups (including EEK, ENA, ESK and EST) (Figure 10.28). There is some overlap between these groupings. The development of the median horn is modelled on this second axis (Figure 10.15). The separate analysis of median horn profiles suggests more strongly that the southern Kenyan and Tanzanian specimens (ESK and EST) tend to separate from the other specimens specifically according to the median horn profile (axes 1 and 3. Figure 10.29 and 10.17).

Within Regions Variation – South

None of the eigenscores showed statistically significant geographic structure with grouped specimens generally mixed in the eigenshape plots (Figure 10.30). There is a suggestion that the specimens of the Luangwa Valley, Zambia population (SZT) tend to group together on the second axis, but these are still mixed up with specimens from other localities.

The separate analysis of the median horn profile segment again indicates that there may be a tendency for the SZT specimens to group together on the second axis (representing the position of the peak of the horn). However, the null hypothesis of no geographic structuring was not rejected for this axis. The third axis, showing covariation with the height of the median horn, does show statistically significant structure within the southern region (Table 10.7). The SCZ population tends to show consistently positive values. However, these were well intermixed with SWC specimens (Figure 10.31).

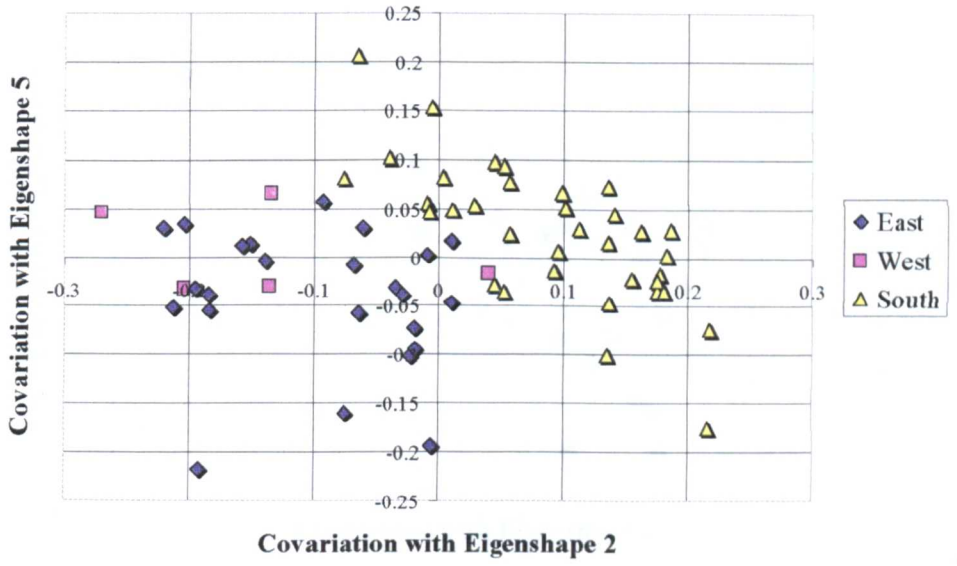


Figure 10.26: *Statistically significant geographic structure in eigenshape covariance values between regions for the complete male skull profile dataset. Significant structuring between regions was demonstrated for eigenshapes 2 and 5 (Table 10.22). See the text for further discussion.*

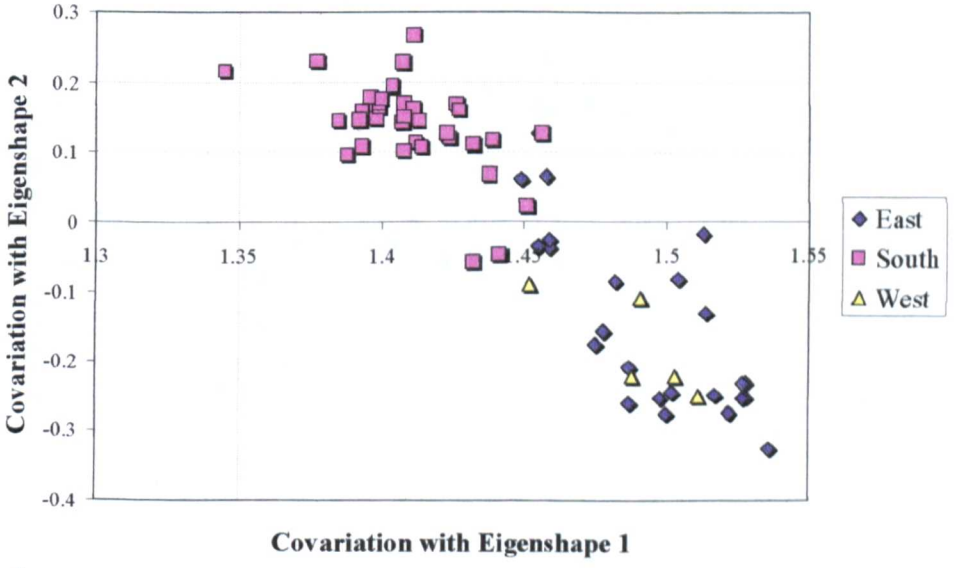


Figure 10.27: *Statistically significant geographic structure in eigenshape covariance values between regions for the male median horn profile dataset. Significant structuring between regions was demonstrated for eigenshapes 1 and 2 (Table 10.22). See the text for further discussion.*

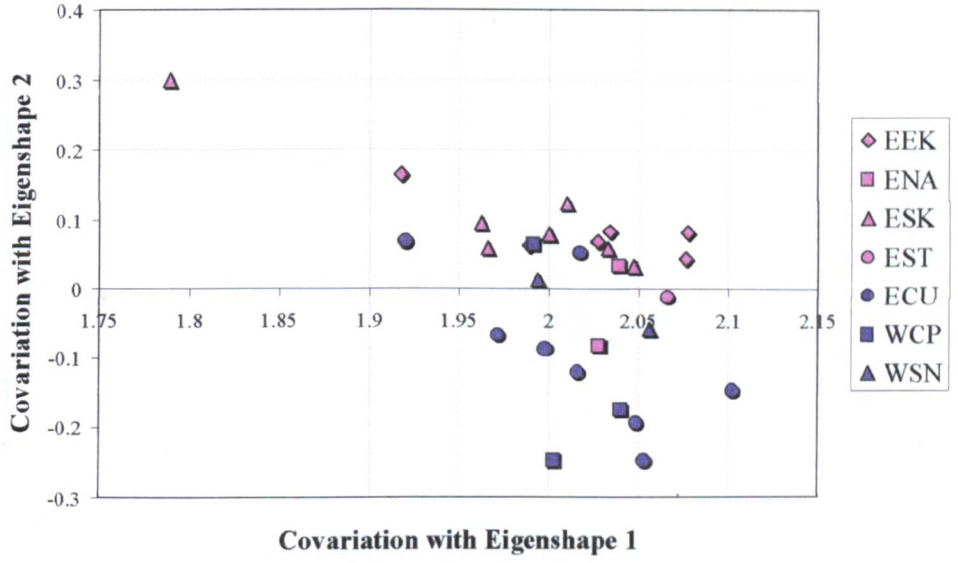


Figure 10.28: *Statistically significant geographic structure in eigenshape covariance values between groups for the northern region, male complete skull profile dataset. Significant structuring between groups was demonstrated for eigenshape 2 (Table 10.22). See the text for further discussion.*

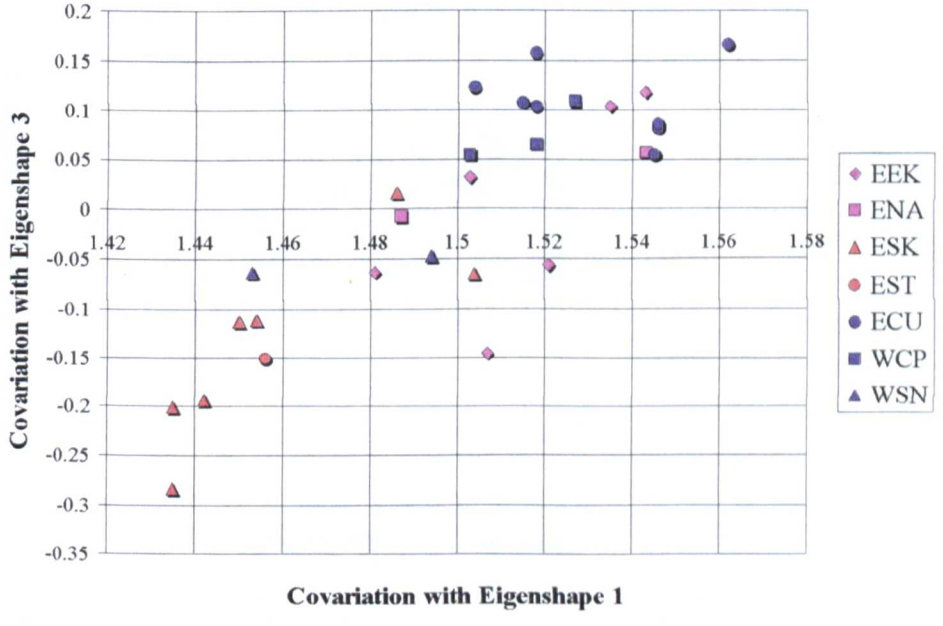


Figure 10.29: *Statistically significant geographic structure in eigenshape covariance values between groups for the northern region, male median horn profile dataset. Significant structuring between groups was demonstrated for eigenshapes 1 and 3 (Table 10.22). Southern Kenyan (ESK) and Tanzanian (EST) specimens are indicated as being different. See the text for further discussion.*

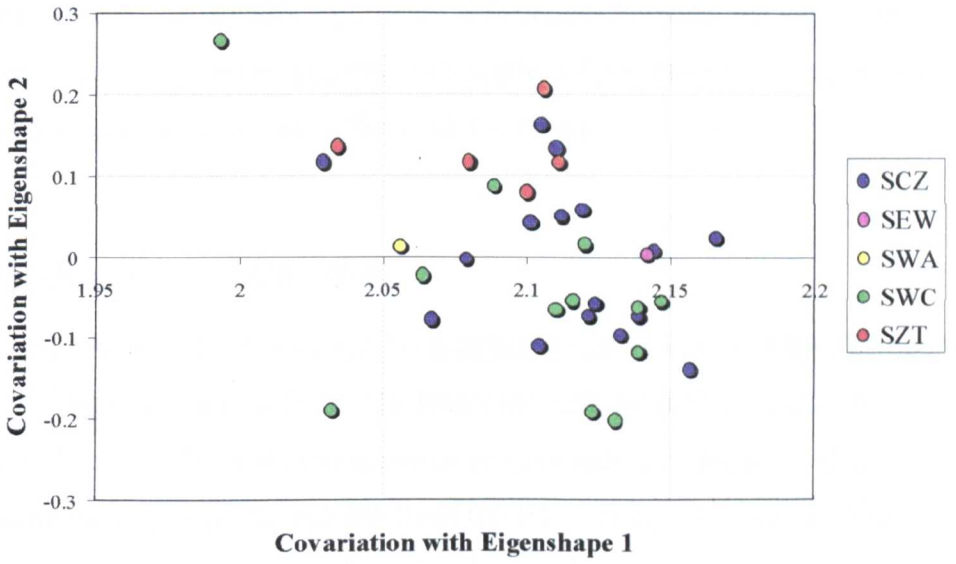


Figure 10.30: *Statistically significant geographic structure in eigenshape covariance values between groups for the southern region, male complete skull profile dataset. Significant structuring between groups was not demonstrated for any eigenshape axis (Table 10.22). However, there may be a trend for Zambian (SZT) populations to group together on the second eigenshape axis. See the text for further discussion.*

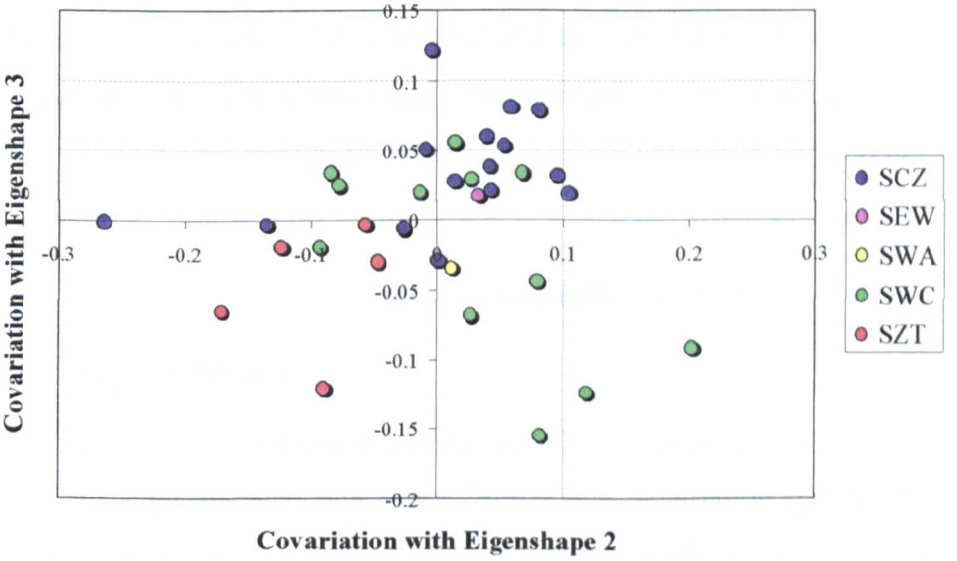


Figure 10.31: *Statistically significant geographic structure in eigenshape covariance values between groups for the southern region, male median horn profile dataset. Significant structuring between groups was demonstrated for eigenshape 3 (Table 10.22). However, there may also be a trend for Zambian (SZT) populations to group together on the second eigenshape axis, although this was not statistically significant. See the text for further discussion.*

Male Parietal Horn Data

Between Regions Variation

There were no statistically significant differences between the eigenshape scores of northern and southern specimens. The scatter of points on the eigenshape score plots show extensive overlap on all axes (Appendix 4.10.4).

Within Regions Variation – North

The only statistically significant geographic structure is shown by eigenscores relating to the fifth eigenshape (Table 10.22). However, this eigenshape accounts for a small amount of variation (0.15%) and demonstrates an asymmetry in the horns (Figure 10.32). Perhaps, by coincidence, three individuals from the ECU group show similar shapes for this asymmetry (Figure 10.32) and so give the results seen.

Within Regions Variation – South

Within the southern region SCZ and SZT specimens are almost completely separated according to their covariation with both eigenshapes 1 and 2 (Figure 10.33) suggesting that the divergence (tip width relative to base width), external width (absolute tip width) and the length of the parietal horns differ between these groups. Specimens from SWC overlap extensively with both groups, though tend to group more with the SCZ individuals.

Male Face and Muzzle Data

Between Regions Variation

Covariation with the third eigenshape is significantly geographically structured (Figure 10.34). This eigenshape accounts for 0.74 per cent of variation and indicates how rapidly the skull narrows from the orbits to the muzzle region (Figure 10.20A). Inspection of the plots shows that there is extensive overlap between the groups.

Within Regions Variation – North

Eastern and western regions are statistically significantly different according to the width of the skull (as demonstrated by eigenshape 1. Figure 10.20B). Comparisons between all northern groups and just the eastern groups additionally show statistically significant structure in eigenshape 3 and 5. Inspection of the plots (Figures 10.35A and B) shows that,

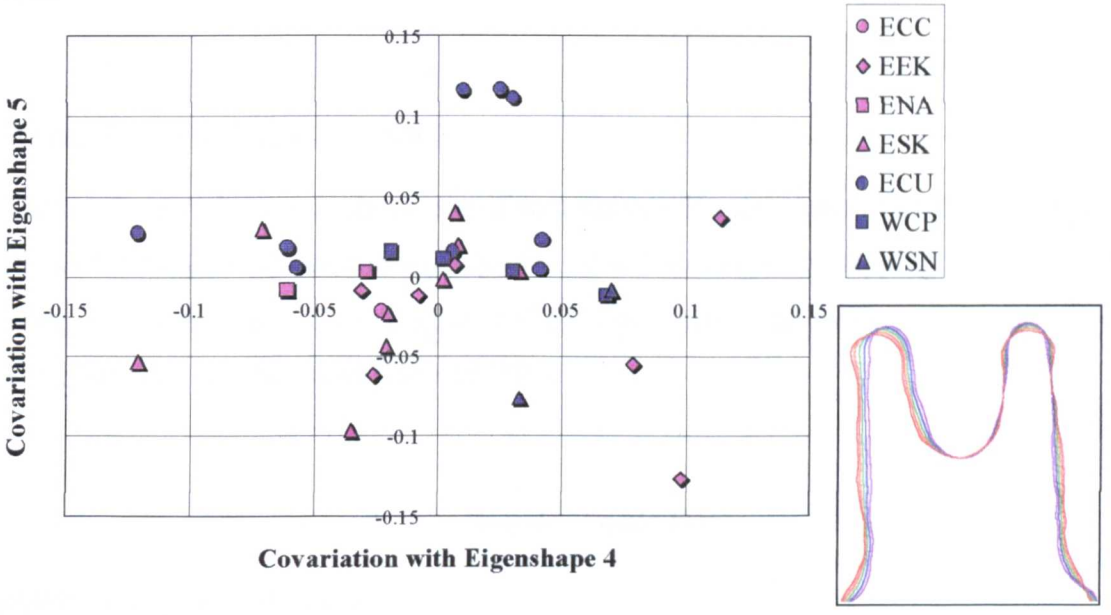


Figure 10.32: *Statistically significant geographic structure in eigenshape covariance values between region for the northern region, male parietal horn outline dataset. Significant structuring between regions was demonstrated for eigenshape 5 (Table 10.22). This axis indicates a slight assymetry between the horns (*Inset*) and may be due to a coincidental individual similarity between three of the ECU individuals.*

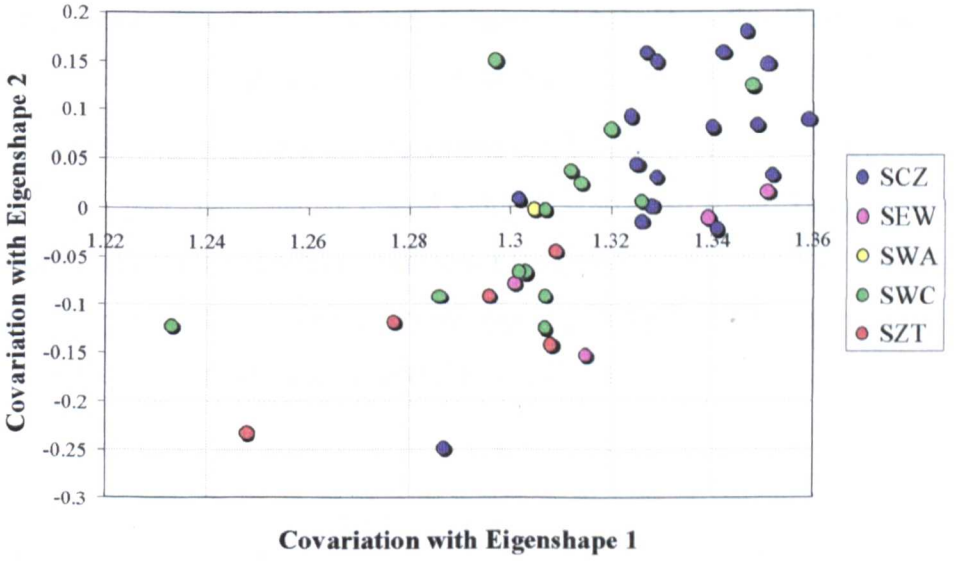


Figure 10.33: *Statistically significant geographic structure in eigenshape covariance values between region for the southern region, male parietal horn outline dataset. Significant structuring between regions was demonstrated for eigenshape1 and 2 (Table 10.22). SCZ and SZT specimens separate almost completely on both axes reflecting differences in divergence, tip width and length (Figure 10.19C). SCZ specimens overlap extensively with both of these groups. See the text for further discussion.*

in all cases, overlap is extensive. The observed effect may be due to the relatively low sample size.

Within Regions Variation – South

The third eigenshape axis shows significant geographic structuring (Table 10.22). The plot shows the SZT group has consistently low values, although overlapping with SCZ and, more extensively, with SWC (Figure 10.36). The shape variation demonstrated by this axis is problematic to describe (Figure 10.20C).

Female Profile Data

Between Regions Variation

No statistically significant geographic variation was found between regional eigenshape scores.

Within Region Variation – North

The eigenshape scores of the second eigenshape show a statistically significant association with the provenance of the specimen (Figure 10.37). This eigenshape relates to the depth of the brain case and the position of the parietal horns (Figure 10.22).

The plot of the northern female specimens can be interpreted with the second eigenshape score values increasing from a lowest value in west African individuals (WSN and WCP), eastwards into Sudan (ENA) and eastern Ethiopia and Eritrea (ENE) and into Uganda (ECU). Then into Kenya, southern Ethiopia and Somalia (EEK) and southwards into southern Kenya and into Tanzania (ESK and EST).

Within Region Variation – South

There are no statistically significant associations. However, the southern sample is dominated by a single locality (SWC; n = 7 of 10). The single SWA individual included falls within the range of the SWC specimens in all plots. The two SCZ individuals show two of the three highest values on the second eigenshape axis, that largely reflects the angle of the parietal horns.

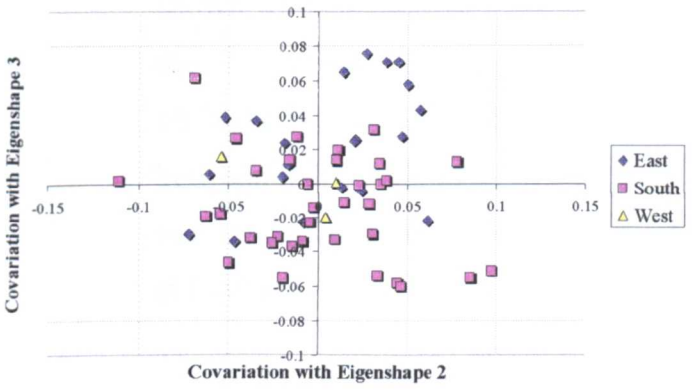


Figure 10.34: *Statistically significant geographic structure in eigenshape covariance values for the complete, male face and muzzle outline dataset. Significant structuring between regions was demonstrated for eigenshape 3 (Table 10.22). However, specimens from the different groups overlap extensively on this axis.*

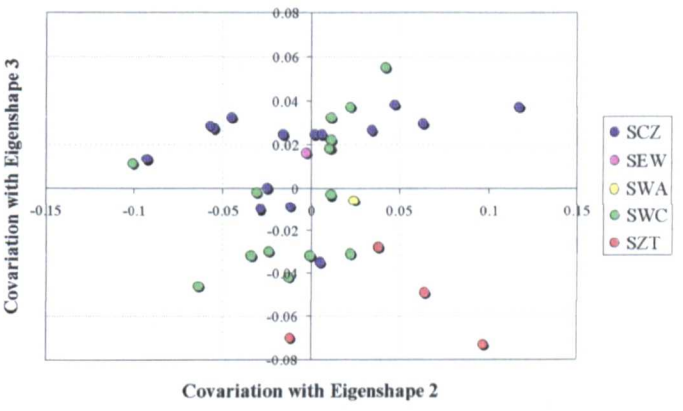


Figure 10.36: *Statistically significant geographic structure in eigenshape covariance values for the southern male face and muzzle outline dataset. Significant structuring between regions was demonstrated for eigenshape 3 (Table 10.22). The individuals from the SZT group have consistently low scores, although they do overlap with SCZ and, more extensively, with SWC individuals. The interpretation of this axis is problemsatic (See Figure 10.20C and the text for further discussion).*

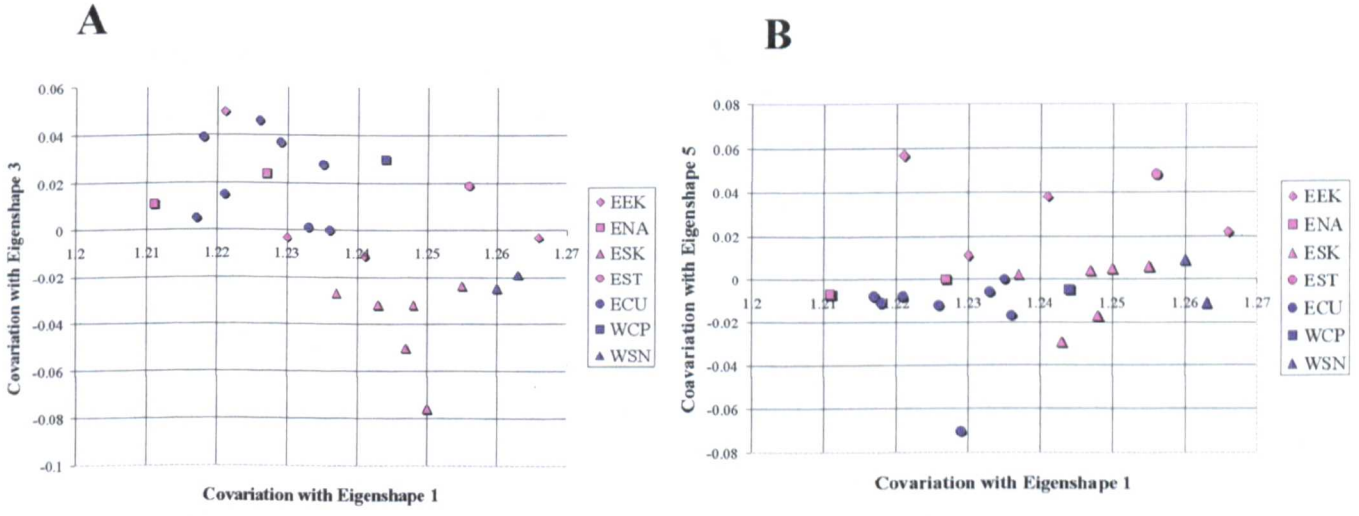


Figure 10.35: *Statistically significant geographic structure in eigenshape covariance values between northern region for the complete, male face and muzzle outline dataset. Significant structuring between regions was demonstrated for eigenshape 3 (Table 10.22). However, specimens from the different groups overlap extensively on this axis.*

Female Parietal Horn Data

Between Regions Variation

Highly significant geographic structure, separating northern and southern specimens, is shown with eigenshape 2 (Figure 10.38). This eigenshape indicates the relative length of the parietal horns and the breadth of the U-shaped region between the horns (Figure 10.24B). Whilst this trend is apparent, examination of the plots shows extensive overlap between individuals of the two regions. Two individuals of the western group are included, one appears intermediate between the two regions and the other group with the southern individuals.

There is a significant relationship with eigenshape 4. However, it seems that this axis models an individual idiosyncrasy with an outlier specimen present (Figure 10.38).

Within Regions Variation – North

While no statistically significant geographic structure has been identified, inspection of the plots suggests that eastern and western specimens are imperfectly separated by both the first and third latent shape trends (Figure 10.39).

Within Regions Variation – South

Again, there are no statistically significant associations found. The two SCZ individuals included in the analyses do pair up to have the lowest scores on the fourth axis.

Female Face and Muzzle Variation

Between Regions Variation

Statistically significant structure between northern and southern regions is found on both axes 3 and 4. Axis 3 appears to represent the concavity of the face reflecting how rapidly the skull narrows into the muzzle (Figure 10.25A). Meanwhile axis 4, arguably, represents whether the end of the muzzle expands slightly into a spatulate form. These axes represent a small fraction of identified variation (0.07% and 0.06% respectively). The plots show extensive overlap of specimens on both axes (Figure 10.40). Hence, these results can be disregarded in terms of providing meaningful characters to identify geographic variation.

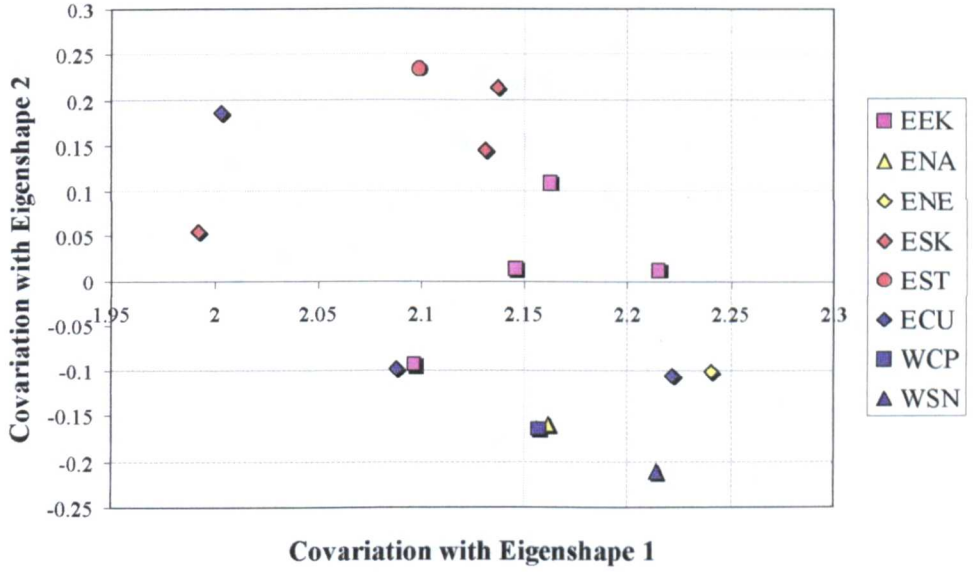


Figure 10.37: *Statistically significant geographic structure in eigenshape covariance values for northern region, female complete profile dataset. Significant structuring between regions was demonstrated for eigenshape 2 (Table 10.22). This axis may be interpreted with values increasing from the west to the east. See the text for further discussion.*

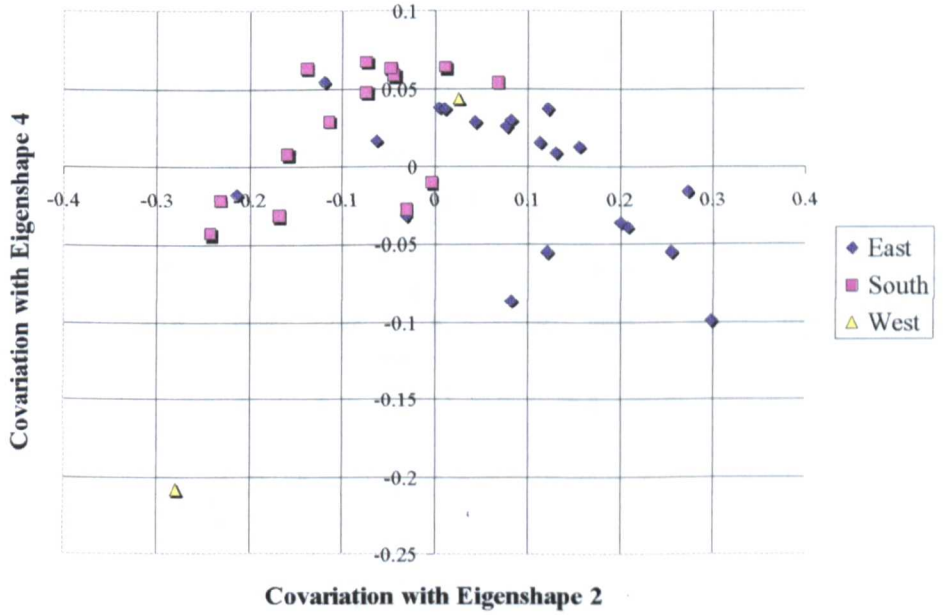


Figure 10.38: *Statistically significant geographic structure in eigenshape covariance values for all regions, female median horn profile dataset. Significant structuring between regions was demonstrated for eigenshapes 2 and 4 (Table 10.22). See the text for further discussion.*

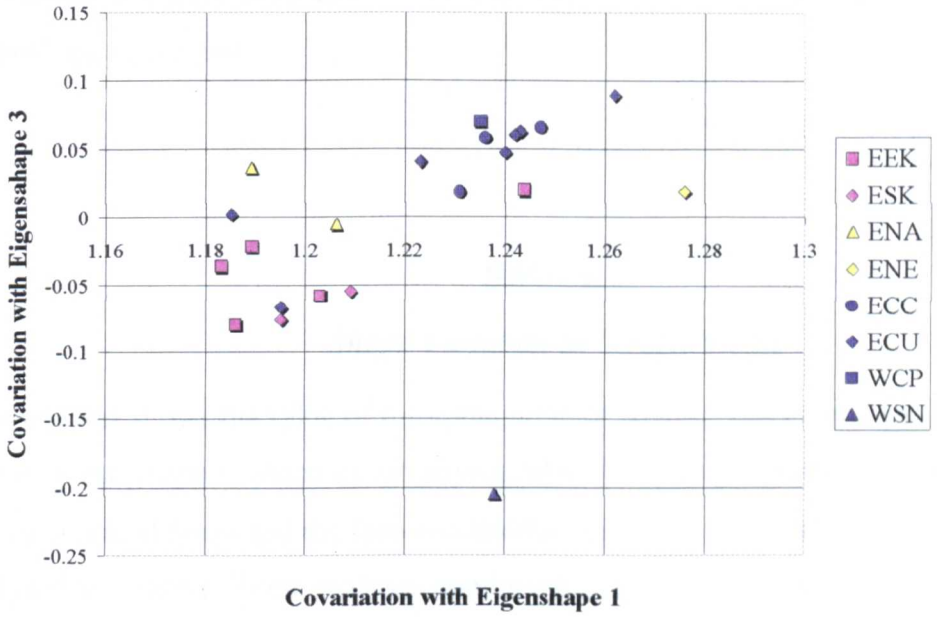


Figure 10.39: *No statistically significant geographic structure in eigenshape covariance values was found for the northern region, female parietal horn outline dataset. However, the plots suggest some structuring between regions. See the text for further discussion.*

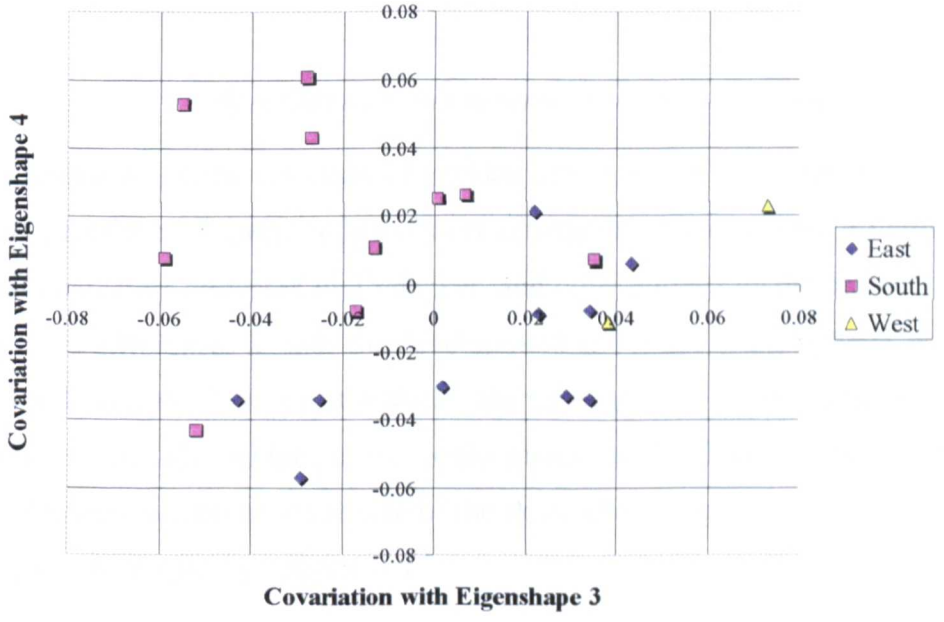


Figure 10.40: *Statistically significant geographic structure in eigenshape covariance values for all regions, female face and muzzle outline dataset. Significant structuring between regions was demonstrated for eigenshapes 3 and 4 (Table 10.22). However, there are difficulties in the interpretation of the shape variation for these axes and extensive overlap of the specimen sets in the plots. See the text for further discussion.*

Within Regions Variation – North and South

No statistically significant geographic structure was identified nor is evident from the eigenshape score plots.

Discussion

Shape Variation in Giraffe Skulls

This chapter shows the value of the application of landmark-restricted eigenshape analysis to the investigation of shape variation in giraffe skulls. Aspects of the shape of the skull profile, parietal horns and the face and muzzle regions for male and female skulls were analysed separately. These analyses combine to give a comprehensive summary of the gross morphology of the skull shape and encompass many of the characters invoked by previous authors as being important in the diagnosis of giraffe subspecies. The eigenshape analysis technique allows the identification and modelling of the major trends of variation within each data set and the ordination of the original specimen outlines according to their covariation with the identified latent shape trends.

Skull Variation in Currently Recognised Subspecies

In his review of giraffe subspecies Lydekker (1904, p. 202) considered that "most, if not indeed all, of the subspecies of giraffe are distinguishable by cranial differences." The cranial characters discussed by Lydekker, and other authors, in the context of species or subspecies differences include the development and shape of the parietal, median and occipital horns, the flexion of the skull[5], the tapering from the orbits to the muzzle, the shape of the muzzle and the general conformation of the skull including relative sizes of skull elements and the absolute size of the skull. (See Chapter 2 and Appendix 1.2.1 for a summary of subspecies differences).

The specimens available to these early authors were limited, introducing the possibility of sampling errors into their designation of taxa. An aim of this project is to investigate the occurrence of geographic structure to morphological variation in the giraffe across its African range. Here I discuss the shape variation identified in the foregoing analyses in the context of previously described morphological variation and considers whether the

[5] The angle made by the basicranial and basifacial planes.

identified shape trends are, indeed, geographically structured or may be attributable to individual variation across the range.

Types of Variation in Giraffe Skull Shape

Geographic variation is a subset of individual variation. However, for this discussion the two terms will be used in a mutually exclusive way. 'Geographic variation' will be used to refer to identified variation that is structured according to the geographical provenance of the specimen. That is, where a set of individuals from a given geographically restricted locality tend to be more like each other than they are to other individuals not included within the geographic confines of that locality. The restriction of such variation to particular localities may be influenced by historical demographic, selective or environmental factors.

The term 'individual variation' is used to refer to all other types of identified morphological variation not included under the definition of geographic variation. This individual variation, therefore, refers to the variation in the phenotype, observed between individuals within the species, and that is distributed in a non-significantly structured manner with respect to the geographic origin of the specimen.

Identified Geographic Variation

Male Specimens

Analysis of male skull profiles show that northern and southern specimens clearly separate according to the development of the median horn. An analysis focussing on the median horn segment only indicates that significant variation between the two regions occurs in both the shape (the curvature of the secondary bone on the nasal bones in the approach to the median horn) and the size of the median horn.

Lydekker (1904. p. 203) suggested that "we notice as we proceed from south to north the gradual passage of a two-horned animal [i.e. the paired parietal horns] into one (so far as the males are concerned) with three horns [i.e. parietals plus median horn]." Similarly, de Winton (1897), in recognising a northern (*G. camelopardalis*) and southern (*G. capensis*) species of giraffe, used the presence or absence of a median horn as a diagnostic character state. He notes that the males of *G. camelopardalis* have " a third horn in the centre of the forehead just above the eyes, cylindrical, from 3 to 5 inches long" (p. 277) and states that "the skull of the male *G. camelopardalis* can of course be at once distinguished by the prominent third horn" (p. 279). Meanwhile, his southern species has on the forehead "a

bump of flattened pyramidal form, larger in the males but never forming anything like a horn" (p. 278). Despite Dagg and Foster's (1982. p. 50) protestations that "we now know that these distinctions are not constant" my results suggest that they are, with few exceptions. These exceptions occur in specimens from southern Kenya and northern Tanzania that represent the previously recognised subspecies *G. c. tippelskirchi* (Figure 10.41). In his description of *G. c. tippelskirchi* Lydekker (1904. p. 219) states (drawing on the observations of Mr. Vaughan Kirby) that "some of the bull giraffes from the same locality [as males with "well-marked" median horns] have little or no third [median] horn." In those *G. c. tippelskirchi* males possessing a median horn it is "decidedly smaller" than in *G. c. rothschildi* (from eastern Kenya, Uganda and southern Sudan). The results of the local group comparisons of the median horn profile within the northern region groups indicate that *G. c. tippelskirchi* individuals (relating to my ESK and EST groups) do indeed show a wide range of variation, and some overlap with other groups, but show consistently less developed median horns (Figure 10.29).

The same profile analysis within the northern region indicates those western skulls, from my ECU, WCP and WSN groups (relating to *G. c. rothschildi* and *G. c. peralta*) possess significantly larger median horns.

Within the southern region the giraffes of the Luangwa Valley in Zambia (n = 5, equivalent to Lydekker's [1911] *G. c. thornicrofti*) consistently separate, with few overlapping specimens, from the southern Zimbabwean group (SCZ n = 17). However, specimens from the wider ranging SWC group (n = 13) overlap extensively with both of these groups. Lydekker's (1911) original description of the Thornicroft's giraffe did not mention the form of the parietal horns. The SZT specimens have shorter, more robust, cylindrical and less divergent parietal horns. The SCZ specimens tend to have longer, divergent horns with a constriction towards the tip, creating a knob at the end of the horn.

Female Specimens

Cranial variation in the female skull has been neglected in previous literature and only described where male specimens are not available (as in the description of *G. c. peralta* from a female specimen. Thomas, 1898) or, in a few cases, in comparison to male specimens.

Variation in skull morphology in female skulls tended not to be discretely geographically structured. The only statistically significant structure found was between the shapes of parietal horns from northern and southern regions. Despite overlap, parietal horns of southern specimens tended to be shorter, more slender and with a rounded U-shape

between them. Northern horns are longer, thicker with a more angular U-shape outline. Interestingly the profile shape models, reflecting a variation in the depth of the skull and position of the parietal horns, showed an apparently clinal trend from the west to the east in the northern region.

Identified Individual Variation

A common feature of both the male and female analyses of the skull profile between all of the comparisons made was the identification of skull flexion as the predominant source of variation between specimen profiles. In no case was the degree of flexion significantly related to geographical provenance. de Winton (1897) used the extent of skull flexion to distinguish between his northern and southern species. He suggests that in the southern species " the base of the brain case is not so much bent down: thus in the northern form the angle formed by the basifacial and basicranial portions of the skull is more acute; this character is more marked in comparing skulls of moderately young animals." (p. 279). de Winton's (1897) sample size was reasonable, with thirteen skulls of each of his recognised species covering a range of ages. Many of these specimens will have been accessioned into the collections of the British Museum of Natural History, and so included in this study (although de Winton did not give accession numbers). However, the results reported here suggest that his assertion was erroneous.

The shape trends shown by the parietal horns (divergence, relative length, breadth of 'U' and 'knobbiness') show limited resolution in characterising geographic groups. The ordinations (Figure 10.33) indicate a difference between specimens from the Luangwa Valley, Zambia (SZT) and those of southern Zimbabwe (SCZ) in horn divergence, length and the presence of a knob but the two groups are extensively overlapped by south-western specimens (SWC). Meanwhile female northern and southern specimens show a statistically significant difference in the relative length of the horns and the breadth of the U-shaped outline between the horns. However, none of the trends detailed in the literature pertaining to the shape of the parietal horns in relation to subspecies were identified.

The analysis of face and muzzle shape only identified the width of the face at the orbits as being statistically significantly different between eastern and western male specimens. However, overlap between the regions was extensive. The expansion of the muzzle was described by Lydekker (1904) as characteristic for his *G. c. rothschildi*, although Thomas (1898) had used this same character in his description of *G. c. peralta* (a single female specimen). No evidence of a geographically structured trend in the shape of the muzzle was found for either male or female specimens.

de Winton (1897) suggested that the skull of southern individuals tends to be broader relative to the length than in northern specimens. No explicit test of such a relationship was made in this study, although a comparison of eastern and western regions suggested a significant difference in absolute width. The first eigenshape of each profile analysis indicated variation in the relative length and depth of the skull. However, this was not significantly related to geographic provenance.

Other Criticisms of Skull Shape Criteria

Singer and Boné (1960) criticise Lydekker's (1904) reliance on the conformation of the horns. They suggest that "it is hardly possible to make any subspecific classification on the basis of the horns" due to the sexual dimorphism shown. Such an assertion makes sense only with a strict typological approach. Singer and Boné sought exceptions to Lydekker's classification, and they found them. For example, they list a group of southern giraffes from the Mabebe Flats in Botswana that have well developed median horns. Unfortunately only one of these specimens was included in the present analysis (due to damage to the other specimens). In the general profile analysis this single specimen (FMNH34422) fell within the scatter of the southern skulls; when restricting the analysis to the median horn profile only, it just overlapped with the northern specimens. Examination of the original specimen indicates that is does indeed have a well developed horn boss (Figure 10.41). However, the nature of the median horn development in this specimen is different to that in northern specimens. In the north the median horn is a smooth, conical projection. The horn of FMNH34422 is well developed, but is a rough low domed structure. Singer and Boné's criticism does not affect the validity of this character for distinguishing northern and southern forms.

Conclusions

In this study extended eigenshape analysis has been used to identify shape trends between sets of specimens and ordinate the specimens according to their covariation with these eigenshape shape trends. Most of the shape variation described in the literature to diagnose subspecies has been detected, though not all of it is consistently geographically structured. Similarly, not all of the identified shape trends that were significantly structured according to geographical provenance can be considered as diagnostic, or even suggestive, of

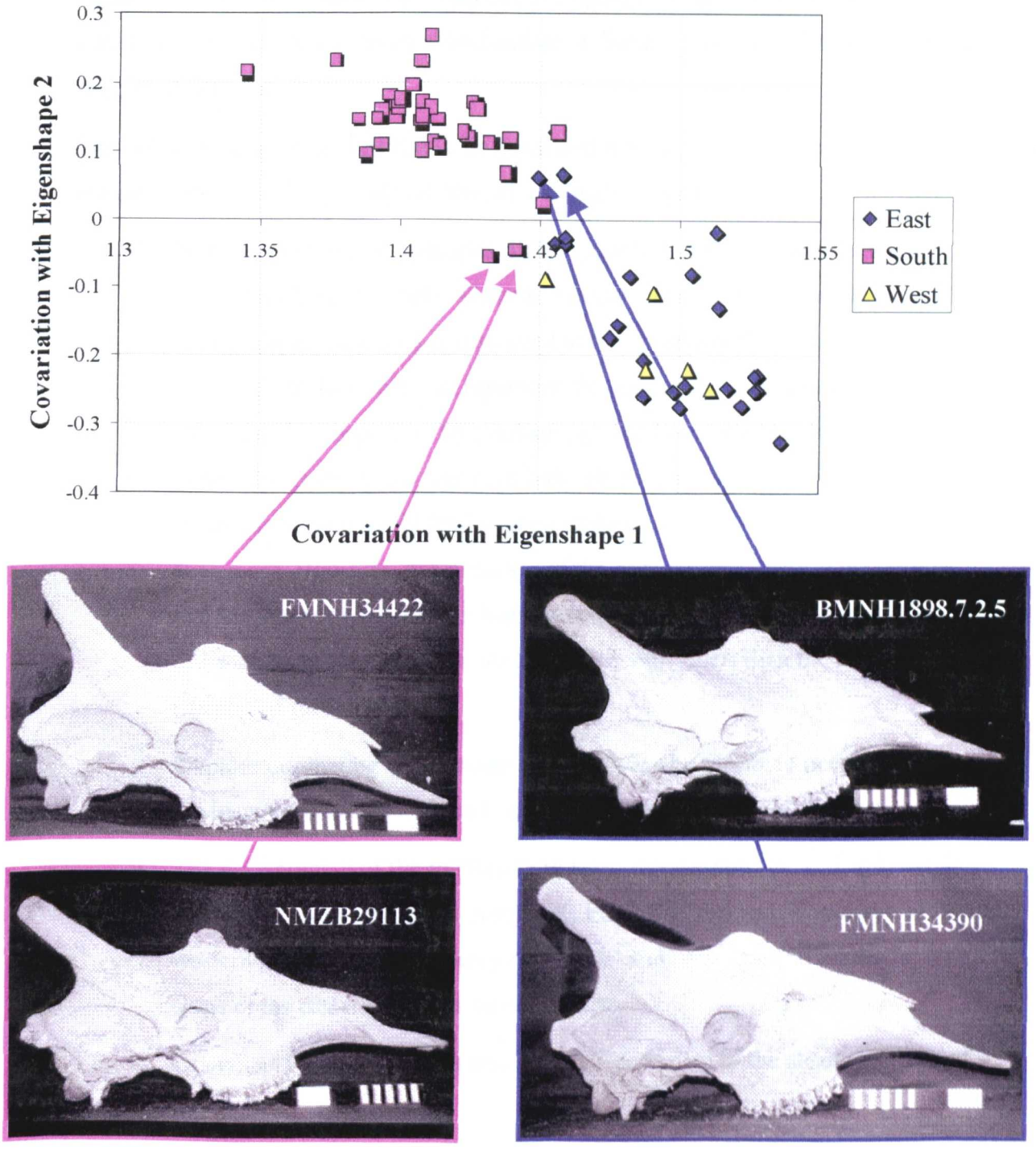


Figure 10.41: *Misclassification of northern and southern skulls according to median horn profile. Skulls on the left (bounded by pink) are southern skulls that overlap into the northern group; skulls on the right (blue) are northern skulls that overlap with the southern group. The two skulls from each region that encroach the greatest into the other group are shown. Both of the northern skulls shown are from southern Kenya (ESK). Of the eight specimens used originating from southern Kenya or Tanzania five overlap with the southern group (BMNH1898.7.2.5; FMNH34930; FMNH127881; PCTan76 and USNM182124). The sixth northern skull that overlaps is from a relatively young animal (PCJ39). The two southern skulls are from Botswana.*

subspecific rank. Some shape trends that provided statistically significant differences between geographic groups could not be interpreted biologically. In some cases statistical significance still left extensive overlap between geographically separate groups. Hence, statistical significance was taken as indicative of, but not presriptive for, geographically structured variation.

The following groups can justifiably be separated according to these results (Unles indicated otherwise these observations refer to male specimens):

1. Northern and southern regions differ according to the presence of a median horn in adult male giraffe. There is a certain amount of overlap. In the northern specimens this is restricted to the specimens from southern Kenya and northern Tanzania that represent the previously recognised *G. c. tippelskirchi* subspecies. This subspecies has been noted for being variable in this character. A few southern individuals have median horn development that might lead to their classification with the northern specimens. However, examination of the original specimens demonstrates that the nature of the median horn development is more indicative of the distinction between northern and southern individuals than the absolute size (height) of the horn.
2. Females originating in the north and south can be separated according to the length and breadth of the U-shape outline of their parietal horns.
3. The development of the median horn separates western (ECU, ENA, WCP and WSN) groups from eastern (EEK, ESK, EST) groups. The shape of the western horns tends to be more cylindrical and parallel-sided while the slope of the eastern horns is more gradual.
4. Eastern and western groups also separate according to the absolute width of the skull.
5. The southern Kenyan and northern Tanzanian specimens (ESK and EST) are variable and, generally, have the least median horn development of all northern groups.
6. In the south, parietal horn differences separate SZT and SCZ population groups. However, these differences are not diagnostic due to the overlap of the neighbouring SWC population. SZT individuals also group together in the profile analysis.

This, and the preceding chapters, have investigated the occurrence of phenotypically structured variation between geographically restricted specimen sets. The following section (Chapters 11 and 12) considers genetic variation between giraffe populations.

CHAPTER 11:

GENETIC VARIATION AND PHYLOGEOGRAPHIC STRUCTURE IN THE HISTORICAL RANGE OF THE GIRAFFE, *GIRAFFA CAMELOPARDALIS*, DETERMINED FROM MITOCHONDRIAL DNA CONTROL REGION SEQUENCE VARIATION

Introduction

Analysis of DNA sequence data has become a popular tool for understanding the evolutionary relationships between organisms at all levels of phylogenetic relatedness (Hillis *et al.*, 1996). The occurrence of comparable molecular markers across taxa and the confidence with which these specific, homologous sequences may be selected from the genome, amplified using the polymerase chain reaction (PCR[1]) and sequenced makes molecular systematics a powerful tool in reconstructing the cladogenic events in the evolutionary history of taxa. At the level of evolutionary organisation at and below the species level, mitochondrial DNA has become the molecule of choice for phylogenetic studies of sequence data (Avise, 1994; Avise, 2000). The term "intraspecific phylogeography" was coined (Avise *et al.*, 1987) to refer to the study of within-species variability in mitochondrial DNA where inferred phylogenetic relationships are combined with the geographic distribution of sequence variants to infer spatial relationships between populations and individuals within a species.

Phylogeography and the Properties of Mitochondrial DNA

General Physical and Biological Properties

Mitochondrial DNA (mtDNA) is a double-stranded circular DNA molecule occurring in the mitochondria of all multicellular animals and some unicellular protozoans. The arrangement of the molecule is remarkably consistent across animal groups with the approximately 15,000 bases typically coding for the same set of 37 genes (22 tRNAs, 13 mRNAs and 2 rRNAs), although the gene order may differ between taxa (Wilson *et al.*,

[1] The principles of PCR were first described by Kleppe *et al.* (1971). See also McPherson *et al.* (1992) and Palumbi (1996).

1985; Avise, 2000.). The genes of the mitochondrion code for many of the polypeptides involved in the electron transport and oxidative phosphorylation processes of respiration and ATP production in the mitochondrion.

A practical benefit is that mtDNA occurs at a high copy number with each cell containing up to ten thousand copies (Page and Holmes, 1998).

Properties as an Intraspecific Marker

The widespread occurrence and stable composition of mtDNA in most eukaryotes allows it to be used as a 'universal' marker across a wide range of taxa. Although, on average, evolving at 5 to 10 times the rate of single copy nuclear DNA (scnDNA, Brown *et al.*, 1979), different regions of the molecule show different rates of accumulation of mutations. Hence, different gene sequences may be used to elucidate relationships at different levels of phylogenetic relatedness. The rapid accumulation of mutations in the control region makes it a useful marker for intraspecific comparisons.

Mitochondrial DNA is overwhelmingly homoplasmic (Avise, 2000) and the mode of transmission is, almost exclusively, maternal[2]. Therefore, in conjunction with the lack of recombination (although see Lunt and Hyman, 1997; Awadalla *et al.*, 1999), the relationships indicated by mtDNA sequences demonstrate the relationships between matrilines in the population. The number of possible transmission routes through an organismal phylogeny is related exponentially to the number of generations in the time span considered. The possible number of pathways is $2^{(G+1)}$, where G = the number of generations (Avise, 1995). Hence, the maternal lineage is only one of the many possible transmission routes. It may be considered disadvantageous to select such a small fragment of the population history for study. However, it is a *known* route that is being traced through the genealogical history of the population and this knowledge should be accommodated in the interpretation of the data. For example, species showing strong female philopatry to natal sites are likely to show well differentiated geographic structure to the distribution mtDNA haplotypes, while species showing female dispersal are likely to be more homogeneous between the regions.

In summary, the ubiquity of mtDNA coupled with the simple structure of the molecule, its maternal mode of transmission and relatively high mutation accumulation rate makes

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[2] Paternal transmission has been identified in some species (e.g. Anderson *et al.*, 1995; Gyllensten *et al.*, 1991; Kondo *et al.*, 1990; Magoulas and Zouros, 1993; Meusel and Moritz, 1993; Satta *et al.*, 1988) but it tends to be rare and transitory (Kaneda *et al.*, 1995; Shitara *et al.*, 1998).

mtDNA a useful phylogeographic marker for examining intraspecific structuring in many animal species.

Phylogeny, Tokogeny and the Recognition of Intraspecific Relationships

Intraspecific phylogeographic analysis of mtDNA data, as originally formulated by Avise *et al.* (1987), is separated into two related objectives;

1. Determining phylogenetic interrelationships among the mtDNA molecules themselves;
2. Describing the geographic distributions of the phylogenetic groupings.

Historically, systematists have often been reluctant to recognise hierarchical, dichotomously branching structure below the level of the species. The tenets of phylogenetic systematics (Hennig, 1950; 1957; 1965; 1966) suggest that phylogenetic, branching relationships occur above the species level, while tokogenetic, reticulating relationships between individuals occur within a species. However, Lansman *et al.* (1981) argued that the lack of recombination (and, hence, the lack of reticulation) in the mtDNA molecule justifies the consideration of the individual organism as the basic operational taxonomic unit (OTU) to act as the terminal in a phylogenetic analysis, rather than the population or species.

The recognition of the transition from phylogenetic relationships between populations to tokogenetic relationships between individuals as representing the defining criterion for the species grade is effectively a reformulation of the biological species concept of Mayr (1942) into the language of cladistics. It defines each species as a gene pool, the individual members of which can interbreed freely, but are reproductively isolated (to some degree) from other individuals outside of the species. Such process-based definitions tend to be appealing as they emulate the biological processes that could lead to species formation. However, the biological species concept has been criticised for the difficulty in applying it to real situations and the same criticisms can be levelled at the supposition that phylogenetic (branching) relationships can not occur below the species level. Numerous lineage splitting events, including rapid vicariance or founder events or geographic isolation of populations, can occur within the range of a species effectively separating breeding populations and creating dichotomies in the evolutionary trajectory of a formerly single lineage. Hence, the use of 'standard' phylogeny reconstruction algorithms, as used for higher taxa comparisons, is valid for intraspecific phylogeographic studies.

Analysis of Sequence Relationships – Tree Building

One of the stated aims of this research is to investigate how different, independent data sets may affect the conclusions drawn regarding the geographic distribution of variation in the giraffe. Also of interest is whether implementation of different analytical procedures, on the same data set, provides different results. Hence, the data will be assessed using Neighbour Joining, Maximum Parsimony and Maximum Likelihood analytical techniques.

Neighbour Joining

Neighbour Joining (NJ) is a distance based method of phylogeny reconstruction. Distance methods make use of an index that represents the degree of similarity and difference between pairs of molecular sequences to create clusters of taxa. This is the 'genetic distance' or 'evolutionary distance'.

The genetic distance is effectively a ratio of the number of substitutions between two sequences versus the total number of base pairs sequenced. In its simplest form this is the proportion of sites that differ between the two sequences (the uncorrected p-distance). However, where the number of differences is large some substitutions may be hidden by the effect of multiple substitutions at the same site. This effect could result in a reversal back to the ancestral state (which may only be read from the data as a plesiomorphic state) or the masking of synapomorphies by subsequent autapomorphic changes. Similarly, true homoplasious substitutions may occur. Numerous authors have suggested models of molecular evolution that attempt to account for such effects and so supply a more accurate reflection of the 'true' evolutionary distances between molecular sequences. Nucleotide substitutions are modelled by paramaterising base frequencies (the proportions of each base in the sequences), the probability of base substitutions (or base exchangeability. i.e. the tendency for a given base to be substituted for any other) and rate heterogeneity of substitutions between sites (Whelan *et al*, 2001). The commonly used models are described and discussed by Nei and Kumar (2000), Page and Holmes (1998) and Whelan *et al* (2001). Computer programs are available that test the data set for the best fitting model (Posada and Crandall, 1998).

The neighbour joining (NJ) algorithm was developed by Saitou and Nei (1987) (also see Studier and Keppler, 1988) and represents a simplified version of the minimum evolution (ME) approach. The ME approach was first suggested by Edwards and Cavalli-Sforza (1963, and see Edwards, 1996) and received further attention with the work of Rzhetsky

and Nei (1992a, 1992b, 1993). ME seeks to minimise the total distance between OTUs in a given set of taxa. The tree containing all of the taxa with the shortest total distance is selected as the ME tree. Branch lengths are calculated according to square distance matrices constructed between all of the included taxa. To obtain the shortest tree topology the length of all possible topologies must be calculated. Hence, ME becomes computationally intensive as the number of possible trees increases rapidly as the number of taxa increases (Felsenstein, 1978a). The NJ algorithm utilises the ME principle at each step in the analysis. Central to the concept of NJ is the concept of 'neighbours'. Neighbours are defined as taxa (terminal units) that are linked at a single node. A NJ analysis starts with a star phylogeny (all branches arising directly from a single central 'hub') for which the 'total distance' is calculated. In the first step each taxon is paired with each other taxon and the new total distance is calculated for each resulting topology. The pair that gives the minimum total distance is retained and the two members of the pair are subsumed into a new terminal unit with their distance values averaged. This pairing algorithm is performed again with the minimum distance tree retained at each step and continues until the tree is fully resolved.

Maximum Parsimony

Maximum parsimony is the term frequently used by molecular geneticists to describe the implementation of cladistic analyses for molecular data. The methods of cladistic analysis (see Kitching *et al.*, 1998) are derived largely from the principles of phylogenetic systematics espoused by the German entomologist Willi Hennig (1950, 1957, 1965, 1966).

Cladistics derives phylogenetic tree topologies by hierarchically grouping taxa according to shared derived character states. In principle phylogenetically related groups may be identified by the distribution of synapomorphies between the taxa in the analysis. An apomorphic character state is derived from an ancestral ('plesiomorphic') character state. A synapomorphy is an apomorphic state shared by two or more taxa due to common ancestry. That is, an ancestral character state transforms into a derived (apomorphic) state through a mutation event. Following subsequent cladogenesis this new character state is passed on to the daughter taxa. The sharing of this character state, then, infers the shared common ancestry and phylogenetic history of the daughter taxa. The distribution of such shared character states between taxa allows hierarchically structured, branching relationships to be hypothesised between the taxa.

However, any arrangement of relationships between taxa, and the resulting character state transformations, can be justified by the erection of the necessary number of *ad hoc*

explanations of character state change. An optimality criterion is required that allows objective comparison of different hypothesised phylogenetic relationships.

The observation of the same character state for a character erects a hypothesis of primary homology (de Pinna, 1991; Brower and Schawaroch, 1996). For molecular data parallel or convergent substitutions invoke hypotheses of primary homology as much as true synapomorphies do. The multiple hypotheses of primary homology that typify a series of compared sequences are analysed via a cladistic analysis. The results of such an analysis are visualised as a branching diagram with taxa at the terminal tips and character states for each character mapped on to the tree. This results in the corroboration of some of the hypotheses of primary homology (the secondary homology of de Pinna, 1991). It will also infer that some of the base substitutions occurred on multiple occasions and are homoplasious (that is they do not originate from a single common ancestor). It is this character state congruence (the corroboration of hypotheses of primary homology) that is the deciding factor in distinguishing homology from homoplasy. The optimality criterion used in cladistic (maximum parsimony) analyses sees the incidence of synapomorphies maximised and the occurrence of homoplasies minimised to explain the distribution of character states between taxa. In practice this means the 'shortest' tree where length is measured in the number of inferred mutation events.

Maximum Likelihood

The maximum likelihood approach applies particular models of molecular evolution in its search for the 'most likely' tree topology (See the NJ section, above). Its complex mathematical nature suits it to the analysis of molecular data sets, where character states are known and are equivalent between characters, but makes it difficult to apply to morphological analyses.

The maximum likelihood approach is primarily a tool for testing models of molecular evolution rather than a direct method of phylogenetic tree reconstruction (Page and Holmes, 1998. p. 201). There are two major, interrelated variables in any ML analysis; the tree and the model of molecular evolution. To derive the tree you need estimates of the probability of evolutionary events. To estimate the probability of these evolutionary events you need a tree. Methods that select the model of molecular evolution that best fits the sampled data are available (Posada and Crandall, 1998), effectively removing one of the uncontrolled variables from the analysis and allowing a phylogenetic tree to be estimated from the data.

Although often couched in terms of probability the fundamental concept of the ML method is likelihood. Although conceptually related, probability and likelihood are fundamentally different. Probability is predictive. It uses either logically derived proportions or empirical observations to predict the outcome of a future event[3]. In contrast, likelihood is retrospective: Given an outcome and a set of circumstances how likely is it that that outcome resulted from those circumstances, rather than another set of circumstances?[4] As probabilities are predictive, and given that the event is going to happen (and so requires an outcome), the probabilities of the outcomes always sum to one. Likelihoods do not have this property and do not sum to one.

ML calculates how likely it is that the observed data set would have resulted from a given tree topology and the imposed model of molecular evolution. The tree topology chosen is the one with the greatest likelihood value. The likelihood value of a tree is calculated from topology and branch length information along with the probabilities of certain nucleotide substitutions given the imposed model of molecular evolution. The likelihood for a given character is the product of the prior probability of the occurrence of a given nucleotide in the hypothesised most recent common ancestor and the probability that that nucleotide will change to each other nucleotide (including staying the same or undergoing a reversal to the original state) on each branch in the tree. The sum of these likelihood values for each nucleotide for that character gives the character likelihood. The likelihood of the tree is then the sum of the character likelihood values.

Materials and Methods

Species and Populations Studied

This study examines the phylogeography of a single species, the giraffe, *Giraffa camelopardalis*. Tissue samples from across the historical range of the giraffe were collected from museum specimens and represent nearly all current populations and many areas that had a historical presence of giraffe but where they now no longer exist. (See

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[3] Logical: A coin has two flat sides that are equally weighted, therefore will have half a chance of landing heads and half a chance of landing tails when tossed fairly.
Empirical: A coin was tossed fairly 100 times, it came down heads 50 times and tails 50 times, therefore it has a 50/100 = 0.5 probability of coming down as a head on the next fair toss.

[4] A fair die is rolled 100 times (circumstances) and turns up a six 90 times (outcome). How likely is this if it is truly a fair die? Not very likely. You would suspect a different set of circumstances, such as the die being loaded, to explain the outcome more adequately.

Appendix 5.11.1 for tissue samples obtained, Appendix 2.6.1 for specimen locations and Figures 2.1 and 2.3 for the historical range).

Collection of Tissue Samples

When using museum collections it is important to cause no damage to the integrity of the specimen that might reduce its value for future scientific work. Hence, each specimen was examined for the presence of dried muscle tissue and small quantities taken only where tissue remained on a skull or other skeletal element. Where available, multiple (up to three) tissue samples were taken from each individual animal, from different locations on a specimen. The source of each tissue sample was recorded accordingly. No skin samples were used in this study.

Tissue samples were stored in air-tight screw capped tubes sealed with a rubber gasket. Each tube was labelled with the unique specimen number on the tube itself and on the cap with an indelible pen. The source of the tissue was also recorded on the tube. A separate record of the source of the tissue sample for each specimen was made. All instruments were cleaned between collecting each tissue sample by wiping with absolute alcohol to remove any contaminants. A list of tissue samples acquired for this study is presented in Appendix 5.11.1.

DNA Extraction, Amplification, Cleaning and Sequencing

Total genomic DNA was extracted from the dry tissue using a protocol based on the tissue protocol given with the QIAmp DNA Mini Kit (Qiagen, 1999. Product Number 51304). The standard fresh tissue protocol was adapted for the dried tissue samples used in this study. The absolute volumes of digestion enzyme and buffer solutions were increased (to allow for absorption into the dry tissue) but concentrations were maintained. The reaction solutions were warmed to 56°c for one hour and then transferred to a revolving wheel to digest for 48 hours. Subsequent steps, including the adsorption of DNA to the purification column and the washing of the DNA, proceeded according to the standard protocol. The complete final protocol used is detailed in Box 11.1. Extraction of DNA from the fresh tissue samples followed the standard protocol.

Where multiple samples were available from the same animal, the sample chosen for extraction was selected subjectively by considering the quantity and apparent quality of each sample. If the first extraction did not yield product following PCR amplification a second extraction, using a different source sample, was performed. For the second

extraction the volume of elution buffer was halved, effectively increasing the concentration of the DNA in solution.

1. Using a clean scalpel and avoiding cross-contamination, cut up to 30-50mg of dry tissue into small pieces and place in a 1.5ml microcentrifuge tube, and add 180µl of Buffer ATL.
2. Add 20µl Proteinase K, mix by vortexing, and incubate at 56°c for a short period (up to one hour) to initiate tissue lysis. Vortex occasionally during incubation to disperse the sample. In some cases the dry tissue soaks up the buffer quickly. In these cases add additional buffer and enzyme to the tissue as necessary. Move to a rotating mixing wheel and leave to digest at room temperature for 48 hours. Mix by vortexing at regular intervals. For samples not completely digested after 48 hours add a further 10µl of proteinase K and incubate the samples at 56°c until the tissue is completely lysed.
3. Briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from the inside of the lid.
4. Add 200µl Buffer AL to the sample, mix by pulse-vortexing for 15seconds, and incubate at 70°c for 10min. Briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from inside the lid.
5. Add 200µl ethanol (96-100%) to the sample, and mix by pulse vortexing for 15sec. After mixing, briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from inside the lid.
6. Carefully apply the mixture from step 5 (including any precipitate) to the QIAamp spin column (in a 2ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000rpm) for 1 min. Place the QIAamp spin column in a clean 2ml collection tube, and discard the tube containing the filtrate.
7. Carefully open the QIAamp spin column and add 500µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 8000rpm for 1min. Place the QIAamp spin column in a clean 2ml collection tube, and discard the collection tube containing the filtrate.
8. Carefully open the QIAamp spin column and add 500µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at 13,200rpm for 3 min.
9. Place the QIAamp spin column in a clean 1.5ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp spin column and add 200µl Buffer AE. Incubate at room temperature for 5 minutes, and then centrifuge at 8,000rpm for 1min.
10. Repeat step 9.

Box 11.1: *DNA extraction protocol adapted for museum samples. This protocol is adapted from the QIAamp® DNA Mini Kit and QIAamp DNA Blood Kit Handbook (Qiagen, 1999. pp 28 - 31). Note that the handbook provides additional notes for each step. Only those notes utilised in the current project are reproduced here.*

Four pairs of giraffe specific PCR primers spanning the entire control region sequence (developed by Dr. Chris Baysdorfer and co-workers, California State University), were tested with the DNA extracted from the museum samples (Table 11.1 and Figure 11.1).

F2 – CCC AAA GCT GAA GTT CTA TT

F3 – TAT CTA CCA TGC CGC GTG AA

F4 – CCT ACC ATC ATT TTT AAC AC

R2 – CAT TTT CAG TGC CTC GCT TT

R3 – GTA TAG TAT AGT GGT TAT GT

R4 – CAA TAA CTG TAT GTA CTA TG

Table 11.1: *Primer sequences of giraffe specific Control Region primers tested in this study.*

Segments of only a few hundred base pairs were amplified from some museum specimens due to the degraded nature of the DNA extracted from the older samples (see Austin *et al.*, 1997). Hence, a primer pair that would consistently amplify a relatively short fragment length was necessary. Fragments from three regions (Table 11.2) were amplified and sequenced to assess which gave the greatest number of informative substitutions in the sample. Good quality DNA amplified from fresh tissue extractions was used for these comparisons. The single giraffe control region sequence available on Genbank (AF151090) was also compared to assess the variability in each sequenced segment.

The F2 to R4 fragment showed the greatest variability of the three fragments tested, despite the fact that it compared fewer individual specimens and was two-thirds the length of the longer F3 to R3 fragment. The F2 to R4 fragment corresponds to the highly variable left domain (also termed the 5' end) (Avise, 2000; Taberlet, 1996). The decision to use the F2 to R4 fragment for this study combines its relatively high variability and its short length (299bp between the primers) that will allow comparison between a greater number of (possibly degraded) museum specimens. Also this region has been used in other studies of African mammal phylogeography (e.g. Arctander *et al.*, 1999; Matthee and Robinson, 1999).

Symmetrical PCR amplifications were carried out in a 20µl reaction volume containing 20pmol of forward (F2) and reverse (R4) primers, 250µmol dNTPs, 2.5mM $MgCl_2$, 1x BIO-X-ACT Optibuffer, 1x BIO-X-ACT Specificity Enhancer and 2 units of BIO-X-ACT DNA Polymerase. BIO-X-ACT polymerase was used as it was found to give cleaner, clearer, more consistent results than other DNA polymerases used. The thermal cycling conditions consisted of one denaturation cycle at 94°c for 5 minutes, then 10 cycles of denaturation (94°c, 1 minute), annealing (46°c, 1 minute) and extension (72°c, 1 minute). Followed by 30 cycles of denaturation, annealing and extension 30 seconds each (i.e.

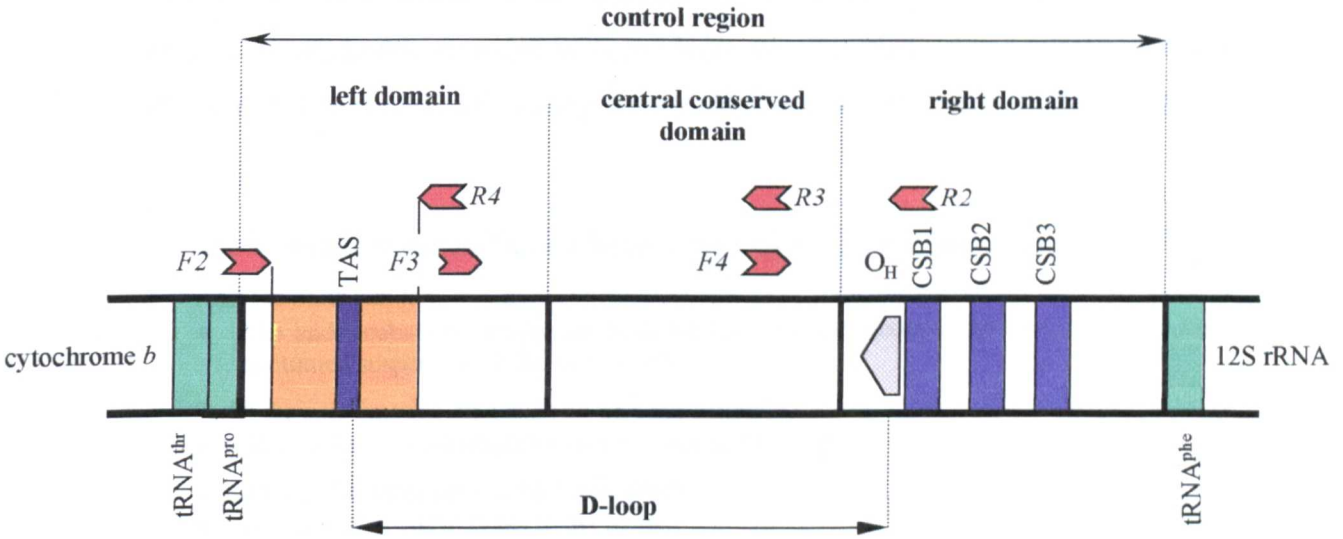

Figure 11.1: *Diagram of mammalian mitochondrial DNA control region showing structure and positions of giraffe specific primers used (red arrows). The region of sequence used in this study is highlighted with an orange block. TAS = termination-associated sequence; O_H = initiation site for heavy strand; CSB = conserved sequence block. Diagram redrawn and adapted from Taberlet (1996).*

Primer Pair	Position in CR	Useful Fragment Length	Variable Bases	% variation	n
F2 – R4	-16 to 283	263	17	6.5%	4
F3 – R3	392 to 813	382	15	3.9%	5
F4 – R2	897 to 1042 (+40)	96	5	5.2%	5

Table 11.2: *Fragment length and proportion of variable bases in fragments derived from control region primer pairs tested. 'Position in CR' refers to the base number corresponding to the attachment of the internal end of each primer. The numbers correspond to the consecutive numbering of bases in the 1002bp giraffe control region sequence. The negative value in F2-R4 indicates that F2 anneals outside the control region (in the $tRNA^{pro}$). The '+40' value in F4-R2 similarly indicates an extralimital annealing site for R2 (in the $tRNA^{phe}$). Specimen sequences compared were A575, A1558, A1559 and A3369 for each comparison. A575 was from Botswana, A1558 and A1559 from Zimbabwe and A3369 from Tanzania. Sequence for A575 was not available for the F2-R4 fragment. The single giraffe control region sequence from Genbank (AF151090), derived from a zoo animal, was also used.*

cycling at 94°c for 30 seconds, 46°c for 30 seconds and 72°c for 30 seconds). A 7 minute extension phase (at 72°c) completed the thermal cycling regime.

PCR reaction mixtures were diluted with loading dye (2µl PCR mixture: 4µl loading dye) and 5µl of this mixture was electrophoresed in a 1% agarose gel containing 0.03% Ethidium Bromide against a size standard. For those specimens showing positive amplification, double stranded PCR products were purified using a BIO101 Geneclean kit (Product Number 1001-600) using the standard protocol (Protocol in Box 11.2).

1. Add 3 volumes of Sodium Iodide solution to the amplification reaction mixture.
2. Add 5µl GLASSMILK.
   - Mix and incubate at room temperature for five minutes, mixing every one to two minutes to ensure continued suspension of the GLASSMILK.
3. Pellet the silica matrix (GLASSMILK) with the bound DNA.
   - Spin in a microcentrifuge for five seconds at 13,200rpm.
4. Wash the pellet three times with NEW Wash.
   - Add 500µl of NEW Wash to the pellet.
   - Resuspend the matrix.
   - Centrifuge at 13,200rpm for five seconds.
   - Discard the supernatant.
   - Repeat wash.
5. After the third wash dry the pellet in a vacuum centrifuge for a few minutes.
6. Elute the DNA from the matrix.
   - Resuspend the DNA in 5µl of Elution Solution.
   - Centrifuge at 13,200rpm for 30 seconds to pellet the matrix.
   - Remove supernatant containing the eluted DNA and put in to a new tube.
   - Repeat the elution a second time.

Box 11.2: *Amplified product cleaning protocol. This protocol is adapted from the GENECLEAN® handbook pp 8 – 11). The handbook offers additional notes following each step and should be referred to for further information.*

Sequencing reactions using a dye terminator cycle sequencing kit, using 2µl of purified template DNA were performed (Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq®. Part Number 402079. PE Biosystems, 1995. Protocol in Box 11.3). Both strands of the PCR products were sequenced and the products resolved on an ABI373 semi-automated DNA sequencer.

1. Mix the following reaction mixture and keep on ice: Terminator Ready Reaction Mix 4.0µl Primer (at 0.8 pmol/µl) 2.0µl Clean Water 2.0µl
2. Vortex the reaction mixture.
3. Add 8.0µl of reaction mixture to 2.0µl of each DNA sample. Keep the tubes on ice.
4. Run tubes through the following thermal cycling regime: 96°c for 10 seconds 50°c for 10 seconds } x 25 cycles 60°c for 4 minutes Hold at 4°c.
Box 11.3: *Cycle sequencing reaction protocol. This protocol is derived directly from the Perkin Elmer cycle sequencing protocol (Perkin Elmer, 1995).*

Sequence Alignment

'Within' Specimens – Forward and Reverse Strands

Forward and reverse sequences for each specimen were aligned using Sequencher 3.1.1 software (Gene Codes Corporation, 1995.) and any ambiguities or conflicts resolved by examining the electropherograms. Where necessary, a second sequence was obtained to clarify the nucleotide sequence. Confirmation of the sequence was obtained from at least two strands for all sample sequences used in the analysis. Analysed sequences for each fragment were defined by the start of the control region at the 5' end (as aligned with Genbank specimen AF151090) and at the 3' end as the extent of the reliable sequence available. No ambiguous bases were left in any of the sequences used in the final analyses.

Between Specimens

The consensus sequences from 98 specimens were aligned automatically using the Sequencher 3.1.1 software and checked by eye. Each sequence was trimmed to 261 or 262bp. Sequence lengths were made equivalent by insertion of gaps during alignment.

Analysis of Sequence Relationships – Tree Building

All analyses were implemented using PAUP* 4.0b4a (Swofford, 1999).

Selecting a Model of Molecular Evolution

The MODELTEST software of Posada and Crandall (1998) was used to establish the model of molecular evolution that best fits the data. The program uses log likelihood scores to compare increasingly complex, hierarchically arranged hypotheses of molecular evolution. The logic is to accept the simplest model that adequately explains the data. Taking the ratio of the log likelihood of the null model (the simpler model) to an alternative, more complex model tests the null hypothesis that using a more complex model does not significantly improve the fit of the data. If the null hypothesis is accepted the simpler model is retained and compared to the next more complex model. If the null hypothesis is rejected the alternative model from the preceding test is tested against the next more complex model.

The chosen model (HKY85 with gamma distribution) was used in NJ and ML analyses.

Neighbour Joining Analysis

NJ analysis was run using the HKY85 with gamma distribution model of molecular evolution and included all 22 haplotypes. Ten thousand bootstrap replicates were made.

Maximum Parsimony Analysis

A branch-and-bound MP analysis of 22 haplotypes used gaps as a fifth character state. Bootstrap support statistics with random addition of taxa were estimated from 10,000 heuristic replicates.

Maximum Likelihood Analysis

Due to the computationally intensive nature of the ML method a heuristic search algorithm was used. One thousand replicated heuristic searches were carried out using the selected model of molecular evolution on 22 haplotypes. For the same reason only 100 bootstrap replicates were performed.

Results

Summary of Genetic Information

Sequence Composition

The mean sequence composition across all haplotypes is 41.0% A, 28.4% T, 20.6% C and 10.0% G. A Chi-square test of homogeneity of base frequencies across taxa indicated no significant differences between the haplotypes ($\chi^2 = 4.985$, df = 63, p = 1.000). No individual sequence was significantly different from the mean composition.

The matrix of uncorrected pair-wise distances (p-values) is given in Table 11.3.

Sequence Alignment

All but a short (6bp) length of sequence aligned unambiguously. Insertion of gaps, for optimal alignment of sequences, gave three possible arrangements. The first inserted a gap at nucleotide position 130 only. The second inserted a single gap per sequence at either position 125 or 130. Meanwhile the third arrangement used at least one gap at either 125 or 130 or inserted two gaps in a sequence at both positions (Table 11.4). The three alternative arrangements gave 28, 28 and 27 parsimoniously informative sites respectively. The third arrangement gave no site with more than two character states per character. The former two arrangements each gave one and two sites with three character states respectively.

Each arrangement was analysed using NJ and MP analyses. In addition sequence data with the ambiguous segment removed (bases 125 to 130 of the 263bp sequence) was analysed. The three alternative arrangements of the complete data set gave identical tree topologies within each type of analysis. The MP approach gave the same 12 most parsimonious trees (MPTs). Comparison of the strict consensus tree of the 12 MPTs (Figure 11.2) and the NJ tree (Figure 11.3) showed that, while the NJ tree was fully resolved, there were no conflicts between the two inferred topologies. The arrangement with the ambiguous bases removed gave an identical NJ tree topology and non-conflicting MP tree topology with slightly lower resolution with a single terminal dichotomy dropped to a more basal polytomy ([H10, A1236]) (See Figure 11.2).

Plotting character state distributions of the ambiguous characters onto the derived trees indicate that, while providing different character support for certain clades, none of the character distributions conflicted (Figure 11.4). The differing distributions of ambiguous characters explains the differing bootstrap support values (calculated from 10,000 heuristic searches each) between the arrangements (Figure 11.2). The lack of conflict in the distribution of characters, coupled with the tree topology congruence of the 'ambiguity

	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11
H01		1	12	5	11	15	13	14	13	15	6
H02	0.0038		11	4	10	14	12	13	12	16	5
H03	0.0461	0.0422		9	1	11	9	12	9	11	10
H04	0.0192	0.0153	0.0345		8	12	12	13	10	14	3
H05	0.0422	0.0383	0.0038	0.0306		10	8	11	8	10	9
H06	0.0575	0.0536	0.0421	0.0460	0.0383		8	7	8	10	13
H07	0.0497	0.0459	0.0345	0.0459	0.0306	0.0306		9	4	8	11
H08	0.0536	0.0498	0.0461	0.0498	0.0422	0.0268	0.0344		9	9	14
H09	0.0498	0.0460	0.0346	0.0383	0.0307	0.0307	0.0153	0.0345		8	9
H10	0.0573	0.0611	0.0422	0.0535	0.0383	0.0382	0.0305	0.0344	0.0305		15
H11	0.0230	0.0192	0.0384	0.0115	0.0345	0.0498	0.0421	0.0536	0.0345	0.0573	
A1236	0.0497	0.0535	0.0422	0.0535	0.0384	0.0382	0.0153	0.0344	0.0229	0.0153	0.0497
AM53546	0.0535	0.0497	0.0383	0.0497	0.0345	0.0344	0.0038	0.0382	0.0191	0.0344	0.0459
BM1898.7.2.5	0.0192	0.0153	0.0346	0.0077	0.0307	0.0460	0.0382	0.0498	0.0307	0.0535	0.0038
EEI2	0.0461	0.0422	0.0076	0.0345	0.0038	0.0421	0.0345	0.0461	0.0346	0.0422	0.0384
EEIGc	0.0499	0.0460	0.0038	0.0383	0.0076	0.0460	0.0383	0.0499	0.0384	0.0460	0.0346
FM127880	0.0345	0.0307	0.0499	0.0230	0.0460	0.0613	0.0613	0.0651	0.0536	0.0689	0.0192
FM27475	0.0536	0.0498	0.0460	0.0498	0.0421	0.0192	0.0344	0.0230	0.0345	0.0420	0.0536
PCNNChad138	0.0535	0.0497	0.0383	0.0497	0.0344	0.0344	0.0038	0.0382	0.0191	0.0344	0.0459
US162017	0.0268	0.0230	0.0422	0.0153	0.0384	0.0536	0.0459	0.0575	0.0383	0.0611	0.0038
US251797	0.0230	0.0192	0.0384	0.0038	0.0345	0.0498	0.0497	0.0536	0.0422	0.0573	0.0153
US251798	0.0230	0.0192	0.0384	0.0038	0.0345	0.0498	0.0497	0.0536	0.0422	0.0573	0.0153

	A1236	AM 53546	BM189 8.7.2.5	EEI2	EEI Gc	FMNH 127880	FMNH 27475	PCCha d138	USNM 162017	USNM 251797	USNM 251798
H01	13	14	5	12	13	9	14	14	7	6	6
H02	14	13	4	11	12	8	13	13	6	5	5
H03	11	10	9	2	1	13	12	10	11	10	10
H04	14	13	2	9	10	6	13	13	4	1	1
H05	10	9	8	1	2	12	11	9	10	9	9
H06	10	9	12	11	12	16	5	9	14	13	13
H07	4	1	10	9	10	16	9	1	12	13	13
H08	9	10	13	12	13	17	6	10	15	14	14
H09	6	5	8	9	10	14	9	5	10	11	11
H10	4	9	14	11	12	18	11	9	16	15	15
H11	13	12	1	10	9	5	14	12	1	4	4
A1236		5	12	11	12	18	11	5	14	15	15
AM53546	0.0191		11	10	11	17	10	2	13	14	14
BM1898.7.2.5	0.0458	0.0421		9	10	6	13	11	2	3	3
EEI2	0.0422	0.0383	0.0346		3	13	12	10	11	10	10
EEIGc	0.0460	0.0422	0.0384	0.0115		12	13	11	10	11	11
FMNH127880	0.0688	0.0651	0.0230	0.0499	0.0461		17	15	6	7	7
FMNH27475	0.0420	0.0382	0.0498	0.0460	0.0498	0.0651		10	15	14	12
PCNNChad138	0.0191	0.0076	0.0421	0.0383	0.0421	0.0574	0.0382		13	14	14
USNM162017	0.0535	0.0497	0.0077	0.0422	0.0384	0.0230	0.0575	0.0497		5	5
USNM251797	0.0573	0.0535	0.0115	0.0384	0.0422	0.0268	0.0536	0.0535	0.0192		2
USNM251798	0.0573	0.0535	0.0115	0.0384	0.0422	0.0268	0.0460	0.0535	0.0192	0.0077	

Table 11.3: *Uncorrected pair-wise distances between haplotypes. Absolute pair-wise distances (base pair differences) between haplotypes are given above the diagonal. Uncorrected 'p' distances are given below the diagonal. Haplotypes separated by a single base pair difference are shaded.*

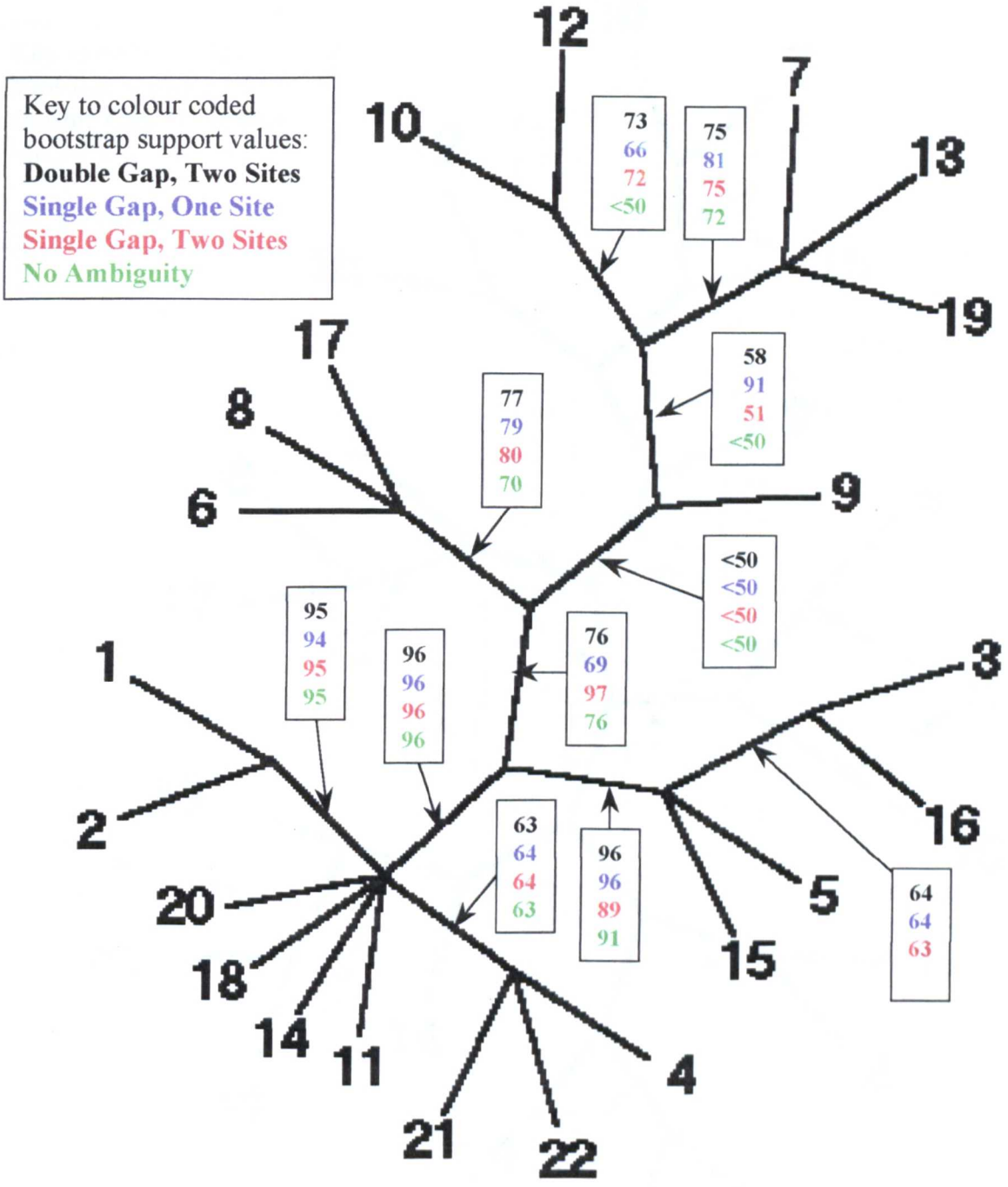

Figure 11.2: *Maximum parsimony analysis of ambiguous alignments. The alignment of genetic sequences provided a short segment (of 6bp) that required the insertion of one or two gaps for alignment. Three alternative arrangements plus the arrangement with the ambiguous section removed were compared to assess the effect of the alterative alignments on the tree topology and on the bootstrap support values. See Tables 11.4 and 11.5 and the text for further information and discussion.*

1 = H01; 2 = H02; 3 = H03; 4 = H04; 5 = H05; 6 = H06; 7 = H07; 8 = H08; 9 = H09; 10 = H10; 11 = H11; 12 = A1236; 13 = AMNH53546; 14 = BMNH1898.7.2.5; 15 = EEI2; 16 = EEIGc94.11.25.6MA; 17 = FMNH27475; 18 = FMNH127880; 19 = PCNNChad138; 20 = USNM162017; 21 = USNM251797; 22 = USNM251798.

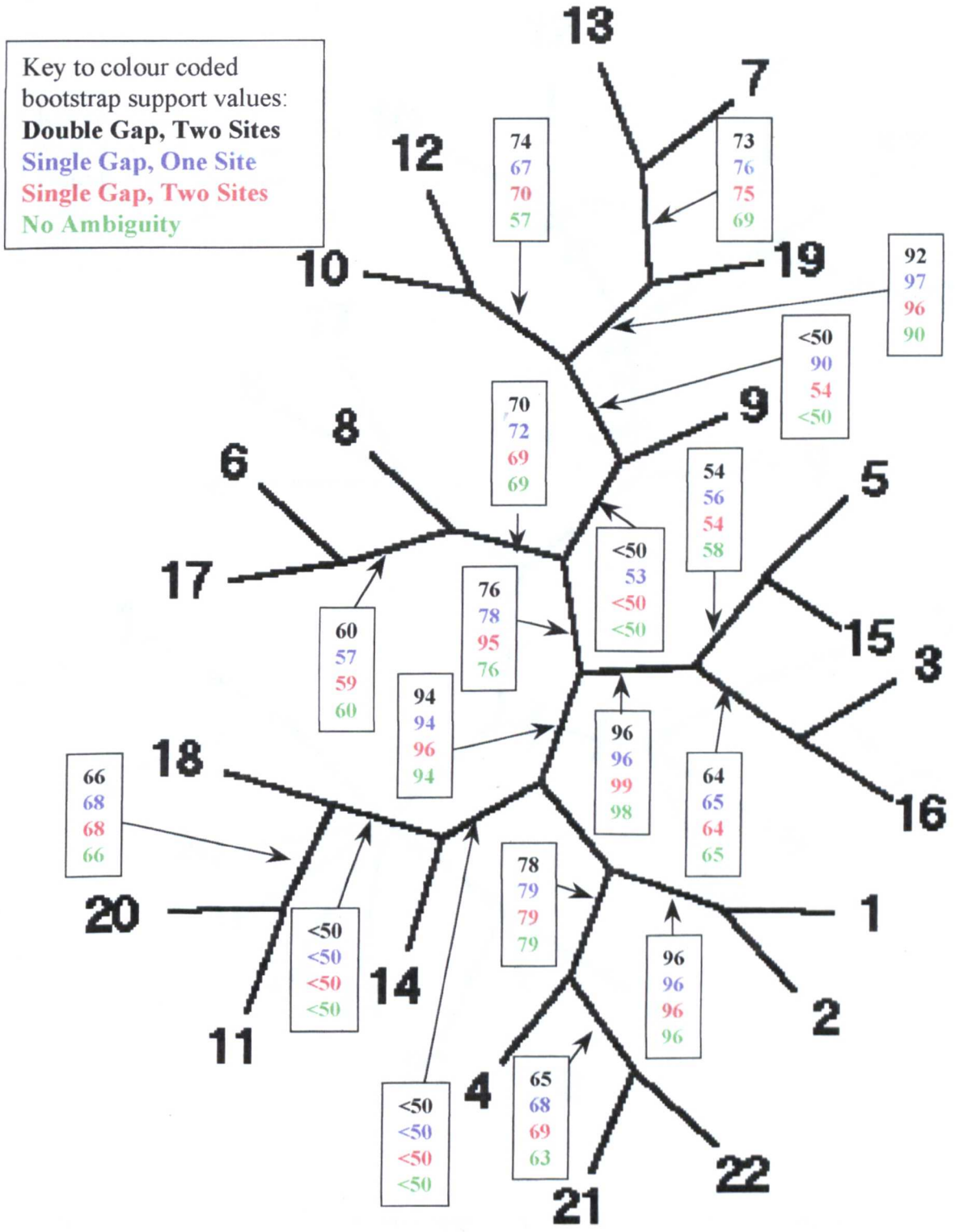

Figure 11.3: *Neighbour Joining analysis of ambiguous alignments. The alignment of genetic sequences provided a short segment (of 6bp) that required the insertion of one or two gaps for alignment. Three alternative arrangements plus the arrangement with the ambiguous section removed were compared to assess the effect of the alterative alignments on the tree topology and on the bootstrap support values. See Tables 11.4 and 11.5 and the text for further information and discussion.*

Haplotypes are numbered as in Figure 11.2.

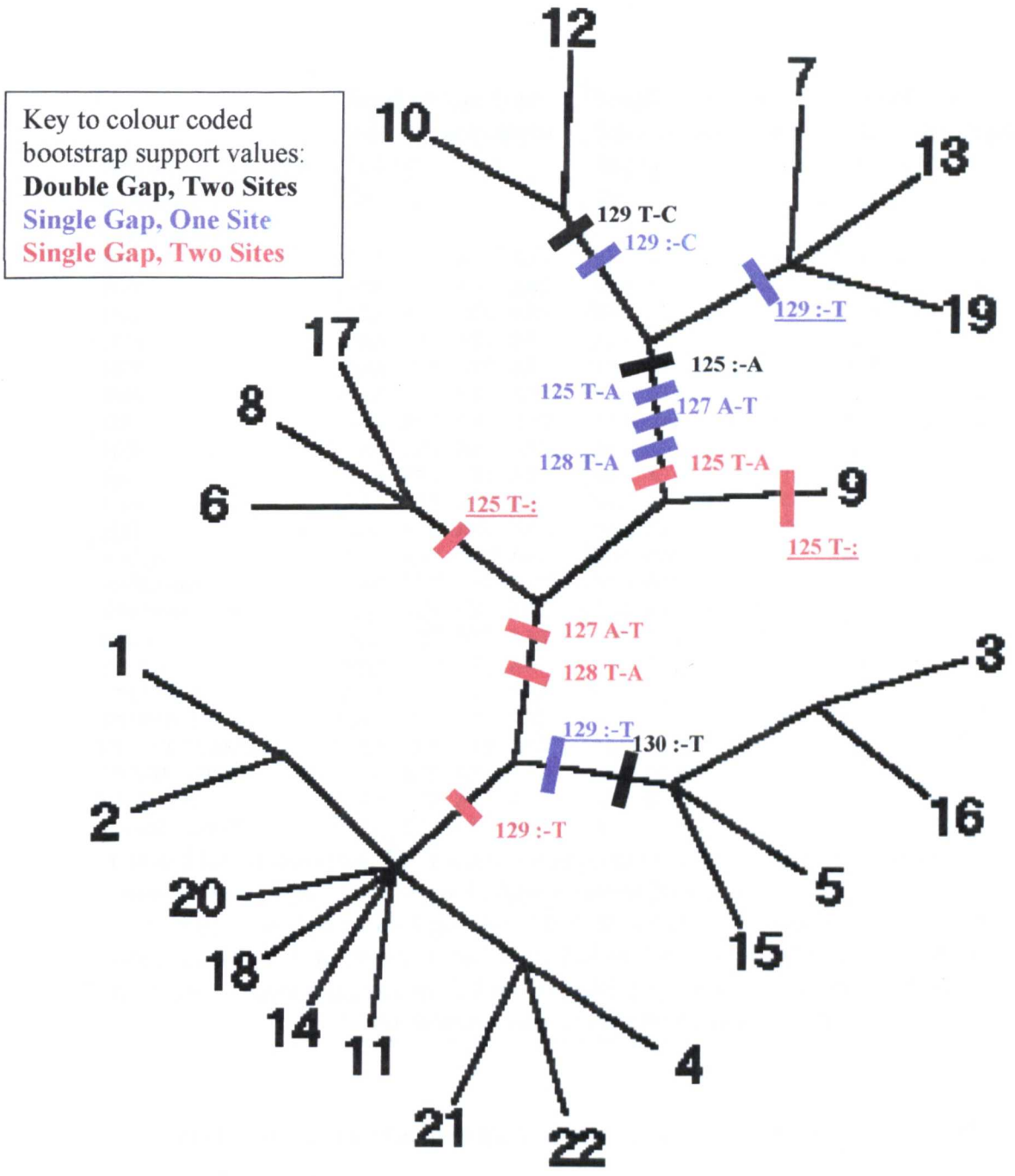

Figure 11.4: *Maximum Parsimony analysis of ambiguous alignments character state map. Alternative arrangements of the ambiguous character states on the strict consensus cladogram. The same twelve trees were derived from each arrangement, providing identical strict consensus tree topologies. The varaition in bootstrap support statistics (Figure 11.2) is explained by the alternative character state substitutions on each branch.*
Haplotypes are numbered as in Figure 11.2.

removed' sequence alignment suggests that the signal provided from the unambiguously aligned data is sufficiently robust to represent the relationships between taxa.

	Single Gap, One Site Arrangement	Single Gap, Two Sites Arrangement	Double Gap, Two Sites Arrangement
Sequence Length	262 bp .	262 bp	263 bp
Informative Characters	28	28	27
H01	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
H02	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
H03	AAA ATT ATT AAT	AAA ATT ATT AAT	AAA A:T TAT TAA
H04	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
H05	AAA ATT ATT AAT	AAA ATT ATT AAT	AAA A:T TAT TAA
H06	AAA ATT AT: AAT	AAA A:T TAT AAT	AAA A:T TAT :AA
H07	AAA AAT TAT AAT	AAA AAT TAT AAT	AAA AAT TAT :AA
H08	AAA ATT AT: AAT	AAA A:T TAT AAT	AAA A:T TAT :AA
H09	AAA ATT AT: AAT	AAA A:T TAT AAT	AAA A:T TAT :AA
H10	AAA AAT TAC AAT	AAA AAT TAC AAT	AAA AAT TAC :AA
H11	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
A1236	AAA AAT TAC AAT	AAA AAT TAC AAT	AAA AAT TAC :AA
AM53546	AAA AAT TAT AAT	AAA AAT TAT AAT.	AAA AAT TAT :AA
BM1898.7.2.5	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
EEI2	AAA ATT ATT AAT	AAA ATT ATT AAT	AAA A:T TAT TAA
EEIGc	AAA ATT ATT AAT	AAA ATT ATT AAT	AAA A:T TAT TAA
FM27475	AAA ATT AT: AAT	AAA A:T TAT AAT	AAA A:T TAT :AA
FM127880	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
PCNNChad138	AAA AAT TAT AAT	AAA AAT TAT AAT	AAA AAT TAT :AA
USNM162017	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
US251798	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA
USNM162017	AAA ATT AT: AAT	AAA ATT AT: AAT	AAA A:T TAT :AA

Table 11.4: *Alternative DNA sequence alignments for eleven haplotype groups and eleven unique haplotypes showing a 12 base segment from positions 121 to 132 only. The first arrangement inserts a single gap, where necessary, at position 130 only. The second inserts a single gap at one of two sites; 125 or 130. The third arrangement inserts one or two gaps in each sequence at 125 and/or 130. The three arrangements result in 28, 28 and 27 parsimony informative characters respectively.*

The decision to use the 'double gap, two sites' arrangement took in to account the following information:

1. The 'ambiguity removed' alignment was not selected as it reduced the resolution of the resultant tree topology;
2. Tree support statistics derived from the four data sets were very similar making a decision based on the level of homoplasy difficult (Table 11.5);
3. Bootstrap support values were most similar between the 'double gap, two sites' arrangement and the 'ambiguity removed' alignment, suggesting that that arrangement most precisely reflected the signal in the unambiguously aligned data;

4. The double gap, two sites offers the most parsimonious interpretation of character state distributions. Each character is represented by only two character states. The alternative arrangements possess one or two characters with three character states. Similarly, the double gap, two sites arrangement provides one fewer parsimoniously informative site, while still maintaining the same MP tree topology with fewer inferred changes in the mapped characters (Figure 11.4).

	Double Gap, Two Sites	**Single Gap, One Site**	**Single Gap, Two Sites**	**No Ambiguity**
Sequence Length	263	262	262	257
Parsimony Informative	27	28	28	24
MPTs	12	12	12	78
Tree Length	56	59	59	53
CI	0.732	0.729	0.746	0.717
RI	0.871	0.874	0.893	0.861
RC	0.637	0.637	0.666	0.617
HI	0.268	0.271	0.254	0.283

Table 11.5: *Tree statistics from three different alignments resulting from a short segment with ambiguous alignment and from the sequences with the ambiguous region removed.*

Sequence differences are summarised in Table 11.6. Aligned sequences are given in full in Appendix 5.11.3.

Haplotype Distributions

The 98 sequences aligned grouped into 22 haplotypes, 11 of these were unique, represented by only a single specimen. Eight of these differed by only a single base change. In such cases all electropherograms were rechecked to ensure that the correct base was called for that site. Haplotype groups varied in size from two to twenty five individuals. The specimens included in each haplotype group, along with their geographic group and specific provenance (with latitude and longitude co-ordinates) are given in Appendix 5.11.2. A summary of the geographic extent (by country and geographic group) of the specimens included in each haplotype group is given in Table 11.7 and Figure 11.5.

				11111	11111	11111	11112	22222	2
	11333	56666	78999	00011	22345	77778	88890	01133	4
	79789	52469	37349	35636	59032	03450	12542	53567	0
Haplotype01	TCCCA	CCTCC	CCACT	AGTTA	:T:TA	AACTC	AGTTT	TAAAT	C
Haplotype02	.....	.....	.....	.....	.....	.....	.....	.....	T
Haplotype03	.....	TT...	.T...	GA..G	:.T..	GG..T	..C..	.G...	T
Haplotype04	....G	.....	.....	.....	.....	.G..T	..C..	.....	T
Haplotype05	.....	T....	.T...	GA..G	..T..	GG..T	.,C..	.G...	T
Haplotype06	...T.	T....	TT.TC	GA.C.	.....	.GT.T	.TC..	.....	T
Haplotype07	.A.T.	T....	TT...	GA.CG	A....	.G..T	...C.	.....	T
Haplotype08	.CCTA	T....	TT.T.	.ACCG	...C.	.GT.C	..C..	.....	T
Haplotype09	...T.	T...T	T....	GA.CG	.....	.G..T	..CC.	.....	T
Haplotype10	...T.	T..T.	TT...	GA.CG	AC.C.	.G..T	..C..	...G.	C
Haplotype11	.....	.....	.....	.....	.....	.G..T	G.CC.	.....	T
A1236	...T.	T....	TT...	GA.CG	AC.C.	.G..T	...C.	.....	.
AM53546	.A.T.	T....	TT...	GA.CG	A....	.G.CT	...C.	.....	T
BM1898.7.2.5	.....	.....	.....	.....	.....	.G..T	..CC.	.....	T
EEI2	..T..	T....	.T...	GA..G	..T..	GG..T	..C..	.G...	T
EEIGc	.....	TT...	.T...	GA..G	..T..	GG..T	G.C..	.G...	T
FM127880	C....	.....	..G..	.....	.....	.G..T	G.C..	..G.C	T
FM27475	...T.	T....	TT.T.	GA.C.	C	.GT.C	..C..	C....	T
PCNNChad138	.A.T.	T....	TTG..	GA.CG	A....	.G..T	...C.	.....	T
USNM162017	.....	.....	.....	.....	.....	.G..T	.GCCC	.....	T
USNM251797	....G	..C..	.....	.....	.....	.G..T	..C..	.....	T
USNM251798	....G	.....	.....	.....	.....	.G..T	..C..	C....	T

Table 11.6: *Summary of giraffe control region DNA sequence variation. The numbers in the top row refer to the position of the base in the 263bp sequence (These numbers must be read vertically). All sequences are referenced to Haplotype01. An identical base to that of Haplotype01 is indicated by a full stop.*

Haplotype Group	n	Country	Geographic Group
Haplotype01	25	Botswana, Zimbabwe, South Africa.	SCZ (1), SEW(4), SWC(19), Unass.(1).
Haplotype02	2	Namibia.	SWC(2).
Haplotype03	20	Namibia, Zimbabwe.	SCZ(14), SWC(6).
Haplotype04	9	Tanzania, Zambia.	ESK(2), EST(1), SZT(6).
Haplotype05	4	Angola, Botswana, Zimbabwe.	SCZ(1), SWA(1), SWC(2).
Haplotype06	3	Kenya.	ESK(2), Unass.(1).
Haplotype07	9	Nigeria, Cameroon, DRC, Sudan, Kenya.	ECC(3), ECU(1), ENA(2), WCP(2), WSN(1).
Haplotype08	8	Kenya, Ethiopia, Somalia.	EEK(7), Unass.(1).
Haplotype09	2	Kenya.	EEK(2)
Haplotype 10	3	Kenya.	ECU(3).
Haplotype 11	3	Kenya, Tanzania.	ESK(1), EST(1), Unass(1).

Table 11.7: *Summary of the geographic extent of each haplotype group.*

Estimated Phylogenetic Relationships Between Haplotypes

The optimal trees resulting from NJ, MP and ML analyses are presented in Figures 11.6, 11.7 and 11.8. The MP tree presented is the strict consensus of 12 most parsimonious trees.

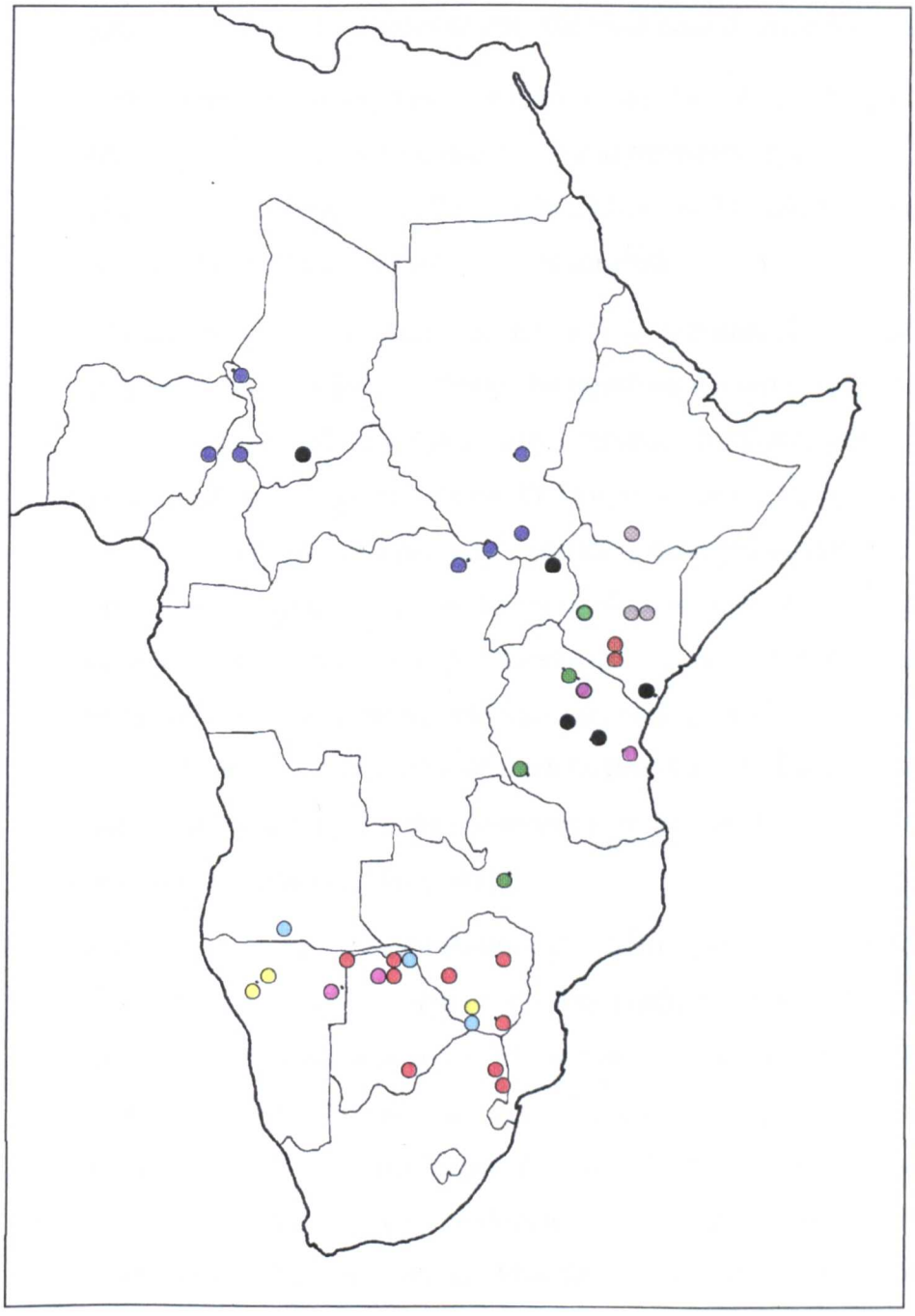

Figure 11.5: *Distribution of giraffe mitochondrial DNA control region haplotypes across the sampled African range. Eleven haplogroups (with more than one identical haplotyoe) and eleven unique haplotypes resulted from the analysis of 98 individual specimens. Note that Haplotype 9 (from the Loroghi Mountains of Kenya) could not be located. Some of the unique haplotypes are not located as they are coincident with other haplotypes. Unique haplotypes occur in north eastern DRC (with haplotype 7); Etosha National Park, Namibia (with haplotype 3); in the Serengeti, Tanzania (with haplotype 4) and in the Kapiti Plateau, Kenya (with haplotype 6). One of the unique haplotypes (FMNH27475) was not located due to an unreliable provenance.*

The trees are presented as unrooted cladograms with branch lengths and bootstrap support values given for each clade. The three trees show similar, non-conflicting topologies. The strict consensus MP tree represents the most conservative estimate of relationships.

These trees are unrooted as no outgroup was used in this analysis. Comparable sequence was obtained from a single okapi (*Okapia johnstoni*) specimen but proved to be difficult to align unambiguously with the giraffe sequences. The okapi is the logical choice of outgroup as it is the only other extant giraffid.

The use of the outgroup rooting criterion for intraspecific studies has been criticised (Castelloe and Templeton, 1994). An outgroup chosen for any study should be phylogenetically closely related to the ingroup, preferably it should be the sister taxon (or taxa if multiple outgroups are used). However, for intraspecific comparisons, at one end of the scale paraphyly and polyphyly resulting from pre-cladogenetic lineage sorting may mean that the gene sequences do not conform to an 'outgroup-ingroup' relationship. At the other end of the scale the sister taxon used may be sufficiently divergent that the genetic distances between outgroup and ingroup, coupled with the effects of homoplasy, may make the unambiguous positioning of the root problematic. The difficulty encountered in unambiguously aligning the sequences may indicate the latter case to be a problem between the giraffe and the okapi.

Calculation of the root probabilities (Castelloe and Templeton, 1994. As implemented by the TCS program of Clement *et al.*, 2000) indicates that haplotype H03 may represent the most basal sequence and so can be used to root the cladogram. The calculation of root probabilities typically uses haplotype frequency data along with sequence information. However, in this case, haplotype frequencies were removed from the analysis as the sampling design was a non-random and was affected by the availability of museum specimens, perhaps introducing bias into the calculation of the root probabilities.

The result of simple mid-point rooting, where the mid-point of the longest branch is used to root the cladogram, is shown in Figure 11.9. This method of rooting provides a credible root and can be interpreted as a geographical split between 'northern' and 'southern' specimens (see below).

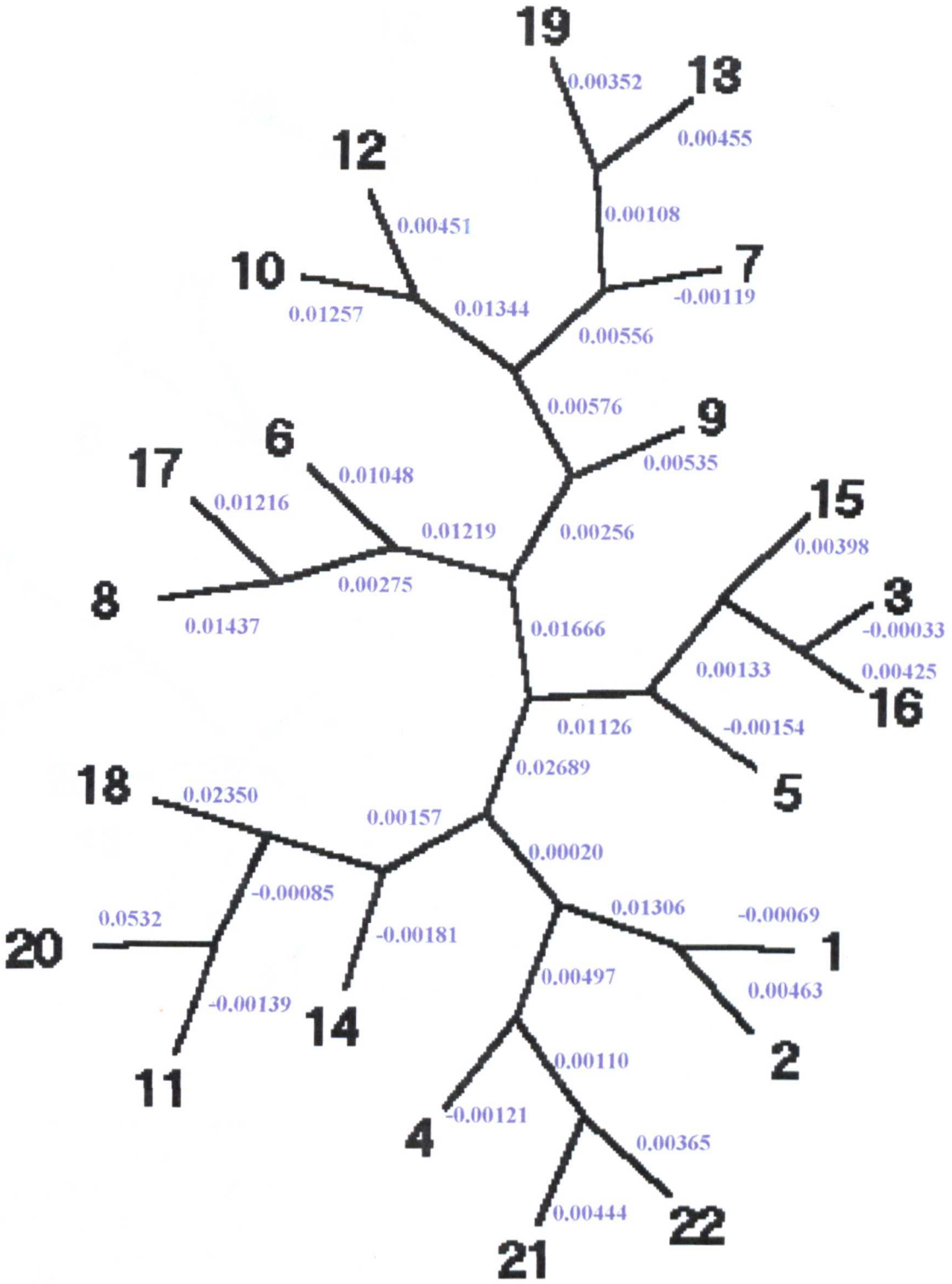

Figure 11.6: *Neighbour Joining Tree with genetic distances (using HKY85 with gamma distribution).*
Haplotypes are numbered as in Figure 11.2.

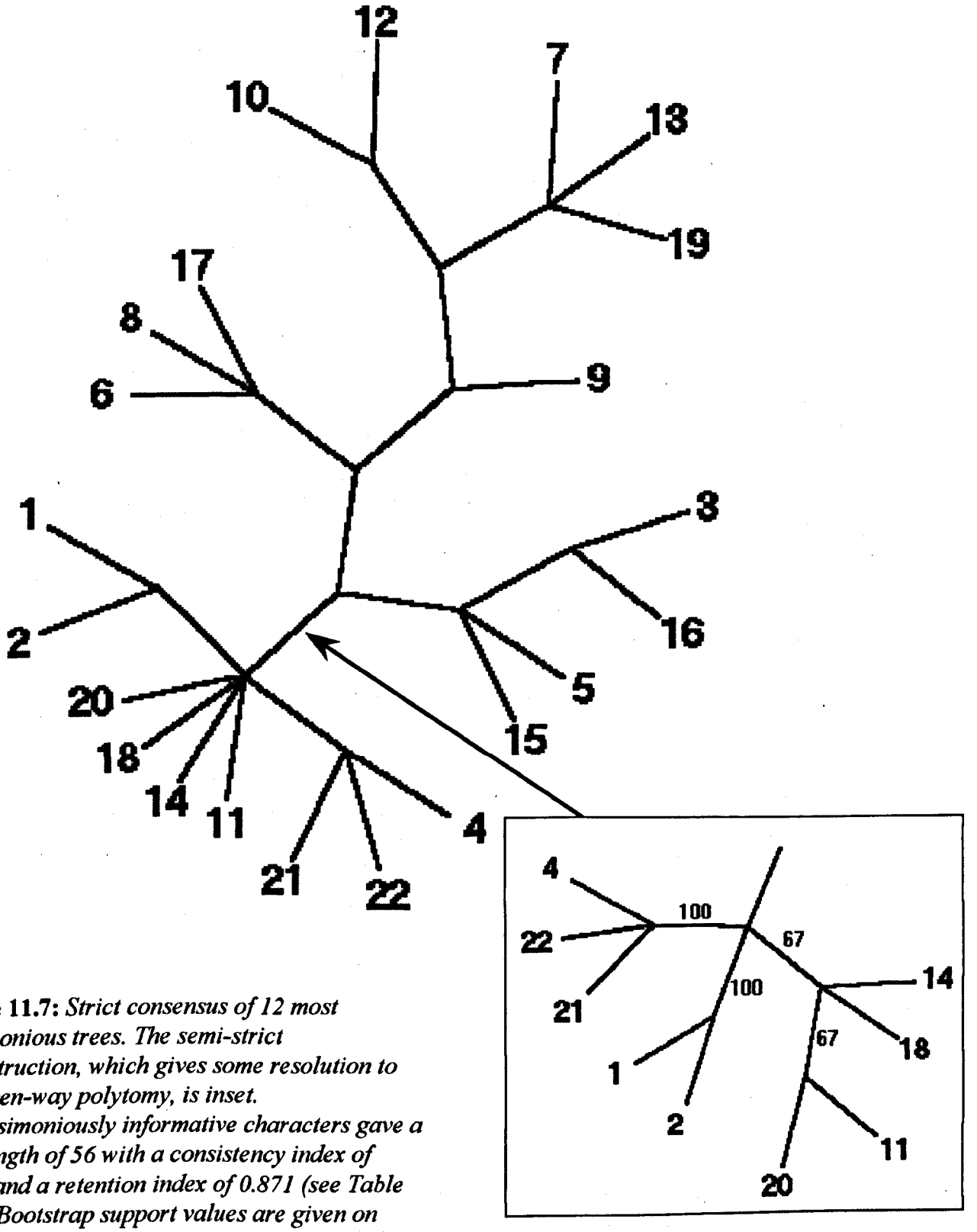

Figure 11.7: *Strict consensus of 12 most parsimonious trees. The semi-strict reconstruction, which gives some resolution to the seven-way polytomy, is inset.*
27 parsimoniously informative characters gave a tree length of 56 with a consistency index of 0.732 and a retention index of 0.871 (see Table 11.5). Bootstrap support values are given on Figure 11.2.
Haplotypes are numbered as in Figure 11.2.

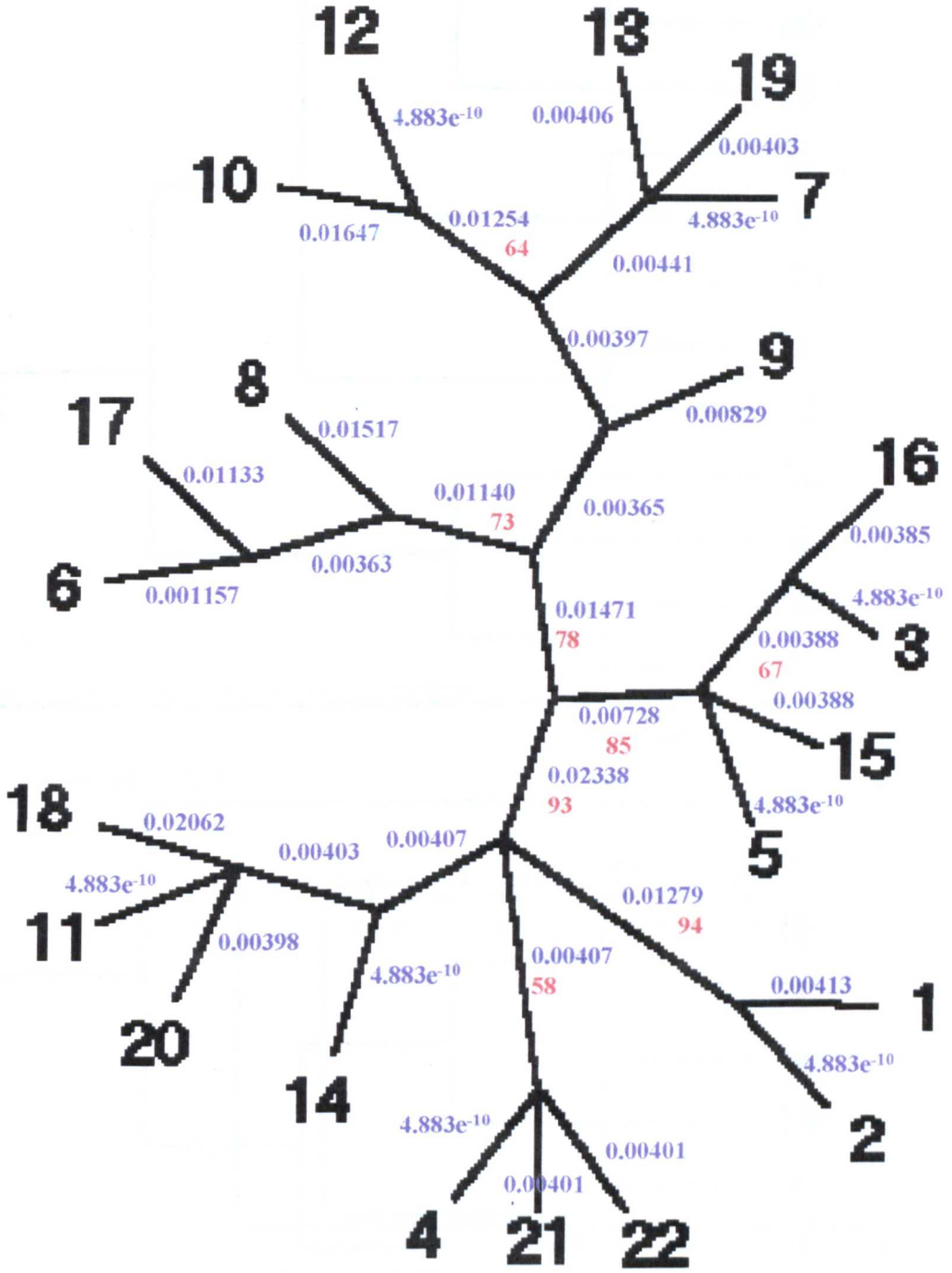

Figure 11.8: *Maximum Likelihood tree with branch likelihood values (in blue). The HKY85 with gamma distribution model was used. A single, identical tree was obtained from each of 1,000 heuristic search replicates, with random addition of taxa. Bootstrap support values (greater than 50%), from 100 bootstrap replicates, are shown in red.*
Haplotypes are numbered as in Figure 11.2.

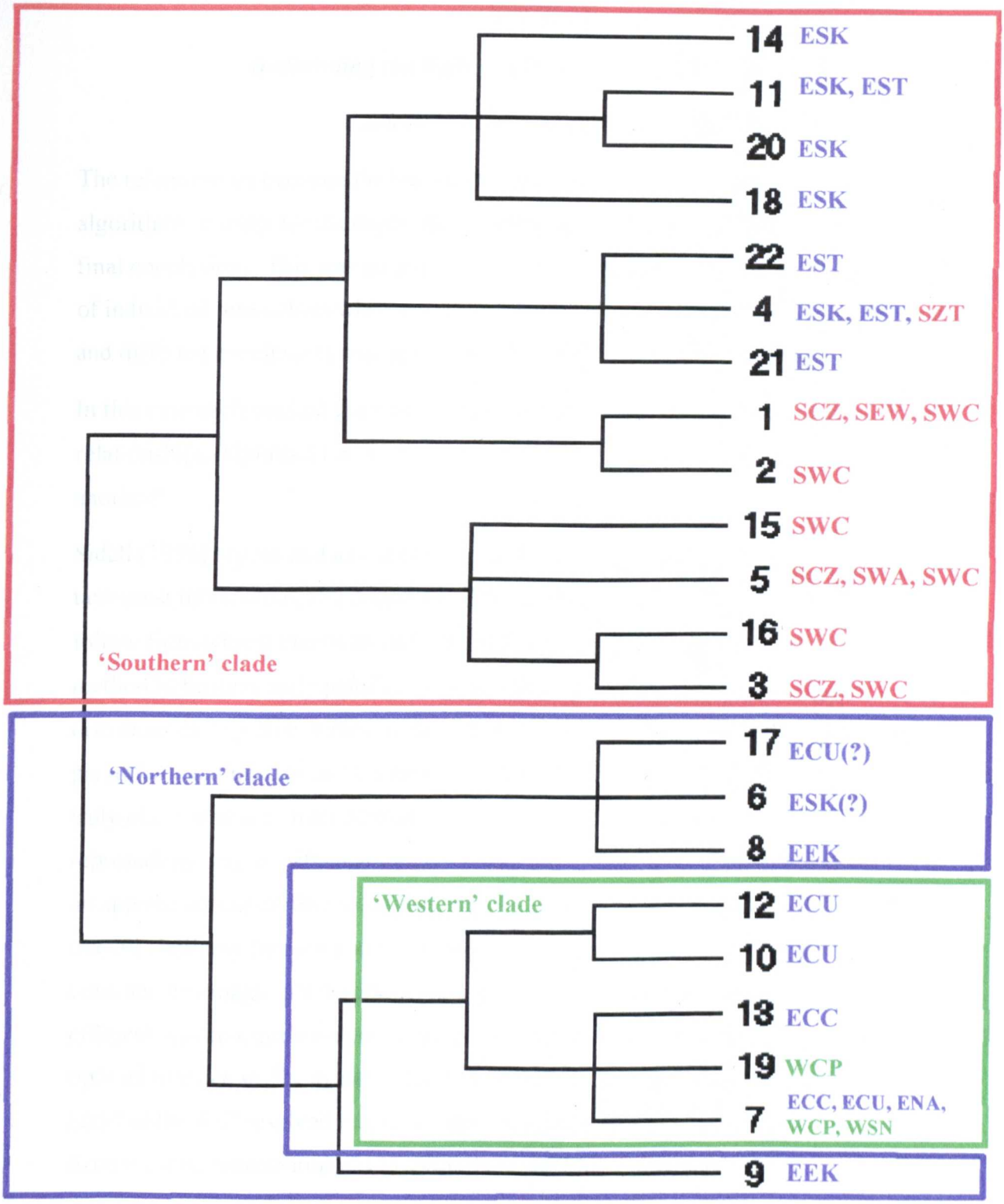

Figure 11.9: *Mid-point rooted semi-strict consensus maximum parsimony tree. The geographic origin of each haplotype is indicated. The three letter codes follow the geographic specimen sets defined in Chapter 6. The colour coding of these letters indicates the* a priori *defined regional groups. The phylogenetic analysis indicates that the* a priori *groups do not accurately reflect the phylogeographic results. The empirically identified clades are indicated in the corresponding colours. Rooting of the tree indicates a clear separation between northern and southern giraffe. Also, a monophyletic western clade and a geographically southern clade basal to the southern Kenyan / Tanzanian clade. Two clades (6 and 17) of doubtful provenance are indicated by (?). See the text for further discussion.*
Haplotypes are numbered as in Figure 11.2.

Discussion

Interpreting the Results of the Tree-Building Algorithms

Selecting a Tree Building Algorithm

The relationships between the haplotype sequences were assessed using NJ, MP and ML algorithms in order to investigate any possible effect of the tree building algorithm on the final conclusions. This was an attempt to emulate the vagaries of the preferences or biases of individual researchers when analysing their data. Might different results be achieved, and different conclusions reached, if different analytical methods are used?

In this case each method gave very similar optimal topologies with the same general relationships (Figures 11.6, 11.7 and 11.8). But are there reasons to prefer one method over another?

Sidall (1998) argues that any attempt to represent the phylogenetic history of a group of taxa must be ontologically consistent, that is "the justification of a method must logically follow from what it claims to achieve." (p. 210). He goes further and asserts that "for a method to be minimally justified, it must claim to be able to *explain* character distributions." (p. 210. Italics in the original). For this reason he suggests that maximum parsimony and maximum likelihood are the only two methods that can be claimed to be truly phylogenetic in their approaches. He dismisses distance based approaches as representing "mere mathematical clustering algorithms" (p. 210) that, at best, only provide an approximation of phylogeny that depends upon a number of assumptions (such as overall similarity being a good indicator of phylogenetic relatedness). Sidall (1998) considers the major utility of distance approaches to phylogeny reconstruction to be an efficient way to estimate a preliminary phylogenetic tree for further scrutiny, rather than an optimal tree *per se*. By its nature the NJ tree will provide resolved relationships between taxa but the well resolved tree may derive as much from the nature of the clustering as from the data representing the phylogenetic history of the taxa studied.

The debate between proponents of maximum parsimony and maximum likelihood has taken the form of theoretical arguments (Felsenstein, 1978; Farris, 1999) and empirical investigation (Huelsenbeck, 1995; Sidall, 1998). Certain 'zones' in the data space where either of the two methods is inaccurate have been identified. Huelsenbeck and Hillis, 1993, used the term the "Felsenstein Zone" to characterise the failure of maximum parsimony, with resulting long branch attraction, when faced with non-sister taxa at the end of long terminal branches. Huelsenbeck (1997) later provided an empirical example of the relationship between the strepsiptera and diptera. Following a similar simulation study Sidall (1998) countered with the recognition of the "Farris Zone" where MP outperforms

ML. Here it is sister taxa that reside at the ends of the long branches. Sidall (1998) points out that Huelsenbeck's (1997) empirical example may equally show erroneous 'long-branch repulsion' between the two groups. The two 'zones' are named after the two major protagonists in the debate.

The conditions under which maximum parsimony and maximum likelihood are deemed to fail, as described by the Felsenstein and Farris Zones, represent extremes that a practising molecular systematist may recognise as problematic from an initial examination of the data. If the mutation rate on a particular branch is that high, the derived sequence may be problematic when it comes to alignment. In such cases the practical alternative of sequencing a different portion of the genome may provide an answer. In the case of intraspecific phylogeographic analysis such long branches are highly unlikely. The results of the simulation studies suggest the two methods to be equally efficacious in the data space expected to be occupied by comparisons at the intraspecific level (assuming that the results of the simulation studies can be validly generalised to real data sets).

So, following Sidall's (1998) suggestion that any method must be ontologically justifiable, maximum parsimony and maximum likelihood are currently the only available methods for reconstructing phylogeny. Beyond this the choice becomes one of the preference of the individual researcher. As the three trees presented here provide similar, non-conflicting topologies, differing only in the resolution of the terminal branches Sidall's advice will be followed with the MP and ML trees discussed further (below).

Interpreting Bootstrap Support Values

Once a tree topology has been generated it is instructive to see how well the data support each of the hypothesised branches. In cladistic analysis resampling procedures are typically used, with the bootstrap support index (Felsenstein, 1985) the most common. Bootstrap support indices are presented here.

The bootstrap procedure randomly samples characters, with replacement, to create a pseudoreplicate data matrix with the same dimensions as the original data set. This effectively randomly removes some characters from the analysis and randomly reweights other characters. Multiple pseudoreplicate data sets are analysed and the percentage of the derived optimal trees that contain each resulting clade is calculated. The most frequently occurring clades, along with their percentage bootstrap occurrence values, are presented in the bootstrap tree.

Although widely used, there are a number of caveats that should be acknowledged when using the bootstrapping technique. If the bootstrap values obtained are to be interpreted in a similar way to statistical confidence limits (e.g. inferring that a bootstrap value of 95% indicates a significant level of confidence in the monophyly of that clade, as suggested by Whelan *et al*, 2001), then the number of informative characters used must be sufficient to generate a meaningful statistical distribution of all possible outcomes, that is 10^3 or 10^4 informative characters (Kitching *et al.*, 1998). Very few data sets are sufficiently large to invoke valid statistical interpretation of the values obtained, so this should be avoided. An alternative interpretation of the bootstrap values is that they provide an indication of the relative support between the clades. That is, clades supported by a large number of characters will be recovered frequently, while clades supported by one or a few characters will be supported less often, particularly if there is homoplasy in the data set. Indeed, due to the random nature of the resampling regime, clades unambiguously supported by one or a few synapomorphies on the preferred tree may not be recovered in the bootstrap analysis at all (or occur at such a low frequency to not be represented in the bootstrap tree). In this case the bootstrap tree should be interpreted as supporting the recovered clades but not necessarily rejecting the non-recovered clades (Kitching *et al.*, 1998). This is of great importance in using a bootstrapping approach to test clade support in intraspecific studies. Genetic differences within a species are, by definition, low. A haplotype may be differentiated by only one or a few mutations. The low levels of bootstrap support given to certain clades in this study must be interpreted in light of this. Rigorously applied population aggregation analysis (Davis and Nixon, 1992) to derive the haplotypes show the differences between haplotypes while other approaches demonstrate the robustness of the phylogeny. The use of exact search algorithms assures that the phylogeny produced is optimal for the chosen selection criteria. A test of the bootstrapping methodology (Hillis & Bull, 1993) demonstrated these caveats empirically.

Geographic Structure to Phylogenetic Relationships

Of the eleven haplotype groups identified only three (H02, H09 and H10) contain specimens from a single geographically restricted locality. These three haplotypes also have the lowest sample sizes (2, 2 and 3 respectively) suggesting that this may be a sampling effect. In contrast only two haplotypes (H04 and H07) occur between two regions (as defined in Chapter 6).

The cladograms demonstrate the following (Figure 11.10):

1. the monophyly of a western region group (although not that which was previously defined according to geographic distributions. See Chapter 6);
2. the paraphyly of the EEK group with respect to this western group; and,
3. the similarity of the Kenyan (ESK) and Tanzanian (EST) specimens and the affinity between these groups and the groups from the south.

I shall now discuss each of these in turn.

The Western Region

The western region was previously defined as consisting of specimens from northern Cameroon, Chad, CAR, (WCP) and Nigeria (WSN). However, the results of these analyses show that all specimens to the west of the Great Rift Valley and the Nile river form a monophyletic clade (including groups ECC, ECU, ENA, WCP and WSN). One haplotype (H07) contains individuals from each of these geographic areas from countries as wide apart as Nigeria, Cameroon, DRC, Sudan and Kenya.

This group is supported by a single synapomorphy; a deletion of a single base (Figure 11.4 and 11.11). This deletion occurs in the short ambiguous region previously investigated. Each of the arrangements of this 6bp ambiguous region provided the same tree topology with one and three synapomorphies supporting the clade (for the 'single gap, one site' and the 'single gap, two sites' arrangements respectively. Figure 11.4). For each of the alternative arrangement homoplasy is introduced for these characters.

Only one ECU individual was classified outside of this 'western group'. The affinities of FMNH27475 are discussed below.

The EEK Group

The EEK group is basal to the western group (as defined from these molecular results) and consists of two haplotypes; H08 (n = 7) and H09 (n = 2). However, these haplotypes differ by nine base substitutions (four synapomorphies and five homoplasies. Figure 11.11). H09 contains the sequence for the type specimen of *G. c. reticulata.*

Grouping with H08 in a monophyletic clade are a unique haplotype and H06. This group is founded upon two synapomorphic base substitutions. The unique haplotype is FMNH27475 and is part of the ECU group coming from northern Uganda. This specimen was presented to the Field Museum of Natural History in 1927 with a provenance given as "Uganda: Northern part of country". Such a locality description is sufficiently vague to

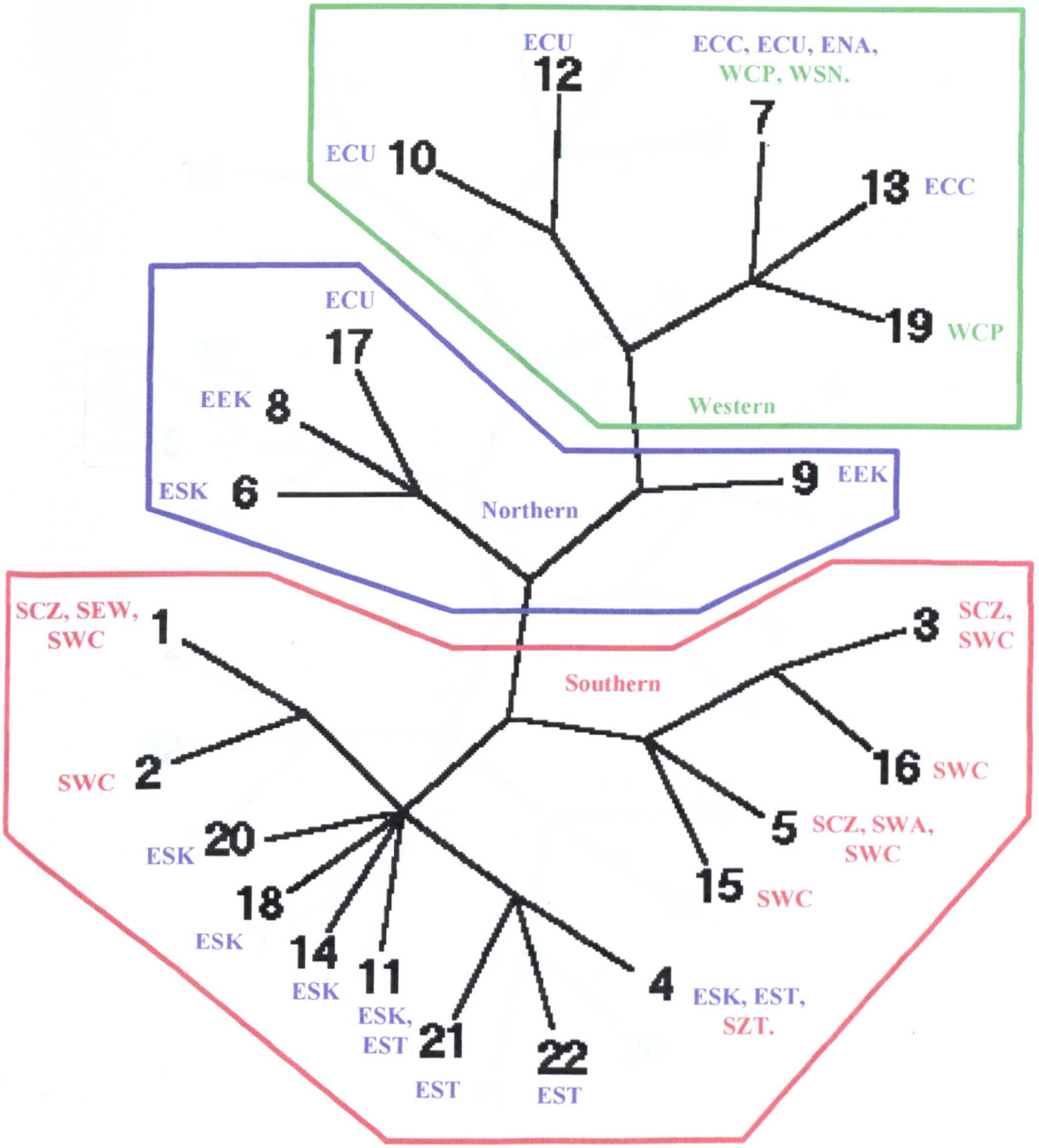

Figure 11.10: *Strict consensus of 12 MPTs showing geographic distributions of haplotypes. Three letter geographic group codes refer to the geographcally restricted specimen sets (see Chapter 6) The colour coding of these letters indicates the* a priori *defined affinities of the specimens. The identified clades are identified by the corresponding colours. Separation between northern and southern groups is indicated, as is the monophyletic 'western' clade.* Haplotypes are numbered as in Figure 11.2.

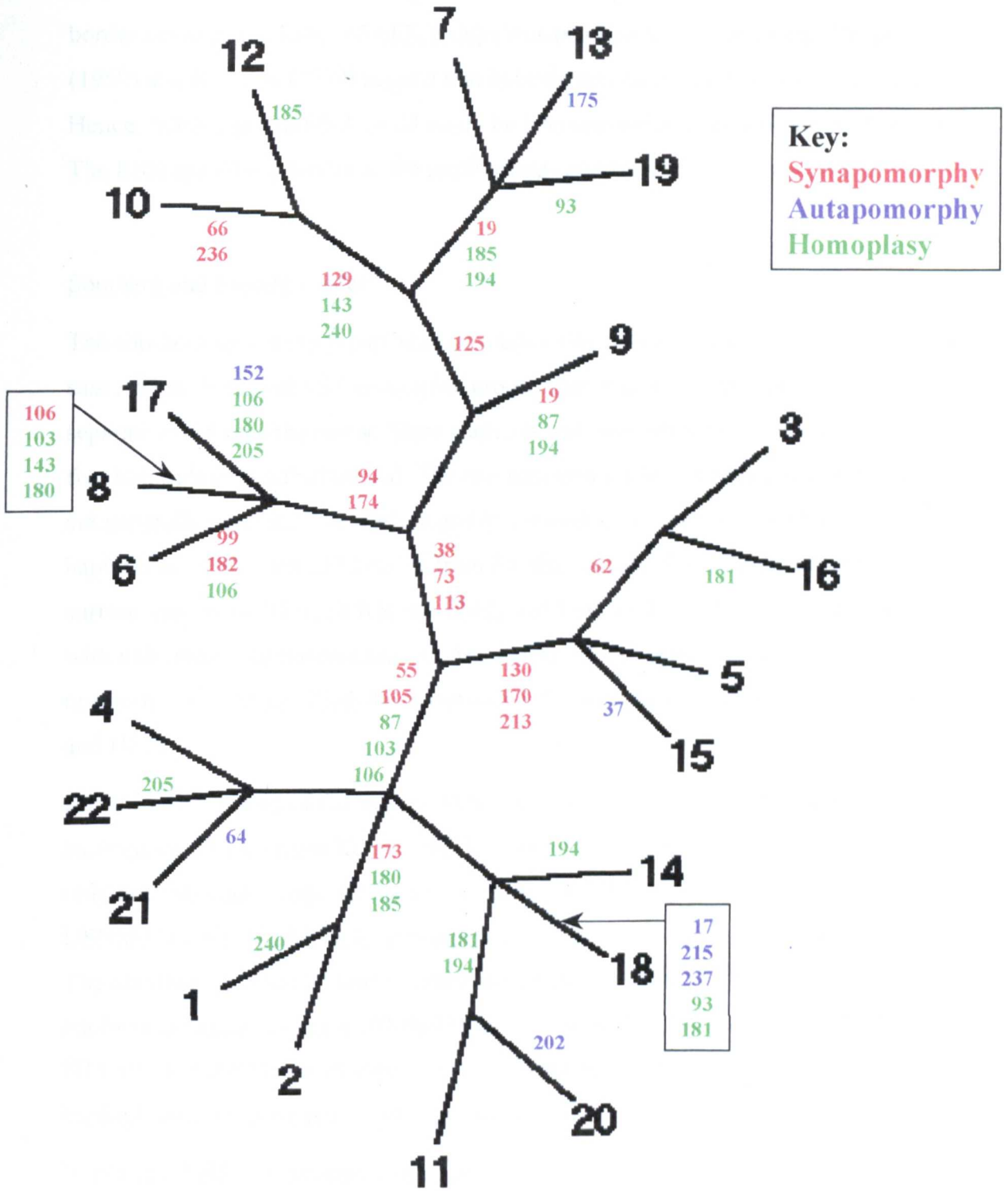

Figure 11.11: *Semi-strict Maximum Parsimony Tree showing the distribution of base substitutions on the tree. Numbers refer to the sequential bases in the 263 base pair sequence of mtDNA control region. Sequence data is summarised in Table 11.6 and presented in full in Appendix 5.11.3.*
Haplotypes are numbered as in Figure 11.2.

raise valid concerns over its accuracy. Meanwhile, H06 contains two individuals included in the ESK group due to their origin to the south of both the Tana and Athi rivers. The border between the ESK and EEK groups was taken to be the Tana river. However, Stott (1959) and Kingdon (1979) suggest that hybrids may occur south of the Tana River. Hence, 'EEK type' mtDNA could easily be introgressed into the northern ESK specimens. The ESK specimens further to the south group separately.

Southern and Eastern Clades

The southern specimens group into two clades, one of which is more closely related to the east African ESK and EST geographic groups than it is to the other southern clade. The separation between the two southern clades is well supported by six synapomorphies (plus five homoplasious substitutions). The two southern clades, although geographically mixed, are generally separate. The western group, consisting of H03, H05 and the two unique haplotypes EEIGc and EEI2 derive from Etosha National Park (Namibia) and the surrounding areas (H03, EEIGc and EEI2) and from southern Zimbabwe (H03 and H05) with individuals from Botswana and Angola (H05). The eastern group extends through northern South Africa, Zimbabwe, Botswana and into the extreme east of Namibia (H01 and H02).

It is the eastern-most of the two southern clades that groups with the east African haplotypes. The southern Kenyan and Tanzanian haplotypes show little resolution with only one sub-clade supported by a single synapomorphy (H04, USNM251797 and USNM251798). The MP strict consensus tree shows a seven-way polytomy at this node. The maximum likelihood resolves the relationships by grouping the three unique haplotypes in that polytomy (BMNH1898.7.2.5, FMNH127880 and USNM162017) with H11 into a clade. However, there is no single character (either synapomorphic or homoplasious) that supports such a grouping.

Haplotype 4 (H04) contained single specimens from southern Kenya (ESK) and Tanzania (EST), as well as all specimens from the Luangwa Valley in Zambia (SZT). The Luangwa Valley giraffe is currently classified as a separate subspecies; *G. c. thornicrofti*.

Interpreting the Phylogeographic Structure

Mid-point rooting of the semi-strict consensus of the 12 MPTs (Figure 11.8) roots the cladogram between northern and southern groups. The northern group then separates a monophyletic western group from a northern Kenya / Ethiopia / Somalia group. The

western group has its eastern boundary at the Nile River in Sudan and the Great Rift Valley in Kenya. All specimens (except one that may have an unreliable provenance) to the west of these physical barriers group as a monophyletic clade. The giraffes of northern Kenya (EEK group) are basal sister taxa to the western group and, although grouping as sisters, do not form a monophyletic cluster corresponding to the EEK group. Therefore these northern Kenyan giraffe are paraphyletic after the removal of the western clade, perhaps suggesting the origin of the western clade from within this northern Kenyan group.

The northern Kenyan and western groups are strongly differentiated from the southern and southern Kenyan / Tanzanian populations (supported by three synapomorphies).

A single haplotype is shared between eastern and southern regions. The inclusion of the southern Kenyan / Tanzanian groups with the southern clades, makes the east African group (as previously defined according to the criteria of geographic proximity) polyphyletic. One of the southern clades groups closely with the southern Kenyan and Tanzanian specimens (supported by two synapomorphies and 94% bootstrap support) while the other is a well-differentiated (five synapomorphies) basal group.

The relationships of the haplotypes within the south are complex. The major haplotype groups ([H01, H02] and [H03, H05, EEI2, EEIGc]) overlap extensively through Botswana and Zimbabwe. As a result, each transgresses formerly recognised subspecies boundaries. The former haplogroup tends to be further east and extends from the Kruger National Park in South Africa, through Zimbabwe and Botswana to the extreme east of Namibia. Meanwhile, the latter group includes an Angolan individual with specimens from Etosha National Park in northern Namibia and extends across through Botswana into southern Zimbabwe. Interpretation of the rooted cladogram suggests a temporal, and concomitant geographical transition, with the 'western southern' haplotypes as basal. The inference from the cladogram is that individuals from this western-southern haplogroup moved eastwards, where the population remained for some time, before expanding its range back towards the west and also migrating northwards, through the Luangwa Valley and into Tanzania and Kenya.

Interpretation as 'Evolutionary Units' and assessment of Conservation Priorities

Three geographically separated monophyletic groups can be identified from the cladograms. The most robust separation is between the northern clade, including individuals from northern Kenya westwards across the Great Rift Valley into Uganda, Sudan, DRC, CAR, Chad and Nigeria, and the southern group including all individuals to the south of southern Kenya and into the southern African sub-region.

Within these broad groups, only the group to the west of the Great Rift Valley forms a widespread monophyletic group that is also geographically isolated from other clades. In the southern group the 'western' clade was strongly differentiated, although this shows extensive geographical overlap with the other southern clades. The separation of these two major southern clades suggests previous fragmentation of the groups with resulting fixation of two mtDNA haplotypes. Subsequent range expansion by one, or both, populations has resulted in phylogenetically differentiated but geographically overlapping clades. The basal position of the western-most group suggests that this may represent an ancestral (or, at least, most closely emulate the ancestral) haplotype that then moved east before expanding its range back to the west and northwards into east Africa. Although 'evolutionarily distinct' according to the mtDNA data it must be remembered that this information pertains only to the maternal line of inheritance. The recent secondary contact and broad range of overlap suggests that the nuclear genome is likely to provide a more homogeneous geographic pattern of genetic variation in southern Africa.

From these genetic data, the northern and southern groups must be considered separate units for conservation as they represent reciprocally monophyletic clades. In the north, the western group is represented by a widespread monophyletic clade. Accordingly all giraffes to the west of the physical barrier of the Great Rift Valley and the Nile River should be considered as a unit. Although a paraphyletic grouping of two (apparently quite divergent) haplogroups, the EEK group of northern Kenya is well differentiated from the specimens from southern Kenya and should be considered as a separate group.

In the south, the western-southern group is basal to a clade containing Southern Kenyan and Tanzanian populations grouped together with Zambian (Luangwa Valley) giraffes and with the other southern giraffe haplogroup in a polytomy. This basal group can be recognised as a separate group by its mtDNA. However, the extensive overlap between the 'eastern-southern' and 'western-southern groups implies interbreeding must be occurring that is not demonstrated by the mtDNA phylogeny. For this reason, despite the differentiation, the two southern groups should be considered as a single unit. However, where one haplogroup is present exclusively (in Namibia for example) care should be taken in any management of the population.

The Luangwa Valley group nests within a clade from southern Kenya and Tanzania. According to genetic criteria the Masaai giraffe of southern Kenya and Tanzania should be grouped with the Thornicroft's giraffe of the Luangwa Valley. However, the geographic isolation of the Zambian population suggests that other criteria could be invoked to recognise the Luangwa giraffe as a separate unit from the east African group.

The next chapter uses nested clade analysis to investigate the nature of population history events using the same genetic data.

CHAPTER 12:

THE POPULATION HISTORY OF THE GIRAFFE IN SUB-SAHARAN AFRICA:

INFERENCE USING NESTED CLADE ANALYSIS OF MITOCHONDRIAL DNA CONTROL REGION SEQUENCES

Introduction

The ease of acquisition of molecular data combined with phylogenetic reconstruction methodology and biogeographic distribution data for conspecific populations has allowed intraspecific population structure to be studied for many taxa. This is the burgeoning field of phylogeography (Avise *et al.*, 1987; Avise, 2000). However, there are some concerns that methods used to reconstruct species level and higher level phylogenies may not be appropriate for reconstruction of intraspecific relationships (Crandall and Templeton, 1996). Also, while the phylogeographic approach does consider spatial and temporal patterns between populations the comparisons made tend to be qualitative and not founded within an explicit statistical framework.

The use of Minimum Spanning Trees (MSTs) or Minimum Spanning Networks (MSNs) in Nested Clade Analysis (NCA) explicitly considers temporal and spatial dimensions within species population histories in a statistical framework. The following section considers the application of NCA analysis to the population history of the giraffe, *Giraffa camelopardalis*.

Concerns with using Phylogenetic Reconstruction Algorithms below the Species Level

Any conclusions drawn from an analysis can only be as good as the information used and the analytical techniques applied. Questions posed and inferences drawn at the intraspecific level rely on a well-founded and reliable estimate of phylogenetic relationships. However, Crandall and Templeton (1996) were concerned that "there are many phenomena that exist at the population level that lead to a lack of resolution of phylogenetic relationships when traditional interspecific methods of phylogeny reconstruction are applied at this level" (p. 82-83). They listed their concerns as:

1. Intraspecific populations, by their nature, have a lower level of phylogenetically informative variation, resulting in fewer characters for the analysis;
2. In higher level phylogenies ancestors are assumed to be extinct. Meanwhile, in genetic studies at the intraspecific level the ancestral haplotypes are expected to persist in the species;
3. Higher level phylogenies are assumed to be bifurcating. However, a given population haplotype may undergo independent mutations producing multiple descendent haplotypes from a single ancestor;
4. DNA segments may undergo recombination. Such a phenomenon is not accounted for in standard phylogeny reconstruction methods.

Lansmann *et al.* (1981) defended the use of the mitochondrial DNA haplotype as the Operational Taxonomic Unit (OTU) in intraspecific analyses while using standard phylogenetic methods citing the lack of recombination in this molecule. This effectively removes the fourth objection. The other three objections are not necessarily valid either, at least for cladistic (maximum parsimony) analysis.

1. Cladistic analysis operates according to the corroboration of hypotheses of primary homology (equivalence of character states between OTUs). The maximisation of the proportion of secondary homologies (corroborated primary homologies) acts as the optimisation criterion for the selection of a phylogenetic tree. A secondary homology on the phylogenetic tree is then considered to be a synapomorphy. It does not matter how many synapomorphies are available, as long as they are consistent. The assumption is made that secondary homologies equate with synapomorphies, rather than with homoplasies that do not trace phylogenetic events. Hence, the only necessary criterion is that the signal resulting from synapomorphy should outweigh the homoplasy signal, rather than it being an issue of absolute numbers. With lower levels of variation any problem of 'long branch attraction' (where homoplasies overwhelm the true phylogenetic signal provided by synapomorphies leading to erroneus hypotheses of relatedness) is unlikely to occur. A problem may arise when trying to assign support values to branches of a phylogenetic tree using a resampling procedure (such as bootstrapping). Clades based upon one or a few synapomorphies may not be represented in the majority rule bootstrap support tree simply due to the sampling procedure.
2. Cladistic analyses do not explicitly state that ancestors are extinct. Contemporary OTUs that emulate ancestral character state sets may well exist (and would be

represented as a terminal, zero-length branch). Cladistic analysis explicitly aims to reconstruct sister taxon relationships. To do this it uses observations of contemporary character states. Logically, contemporary OTUs can not have an ancestor – descendent relationship. What cladistic analysis does do is to reconstruct relationships according to hypothesised sharing of synapomorphic character states, and so reconstructs hypothesised ancestors at nodes in the cladogram. Any terminal taxon (particularly, but not necessarily, if it is lacking characteristic autapomorphies) may represent the ancestral form of its sister taxon; equally, it may not. Cladistic analysis does not assume the extinction of ancestors, nor does it preclude the contemporary occurrence of an 'ancestral' form. The assumptions and results of the analysis are neutral in this regard, any suggestion of ancestor – descendent relationships that may be implied are value judgements made by the researcher following interpretation of the phylogeny and other data.

3. The assumption of a bifurcating tree is a convention for cladistics, not a requirement. Where 'soft' polytomies occur (those that occur due to the lack of resolution in the data) additional data should be sought to resolve them. 'Hard' polytomies, resulting from multiple independent mutations of the same ancestral form, should not be resolvable by the addition of further data. However, if the balance of evidence at a hard polytomy falls one way or the other simply by chance sampling then the maximum parsimony algorithm 'prefers' a bifurcating topology. The methodology makes no assumption of the evolutionary process being strictly bifurcating, the bifurcating model is simply chosen as the simplest representation of a branching tree. Again, the interpretation of the tree is important. A lineage could remain unchanged (in terms of its character states) along a branch where cladogenetic events are 'budding off' differentiated populations. Such a scenario can be interpreted as an 'ancestral' stock producing multiple daughter populations.

The criticisms may be valid for distance and maximum likelihood phylogeny estimation techniques used at the intraspecific level as neither technique makes explicit hypotheses of relationship. Distance methods, such as Neighbour Joining, are, essentially, mathematical clustering algorithms and rely on the assumption that overall similarity indicates proximity of evolutionary relatedness. Hence, plesiomorphic and homoplasious similarities are counted as indicating relationship as much as synapomorphies. Where phylogenetically informative variation is low the phylogenetic signal (synapomorphies) may be outweighed by the plesiomorphic and homoplasious similarities leading to erroneous relationships being hypothesised, based on a single distance measure.

In accord with the distance methods Maximum Likelihood algorithms do not erect hypotheses of primary homology but instead uses a probability based approach to decide on the most likely tree topology. In this way, the maximum likelihood algorithm may be similarly affected by homoplasious and plesiomorphic data overwhelming the true phylogenetic signal from the synapomorphies where variation is low.

The nature of the Neighbour Joining algorithm always provides a fully resolved, bifurcating tree with Operational Taxonomic Units (OTUs) positioned at the tips. Maximum Likelihood also uses the bifurcating tree convention with terminal OTUs. This neither assumes that evolution is consistently bifurcating nor that all ancestral taxa are extinct but are simply conventions of the technique. The interpretation of the tree topology is the responsibility of the researcher.

The problem with using standard phylogeny reconstruction techniques at the intraspecific level is that the relationships expressed refer explicitly to nested levels of sister taxon relationships. Any additional relationships inferred (e.g. ancestor – descendent) are suppositional and based upon other information. The true value of the phylogeny reconstruction algorithms directed at the intraspecific level that include extant OTUs as nodes in the tree is that they make the nature of the relationships explicit and so allow the testing of a wider range of hypotheses.

Minimum Spanning Trees and Minimum Spanning Networks

A minimum spanning tree seeks to join all taxa together according to a given optimality criterion. Typically the optimality criterion minimises a distance-based or parsimony-based measure. Rather than restricting taxa to terminal tips of branches (as in standard phylogenetic reconstruction methods) MSTs allow sample taxa to be placed at interior nodes. Hence, explicit hypotheses of ancestor – descendent relationships are constructed. MSNs extend this idea and portray ambiguities by allowing looped relationships between taxa. These loops may be a result of genetic recombination events through evolutionary time or may represent ambiguities due to homoplasious mutations. Methods of MST/MSN construction are reviewed by Posada and Crandall (2001).

Nested Clade Analysis

NCA (Templeton, 1998) combines temporal and spatial data with phylogenetic information to allow evolutionarily (temporal) nested analyses of spatial structuring of

genetic variation. Different processes in a population's history leave different 'signatures' in the geographic distribution of haplotypes (Templeton *et al.*, 1995). The contemporary temporal and spatial conditions at the time of a mutation event leave a record embedded in the phylogenetic history of the taxon. NCA seeks these patterns and, using general observations concerning the age and geographical extent of each clade[1], implies certain processes in the evolutionary history of a clade (Templeton *et al.*, 1995).

With restricted gene flow the geographical extent of a haplotype is closely correlated to its age (Nath and Griffiths, 1993; Neigel *et al*, 1991; Neigel and Avise, 1993; Slatkin, 1991; 1993). Relative ages are inferred from the nesting structure. By definition, clades that nest within other clades are younger, occurring later in evolutionary time. Various combinations of these two factors compared within clades and between nested clades allow the testing of the null hypothesis of no genetic structuring with respect to geographical location. The rejection of the null hypothesis leads to the inference of geographically structured genetic variation. NCA now allows the discrimination among various potential biological explanations (e.g. restricted gene flow, population fragmentation or range expansion) for this geographic structure (Templeton *et al.*, 1995; Templeton, 1998).

This chapter examines the inferred population history of a single species; the giraffe, *Giraffa camelopardalis*, using mtDNA control region sequence variation.

Material and Methods

Tissue Samples Used

Tissue samples were collected from museum specimens and represent individuals from across the contemporary and recent historical range. The collection, handling and storage of tissue samples are described in Chapter 3. A list of tissue samples acquired for this study is presented in Appendix 5.11.1.

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[1] In nested clade analysis the term clade is used to refer to the nesting units derived from the minimum spanning tree. It should be noted that this is an incorrect use of the term that, correctly, refers to a monophyletic group resulting from a splitting (cladogenic) event on a cladogram, and includes the ancestor and all descendant taxa. However, it will be used here for consistency.

DNA Extraction, Amplification, Cleaning, Sequencing and Sequence Alignment

The extraction, amplification and sequencing of DNA from the museum specimens is described in the Chapter 12. Sequence alignment and the resolution of ambiguities is also described in Chapter 12.

Nested Clade Analysis

Estimation of the Minimum Spanning Tree

The statistical parsimony based methodology of Templeton *et* al. (1992), implemented by the TCS program (version 1.06. Clement *et al.*, 2000), was used to build a minimum spanning network for 22 haplotypes (Figure 12.1). This software was preferred over other MST constructing algorithms (see Posada and Crandall, 2001) as it indicates 'missing' haplotypes that are not represented in the data set. Missing haplotypes are those haplotypes that are a single substitution step away from a represented haplotype and so form 'single step links' between the represented haplotypes where these differ by more than a single mutation. These missing haplotypes are necessary for consistency in the nesting procedure.

Nesting Clades (Nested Statistical Design)

The standard nesting rules of Templeton *et al.* (1987), Templeton and Sing (1993) and Crandall (1996) were used to progressively nest haplotypes. The nesting levels refer to the number of substitution steps between the haplotype groups in the MST. By definition, individuals of the same haplotype form zero-step clades. Starting from the tips of the MST and progressing along the branches, haplotypes differing by a single substitution are grouped to form 1-step clades. The 1-step clades then become the grouping unit and are grouped in the same way to give 2-step clades. This procedure continues until the entire MST is enclosed within a single group. Any ambiguities are resolved according to the standard nesting rules. The final nested MST is given in Figure 12.2.

Additional Information

Topological information for each nesting unit indicating its position on a tip or as an internal node is also gleaned from the MST.

The input data is given in Appendix 5.12.1.

Nested Statistical Analyses

Hypotheses of geographic structure to haplotype distributions can be tested in two ways; nested geographical contingency analysis and nested geographical distance analysis.

Nested Geographical Contingency Analysis

Nested geographical contingency analysis (Templeton and Sing, 1993) is the simpler of the two approaches. It uses estimation of χ^2 statistics from an exact $c \times l$ permutational contingency test (using a contingency table with c clades and l locations) to test the null hypothesis of no association of clades with geographic localities. As such it treats localities as categorical variables and does not take in to account the distances between geographically restricted haplotype groups.

Nested Geographical Distance Analysis

The inclusion of geographical distance data with the nested clade design allows more detailed spatial, as well as temporal, patterns of genetic variation to be investigated (Templeton, 1998). Geographical data was included in the current analysis as the latitude-longitude co-ordinates of the centroid of each geographically restricted specimen set (see Chapter 6). The centroid was determined empirically for the available specimens by calculating the average latitude-longitude co-ordinates from the specimens in each geographic set.

The geographical data are quantified in two ways: The clade distance, D_c, measures the range of a particular clade by calculating the average distance that each individual from the given clade lies from the geographical centre of all individuals in that clade. The nested clade distance, D_n, indicates the position of a clade relative to its neighbours by taking the average distance that individuals from a given clade lie from the geographical centre of all individuals from the next higher level clade. These distances, coupled with the topological information for each nesting unit[2], allow the calculation of two further statistics: The difference between the interior and tip values for the both the clade distances and the nested clade distances for each nested clade.

[2] Is each clade an interior (I) or tip (T) clade?

The results of the nested geographical distance analysis were interpreted using the key provided by Templeton (1998[3]).

Both analyses are implemented using the GeoDis software of Posada *et al.* (2000).

Results

Genetic Information

Genetic information, including sequence alignments (Appendix 5.11.3) and haplotype groups (Appendix 5.11.2), is reported in the preceding section. The distribution of haplotypes between geographically restricted specimen sets is given in Table 12.1.

Haplotype Groups	ECC	ECU	EEK	ENA	ESK	EST	SCZ	SEW	SWA	SWC	SZT	WCP	WSN
H01							1	4		19			
H02										2			
H03							14			6			
H04					2	1					6		
H05							1		1	2			
H06					2								
H07	3			2								2	1
H08			7										
H09			2										
H10		3											
H11					1	1							
Unique Haplotypes													
A1236		1											
AMNH53546	1												
BMNH1898.7.2.5					1								
EEI2										1			
EEIGc										1			
FMNH127880					1								
FMNH27475		1											
PCNNChad138												1	
USNM162017					1								
USNM251797						1							
USNM251798						1							

Table 12.1: *Distribution of haplotypes between geographically restricted specimen sets. Geographic specimen sets are described in the Chapter 6.*

[3] Updated at http://bioag.byu.edu/zoology/crandall_lab/geodis.htm

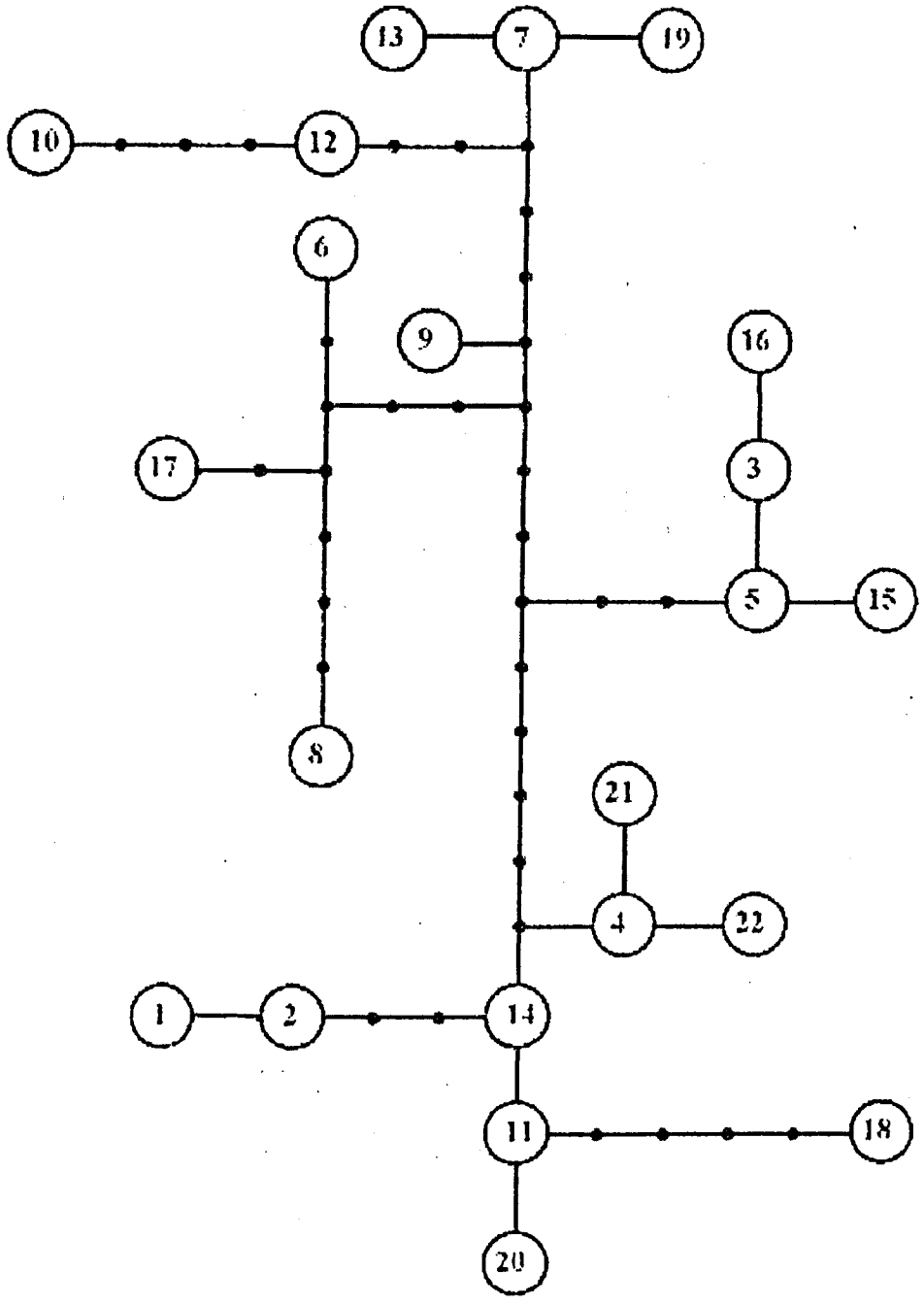

Figure 12.1: *Minimum Spanning Tree of the 22 haplotypes derived from 98 giraffes. Open circles indicate haplotypes (listed below). Black dots represent 'missing' haplotypes not represented in the sampled individuals but hypothesised to exist as intermediate mutational stages.*

1 = H01; 2 = H02; 3 = H03; 4 = H04; 5 = H05; 6 = H06; 7 = H07; 8 = H08; 9 = H09; 10 = H10; 11 = H11; 12 = A1236; 13 = AMNH53546; 14 = BMNH1898.7.2.5; 15 = EEI2; 16 = EEIGc94.11.25.6MA; 17 = FMNH27475; 18 = FMNH127880; 19 = PCNNChad138; 20 = USNM162017; 21 = USNM251797; 22 = USNM251798.

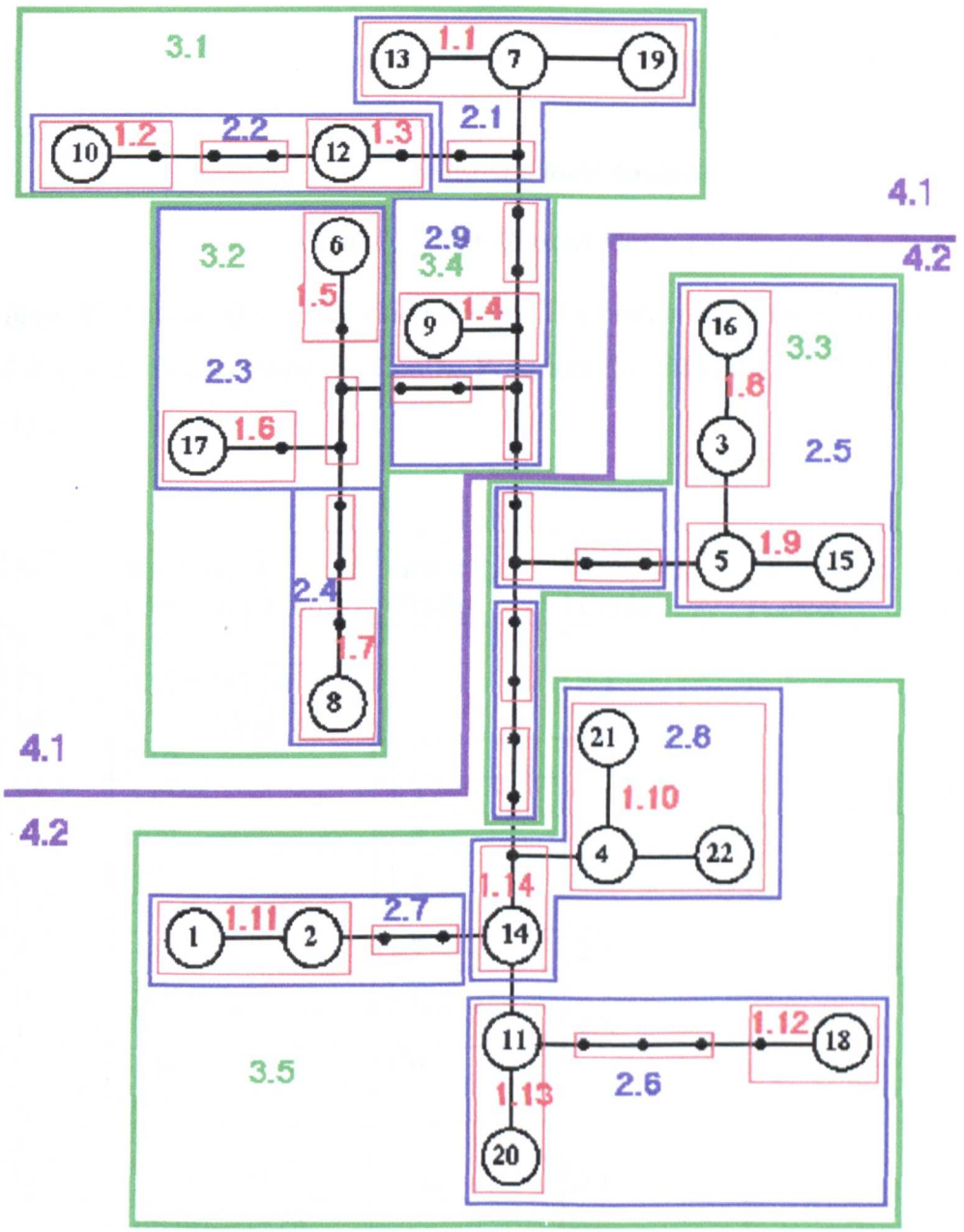

Figure 12.2: *Nested Clade Structure. Hierarchical groups represent the nested statistical design used for nested clade analysis. Nesting rules follow Templeton* et al., *(1987), Templeton and Sing (1993) and Crandall (1996). See Figure 12.1 for the key to the haplotypes.*

Minimum Spanning Tree

The minimum spanning tree is given in Figure 12.1. The MST topology is congruent with the phylogenetic reconstructions presented in the previous section (Figures 11.5, 11.6 and 11.7) at higher levels of relatedness and non-conflicting at lower levels (closer to the tips).

Nested Clade Analysis

Nested Clades (Nested Statistical Design)

Figure 12.2 shows the nested clade design. The nesting design is summarised in Table 12.2. Output results (from the GeoDis Program, Posada *et al.*, 2000) are given in Appendix 5.12.1.

No.	Haplotypes (Zero-step Clades)	One-step Clades	Two-step Clades	Three-step Clades	Four-step Clades
7	H07	1.1	2.1	3.1	4.1
13	AMNH53546				
19	PCNNChad138				
10	H10	1.2	2.2		
12	A1236	1.3			
9	H09	1.4	2.9	3.4	
6	H06	1.5	2.3	3.2	
17	FMNH27475	1.6			
8	H08	1.7	2.4		
16	EEIGc	1.8	2.5	3.3	4.2
3	H03	1.9			
5	H05				
15	EEI2				
4	H04	1.10	2.8	3.5	
21	USNM251797				
22	USNM251798				
14	BMNH1898.7.2.5	1.14			
1	H01	1.11	2.7		
2	H02				
18	FMNH127880	1.12	2.6		
11	H11	1.13			
20	USNM162017				

Table 12.2: *Summary of nested clade design. The minimum spanning tree is given in Figure12.1. The nested clade diagram is given in Figure 12.2.*

The penultimate step of the nesting procedure (4-step clade) separated northern and southern groups. Only one haplotype (H05) was shared between these two groups.

In the north, the three-step clades showed a separation between western geographical groups (ECC, ECU, ENA, WCP and WSN) and eastern groups (EEK, ESK. One ECU individual was included here). Two distinct EEK groups were maintained in the northern three step clades (clade 3.2 with haplotypes 6, 8 and 17; clade 3.4 with haplotype 9). In the southern three-step clades the two clades overlapped geographically but were dominated by SCZ and SWC individuals respectively.

One- and two-step clades were largely congruent due to the typical multiple substitutions between haplotypes. As a result one-step clades tended to represent single haplotypes while two-step clades either clustered haplotypes within a single geographically defined set or between geographically contiguous sets. In the north the extreme western groups (ECC, ENA, WCP and WSN) grouped together at the one- and two-step clade level (1-1 and 2-1). The other two step clades brought together individuals from single groups (although 2-3 had the single ECU individual of doubtful provenance grouped with ESK individuals). In the south the two-step clade separation tended to be between SCZ dominated clades, SWC dominated clades and the ESK/EST/SZT clade.

Nested Geographical Contingency Analysis

The null hypotheses of no association between clades and geographic location were rejected for comparisons within clades 3-1, 3-2, 3-5, 4-1, 4-2 and the total cladogram (Table 12.3).

Clade	Permutational χ^2 Statistic	Probability
1-1	4.063	0.873 ns
1-8	2.100	0.326 ns
1-9	0.833	1.000 ns
1-10	6.519	0.180 ns
1-11	0.516	1.000 ns
1-13	0.750	1.000 ns
2-5	6.471	0.063 ns
2-6	4.000	0.499 ns
2-8	3.723	0.246 ns
3-1	14.000	<0.001 ***
3-2	10.000	0.015 *
3-5	56.700	<0.001 ***
4-1	24.168	0.017 *
4-2	35.345	<0.001 ***
Total Cladogram	86.503	<0.001 ***

Table 12.3: *Nested geographical contingency analysis. Clades with no genetic or geographical variation or that consist entirely of a lower level clade are not shown. The permutational χ^2 statistic derives from 1,000 permutations.*

Table 12.4 gives the composition of the significant clades along with the geographic groups represented in each nested clade.

Inspection of Table 12.4 demonstrates that, while some sub-clades overlap in their geographical extent, there is a general lack of congruence between the sub-clades within each significantly different clade.

Clade	Sub-clades	Geographic Groups
3-1	2-1	ECC(4), ENA(2), WCP(3), WSN(1).
	2-2	ECU(4).
3-2	2-3	ESK(2), ECU(1).
	2-4	EEK(7).
3-5	2-6	ESK(3), EST(1).
	2-7	SCZ(1), SEW(4), SWC(21).
	2-8	ESK(3), EST(3), SZT(6).
4-1	3-1	ECC(4), ECU(4), ENA(2), WCP(3), WSN(1).
	3-2	ECU(1), EEK(7), ESK(2).
	3-4	EEK(2).
4-2	3-3	SCZ(15), SWA(1), SWC(10).
	3-5	ESK(6), EST(4), SCZ(1), SEW(4), SWC(21), SZT(6).
Total	4-1	ECC(4), ECU(5), EEK(7), ENA(2), ESK(2), WCP(3), WSN(1).
	4-2	ESK(6), EST(4), SCZ(16), SEW(4), SWA(1), SWC(31), SZT(6).

Table 12.4: *Clades showing significant geographical structure in genetic variation according to nested geographical contingency analysis. The geographic groups represented in each clade are listed, along with the sample size from each location.*

Nested Geographical Distance Analysis

Four clades showed significant structure between clades and geographical distribution (i.e. allowed statistically significant rejection of the null hypothesis of no geographic structuring to genetic variation); 2-5, 3-1, 3-5 and 4-2. The results of the nested geographical distance analysis are presented in Table 12.5. The decision making process is detailed in Table 12.6.

Clade 2-5 contained two sub-clades (1-8 and 1-9). Each contained two haplotypes from three localities. The geographic ranges of the haplotypes overlapped extensively with two of the three localities present in each nested clade. However, clade 1-8 was dominated by individuals from southern central Zimbabwe (SCZ. 14 of 21 individuals) while clade 1-9 had mainly south western African individuals (SWC. 3 of 5). Restricted gene flow with isolation by distance was inferred from the key (Templeton, 1998) as separating these clades.

Zero-step Clades			One-step Clades			Two-step Clades			Three-step Clades			Four-step Clades		
Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n
7	956	957												
13	0	849	1-1											
19	0	745												
I-T	956	160												
16	0	588												
3	219	230	1-8											
I-T	219	-358												
5	208	220												
15	0	542	1-9											
I-T	208	-323												
4	427	427												
21	0	8142	1-10											
22	0	8142												
I-T	427	-7715												
1	504	510												
2	0	658	1-11											
I-T	-504	148												
11	0	0												
20	0	0	1-13											
I-T	0	0												
			1-8	241	591									
			1-9	228	634	2-5								
			I-T	-12	43									
			1-10	427	485									
			1-14	0	972	2-8								
			I-T	-427	487									
			1-12	0	263									
			1-13	0	210	2-6								
			I-T	0	-53									
						2-1	932	938						
						2-2	0	1279	3-1					
						I-T	932	-341						
						2-3	0	0						
						2-4	0	0	3-2					
						I-T	0	0						
						2-6	233	1707						
						2-7	515	1029	3-5					
						2-8	529	560						
						I-T	52	-559						
									3-1	0	0			
									3-2	0	0	4-1		
									3-4	0	0			
									I-T	0	0			
									3-3	613	822			
									3-5	934	999	4-2		
									I-T	-321	-177			
												4-1	0	0
												4-2	0	0

Table 12.5: *Results of nested geographical distance analysis. The nested design is summarised in Table 12.2 and show in Figure 12.2. Composite clades (consisting of two or more clades or haplotypes) are signified by the nesting level followed by a sequential, ordinal number. Internal nodes in each clade are shaded. The superscript 'S' indicates the distance measure to be statistically significantly small (at the 5% level), while the superscript 'L' indicates it to be significantly large. The interpretation of these results is given in Table 12.6.*

Clade 3-1 contains two sub-clades (2-1 and 2-2). Clade 2-1 represents individuals from the west bank of the Nile across central and western Africa (groups ECC, ENA, WCP and WSN). Meanwhile clade 2-2 consists of individuals from Uganda, western Kenya and southern Sudan. The NCA results suggest that western African giraffe have differentiated from Ugandan giraffe following an allopatric fragmentation event at some time in their history.

Clade 3-5 contains haplotypes from southern and eastern Africa. Three sub-clades include individuals from southern Kenya and Tanzania and the Luangwa Valley in Zambia (2-8), Namibia, Botswana, Zimbabwe and South Africa (2-7) and southern Kenya and Tanzania (2-6). A contiguous range expansion event is suggested for this clade.

Clade 4-2 contains clades 3-3 and 3-5. Clade 3-3 is equivalent to clade 2-5, which is described above, as is clade 3-5. The process between these clades is ambiguous with the key unable to discriminate between range expansion / colonisation and restricted dispersal / gene flow.

Table 12.6 (below): *Inference of population processes by clade. The key steps with additional notes, including the thought processes that went in to making the decisions, are presented below (notes are in italics). Interpretations are only made for clades that have some significant values (an affirmative answer to the first question). Clades with no statistically significant values all answer no to the first question and are not presented. Statistical significance is determined from 1,000 data permutations. The key derives from Templeton (1998).*

Clade 2-5

	Question	Answer	Go to
1.	Are there any significant values for D_c, D_n or I-T within the clade? *The D_c value of tip clade 1-8 is significantly small.*	Yes	2
2.	Is at least one of the following conditions satisfied?		3
	a. The D_cs for some tips are significantly small and the D_cs for the interiors are significantly large or non-significant.	Yes	
	b. The D_cs for tips are significantly small or non-significant and the D_cs for some but *not* all of the interiors are significantly small.	No	
	c. The I-T D_c is significantly large.	No	
3.	Are any D_n and/or I-T D_n values significantly reversed from the D_c values, and/or do one or more tip clades show significantly large D_ns or interior clades significantly small D_ns or I-T significantly small D_n with the corresponding D_c values being non-significant?	No	4
4.	Do the clades (or two or more subsets of them) with restricted geographical distributions have ranges that are completely or mostly non-overlapping with the other clades in the nested group (particularly interiors), and does the pattern of restricted ranges represent a break or reversal from lower level trends within the nested series (applicable to higher level clades only)?	No	End
	Conclusion: Restricted Gene Flow with Isolation by Distance. This inference is strengthened if the clades with restricted distributions are found in diverse locations, if the union of their ranges roughly corresponds to the range of one or more clades (usually interiors) within the same nested group (applicable only to nesting clades with many clade members or to the highest level clades regardless of number), and if D_c values increase and become more geographically widespread with increasing clade level within a nested series (applicable to lower level clades only).		

Table 12.6 continued.

Clade 3-1

	Question	Answer	Go to
1.	Are there any significant values for D_c, D_n or I-T within the clade? *Tip clade 2-2 has a significantly small D_c value and a significantly large D_n value. The I-T D_c value is significantly large while the D_n value is significantly small.*	Yes	2
2.	Is at least one of the following conditions satisfied?		3
	a. The D_cs for some tips are significantly small and the D_cs for the interiors are significantly large or non-significant.	Yes	
	b. The D_cs for tips are significantly small or non-significant and the D_cs for some but *not* all of the interiors are significantly small.	No	
	c. The I-T D_c is significantly large.	Yes	
3.	Are any D_n and/or I-T D_n values significantly reversed from the D_c values, and/or do one or more tip clades show significantly large D_ns or interior clades significantly small D_ns or I-T significantly small D_n with the corresponding D_c values being non-significant?	Yes	5
5.	Do the clades (or two or more subsets of them) with restricted geographical distributions have ranges that are completely or mostly non-overlapping with the other clades in the nested group (particularly interiors), and does the pattern of restricted ranges represent a break or reversal from lower level trends within the nested series (applicable to higher-level clades only)? *The two clades nested in clade 3-1 represent the ECU group and the rest of the western groups respectively. The ranges of the two clades are mutually exclusive.*	Yes	15
15.	Are the different geographical clade ranges identified in step 5 separated by areas that have not been sampled?	Yes	16
16.	Is the species absent in the non-sampled areas? *The contemporary distribution suggests that giraffe are absent from intervening areas. However, the historical range is likely to have seen giraffe in between currently recognised ranges. The species is taken to not occur between the ranges.*	Yes	End
	Conclusion: Allopatric Fragmentation. (If inferred at a high clade level, additional confirmation occurs if clades displaying restricted and, at least partially, non-overlapping distributions are mutationally connected to one another by a larger than average number of steps.) *The alternative interpretation, taking a continuous historical distribution in to account, would see an ambiguous conclusion where the geographical sampling scheme would be considered to be inadequate to discriminate between possible population processes. However, as it is now impossible to sample the intervening ranges the conclusion of allopatric fragmentation, based on current ranges, is preferred.*		

Clade 3-5

	Question	Answer	Go to
1.	Are there any significant values for D_c, D_n or I-T within the clade? *The Dc value of tip clade 2-6 is significantly large while that of tip clade 2-7 is significantly small. Both of the D_c and D_n values of the interior clade 2-8 are significantly small. The I-T D_n value is significantly small.*	Yes	2
2.	Is at least one of the following conditions satisfied?		11
	a. The D_cs for some tips are significantly small and the D_cs for the interiors are significantly large or non-significant.	No	
	b. The D_cs for tips are significantly small or non-significant and the D_cs for some but *not* all of the interiors are significantly small.	No	
	c. The I-T D_c is significantly large.	No	
11.	Is at least one of the following conditions satisfied?		12
	a. The D_c value(s) for some tip clade(s) is/are significantly large.	Yes	
	b. The D_c value(s) for all interior(s) is/are significantly small.	Yes	
	c. The I-T D_c is significantly small.	No	
	Conclusion: Range Expansion.		
12.	Are the D_n and/or I-T D_n values significantly reversed from the D_c values?	No	End
	Conclusion: Contiguous Range Expansion. *Clade 3-5 combines clades 2-6, 2-7 and 2-8 that contain ESK/EST,*		

SCZ/SEW/SWC and ESK/EST/SZT individuals respectively. As clade 2-8 is interior this suggests a range expansion from east Africa (southern Kenya or Tanzania) into southern Africa.		

Clade 4-2

	Question	Answer	Go to
1.	Are there any significant values for D_c, D_n or I-T within the clade? *The D_c value for interior clade 3-3 is significantly small.*	Yes	2
2.	Is at least one of the following conditions satisfied?		5
	a. The D_cs for some tips are significantly small and the D_cs for the interiors are significantly large or non-significant.	No	
	b. The D_cs for tips are significantly small or non-significant and the D_cs for some but *not* all of the interiors are significantly small.	No	
	c. The I-T D_c is significantly large.	No	
5.	Do the clades (or two or more subsets of them) with restricted geographical distributions have ranges that are completely or mostly non-overlapping with the other clades in the nested group (particularly interiors), and does the pattern of restricted ranges represent a break or reversal from lower level trends within the nested series (applicable to higher-level clades only)?	No	6
6.	Do clades (or haplotypes within them) with significant reversals or significant D_n values without significant D_c values define two or more geographically concordant subsets, or are they geographically concordant with other haplotypes/clades showing similar distance patterns?	Too few clades.	7
	TOO FEW CLADES (≤2) TO DETERMINE CONCORDANCE – **Insufficient Genetic Resolution to Discriminate between Range Expansion/Colonisation and Restricted Dispersal/Gene Flow.**		
7.	Are the clades with significantly large D_ns (or tip clades in general when D_n for I-T is significantly small) separated from the other clades by intermediate geographical areas that were sampled?	No	8
8.	Is the species absent in the non-sampled areas?	No	**End**
	Conclusion: Sampling Design Inadequate to Discriminate between Isolation by Distance (Short Distance Movements) versus Long Distance Dispersal.		
	The key seems to be inconsistent for this particular process. Clade 4-2 contains a significantly small D_c value for nested clade 3-3 and no other significant values. From step 6 this pattern is not identified in the key steps making interpretation difficult. The conclusion reached at step 6 is, therefore, considered sufficient.		

Table 12.6 continued: *Inference of population processes by clade.*

Discussion

The nested clade analysis identified three population events, including restricted gene flow, range fragmentation and range expansion, as important in the population history of the giraffe. A comparison of the results of the NCA is made with the results of the phylogeographic analysis.

Minimum Spanning Tree

The MST provided a fully resolved topology with eight of the 22 haplotypes occurring on internal 'ancestral' nodes. The relationships indicated were largely concordant, and did not conflict, with those of the phylogenetic reconstructions presented in the preceding section.

Inferred Population History of the Giraffe

According to nested clade analysis three historical events have left interpretable traces in the genetic variation of giraffe across their sub-Saharan African range.

In the southern region, while two major haplotype groups ([H01, H02] and [H03, H05, EEI2, EEIGc]) overlap extensively through Botswana and Zimbabwe restricted gene flow with isolation by distance has affected the distribution of haplotypes between these two groups. The southern Zimbabwean population (SCZ) is shown as geographically separate from the more westerly group (SWC) on many continental distribution maps (e.g. Dagg, 1962; Kingdon, 1979; Skinner and Smithers, 1990. See Figure 6.1) as well as on detailed surveys of Zimbabwean wildlife (Child and Savory, 1964). Some authors consider the giraffe of western and southern Zimbabwe to represent two separate subspecies (e.g. Meester *et al.*, 1986). Communal lands, with subsistence agriculture or commercial farms, now cover the land between the two populated areas in Zimbabwe making current migration between the populations unlikely. These results suggest that this separation is either sufficiently recent that fragmentation has not yet allowed fixation (or near fixation) of haplotypes in each population or that gene flow, albeit restricted, has continued into recent times.

Within the western haplotype group, the specimens originating in Uganda and surrounding areas (the ECU group) are separated from other western individuals. This event is interpreted as allopatric fragmentation. This result relies on the species being absent from intervening areas. Although this is almost certainly true now with the probable extinction of the giraffe through much of Sudan, to the north (East, 1999), and the next population to the west in the Garamba National Park in northern DRC, historically this is unlikely to have been the case. Hence, the interpretation of these results depends upon the time scale used. This analysis did include two individuals from western Sudan (ENA) and four from Garamba National Park (ECC). The alternative interpretation, assuming populations in intervening areas, gives an ambiguous result that is unable to differentiate between fragmentation, expansion and isolation by distance. With the current range clearly fragmented with giraffe populations absent for large areas to the north and west of the ECU group, the interpretation of the ECU group being different due to allopatric fragmentation is accepted.

Perhaps the most interesting of the inferred population events is that linking the southern Kenyan and Tanzanian populations of east Africa with the more westerly of the southern groups, via the Zambian population in the Luangwa Valley. A contiguous range expansion is inferred. The placement of one of the east African clades as internal to the southern

clade and the other east African clade suggests that the expansion came southwards through the Luangwa Valley and into the southern African sub-region. This haplotype now spreads from South Africa, through Zimbabwe and Botswana into the extreme east of Namibia.

The fourth significantly different clade indicated differences between the overlapping 'eastern' and 'western' groups in southern Africa. However, the conclusion drawn was that the sampling design was inadequate to discriminate between range expansion / colonisation and restricted gene flow.

Comparison with Phylogeographic Results

The interpretation of the nested clade analysis largely reinforces the inferences derived from the phylogeographic analysis. The separation of the northern and southern groups is clearly demonstrated in the rooted cladogram (Figure 11.8) and as the paired 4-step clade grouping in the nested clade design (Figure 12.2 and Table 12.2).

The nested clade analysis did not find a statistically significant event separating the northern Kenyan (EEK) individuals from the western group. This may have been because of the quality of the data. Within the 'EEK group' there were two haplotypes included that may have provided confounding information to the analysis. The individual FMNH27475 is recorded as being from northern Uganda. However, this individual groups apart from other Ugandan individuals, with specimens from northern Kenya. The specimen was collected in 1927, with a sufficiently vague locality to allow doubts as to its accuracy. The two individuals included within H06 are assigned to the southern Kenyan (ESK) group. However, they occur at the northern limit of this group, neighbouring the EEK group, and distant from the main range of the ESK group. It is likely that introgression of EEK mtDNA has occurred into the ESK group in this area.

The separation of the ECU group by allopatric fragmentation in the nested clade analysis is reflected by the monophyly of this group with respect to the other western African haplotypes in the phylogenetic analysis.

Finally, the relationship of the southern groups to the southern Kenyan and Tanzanian groups is demonstrated by both analyses. The 'western-most' of the southern groups is a monophyletic clade that is basal to the other southern group and the southern-eastern group. The results of the nested clade analysis demonstrated this difference but were unable to infer any population process causing the differentiation (within clade 4-2; western southern clade = 3-3; eastern southern/southern eastern = 3-5).

A polytomy in the phylogenetic reconstruction (Figure 11.8) between the 'eastern-southern African' group (SCZ, SEW, SWC) and a mixed 'southern/eastern African group' (SZT, ESK, EST) does not allow the direction of the range expansion between the 'eastern-southern' and the 'southern-eastern' populations to be polarised. However, the basal position of the western-southern clade (SCZ, SWA, SWC) and the tip position of a 'southern-eastern group' (ESK, EST) suggests that an ancestral southern population spread from western southern Africa eastwards before expanding northwards into Zambia, Tanzania and Kenya. The nested clade analysis indicates the opposite view that the movement came from Kenya and Tanzania, through Zambia and into the south. The resolution of this controversy requires further data.

Both the phylogeographic and nested clade analysis approaches provide spatial and temporal dimensions to the understanding of intraspecific variation. The major difference is in the rigor of each approach. Interpretations of phylogeographic analyses are, to some extent, subjective relying on the interpretative skills of the researcher. In contrast the approach of nested clade analysis uses more explicit hypotheses of relationship (including clade membership, topographic position and ancestor-descendent relationships) and uses them within an explicit statistical framework to test models of historic population events.

Phylogeographic analyses do allow the inference of spatial and temporal patterns and, where such patterns are adequate to resolve the questions of interest, can be used (as is demonstrated by the extensive literature in the discipline of phylogeography. Avise, 2000). However, the approach of nested clade analysis is clearly advantageous where process based inferences regarding population structure and history are required. As Templeton (1998. p. 384) asserts "the primary advantage of using the haplotype tree information [i.e. nested clade analysis] is not the quantitative advantage of enhanced power and precision; rather, it is the qualitative advantage of discriminating among various biological explanations for any detected geographical association." Both methods are powerful interpretative tools in their own right and should be utilised according to the requirements of the questions in hand.

Three independent data sets have now been examined using a variety of analytical methods, to determine the extent of geographically structured variation in the range of the giraffe. The final discussion chapter summarises the results of each analysis and considers the implications for the subspecific status of the giraffe, as well as the conservation implications of subspecific taxonomy in general.

CHAPTER 13:

TAXONOMY, BIODIVERSITY AND CONSERVATION: A SYNTHESIS AND DISCUSSION OF PRACTICAL AND THEORETICAL ISSUES

Subspecific Biodiversity

That heritable variation occurs within species is a requirement of Darwinian evolution by natural selection. It is a logical corollary that, where locally beneficial, fitness-enhancing variation is present and selected for, geographically structured phenotypic or genotypic variation will be found within a wide-ranging species. Equally, population history, with the effects of vicariance events, founder populations and random drift, may lead to differentiation between conspecific populations. Where geographically discontinuous patterns occur, subspecific groupings may be identified. The discovery of such patterns suggests that historical processes acted upon local populations. Recognition of these patterns logically precedes any investigation of the processes involved. The formal taxonomic treatment of differentiated subspecific groups provides a rigorous framework for the recognition and conservation of within species biodiversity in both biological and legislative contexts.

As a basis on which to explore such patterns, this thesis has examined the occurrence of phenotypic and genotypic variation in a single species, the giraffe (*Giraffa camelopardalis*) across its entire recent African range using phenotypic and genetic data sets and differing methodologies. This species was chosen for study as its taxonomic status required revision, having been altered (Dagg, 1971) since the last formal taxonomic revision (Lydekker, 1904). Also it provided three independent data sets with which to examine geographically structured patterns allowing the exploration of any conflicts that might occur between these data sets and the analytical techniques used. However, the results indicate a great deal of congruence between the results.

Subspecific Variation in the Giraffe, Giraffa camelopardalis

Giraffe specimens from museums were examined for pelage pattern, morphological and molecular variation (Sections 3, 4 and 5, respectively). The geographic structure of any variation was explored using geographically delimited specimen sets. This approach was taken as, within the context of this study, it was necessary to obtain comparable

information from the same set of individuals for inclusion in each analysis. To this end museum specimens were used. Museum collections represent historically gathered specimens, allowing the investigator no control over the sampling regime. This must be taken into account when designing the analyses. Specimens were grouped objectively using historical range maps and features of physical geography to delimit potentially restricted populations. A conservative approach, minimising the range of each population, allowed neighbouring sets to be grouped together if they showed no difference in the analyses (see Chapter 6).

Summary of Pelage Variation

Variation in pelage patterns between geographically delimited populations was examined using forty pelage characters extracted from literature descriptions of giraffe subspecies (de Winton, 1897; 1899; Lydekker, 1904; 1911; Thomas 1894; 1901). The consistency of each character was assessed within and between geographic specimen sets. In addition, the characters providing consistent character states within each set were compared in a hierarchical variation of population aggregation analysis (Davis and Nixon, 1982) (See Chapter 7).

Each of the specimen sets representing previously described subspecies provided a diagnostic combination of character states allowing their separation from other populations. In combination with range information, each population could be identified to their geographic region of origin. However, some of the characters that were 'diagnostic' were found to be variable between neighbouring populations. With these removed from the analysis, neighbouring populations agglomerate into recognised subspecies groups (e.g. ECU and ECC; SWA and SCZ/SEW/SWC). Some of the original groups consisted of relatively small sample sizes (two groups consisting of a single specimen), while other subspecies were not represented at all. Six of the currently recognised subspecies were supported by the pelage pattern analysis (Chapter 7).

The nature of the analysis undertaken is population based. Groups were separated or agglomerated at sequential hierarchical levels depending upon conflicting or shared character states. The selective nature of the comparisons made at each level, and the occurrence of individual variation in other geographical specimen sets, means that, while the method allows separation of population groups, it does not allow subsequent classification of unknown specimens. Hence, the results are locally optimal and relevant only to the specimens included. These results can not be generalised, with absolute confidence, to the diagnosis of individuals to subspecies.

Summary of Morphological Variation

Multivariate discriminant analysis results supported the recognition of four geographically separate giraffe groups (Chapter 9). Males of northern and southern specimens included in my samples are clearly separated according to the development of the median horn. Specimens where the height of the skull, including the median horn, was greater than the greatest width of the skull, at the orbital rims, come from the north.

Within the northern group, western specimens (from the Great Rift Valley of northern Kenya and the Nile River westwards: ECU, WCP, WSN) differed from northern Kenyan specimens (from east of the Great Rift Valley: EEK) by the width of the skull. Finally, southern Kenyan and Tanzanian specimens (ESK, EST) were characterised by their relatively reduced development of the median horn (as compared to other eastern, northern specimens).

In the southern group (SCZ, SWC), little variation was found between specimens in the main southern range, allowing these populations to be recognised as a single entity with little or no geographically structured variation. Individuals from the Luangwa Valley of Zambia (SZT) tend to have more robust skulls with shorter parietal horns. However, the sample size was small. The Luangwa Valley giraffe deserve greater attention and can be maintained only as a provisional subspecies based on these results.

Shape analysis (Chapter 10) separates northern and southern specimens according to the development of the median horn in males. Northern and southern female giraffe may be differentiated according to the form of the parietal horns.

Within the north the shape of the median horn and the width of the skull separates the western (ECU, WCP, WSN) and eastern (EEK, ESK, EST) groups. Southern Kenya and Tanzanian forms (ESK, EST) show the least development of the median horn.

In the south, Zambian (SZT) and southern Zimbabwean (SCZ) giraffe separate according to parietal horn variation, but both groups overlap with other giraffe from the region (SWC), suggesting no geographically characteristic differentiation in the skulls shapes examined.

Summary of Molecular Results

Phylogenetic reconstruction of molecular data clearly separates northern and southern clades (Chapter 11), but not in the same way as the morphological analyses. The

geographic content of the compared groups was imposed upon the morphological analyses and separated northern and southern specimens according to the major gap in the distribution. These molecular results suggest that the genetic separation of the northern and southern forms occurs in Kenya, with the giraffes of southern Kenya (ESK) and Tanzania (EST) included within the southern African clade.

In the northern clade, a monophyletic western group (including ECC, ECU, ENA, WCP and WSN individuals) derives from a basal, paraphyletic northern Kenyan group (EEK).

In the south, two geographically overlapping haplogroups were identified. The first is slightly more western in its range and extends from northern Namibia, through Botswana across to southern Zimbabwe. The second tends to be more eastern in distribution and extends from northern South Africa and Southern Zimbabwe westwards into the extreme east of Namibia only. The western group is basal to the more easterly clade. The eastern group is contained within an unresolved polytomy containing the east African haplotyes and the single haplotype from the Luangwa Valley, Zambia (SZT).

Inference of events in the population history of the giraffe, using nested clade analysis (Chapter 12), suggests that isolation by distance, range fragmentation and range expansion events account for the observed pattern of mtDNA diversity.

Congruence and Conflict between Pelage, Morphology and Molecular Data

The results derived from the three independent data sets are remarkably congruent with only one conflicting result. Whereas some of the ESK/EST individuals from southern Kenya and Tanzania share a mtDNA haplotype with all individuals sequenced from the Luangwa Valley in Zambia (SZT), morphological and pelage data indicate that these populations are phenotypically distinct. The populations involved were part of a range expansion event (according to the nested clade analysis results). A plausible scenario, based on the phylogenetic analysis would see female migration from Zambia (where the haplotype occurs in all sampled individuals) into Tanzania and southern Kenya (where it occurs in a fraction of the population). Hence, introgression of the southern mtDNA into the phenotypically different population occurred without 'diluting' the locally adapted phenotype. The minimum spanning tree suggests an opposite direction of movement with the eastern individuals placed internally on the tree. More data is required to resolve this issue, although it is felt that the south to north east move is the more likely. Interestingly the development of the median horn, distinctive for northern forms and reduced in southern

specimens, is variable in the very area where mtDNA introgression from the south into the north occurs.

Interpretation with respect to Currently Recognised Subspecies

Giraffa camelopardalis camelopardalis (ENE)

Occupying eastern Sudan and the extreme west of Ethiopia, the nominate subspecies was only included in the mtDNA analysis. It shared a haplotype with a widespread 'western' group. Specimens of this subspecies are required for morphological and pelage analysis.

Giraffa camelopardalis angolensis (ENA)

A single specimen from the range in southern Angola was included in the analysis of pelage patterns. This specimen differed from the other southern specimens by the larger size of its body spots. However, without further specimens available to corroborate the consistency of this characterisation of the body spots, it is not possible to definitively separate the Angolan subspecies from the other southern subspecies. Further specimens are required for examination.

Giraffa camelopardalis antiquorum (ENA)

Restricted to western and south western Sudan this subspecies was included only in the genetic analysis where it shared a haplotype with the western haplogroup. Further specimens are required to determine the affinities of this subspecies.

Giraffa camelopardalis giraffa (SCZ, SEW, SWC)

This putative subspecies is widespread across southern Africa. It extends from northern Namibia in the west across the northern half of the country into Botswana and across to western and southern Zimbabwe into northern South Africa. Some authors maintain that the range splits in Zimbabwe with the individuals from western Zimbabwe westwards belonging to this subspecies and those of southern Zimbabwe, northern South Africa and southern Mozambique making up *G. c. wardi*. Molecular, morphological and pelage pattern variation suggest that individuals from across this entire southern African range belong to a single subspecies. No geographically structured phenotypic variation was evident, while two genetic haplotypes in the area overlapped extensively.

Giraffa camelopardalis peralta (WCP, WSN)

Giraffe from throughout the range in western central and western Africa share a single haplotype and group into a monophyletic clade with all other giraffe from west of the Great Rift Valley and the Nile River. Morphological analysis concurs with the genetic analysis in grouping western giraffes with specimens as far east as Uganda and western Kenya. Pelage patterns are distinctive with sharp edged spots with wide interspot lines in contrast to the body spot colour.

Giraffa camelopardalis reticulata (EEK)

The distinctive regular, polygonal spots of this northern Kenyan giraffe previously saw it recognised as a separate species (de Winton, 1897; Lydekker, 1904). It is now clearly a separate subspecies. Skull morphology and genetic analyses also suggest that it is separate from other subspecies.

Giraffa camelopardalis rothschildi (ECU)

Morphologically and genetically this purported subspecies shows an affinity to other populations lying to the west of the Great Rift Valley and the Nile River. Deriving from Uganda, western Kenya and southern Sudan specimens share a monophyletic clade with other 'western' haplotypes but, within this group, are monophyletic. Nested clade analysis indicates a fragmentation event between this and the other western populations. The pelage pattern of this group is distinctive due to the large bold body spots, many of which have darker centres.

Giraffa camelopardalis thornicrofti (SZT)

The sharp edges of the irregular body spots, large on the fore flanks, but small on the rump, distinguish this population from other southern groups. Isolated in the Luangwa Valley of Zambia, the Thornicroft's giraffe shows a tendency to be morphologically differentiated with a more robust skull with shorter parietal horns. However, more specimens are needed to investigate any morphological trends more thoroughly. Genetically all specimens from this locality were identical and shared this haplotype with some individuals from Tanzania and southern Kenya. Nonetheless, it differs morphologically from these more northerly individuals. The tri-lobed lower canine tooth, previously suggested as diagnostic for this

subspecies, is not a reliable indicator. This tooth morphology does not occur consistently in Thornicroft's specimens and does occur, on occasion, in other areas.

Giraffa camelopardalis tippelskirchi (ESK and EST)

From southern Kenya and Tanzania this subspecies shows a greater affinity to the southern haplotypes than to northern genetic sequences. However, despite the variable development of the median horn these individuals differ in skull morphology from more southerly specimens. The 'splintered' stellate spots are clearly distinctive for this subspecies.

Subspecies Summary – Definitive and Provisional

Southern Giraffe

Most southern giraffes may all be subsumed into a single definitive subspecies, *G. c. giraffa*. It is possible, although unlikely, that the Angolan giraffe may be subspecifically different, but further specimens are required to test this possibility. For this reason the subspecies *G. c. angolensis* should be maintained as a provisional subspecies only.

The Thornicroft's giraffe of the Luangwa Valley of Zambia should be maintained as a definitive subspecies, *G. c. thornicrofti*, recognising that this rank is justifiable primarily by the distinctive pelage pattern rather than by morphological differences. Its isolation from other populations and its potential importance in understanding historical giraffe movements makes the Luangwa Valley giraffe population biologically important. The haplotype shared with east African individuals is worthy of further investigation.

East African Giraffe

The reticulated and Masaai giraffes (*G. c. reticulata* and *G. c. tippelskirchi*) are immediately identifiable by their pelage patterns, differentiable by their skull morphology and genetically distinct. Hence, both are deserving of recognition as definitive subspecies.

The Rothschild's giraffe (*G. c. rothschildi*) differs morphologically from other east African giraffes although it is indistinguishable from those further to the west. Genetically, Rothschild's individuals form a monophyletic group within a higher level, monophyletic western clade made up of specimens from Africa west of the Great Rift Valley and the Nile River. Nested clade analysis indicates a historical fragmentation event between Rothschild's giraffes and other western giraffe populations. Their pelage pattern is distinctive, and the Rothschild's giraffe should be maintained as a definitive subspecies.

The populations from Sudan, including purported *G. c. camelopardalis* and *G. c. antiquorum* were inadequately sampled. Haplotypes from both of these subspecies were identical to those from the widespread western haplotype, with one ECC individual (assumed *G. c. camelopardalis*) differing by a single substitution. Neither subspecies was included in analyses of skull morphology. Meanwhile, head and neck mounts of two *G. c. antiquorum* individuals provided insufficient data for the pelage analysis, although the sharp-edged spots and wide interspot lines on the necks are similar to those of western giraffes. While both subspecies need to be sampled more adequately, it is imperative that specimens of the nominate subspecies be examined to allow the accurate allocation of the nominate subspecies name. It is recommended that both subspecies names be maintained as provisional subspecies while further data is obtained. It seems likely that the nominate subspecies will be revised to contain *G. c. antiquorum* and the west African subspecies, *G. c. peralta*.

West African Giraffe

West African giraffe (WCP and WSN) represent a single definitive subspecies forming a monophyletic clade, according to genetic data, and being indistinguishable morphologically and by their pelage patterns. It is felt that the *G. c. antiquorum* subspecies may be synonymous with this subspecies, and both may be subsumed into the nominate subspecies.

Molecules and Morphology: Information Content and Quality

This study has utilised multiple, independent data sources to consider the subspecies status of the giraffe, reflecting the holistic approach advocated by such authors as Ryder (1986), Waples (1991) and Paetkau (1999). There has been a concern expressed that some information sources may be regarded as being 'better' than others. For example Dizon *et al.* (1992. p. 28) suggest that "The evidence obtained from genetic methods is considered by resource managers as the most unequivocal for differentiating species and their intraspecific structure". Meanwhile Crandall *et al.* (2000. p. 290) state "The widespread use of molecular genetic markers in the context of Moritz's ESU concept has led many investigators to regard these data as essential to conservation management, often to the exclusion of other data."

The reasons for this advocacy of molecular results over other data sources may include the apparently definitive nature of genetic data. Given good laboratory and data acquisition practice, genetic data is typically known unequivocally, or, at least, with a high degree of confidence, for a given specimen. In sequence data the characters are discrete and the results are consistently reproducible. In contrast, morphological, behavioural or ecological data are more variable in nature with a demonstrable measurement error that needs to be accounted for. Hence, the nature of the data may be affecting the perception of the results.

Also persuasive are the process-based arguments that any characters used to diagnose evolutionary units should be demonstrably heritable (Dizon *et al.*, 1992; Vogler and DeSalle, 1993). Clearly genetic characters are, by definition, heritable, whereas morphological, behavioural and ecological characters are more complex and may be more labile and influenced by the environment. Vogler and DeSalle (1993) make stringent recommendations regarding the quality of the characters used in the analysis indicating that the "heritability of characters is ... required to avoid the recognition of environmentally controlled or age-related variation" (p. 357). They suggest that 'bad' characters include "continuous morphological characters", as they are difficult to score for cladistic analysis, and "behavioral and ecological characters for which evidence of heritability cannot be provided unequivocally" (p. 357). Dizon *et al.* (1992) are somewhat less restrictive in their recommendations but do note, when discussing phenotypic variation, that "some morphological patterns ... may be ecophenotypic, that is, not stable to environmental variation" (p. 28). However, they recognise that "a population's life histories and behavioral traits may be modified by the environment through density dependent control mechanisms" (p. 27). They advocate the use of such variables for defining separate populations for management. For example, the simple population response of an alteration in the timing of breeding provides an obvious prezygotic reproductive barrier between populations. The focus on process-based inheritance may misidentify units of biodiversity.

Environmentally induced variation is as valid as inherited variation if this is geographically consistent. Such a holistic approach includes those aspects of the contemporary environment that induce these effects. An easy counterpoint against this argument is that conditions may change in the future. In fact, within a framework of systematic biology, this is not a useful argument but represents a misunderstanding of the practices of systematic biology. Any named taxon represents a hypothesis of relationships. Such hypotheses should be under regular scrutiny and can be changed readily if patterns change. As such, 'units' of biodiversity are dynamic.

Whatever the perceptions, the example of the Cryan's buckmoth (Legge *et al.*, 1996) is a clear example where the genetic results did not reflect an obvious ecological differentiation between populations within a species complex (see Chapter 1).

The integration of the principals of systematic biology into the objectives and practice of describing subspecific biodiversity units is vital. Only a systematic biology approach to subspecific diversity will take account of the range of genotypic, phenotypic, behavioural, ecological and environmental data that is required to adequately delineate realised, or potential, phylogenetic biodiversity. But first it is necessary to consider whether each type of data provides equivalent information.

Genotypic Data: Mitochondrial DNA Control Region Sequences

The mtDNA control region does not code for a protein sequence. Instead it functions as the site of initiation of DNA replication of the circular mitochondrial DNA molecule. This lack of protein coding means it can be considered to be a 'neutral' marker. That is, it is not under directed selection pressure and, beyond the normal fidelity of DNA replication, may accumulate and fix base substitutions in a manner that reflects the population history of a species. Mitochondrial DNA is a maternally inherited molecule.

Mitochondrial DNA sequences, therefore, provide information pertaining to the phylogeny of female mediated gene flow. Mutation, cladogenesis, vicariance, migration, founder effects, bottlenecks and other historical population processes are embedded in the history of a species and may be detected from data derived from the mtDNA. The data obtained from mtDNA simply traces the temporal and spatial 'flow' of female individuals through the history of a species.

Phenotypic Data: Skin and Bones

The within species variation detected in phenotypic data derives from two sources:

1. Directed change mediated by natural selection or sexual selection pressures;

   or

2. Random drift whereby selectively neutral variation occurs and approaches or reaches fixation in a population.

Phenotypic variation may reflect adaptations to local environmental conditions, including adaptations for local food acquisition, local climate or local predation pressures. In this

way phenotypic data may represent a more general and more complete representation of the significance of geographical variation in populations.

Reconciling Molecules and Morphology at the Subspecies Level: What are we Trying to Conserve?

Molecular and morphological approaches to recognising geographically structured patterns complement each other at the subspecific level and both are necessary to investigate species variation adequately. The use of one source of information to the exclusion of the other loses useful information from the analysis. Moritz's (1994) discussion of molecular indicators of subspecific units (ESUs) for conservation has lead to some conservation biologists to seek molecular genetic data on which to formulate management policy to the exclusion of other data sources (Crandall *et al*, 2000); an approach that is excessively restrictive. In his discussion of Evolutionarily Significant Units Moritz (1994. p. 373) states that the purpose of defining subspecific units for conservation is "to ensure that evolutionary heritage is recognised and protected and that the evolutionary potential inherent across the set of ESUs is maintained". He rightly points out that the future of a lineage can not be predicted unequivocally, but that phylogenies can be reconstructed to infer historical evolutionary events. In this context he asserts that 'significant units' should indicate "that the set of populations has been historically isolated and, accordingly, is likely to have a distinct potential" (p. 373), thereby emphasising history over current adaptation. Moritz even considers that conservation of the full array of subspecific variation "negates the evolutionary process that we seek to maintain, insofar as preservation of variants adapted to previous conditions may retard the response to natural selection" (p. 373).

I suggest that such an argument is fallacious and illogical in a number of respects. Populations are adapted to current conditions, not to 'previous' conditions. A population adapts, over the generations, according to the changing local conditions. Who better to conform to the future, local conditions than a population already adapted to the current environment? The suggestion that 'current adaptation' is somehow a handicap to future evolutionary change makes little sense. The time since cladogenesis (Moritz's 'historical isolation') has no bearing on a population's ability to adapt to a changing environment. Natural Selection works upon natural variation in the phenotypic expression of the genotype; genetic variation is the prerequisite for future adaptation. Perhaps it could be argued that the longer the time since cladogenesis, the more time there has been for new mutations to accrue, providing potential sources of adaptation in a new environment. But

this misses the simple point that genetic variation was already present in the common ancestor and, barring significant founder effects seriously compromising the variation in a daughter lineage, is available for future selection in both daughter taxa.

This restricted approach advocated by Moritz (1994) has been criticised further by Paetkau (1999) and Crandall *et al.* (2000) (see Chapter 1). Both of these authors raised practical and theoretical objections to the limited genetic approach and agreed with previous authors (Ryder, 1986; Ryder *et al.*, 1988; Waples, 1991) that a more holistic approach, encompassing all available information, is necessary to understand subspecific variation.

Similarly, the suggestion that only demonstrably inherited variation should be considered as valid information for conservation decisions (Vogler and DeSalle, 1994) is unworkable and removes potentially important sources of informative variation. Vogler and DeSalle (1994. p. 357) assert that "It is necessary that the characters used can be inferred to have a heritable basis, because they are taken to reflect the common descent of organisms. Heritability of characters is also required to avoid recognition of environmentally controlled or age-related variation." The first statement is valid in the context of reconstructing the intraspecific phylogeny or population history of the species. But phylogenetic information must be interpreted in the light of other information and should not necessarily be the end in itself. However, the second statement is not correct, in my view. The environment affects (and is affected by) an organism at all stages in its life. Where environmental conditions alter ontogenetic development, and do so in a consistent way for each organism in a conspecific population, the variation observed indicates naturally occurring, biologically important variation in just the same way that inherited variation does.

A consideration of non-inherited factors in the recognition of subspecific diversity extends the holistic approach to determining subspecific groups for conservation. Such an approach moves beyond the use of multiple data sets pertaining to the individuals in the population, to encompass the environment more generally. A criticism of such an approach could be that in a single location (or area), where conditions are variable between breeding periods, the variation in the environment might cause differential phenotypic expression in a single lineage between generations. However, this would not be a criticism of the concept, but a criticism of its application.

Fundamentally, the concept espoused here for the recognition of units of biodiversity is that it is important to recognise of the pattern of variation. The occurrence of a pattern logically infers that a process has taken place. Undoubtedly, the investigation and elucidation of these processes are often of great biological interest in themselves, and may

have a bearing on the efficacy of any conservation policy proposed. However, if our aim is to maximise the recognition and conservation of biodiversity, the first step is to recognise the pattern of variation within a holistic framework.

Formal Taxonomy: Using Subspecies Names in Conservation

In discussing and communicating our knowledge of biology we use names. In the context of biodiversity conservation, the way in which organisms are classified and 'labelled' has implications for the way we quantify biodiversity (Cracraft, 1992), how we prioritise each taxon (e.g. Daugherty *et al.*, 1990; May, 1990; Vane-Wright *et al.*, 1991) and how we legislate for conservation (Geist, 1992; Committee on Scientific Issues in the Endangered Species Act, 1995). Hence, the naming of species and subspecies through the formal process of taxonomy may have fundamental importance for the conservation of low-level phylogenetic biodiversity.

Wilson and Brown (1953) raised questions as to the biological validity of the subspecies and recommended the abandonment of the term. However, they did recognise the occurrence of geographically structured variation within species but, instead of the formality of the trinomial, suggested a vernacular, descriptive system using locality names to indicate variants. The introduction of the 'Evolutionarily Significant Unit' (Ryder, 1986) was due, in large part, to frustration with the inappropriate application of subspecies names. Cracraft (1998), similarly, expresses the reservations of many biologists: "Subspecies ... are not easily used because although some are distinct, geographically localised units, others are arbitrary sub-divisions of continuously distributed geographic variation and are not distinct units." However, the objections raised by each of these authors reflect the poor application of the subspecies concept in the past rather than a failure of the concept itself. In particular, it has been realised only recently, due to our greater knowledge and understanding of the distribution and variation within many taxa, that previously recognised subspecies are, in many cases, poorly described and delimited (both taxonomically and geographically).

Any system of classification provides greatest service when its information content is maximised. Advocacy of the phylogenetic species as the terminal taxonomic unit with the concomitant rejection of the subspecies loses information. De Laubenfels (1953. p. 43) stated that "my contention is that scientific nomenclature should show degree of difference as well as the fact of relationship." A promotion of those subspecies deemed suitable for

recognition as valid phylogenetic species and the rescindment of any kind of formal taxonomic status for those that do not meet these criteria loses information and impoverishes our ability to recognise, and classify, geographic variation within species. The species is the fundamental unit of taxonomy. Subspecies indicate variation within the species. A system that encompasses generic, specific and subspecific levels contains nested information at each level. All individuals within a given species are more closely related to members of there own species than to individuals of other species. Moreover, individuals of a given subspecies are more closely related to those of another conspecific subspecies than to individuals of another species. The refusal to recognise subspecies and the use of a purely binomial system using only the genus and species loses two levels of useful information: Firstly, using only the classification there would be no way to know which of the 'species' within the genus were more closely related to each other. Secondly, geographically structured variation in character states that do not have completely discrete distributions would not be recognised. The use of an additional class below the rank of the species clearly demonstrates the "fact of relationship" but also indicates that there is a "degree of difference" within the species.

I agree with Cracraft (and co-workers; Cracraft, 1997; Cracraft *et al.*, 1998) that the goals of taxon recognition and conservation should be met through the use of the formal framework of systematics and taxonomy. He criticises the use of ESUs in conservation biology, as a vehicle for legislative protection of taxa, pointing out that the concept has no "international standards of scholarship" nor "formal nomenclatural rules" (Cracraft, 1997. p. 335). Meanwhile, formal taxonomy provides a "formal, universal language for taxonomic diversity" that "historically underpins ... biodiversity science and is now recognised within legal frameworks". Furthermore "it is backed-up by centuries-old scholarship, tradition and widely accepted rules of procedure" (All quotes Cracraft *et al.*, 1998. p. 147).

Species, Subspecies, Conservation and Legislation

Although debate still rages regarding our understanding of the true nature of species and how best to delineate them (e.g. Wheeler and Meier, 2000) the species is consistently defined as representing a 'fundamental' biological and taxonomic unit. Whatever the specifics of the species concept used, well-known, geographically wide-ranging species are often shown to vary across their range. In such cases, where should legislative efforts be targeted for effective enforcement of conservation ideals?

At present, most legislation that purports to target taxa at and below the species level use the term species throughout and give a *de facto* recognition to the occurrence of subspecific variation by recognising "within species" diversity (UNEP, 1992), "subspecies or geographically separate population[s]" (CITES, 1973) or "smaller taxa [than species]" (U.S. House of Representatives, 1973). However, the needs of a biological classification system may not readily lend themselves to the rigorous requirements of the legal system. How might taxonomic designations be used to implement conservation legislation?

The fundamental nature of the species and subspecies taxa will affect the utility of each taxon in conservation legislation. In this discussion I follow a Phylogenetic Species Concept (PSC) approach to the recognition of species (see Chapter 1). That is, a species is a fundamentally diagnosable unit consisting of individuals that may be recognised and grouped by a shared, concordant pattern of characters and character states. Cracraft *et al* (1998), in a discussion of the PSC as a diagnosable unit for conservation, states that "the PSC is an evolutionary lineage concept, and a populational concept, thus it cannot be applied to single individuals ... in other words it is a taxic concept" (p. 148). The contention here is that a species is a hypothesis of relationships whereby any individuals included in the group are more closely related to other members of that group than to any individual outside of the group. In this way, a species is a population of similar individuals. A species then is a relational concept, in that individual members of any species are, of necessity, compared to all other individuals in the same and similar taxa to establish group membership. However, this represents the species description phase of taxonomy. Once a species has been recognised, it may be diagnosed and those features recognised as characteristic of the species may be used to classify subsequent individuals as members of that species. In these practical terms, I consider the species to be an individual-based concept. That is, given adequate descriptions of taxa and sufficient material, it is possible to assign any individual specimen to its species.

The PSC uses qualitative assessment of characters and character states to describe and assign individuals to species. Within these character states there is scope for continuous, quantitative variation of traits. Where geographic structure is shown, subspecies taxa may be described and classified. Subspecies, therefore, indicate trends in the population and rely upon adequate sampling and statistical testing to provide robust and reliable conclusions regarding their level of differentiation. The overlap of character state parameters may be allowable in the recognition of subspecies (Rand and Traylor, 1950). Hence, subspecies represent a population-based concept in which no individual can be assigned to its correct subspecies with certainty (only with a certain probability that may vary between subspecies and species, Zusi, 1982.). Only species in which the geographic

range and the phenotypic variation of phena and individuals occurring in that range are well known, can be sub-divided into subspecies (Corbet, 1970; Blackwelder, 1967).

Legislation requires unambiguous definitions to allow clear and unequivocal interpretation of its regulations. For this reason, the legislative treatment of individual-based and population-based concepts must be different to reflect the difference in information content of the two taxa. A simple separation of legislation with differing aims is instructive; that of *in situ* conservation and *ex situ* regulation. In this context, *in situ* conservation legislation includes any ecosystem or habitat based conservation program aiming to conserve organisms remaining in their natural environment. Meanwhile, *ex situ* conservation legislation includes laws restricting trade in whole animals or animal parts (e.g. CITES) where specimens of named taxa must be identified for effective control.

Individual-based Concepts and Conservation Legislation

Individual-based concepts provide the unambiguous classification of individuals to their correct taxon and so offer a simple device for direct use in conservation legislation. Valid species, recognised by adequately defined characters and character states, can be recognised in both *in situ* and *ex situ* circumstances. For *in situ* conservation species ranges and distributions within that range (largely reflecting habitat requirements) must be known. For *ex situ* conservation, the ability to diagnose a specimen to its species is necessary for effective implementation of legislation.

Population-based Concepts and Conservation Legislation

Population-based concepts do not necessarily allow unequivocal allocation of a specimen to its correct taxon. At best a specimen can be assigned with a certain confidence level. This will cause problems for legislation where unequivocal recognition of similar named taxa is required, making the use of subspecies in *ex situ* legislation unworkable.

There may be circumstances where subspecies could be identified and used. Where morphologically defined subspecies are completely coincident with non-ambiguous molecular markers genetic tests may be used to allocate subspecies. However, a number of conditions must be met in order for such molecular tests to be effective:

1. Sufficiently large samples of each subspecies must be tested in order to develop the necessary molecular markers and prove beyond reasonable doubt that the proposed molecular motifs are indeed unique to the prescribed subspecies.

2. Introgression of nuclear or, particularly, mitochondrial DNA from one subspecies to another may occur, effectively obscuring the differences between the subspecies and providing erroneous identifications, and must be accounted for.

Such tests are time consuming and expensive and may only be viable for high profile species with particular conservation problems. An example would be identifying the origin of animals poached from the wild entering the black market for exotic pets or medicinal animal parts.

Subspecies are defined adequately and unambiguously by two factors: (1) the description of the subspecies, and (2) the range of the subspecies. The range of the subspecies then acts as the definitive element of the subspecies diagnosis for implementation of *in situ* conservation action. With known ranges local conservation action can be implemented (in exactly the same way as for the species taxon) while subspecies designations may be used in identifying biodiversity hotspots and priority areas for conservation or for inferring isolated areas and/or areas rich in divergent (speciating?) populations.

Species and Subspecies in Taxon and Habitat Conservation

The biological nature of the species and the subspecies concepts, then, have an impact on the formulation, implementation and interpretation of conservation legislation. I suggest that the species should be regarded as the fundamental unit for the conservation legislation to recognise in terms of taxon conservation. The range and inherent variation (and its partitioning between individual and geographic sources) of many species are not adequately known to separate them in to subspecies that may then be utilised for conservation legislation.

Subspecies should only be recognised where the geographic range of a species is well known and is adequately sampled. The utility of the subspecies, in conservation terms, should be in the investigation of large scale patterns of variation and in the identification of regions rich in subspecific variation that may represent areas of ongoing speciation or locally varying environments. The geographic range (habitat) is then conserved, effectively protecting the taxon and its ecosystem, rather than the taxon itself being afforded protection.

Applying Taxonomy to Conservation

One of the stated aims of this project was to compare the conclusions that could be drawn from using different data sets to derive subspecific units of the giraffe. For this reason, this project undertook and has reported the three studies, focussing on pelage pattern, morphological and molecular data, separately. This approach sought to emulate the possible scenario of different researchers presenting the three sets of results independently, and then of the conservation manager needing to reconcile the conflicts or congruence between the data sets into a coherent conservation policy.

A retrospective view of the project allows specific recommendations for the intended continuation of work on giraffe subspecies. General recommendations can also be made for other projects using a holistic approach to the recognition and delimitation of subspecies taxa.

Giraffe Subspecies: In Retrospect and in the Future

The major work describing the subspecies of the giraffe was undertaken nearly a century ago using a limited sample of available material (Lydekker, 1904). The classification at that time recognised two species; *Giraffa reticulata* and *G. camelopardalis*; with the latter containing ten named subspecies (see Figure 2.6). With the recognition of additional subspecies (Lydekker, 1911) and the amalgamation of others, nine subspecies in a single species have been recognised recently (Dagg, 1971. Table 2.2 and Figure 2.1). However, no taxonomic revision of the status of giraffe subspecies had taken place since 1939 (Krumbeigel, 1939). This project undertakes the first, extensive, objective revision of giraffe taxonomy since this time.

Future Work on Giraffe Subspecies

Notwithstanding the work undertaken in this thesis, more work needs to be done to further clarify the patterns of subspecific variation in the giraffe. Certain of the currently recognised subspecies were under-represented in the specimen sampling and are maintained as provisional subspecies, only due to taxonomic priority. European museum collections were not included in the sampling for this study and offer additional specimens for inclusion. Both morphological and genetic data from these collections will be sought in the continuation of this work.

In particular, the resolution of the relationships between southern and east African clades requires closer examination. The phylogeographic approach provides an unresolved polytomy at the node joining the eastern and southern clades. The occurrence of a second southern clade basal to this node suggests the migration of a southern population northwards into east Africa. However, the Minimum Spanning Network shows members of the eastern clade to be ancestral to the southern clade, suggesting the movement to be a southwards migration out of east Africa. An increase in the volume of data, by extending the region of DNA sequenced, may resolve this inconsistency.

Furthermore, data from nuclear genes are required. Mitochondrial DNA provides data pertaining to the maternal line of inheritance only. The only conflict between the three independent data sets results from a shared haplotype between geographically separated populations not being reflected by phenotypic (morphological or pelage) variation. The nested clade analysis suggests that the distribution of haplotypes in the region in question (northern Zambia, Tanzania and southern Kenya) resulted from a contiguous range expansion. The conflict may be explained if females migrated into an area already populated by males and their 'foreign' mtDNA became introgressed into the population. Data from nuclear DNA would go some way to resolve the true nature of this conflict.

A Holistic Approach to Subspecies Description

Projects seeking to examine subspecific variation must complement genetic and phenotypic data within the same study. Genetic data give information pertaining to phylogeny, cladogenesis and population history events. Meanwhile, morphology and pelage patterns contribute additional information regarding local adaptation and may lead to an understanding of differing local selective pressures.

The value of museum collections to fundamental systematic research, that underpins so many other disciplines, is paramount. Museum collections represent the single most important resource in the investigation of subspecific taxonomy (beyond new sampling).

The geographical grouping approach (Chapter 6) taken here, whereby specimens were grouped into minimum sets that were geographically delimited according to published, historical range maps, is valid in circumstances where the sampling regime is already prescribed for the researcher (as in this case where museum specimens were used). These sets can then be used as groups to test null models of geographical variation in phenotypic characters. The hierarchical grouping of the local specimen sets into regional groups for comparisons was done according to geographic locality.

Under this analytical regime, and as a result of this study, I recommend that molecular investigations should be undertaken, and completed, before the analysis of phenotypic data. The genetic results derived for this study indicated that the separation of these geographically delimited regions was not concordant with the identified major clades. This, perhaps, compromised the power of some of the interregional comparisons made. However, in this study, local contrasts of paired, neighbouring sets provided more detailed comparisons of phenotypic variation and were not affected by such arbitrary grouping. Hence, in future studies, I suggest that genetic analyses be undertaken first in order to clarify the major phylogenetic clades. These results, in conjunction with the geographic distributions of specimens, can then be used to group specimens for statistical analysis.

Beside this one alteration, the methodology followed in this study represents a robust, coherent approach to examining subspecific variation in a widespread species.

Further Research on Giraffe and Other Mammalian Taxa

Beyond the recommendations for investigations of subspecific variation discussed above, the approach taken and the methods developed during this research lead on to a number of research opportunities and directions, both with giraffe in particular and with other mammalian taxa more generally. Two projects will continue the work to elucidate giraffe geographic variation and morphological development.

Giraffe Specific Projects

Pelage Pattern Analysis using Erosion-Dilation Cycling

The pelage pattern analysis undertaken in this thesis (Chapter 7) characterised pelage patterns according to character states that were consistent within geographically delimited groups but varied between them. Such a categorical, character based approach does not allow for the quantification of individual variation. Pelage patterns will be further investigated using the related approaches of erosion cycling and erosion-dilation cycling (Ehrlich *et al.*, 1984) to investigate aspects of pelage patterns including spot size and, particularly, the subtleties of spot shape.

Shape Analysis of Ontogenetic Development of Giraffe Skulls

The shape analysis approach used here (Chapter 10) to examine geographically structured variation between giraffe populations will be used to examine the developmental trajectories of giraffe skulls. Coupling shape data with age estimates (Chapter 4) allows the quantification and ordination of skull shape at different ages. Differences between male and female development (in this sexually dimorphic species, see Chapter 5) and between geographic morphotypes (e.g. northern and southern forms, differing with the development of the median horn) will be studied.

General Recommendations for Studies of Mammalian Taxa

The approach taken here may also be generalised to the study of subspecific variation in other taxa.

Parameter Selection

The 'traditional' analysis of morphometric measurements using multivariate statistical approaches remains a strong research tool. However, the selection of (typically, for mammalian studies at least) skull dimensions for analysis is often arbitrary and represents the preference of the researcher. This project reverts to the principles of morphological integration (Olson & Miller, 1958) to aid in the objective, empirical selection of a set of phenotypic-functional measurements (Chapter 8). In doing so this method improves on the approach of Chimimba and Dipenaar (1995), Chimimba *et al.* (1999) and Taylor and Meester (1993). The method outlined in this thesis provides an objective, empirically-based, repeatable technique to allow for the selection of the final data set and is recommended for other similar studies.

A Framework for the Study of Geographic Variation

This study provides a framework for the study of geographic variation in mammalian species. Museum collections around the world hold an immense quantity of information that can, and should, make a vital contribution to the science of biodiversity conservation. This thesis provides a framework whereby collections-based studies of mammalian subspecific variation can be carried out.

As discussed, investigations of subspecific variation, whether for the practical necessity of formulating conservation policy, the academic pursuit of describing phylogeographic

patterns or for the pure interest of delimiting an accurate taxonomy, should utilise all available information. Molecular data add a temporal, historical dimension to the analysis by hypothesising past relationships and cladogenetic events. Meanwhile, morphological information identifies current adaptation. Both are of value in understanding subspecific variation and should complement each other in any analysis of subspecific variation.

In conclusion, this thesis provides a case study that shows (at least one way) that the academic traditions of morphological and molecular biologists can be brought together to improve our understanding of systematic and taxonomic knowledge and to inform the needs of a third group; the conservation biologists. The acceptance of the formal taxonomic grade of the subspecies as a fundamental unit for conservation will do much to enhance the pursuit of biodiversity conservation.

Patterns of Subspecies Diversity in the Giraffe.

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Patterns of Subspecies Diversity in the Giraffe.

APPENDICES

APPENDIX 1.2.1:

SUMMARY OF SUBSPECIES DESCRIPTIONS

These tables summarise the descriptions of the nine currently recognised giraffe subspecies (Dagg, 1971) and use the original descriptions. Descriptions of previously recognised and subsequently synonymised subspecies have been included as notes where they are recorded as differing from their senior synonym taxon.

A standard series of headings have been devised to categorise the quotations from the original descriptions or reviews. It is the nature of subspecies descriptions that only differences are mentioned and similarities are omitted. Therefore, where no comment on a particular character state was made, that heading has been removed from the table for clarity.

Giraffa camelopardalis camelopardalis (L.) 1758: 66.

N.B. Lydekker (1904) used the name *G. c. typica* for this subspecies.

Character	*Character State Description*	*Author*
Sexual Dimorphism	"Sexes nearly alike in respect to form and colour of markings."	Lydekker, 1904. p. 205.
	"In the female the spots are smaller and more numerous, this being especially noticeable on the hind-quarters and the upper parts of the fore-legs."	Lydekker, 1904. p. 205.
	"It is important to notice that the general pattern of the coloration is the same in both sexes."	Lydekker, 1904. p. 205.
Skull		
Occipital Horns	"No prominent occipital (posterior) horns."	Lydekker, 1904. p. 205.
Pelage		
Interspot Network	"... a coarse network of comparatively narrow light lines, which are buffish white in immature bulls, and nearly white in cows of the same age."	Lydekker, 1904. p. 205.
Body Spots	"Spots large, apparently chestnut coloured at all ages, more or less distinctly quadrangular in form,..."	Lydekker, 1904. p. 205.
Head Spots	"Front of face in bulls somewhat spotted, and sides fully spotted."	Lydekker, 1904. p. 205.
Belly Spots	"The underparts are comparatively free from spots"	Lydekker, 1904. p. 205.
Limb Spots	"On the outer side of the forelimb the spots extend well down to the knee, and in the hindlimb a considerable distance down to the cannon bone."	Lydekker, 1904. p. 205.
	"... the inner surface of the upper segments of both limbs" are "comparatively free from spots".	Lydekker, 1904. p. 205.

Notes on Synonyms:

Giraffa camelopardalis congoensis

Dagg (1971) considers *G. c. congoensis* to (perhaps) be a synonym of *G. c. camelopardalis*. Lydekker (1904) considers *G. c. congoensis* to be "specially characterised by the well-developed frontal horn, coupled with the

full spotting of the lower portion of the limbs (especially the hind pair), of which the ground colour is grey-fawn, and the large size and subquadrangular form of the body-spots, which show no tendency to split up into stars." Lydekker's (1904) separation of *G. c. congoensis* from the nominate subspecies on these characters may not be justified, even by his own description. He offers no description of median horn development in *G. c. camelopardalis* while the ground colour and spot pattern does not seem to differ greatly (at least in the interpretation of his descriptions). The extent of the spotting on the legs may be a diagnostic characters, but has been shown to be variable in other subspecies.

Lydekker (1904. p. 220) goes on to describe "the sides of the head [as] much more fully spotted than in the Cape form" and "the tail is remarkale for the great fullness of its terminal tuft".

Giraffa camelopardalis angolensis Lydekker, 1903: 121.

Character	*Character State Description*	*Author*
Skull		
Median Horn	"Frontal [median] horn represented by a low tuberosity or swelling."	Lydekker, 1904. p. 221.
Occipital Horns	"Whether posterior horns were developed, I have not been able to ascertain."	Lydekker, 1904. p. 221.
Pelage		
Interspot Network	"Ground colour white or whitish..."	Lydekker, 1904. p. 221.
Body Spots	"Markings more of the network type..."	Lydekker, 1904. p. 221.
	"Body-spots large, with ill-defined margins, and brown in colour..."	Lydekker, 1904. p. 221.
Head Spots	"Spots on face confined to an area lying below a longitudinal line running beneath the eye to the angle of the mouth."	Lydekker, 1904. p. 221.
Ear Patch	"A small and indistinct triangular area below the ear in which the ground colour is white."	Lydekker, 1904. p. 221.
Limb Spots	"... a sudden break into smaller spots about the middle of the thigh..."	Lydekker, 1904. p. 221.
	"... legs fully spotted to the hoofs, with the ground colour of their lower portion tawny."	Lydekker, 1904. p. 221.

Giraffa camelopardalis antiquorum (Jardine) 1835: 187.

Character	*Character State Description*	*Author*
Skull		
Occipital Horns	"Horns as in Nubian Giraffe [*G. c. camelopardalis*]."	Lydekker, 1904. p. 206.
Pelage		
Belly Spots	See Limb Spots.	
Limb Spots	"Nearly allied to [*G. c. camelopardalis*] from which it is easily distinguishable by the circumstance that	Lydekker, 1904. p. 206.

Character	*Character State Description*	*Author*
	in the fore-limb from just above the line of the abdomen, and on the hind-limb halfway up the thigh, the spots suddenly break up into a series of very small spots of irregular shape, similar spots occurring on the underparts and inner side of limbs."	

Giraffa camelopardalis giraffa Schreber, 1784: pl. 255.

Lydekker referred to *G. c. giraffa* as *G. c. capensis.*

Character	*Character State Description*	*Author*
Sexual Dimorphism	"... with the sexes alike with regards the pattern of the spots, but the old bulls much darker than the cows."	Lydekker, 1904. p. 225.
Skull		
Median Horn	"... the frontal [median] horn is rudimentary."	Lydekker, 1904. p. 225.
Occipital Horns	"... without posterior (occipital) horns ..."	Lydekker, 1904. p. 225.
Pelage		
Interspot Network	"... a tawny ground."	Lydekker, 1904. p. 225.
Body Spots	"A large and very dark coloured giraffe..."	Lydekker, 1904. p. 225.
	"... displaying the 'blotched type' of coloration in the most pronounced form ..."	Lydekker, 1904. p. 225.
	"As regards the distinctive features of the spots, or blotches, it may be observed that the large chocolate-brown, or almost black, body spots of the old bulls are more or less quadrangular in shape, without showing any tendency to split up into stars, and form conspicuous dark blotches..."	Lydekker, 1904. p. 225.
Ear Patch	"A white area ... on the sides of the head and neck below the ear."	Lydekker, 1904. p. 226.
Limb Spots	"The legs are fully spotted and dark coloured throughout..."	Lydekker, 1904. p. 225.
	"On the upper part of the limbs the spots tend to become somewhat irregular and jagged in outline, and they generally decrease in size as the hoofs are approached."	Lydekker, 1904. p. 226.

Notes on Synonyms:

Giraffa camelopardalis wardi

G. c. wardi resembles *G. c. giraffa* in the dark chocolate brown colour of the spots and the low median horn. In contrast to *G. c. giraffa*, *G. c. wardi* has the occipital horns "enormously developed" (Lydekker, 1904. p. 221). Lydekker (1904. p. 222) goes on to stress this point noting that the skull is "remarkable for the extraordinary development of the posterior, or occipital horns." Such is their development, coupled with the reduced size of the median horn, that Lydekker (1904. p. 223) considers the 'Four-horned Giraffe' to be an appropriate name. Also remarkable is the "generally large dimensions" of the skull and the "massiveness of the main [parietal] horns, of which the extremities are expanded in a knob-like manner." The body spots are broken up into irregular stars reminiscent of *G. c. tippelskirchi*. Lydekker (1904) points out that the "stellate

character of these spots widely distinguishes the race from the Cape Giraffe" (p. 222) and that "compared with the Cape Giraffe the spots are much more irregularly formed and star-like" (p. 224) making Dagg's (1971) reason for inclusion of *G. c. wardi* in *G. c. giraffa* unclear.

Giraffa camelopardalis peralta Thomas 1898: 40.

Both Thomas (1898) and Lydekker (1904) only had access to the skull and cannon bones of a single individual female.

Character	*Character State Description*	*Author*
Skull		
General Comments	""The skull was clearly ... that of a female, but was actually longer than any [available male skull] and ... considerably longer than any female skull..."	Thomas, 1898. p.39.
	"The skull, although unfortunately that of a female, apparently indicates a three-horned race."	Lydekker, 1904. p. 226.
Parietal Horns	"... widely divergent instead of parallel when viewed from the front, and, when viewed from the side, more vertically upright, instead of lying back in the plane of the forehead."	Thomas, 1898. p. 40.
Median Horn	"The third horn, for a female, was well developed, its bony core forming an obviously distinct ossification on the top of the swollen frontals."	Thomas, 1898. p. 40.
Face and Muzzle	"While in the ordinary Giraffe the tapering forward of the face from the orbits to the muzzle was even and gradual, in the present skull it was exceedingly abrupt at first, from the very broad orbital region to a point above the anterior premolars; then from this point forward the muzzle was very narrow and slender, almost parallel sided, broadening again in the region of the very large spatulate nasal opening."	Thomas, 1898. p. 39.
Skeleton		
Limb Bones	"... although its cannon bones still had their epiphyses separate, their total length exceeded that of the cannon bone (with ankylosed epiphyses) of a female Abyssinian Giraffe by nearly three inches, and scarcely fell short of that in an old male Giraffe from the same region."	Thomas, 1898. p.39.
	"The limb bones indicate an animal of great bodily height."	Lydekker, 1904. p. 226.

Giraffa camelopardalis reticulata de Winton 1899: 212.

Character	*Character State Description*	*Author*
Skull		
Median Horn	"The males have a third horn on the centre of the forehead just above the eyes, cylindrical, from 3 to 5 inches long..."	de Winton, 1897. p. 277
Pelage		
Interspot Network	"The ground-colour varies from white to fawn..."	de Winton, 1897. p. 277
	"... narrow pale lines..."	de Winton, 1899. p. 212

Character	*Character State Description*	*Author*
	"... a coarse network of narrow white lines..."	de Winton, 1899. p. 212
	"... the spaces between the dark patches are generally narrower and always far more clearly defined in aged animals than in those of a similar age in the Southern species."	de Winton, 1897. p. 277
Body Spots	"... the dark marks were large, sharply defined, and only separated from each other by narrow pale lines ..."	Thomas, 1894. p.135.
	""... the dark polygonal markings vary from orange-red to red-chocolate, the edges being evenly and sharply defined	
	"... a liver-red animal with a coarse network of narrow white lines dividing the body-colour into large sharply defined patches..."	de Winton, 1899. p. 212
Ear Patch	"... the white ears..."	de Winton, 1899. p. 212
Limb Spots	"The legs below the knees and hocks are white."	de Winton, 1897. p. 277
	"... the white ... legs below the knees and hocks..."	de Winton, 1899. p. 212

Notes on Synonyms:

Giraffa camelopardalis nigrescens

Lydekker (1911) described a subspecies of 'netted' giraffe from Somalia and Kenya. *Giraffa reticulata nigrescens*, as he named it, was based on a fragment of tanned skin. Lydekker (1911) reported that *G. r. nigrescens* differs from the nominate subspecies of reticulated giraffe in that "the white lines are rather wider and the dark areas smaller and brownish rufous, with a tinge of blackness, and a distinct blackish streak or star in the centre."

Giraffa camelopardalis rothschildi Lydekker, 1903: 122.

G. c. cottoni is included as a synonym by Dagg (1971).

Character	*Character State Description*	*Author*
General Comments	"... Giraffes of an unusually large size..."	Thomas, 1901. p. 474.
Sexual Dimorphism	"... the sexes, in the early adult condition at least are markedly different as regards both the form and colour of the spots..."	Lydekker, 1904. p. 210.
	"The marked discrepancy in the colouration of the two sexes is therefore a very distinctive feature of this race of giraffe at this age."	Lydekker, 1904. p. 212.
Skull		
General Comments	"A three horned Giraffe"	Lydekker, 1904. p. 210.
Parietal Horns	"The main [parietal] horns are large, and quite normal in position."	Thomas, 1901. p. 475.
Median Horn	"The anterior median horn is heavily developed in the males"	
Occipital Horns	"... peculiar projections on the sides fof the occiput ... on which account he called the animal a Five-	Thomas, 1901. p. 475.

Character	*Character State Description*	*Author*
	horned Giraffe..."	
	"Five horns generally or invariably present in old bulls, owing to the development of the posterior, or occipital, pair."	Lydekker, 1904. p. 210.
	Lydekker (1904) recognises individual variation in the development of the occipital horns.	Lydekker, 1904. p. 212.
Face and muzzle	"... marked lateral expansion of the premaxillary region..."	Lydekker, 1904. p. 209.
Pelage		
General Comments	"...both males and females of this race of Giraffe were often so dark in colour that they appeared to be nearly black, with white bellies and legs; this deepening of coloration being ... apparently coincident with advancing age."	Lydekker, 1904. p. 212.
Interspot Network	"[in adult bulls] ...the light interspaces yellowish fawn, forming narrow network-lines on the body, but becoming much broader on the neck..."	Lydekker, 1904. p. 210.
	"[In females] ... a light orange-fawn ground. The light areas on the neck very wide..."	Lydekker, 1904. p. 210.
Body Spots	"The blotches in young specimens are reddish fawn, darkening in the centre to deep blackish brown, and this darkening spreads in old specimens, until the blotches are wholly blackish."	Thomas, 1901. p. 475.
	"...the spots in adult bulls large and very dark coloured, showing a tendency to split up into stars, as indicated by lighter tripartite radiating lines in the larger ones..."	Lydekker, 1904. p. 210.
	"...some full grown bulls are decidedly lighter than the type, and exhibit distinctly star-like and irregular spots."	Lydekker, 1904. p. 212.
	In females the spots are much more irregular, jagged, and star like, reddish chestnut in colour..."	Lydekker, 1904. p. 210.
Neck Spots	"[In adult bulls] ... on the neck, ... the spots assume a more irregular and somewhat jagged contour."	Lydekker, 1904. p. 210.
Head Spots	"[In adult bulls] Sides of the face fully spotted with black."	Lydekker, 1904. p. 210.
	"From the strong spotting of the face in young adult bulls, this race might well be called the Spotted-faced Giraffe."	Lydekker, 1904. p. 212.
	"[In females] ... sides of face sparsely spotted..."	Lydekker, 1904. p. 210.
Ear Patch	"[In adult bulls] ... a triangular white area in the neighbourhood of the ear.."	Lydekker, 1904. p. 210.
	"[In females] ... white area round ear small..."	Lydekker, 1904. p. 210.
Limb Spots	"[In adult bulls] ...the lower part of the legs pure white and unspotted..."	Lydekker, 1904. p. 210.
	"[In adult bulls] ... Above the knees and hocks the spots are chestnut, these chestnut spots extending higher upon the hind than on the fore limbs."	Lydekker, 1904. p. 210.
	"[In females] ... the spots on the legs very small..."	Lydekker, 1904. p. 210.

Notes on Synonyms:

Giraffa camelopardalis cottoni

Lydekker (1904. p.207) based this subspecies on the evidence of a single male specimen. He noted that it was "apparently very closely related to the Baringo race" (*G. c. rothschildi*). According to Lydekker (1904. p. 207) this subspecies differs from *G. c. rothschildi* as "the spots on the neck are deep chestnut-brown instead of black, and show no tendency to split up into smaller spots by the development of lighter lines radiating from the centre." Also "the spots themselves are of more regular and more squared form, those on the lower part of the neck being so arranged that the fawn-coloured interspaces form continuous transverse bands." The face spotting differs and is restricted (in *G. c. cottoni*) to "an area lying considerably below a longitudinal line drawn through the eye" with "the spots between the eye and the ear ... smaller" and not extending over the parietal horns or the top of the head. Similarly there are no spots under the chin. All head spots are brown instead of black. The body spots and spots of the upper leg are smaller and more numerous in *G. c. cottoni* than in *G. c. rothschildi*. Meanwhile, the spots on the front and inner side of the legs are paler. The parietal and occipital horns are smaller. The single skull of *G. c. cottoni* has a 'orbital horn' projecting over the right orbit. The collector assured Lydekker that all male giraffe from this locality posses such a 'horn' on the right side, and suggested that some male *G. c. rothschildi* have an orbital horn on the left side. The skull of *G. c. cottoni* is also lower and narrower without the expansion of the premaxillae.

Lydekker acknowledges the difficulty of recognising a subspecies from a single specimen and notes that this subspecies is "therefore of necessity somewhat provisional and liable to revision". Indeed his distinctions are inconsistent. In considering the coloration of the two subspecies he considers *G. c. rothschildi* to be the far darker animal although in his description of this subspecies he describes the extreme darkness (blackness) as a trend, not as an absolute. Clearly features such as the horizontal banding of the network on the neck and the presence of the orbital horn over the right eye may be due to individual variation.

Lydekker (1904) does recognise that *G. c. cottoni* may represent a transition between *G. c. rothschildi* and *G. c. antiquorum* (p. 210). Despite this, he (on p. 209) justifies his decision because of "the general tone, form, and mode of the arrangement of the spotting". He stresses the pattern of the facial spots, the spots on the top of the head and parietal horns and on the forelimb.

Giraffa camelopardalis thornicrofti Lydekker, 1911: 484.

Character	*Character State Description*	*Author*
General Comments	"... appears to be related to ... *G. c. tippelskirchi* but differs by the more compact frontal horn, the brown, in place of grey forehead, and the uniformly fawn lower part of the legs, the latter being whitish in adult bulls, but fawn and spotted in cows and young bulls."	Lydekker, 1911. p. 484.
Skull		
Median Horn	"... characterised by the low and conical frontal horn ..."	Lydekker, 1911. p. 484.
Pelage		
Interspot Network	"... a yellowish-fawn ground..."	Lydekker, 1911. p. 484.
Body Spots	"... absence of a distinctly stellate pattern in the neck and body spots, which are light brown on a yellowish-fawn ground .."	Lydekker, 1911. p. 484.

Character	*Character State Description*	*Author*
Neck Spots	See 'Body Spots'.	
Head Spots	"... grey colour and scattered spotting of the sides of the face, the chestnut brown forehead, deepening into black on the tips of the horns... "	Lydekker, 1911. p. 484.
Limb Spots	"... uniformly tawny colour of the lower portion of the limbs."	Lydekker, 1911. p. 484.

Giraffa camelopardalis tippelskirchi Matschie, 1898: 78.

Character	*Character State Description*	*Author*
Sexual Dimorphism	"... the sexes nearly alike in the form and colour of the spots..."	Lydekker, 1904. p. 215.
Skull		
General Comments	"...three-horned Giraffe..."	Lydekker, 1904. p. 215.
Median Horn	"... we have no evidence as to whether the male of the present race has a third horn."	Lydekker, 1904. p. 219.
	"That such an appendage [median horn] was present is ... demonstrated by sketches of a Giraffe's head and skull, having the type of coloration characteristic of the present form and carrying a well-marked third horn on the forehead."	Lydekker, 1904. p. 219.
	"... this third [median] horn appears to be decidedly smaller than in *G. c. rothschildi*..."	Lydekker, 1904. p. 219.
	"... some of the bull giraffes from the same locality have little or no third [median] horn."	Lydekker, 1904. p. 219.
Occipital Horns	"It is not known whether or no the posterior, or occipital, horns are developed."	Lydekker, 1904. p. 215.
Pelage		
Body Spots	"The spots in both sexes very irregular and jagged in contour, often displaying a distinctly star-like shape."	Lydekker, 1904. p. 215.
Limb Spots	"... the lower part of the legs (at least generally) more or less spotted and either whitish or olive coloured..."	Lydekker, 1904. p. 215.

Notes on Synonyms:

Giraffa camelopardalis schillingsi

The holotype of *G. c. schillingsi* is a skin of an adult female from the Kilimanjaro District of Tanzania and figured by Lydekker (1904. His text figures 29 and 30). Lydekker described it as "characterised by the white, unspotted legs, the light forehead, sparsely spotted cheeks, and the jagged, irregular, and somewhat star-like form of the chestnut spots, which are widely separated on the neck." Lydekker considers that *G. c. tippelskirchi* and *G. c. schillingsi* can not be separated. He cites a male skin, also from Kilimanjaro (in the BMNH, but gives no accession number), with typical *G. c. schillingsi* markings that has "the lower part of the fore-legs ... fawn-coloured, and spotted almost or quite down to the hoofs, after the fashion of the type of *tippelskirchi*." Hence, there is an individual of intermediate form occurring at the type locality of *G. c. schillingsi*, which Lydekker (1904. p. 219) considers to be "sufficient to show that the two are identical."

APPENDIX 2.3.1:

COMPLETE LIST OF SPECIMENS EXAMINED BY INSTITUTION

This is a complete list of all specimens examined, from eleven museums and research institutions in southern Africa, the U.K. and the U.S.A. Specimens from a total of 355 individual animals were examined. Not all specimens were used in all analyses. The particular specimens used in each analysis are listed in the relevant chapters.

U.K. Institutions

The Natural History Museum, London

BMNHALButler	BMNH1901.8.9.49	BMNH1912.2.24.1	BMNH1962.220
BMNH671a	BMNH1901.8.9.50	BMNH1912.2.24.2	BMNH1964.225
BMNH671b	BMNH1902.11.12.1	BMNH1912.2.24.3	BMNH1966.429
BMNH671c	BMNH1902.11.13.1	BMNH1912.2.24.4	BMNH1972.1330
BMNH671d	BMNH1903.11.18.1	BMNH1912.2.24.5	BMNH1972.811
BMNH1842.12.6.16	BMNH1903.4.15.1	BMNH1919.7.15.445	BMNH1972.812
BMNH1889.4.11.1	BMNH1903.4.16.1	BMNH1923.1.17.8	BMNH1976.347
BMNH1896.2.29.1	BMNH1903.8.13.1	BMNH1923.10.20.8	BMNH1986.1604
BMNH1898.4.28.1	BMNH1904.11.2.1	BMNH1923.10.20.19	BMNH1986.2500
BMNH1898.7.2.4	BMNH1904.11.2.2	BMNH1925.2.20.16	BMNHNN1
BMNH1898.7.2.5	BMNH1906.10.26.1	BMNH1925.2.20.17	BMNHNN2
BMNH1898.7.2.6	BMNH1906.2.12.1	BMNH1925.2.20.18	BMNHNN3
BMNH1899.12.10.1	BMNH1906.2.12.2	BMNH1926.12.13.15	BMNHNN4
BMNH1899.12.10.2	BMNH1907.12.15.2	BMNH1928.11.11.24	BMNHNN5
BMNH1899.7.8.5	BMNH1907.2.4.15	BMNH1931.2.1.48	BMNHNN6
BMNH1900.1.3.3	BMNH1907.7.8.255	BMNH1933.4.2.2	BMNHNN7
BMNH1900.2.25.1	BMNH1908.7.5.1	BMNH1938.7.8.21	BMNHNN8
BMNH1900.3.18.3	BMNH1908.8.12.2	BMNH1938.7.8.22	BMNHNN9
BMNH1900.4.3.1	BMNH1908.8.12.21	BMNH1939.4328	BMNHNN10
BMNH1901.5.14.1	BMNH1909.11.27.1	BMNH1939.4329A	BMNHNN11
BMNH1901.8.9.47	BMNH1910.10.17.1	BMNH1939.4804	
BMNH1901.8.9.48	BMNH1910.5.16.2	BMNH1959.440	

The Powell-Cotton Museum, Birchington, Kent.

PCCongo2A	PCMN275	PCMN581	PCSWA11
PCCongoNoNum1	PCMN277	PCNNChad138	PCTAN76
PCCongoNoNum2	PCMN278	PCNNChad142	PCTAN89
PCCAMII138	PCMN279	PCNNChad147	PCTAN102
PCCAMII139	PCMN280	PCNNChad209	PCUganda259
PCJ39	PCMN578	PCSudan16	PCUganda260
PCJ66	PCMN579	PCSWA9	PCUganda261

North American Institutions

American Museum of Natural History, New York.

AMNH14135	AMNH53545	AMNH81820	AMNH83460
AMNH24290	AMNH53546	AMNH81821	AMNH83605
AMNH24291	AMNH53547	AMNH81822	AMNH99493
AMNH24292	AMNH53548	AMNH81823	AMNH139695
AMNH24293	AMNH53549	AMNH81824	AMNH139696
AMNH27675	AMNH53550	AMNH81825	AMNH165051
AMNH27752	AMNH54122	AMNH81826	AMNH165052
AMNH27753	AMNH54123	AMNH82001	AMNH202438
AMNH35536	AMNH54326	AMNH82002	AMNH202884
AMNH35628	AMNH69403	AMNH82003	
AMNH53543	AMNH80146	AMNH83458	
AMNH53544	AMNH80146	AMNH83459	

Field Museum of Natural History, Chicago.

FMNH15576	FMNH34422	FMNH34429	FMNH127881
FMNH27475	FMNH34423	FMNH34930	FMNH127882
FMNH29515	FMNH34424	FMNH53765	FMNH127883
FMNH32901	FMNH34425	FMNH54251	FMNH127884
FMNH32902	FMNH34426	FMNH127878	FMNH127885
FMNH32904	FMNH34427	FMNH127879	FMNH127886
FMNH32905	FMNH34428	FMNH127880	FMNH127887

United States National Museum, Washington D.C.

USNM14411	USNM162989	USNM251797	USNM296145
USNM15339	USNM163112	USNM251798	USNM299998
USNM121010	USNM163113	USNM251799	USNM304612
USNM154033	USNM163312	USNM251800	USNM304613
USNM155438	USNM163324	USNM252549	USNM308877
USNM162016	USNM182124	USNM252585	USNM314984
USNM162017	USNM182125	USNM270594	
USNM162018	USNM182192	USNM277250	
USNM162988	USNM200151	USNM279405	

Southern African Institutions

Etosha Ecological Institute, Namibia

EEI1	EEI4	EEI7	EEIGc94.11.25.6MA
EEI2	EEI5	EEI8	Etosha1
EEI3	EEI6	EEI9	Etosha2

Note: The two specimens prefixed with 'Etosha' are field collected specimens from carcasses found in the Etosha National Park, Namibia.

Kruger National Park, South Africa.

KNP1	KNP3	KNP5	KNP7
KNP2	KNP4	KNP6	KNP8

National Museum, Bloemfontein, R.S.A.

NMB?	NMBNN5	NMB768?	NMB9337
NMBNN1	NMBNN6	NMB9337	NMB9380
NMBNN2	NMBNN7	NMB6036	NMBA2830
NMBNN3	NMB271	NMB6062	
NMBNN4	NMB274	NMB8738	

National Museum of Natural History, Bulawayo, Zimbabwe.

NMZB11525	NMZB23985	NMZB26865	NMZB29118
NMZB11533	NMZB23986	NMZB27144	NMZB29121
NMZB11544	NMZB23987	NMZB27451	NMZB58342
NMZB20221	NMZB26177	NMZB29099	NMZB58343
NMZB20382	NMZB26178	NMZB29100	NMZB59379
NMZB22635	NMZB26179	NMZB29101	NMZB60800
NMZB22862	NMZB26180	NMZB29102	NMZB60801
NMZB22947	NMZB26181	NMZB29103	NMZB60802
NMZB22957	NMZB26182	NMZB29104	NMZB60803
NMZB22958	NMZB26183	NMZB29105	NMZB60804
NMZB22959	NMZB26184	NMZB29106	NMZB60805
NMZB23977	NMZB26185	NMZB29107	NMZB60806
NMZB23978	NMZB26186	NMZB29108	NMZB60807
NMZB23979	NMZB26187	NMZB29109	NMZB60808
NMZB23980	NMZB26188	NMZB29110	NMZB60809
NMZB23981	NMZB26189	NMZB29111	NMZB60810
NMZB23982	NMZB26190	NMZB29112	NMZB60811
NMZB23983	NMZB26191	NMZB29113	NMZB60812
NMZB23984	NMZB26200	NMZB29114	NMZB60813

South African Museum, Cape Town, R.S.A.

ZM17176	ZM35364	ZM35367	ZM37058
ZM33540	ZM35365	ZM36654	ZM39692
ZM35363	ZM35366	ZM36851	ZM39827

Archaeology Section, Transvaal Museum, Pretoria, R.S.A.

AZ121	AZ635	AZ2154	AZ2556
AZ448	AZ1948	AZ2336	AZ2888

Mammal Section, Transvaal Museum, Pretoria, R.S.A.

TM12141

APPENDIX 2.3.2:

GIRAFFE SKULL MEASUREMENTS DATA SHEET

SHEET NUMBER: DATE: MUSEUM:

Accession Number								
Locality								
Sex								
Other label information								
Weight								
1 Weight								
Greatest Lengths	L	R	L	R	L	R		
2 Premaxillae to Parietal horns								
3 Premaxillae to Occipital ridge/horns								
4 Premaxillae to Condyles								
5 Nasal notch to Condyles								
Lengths	L	R	L	R	L	R		
6 Nasal Notch to anterior Orbit								
7 Premaxillae to anterior Orbit								
8 Posterior Orbit to Occipital ridge								
Widths								
9 Zygomatic width								
10 Minimum Maxillary width								
11 Maximum premaxillary width								
12 External auditory meatus width								
13 Maximum Orbital width								
14 Lacrimal width								
Heights								
15 Posterior M^3 to tip of Median horn								
16 Bottom of brain case to base of Parietal horns								
Parietal Horns	L	R	L	R	L	R		
17 Antero-posterior diameter of tip								
18 Lateral diameter of tip								
19 Tip Circumference								
20 Narrowest Circumference								
21 Height (from saggital point)								
22 External width of bases								
23 External width of tips								
24 Internal width of tips								

	L	R	L	R	L	R
Back of skull						
25 Top of Foramen magnum to top of skull						
26 Top of foramen magnum to tip of Parietal horns						
27 Top of Foramen magnum to top of occipital ridge.						
28 External width of occipital ridge						
29 Maximum width (if different)						
Base of Skull	L	R	L	R	L	R
30 Toothrow length (maxillary)						
31 Pterygoids to posterior of M^3						
32 Post glenoid process width						
33 Post glenoid process to posterior of M^3						
34 Tip of maxillae to PM^2						
35 Interpterygoid width						
36 Snout width at M1						
37 Palatine to Premaxillae length						
38 Palatine-Condyle length						
Mandible	L	R	L	R	L	R
39 Mandible height						
40 Mandible length						
41 Toothrow length (mandibular)						
42 Diastema length						
43 Canine width						
44 Width of mandibular symphysis posterior to canine alveoli						
45 Length of mandibular symphysis						
46 Incisiform toothrow length						
Post – cranial skeleton	L	R	L	R	L	R
47 Humerus length						
48 Radius/Ulna length						
49 Metatarsal length						
Maxillary M^1	L	R	L	R	L	R
50 Buccal Length						
51 Lingual Length						
52 Buccal Height – Front						
53 Buccal Height – Back						
54 Lingual Height – Front						
55 Lingual Height – Back						
56 Proximal Width						
57 Distal Width						
58 Lingual Width – Front						
59 Lingual Width - Back						

Additional Notes:

APPENDIX 2.3.3:

LIST OF MEASUREMENTS TAKEN WITH ABBREVIATIONS AND DESCRIPTIONS

All measurements are made in millimetres, except for MASS which is in kilograms. See Appendix 2.3.4 for an alphabetical list of measurement acronyms. See Appendix 2.3.6 for a figure of all measurements.

1 *Mass* – **MASS**
The mass of the cranium recorded in kilograms.

2 *Premaxillae to parietal horns* – **PPHL**
Measured bilaterally from the anterior tip of the premaxillae to the furthest point of the tip of the parietal horns.

3 *Premaxillae to occipital ridge/horns* – **POL**
Measured bilaterally from the anterior tip of the premaxillae to the furthest point of the occipital ridge.

4 *Premaxillae to condyles* – **PCL**
Measured bilaterally from the anterior tip of the premaxillae to the top of the articular surface of the occipital condyles where the top of the condyle meets the occipital bone.

5 *Nasal notch to condyles* – **NCL**
Measured bilaterally from the most posterior angle of the nasal notch to the articular surface of the occipital condyles where the top of the condyle meets the occipital bone.

6 *Nasal notch to anterior orbit* – **NOL**
Measured from the most posterior angle of the nasal notch to the most anterior margin of the orbit.

7 *Premaxillae to anterior orbit* – **PAOL**
Measured bilaterally from the anterior tip of the premaxillae to the most anterior margin of the orbit.

8 *Posterior orbit to occipital ridge* – **OOL**
Measured bilaterally from the most posterior margin of the orbit to the furthest point of the occipital ridge.

9 *Zygomatic width* – **ZGW**
Measured across the widest breadth of the zygometic ridges, typically (though not necessarily) close to the symphysis of the jugal and squamosal bones.

10 *Minimum maxillary width* – **MMW**
The minimum width across the maxillary bones anterior to the molariform teeth.

11 *Maximum premaxillary width* – **MPW**
Maximum width across the premaxillary bones.

12 *External auditory meatus width* – **EAMW**
Maximum width across the external auditory meatae.

13 *Maximum orbital width* – **MOW**
Maximum width across the frontal bones forming the dorsal margin of the orbit.

14 *Lacrimal width* – **LAW**
Minimum width across the lacrimal bones forming the anterior margin of the orbit.

15 *Posterior M^3 to tip of median horn* – **MMH**
Maximum height of the skull from the most ventral edge of the maxillary bone immediately posterior to the third maxillary molar to the highest point on the mid line of the median horn.

16 *Bottom of brain case to base of parietal horns* – **BPH**
Minimum height of the skull from the base of the braincase to the base of the parietal horns.

17 *Antero-posterior diameter of tip* – **APD**
Measured bilaterally, as the maximum diameter of the tip of the parietal horns in the antero-posterior orientation.

18 *Lateral diameter of tip* – **LAD**
Measured bilaterally, as the maximum diameter of the tip of the parietal horns in the lateral orientation, typically (though not necessarily) at right angles to the antero-posterior plane.

19 *Tip circumference* – **TIC**
Maximum circumference around the tip, measured for both horns.

20 *Narrowest circumference* – **NAC**
The minimum circumference of the parietal horns anywhere along their length. Measured for both horns.

21 *Height (from sagittal point)* – **HSP**
The height of the parietal horns measured to the top of the skull. A straight edge was rested across the tops of both horns and the height to the closest point of the skull was measured from the centre point of the straight edge.

22 *External width of bases* – **EWB**
The maximum width of the base of the parietal horns, including secondary bone deposition.

23 *External width of tips* – **EWT**
The maximum width measured to the outside surface of the tips of the parietal horns.

24 *Internal width of tips* – **IWT**
The maximum width measured to the inside surface of the tips of the parietal horns.

25 *Top of foramen magnum to top of skull* – **FMS**
Height of the skull measured from the top of the foramen magnum to the top of the skull. The point on the top of the skull located at the intersection of the medial longitudinal plane of the skull and the medial plane of the parietal horns.

26 *Top of foramen magnum to tip of parietal horns* – **FMP**
Height from the top of the foramen magnum to the tip of the parietal horns. Measured bilaterally.

27 *Top of foramen magnum to top of occipital ridge.* - **FMO**
The height from the top of the foramen magnum to a point on the top of the occipital ridge. This point located on the medial longitudinal plane of the skull and on the 'edge' of the ridge when sharp or on the maximum of curvature if rounded.

28 *External width of occipital ridge* – **EWO**
The maximum width of the occipital ridge. Typically (though not necessarily) measured to the occipital / squamosal suture on each side.

29 *Maximum width of the occipital ridge* – **MWO**
This measurement was taken if the secondary bone deposition on the occipital ridge created 'occipital horns' which were wider than the supporting occipital ridge. Measured to the maximum width of the secondary bone growth. If no secondary bone growth was present the value for **EWO** was used here.

30 *Toothrow length (maxillary)* – **MXTL**
The length of the maxillary toothrow from the anterior margin of the PM^2 alveolus to the posterior margin of the M^3 alveolus. The measurement was made to the edges of the alveoli, rather than to the teeth themselves so that the same measurement could be taken on skulls with missing teeth.

31 *Pterygoids to posterior of M^3* – **PPM**
Minimum distance from the pterygoid process to the closest margin of the M^3 alveolus. Measured bilaterally.

32 *Post glenoid process width* – **PGW**
The minimum distance between the tips of the post glenoid processes.

33 *Post glenoid process to posterior of M^3* – **PGM**
Minimum distance between the tip of the post glenoid process to the closest (posterior) margin of the M^3.

34 *Tip of premaxillae to PM^2* – **TMP**
Distance from the tip of the premaxillae to the anterior margin of the PM^2 alveolus. Measured bilaterally.

35 *Interpterygoid width* – **IPW**
Minimum distance between the pterygoid processes.

36 *Snout width at M^1* – **SWM**
Minimum distance across the palate measured between the M^1 alveoli.

37 *Palatine to premaxillae length* – **PPL**
Distance from the most posterior margin of the palate in the medial plane to the tip of the premaxillae. Measured bilaterally.

38 *Palatine-condyle length* – **PACL**
Distance from the most posterior margin of the palate in the medial plane to the posterior edge of the occipital bone in the medial plane between the occipital condyles.

39 *Mandible height* – **MAH**
The maximum height of the mandible from the base to the tip of the vertical ramus. Measured bilaterally.

40 *Mandible length* – **MAL**
Maximum length of the mandible from the most posterior point of the vertical ramus to the anterior of the symphysis of the dentaries (between the left and right I_1).

41 *Toothrow length (mandibular)* – **MDTL**
The length of the mandibular toothrow from the anterior margin of the PM_2 alveolus to the posterior margin of the M_3 alveolus. The measurement was made to the edges of the alveoli, rather than to the teeth themselves so that the same measurement could be taken on jaws with missing teeth.

42 *Diastema length* – **DIL**
Minimum distance between the anterior margin of the PM_2 alveolus and the posterior of the C_1 alveolus. The measurement was made to the edges of the alveoli, rather than to the teeth themselves so that the same measurement could be taken on jaws with missing teeth.

43 *Canine width* – **CAW**
The maximum length of the occlusal surface of the canine.

44 *Width of mandibular symphysis posterior to canine alveoli* – **WMS**
The maximum width of the dentaries immediately posterior to the canine alveoli.

45 *Length of mandibular symphysis* – **LMS**
The maximum length of the symphysis of the dentaries in the medial plane from between the left and right I_1.

46 *Incisiform toothrow length* – **ITL**
The length of the incisiform toothrow from the anterior margin of the I_1 alveolus to the posterior margin of the C_1 alveolus. The measurement was made to the edges of the alveoli, rather than to the teeth themselves so that the same measurement could be taken on skulls with missing teeth.

47 *Humerus length* – **HUL**
Total length of the humerus measured centrally down the anterior face of the bone.

48 *Radius / ulna length* – **RUL**
Total length of the radius measured centrally down the anterior face of the bone.

49 *Metatarsal length* – **MEL**
Total length of the metatarsal measured centrally down the anterior face of the bone.

50 *Buccal length* – **BUL**
The greatest (antero-posterior) length of the buccal occlusal surface of the first maxillary molar (M^1).

51 *Lingual length* – **LIL**
The greatest (antero-posterior) length of the lingual occlusal surface of the first maxillary molar (M^1).

52 *Buccal height; front* – **BHF**
Greatest height of the anterior buccal cusp of the first maxillary molar (M^1) measured from the highest point of the cusp directly down to the cingulum.

53 *Buccal height; back* – **BHB**
Greatest height of the posterior buccal cusp of the first maxillary molar (M^1) measured from the highest point of the cusp directly down to the cingulum.

54 *Lingual height; front* – **LHF**
Greatest height of the anterior lingual cusp of the first maxillary molar (M^1) measured from the highest point of the cusp directly down to the cingulum.

55 *Lingual height; back* – **LHB**
Greatest height of the posterior lingual cusp of the first maxillary molar (M^1) measured from the highest point of the cusp directly down to the cingulum.

56 *Proximal width* – **PXW**
Greatest width of the posterior occlusal surface of the first maxillary molar (M^1).

57 *Distal width* – **DSW**
Greatest width of the anterior occlusal surface of the first maxillary molar (M^1).

58 *Lingual width - front* – **LWF**
Greatest width of the anterior lingual occlusal surface of the first maxillary molar (M^1).

59 *Lingual Width - back* – **LWB**
Greatest width of the posterior lingual occlusal surface of the first maxillary molar (M^1).

APPENDIX 2.3.4:

ALPHABETICAL LIST OF MEASUREMENT ABBREVIATIONS

All measurements are made in millimetres, except for **MASS** which is in kilograms.

Abbrev.	No.	*Description*
APD	17	*Antero-posterior diameter of tip*
BHB	53	*Buccal height – back*
BHF	52	*Buccal height – front*
BPH	16	*Bottom of brain case to base of Parietal horns*
BUL	50	*Buccal length*
CAW	43	*Canine width*
DIL	42	*Diastema length*
DSW	57	*Distal width*
EAMW	12	*External auditory meatus width*
EWB	22	*External width of bases*
EWO	28	*External width of occipital ridge*
EWT	23	*External width of tips*
FMO	27	*Top of foramen magnum to top of occipital ridge.*
FMP	26	*Top of foramen magnum to tip of parietal horns*
FMS	25	*Top of foramen magnum to top of skull*
HSP	21	*Height (from sagittal point)*
HUL	47	*Humerus length*
IPW	35	*Interpterygoid width*
ITL	46	*Incisiform toothrow length*
IWT	24	*Internal width of tips*
LAD	18	*Lateral diameter of tip*
LAW	14	*Lacrimal width*
LHB	55	*Lingual height – back*
LHF	54	*Lingual Height – front*
LIL	51	*Lingual length*
LMS	45	*Length of mandibular symphysis*
LWB	59	*Lingual width – back*
LWF	58	*Lingual Width – front*
MAH	39	*Mandible height*

Abbrev.	No.	*Description*
MAL	40	*Mandible length*
MASS	1	*Mass*
MDTL	41	*Toothrow length (mandibular)*
MEL	49	*Metatarsal length*
MMH	15	*Posterior M^3 to tip of median horn*
MMW	10	*Minimum maxillary width*
MOW	13	*Maximum orbital width*
MPW	11	*Maximum premaxillary width*
MWO	29	*Maximum width of the occipital ridge*
MXTL	30	*Toothrow length (maxillary)*
NAC	20	*Narrowest circumference*
NCL	5	*Nasal notch to condyles*
NOL	6	*Nasal notch to anterior orbit*
OOL	8	*Posterior orbit to occipital ridge*
PACL	38	*Palatine-condyle length*
PAOL	7	*Premaxillae to anterior orbit*
PCL	4	*Premaxillae to condyles*
PGM	33	*Post glenoid process to posterior of M^3*
PGW	32	*Post glenoid process width*
POL	3	*Premaxillae to occipital ridge horns*
PPHL	2	*Premaxillae to parietal horns*
PPL	37	*Palatine to premaxillae length*
PPM	31	*Pterygoids to posterior of M^3*
PXW	56	*Proximal width*
RUL	48	*Radius / ulna length*
SWM	36	*Snout width at M^1*
TIC	19	*Tip circumference*
TMP	34	*Tip of premaxillae to PM^2*
WMS	44	*Width of mandibular symphysis posterior to canine alveoli*
ZGW	9	*Zygomatic width*

APPENDIX 2.3.5:

DESCRIPTION OF MEASURING DEVICES USED.

The variables measured by each measuring device are listed.

I *Somet Inox Engineering Callipers 15cm.*

Both internal and external measurements could be taken. Range 0 to 15.3 mm. Accurate to 0.05mm.

Used for: **APD, LAD, PPM, IPW, CAW, WMS, ITL, BUL, LIL, BHF, BHB, LHF, LHB, PXW, DSW, LWF, LWB.**

II *RS Components Ltd. Engineering Callipers 30cm.*

Internal and external measurements. Range 0.0 to 30.2mm. Accurate to 0.02mm.

Used for: **OOL, MMW, MPW, EAMW, HSP, EWB, EWT, IWT, FMO, EWO, MWO, MXTL, PGW, PGM, TMP, SWM, PACL, MAH, MDTL, DIL, LMS,**

III *Purpose made calipers by Mr Selwyn Mundy, senior technician, Institute of Zoology, Zoological Society of London.*

External measurements only. Range 0 to 883 mm (approx). Scale demarcated at 5mm intervals. Estimates of between mark distances were made, hence accuracy of approximately 1mm.

Used for: **PPHL, POL, PCL, NCL, NOL, PAOL, ZGW, MOW, LAW, MMH, BPH, FMS, FMP, PPL, MAL, HUL, RUL, MEL,**

IV *Dean re-inforced textile Tailor's Measuring Tape*

Used for 'curved' measurements. Range 0 to 100cm. Demarkated at 1mm intervals.

Used for: **TIC, NAC.**

Balance.

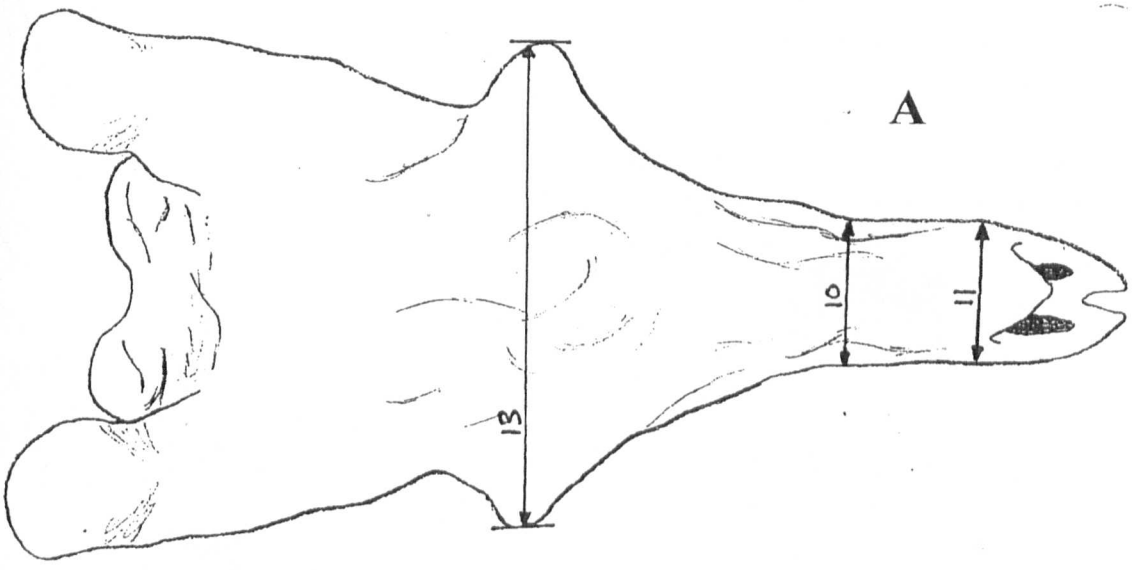
V *Salter 'Super Samson' Spring Balance*

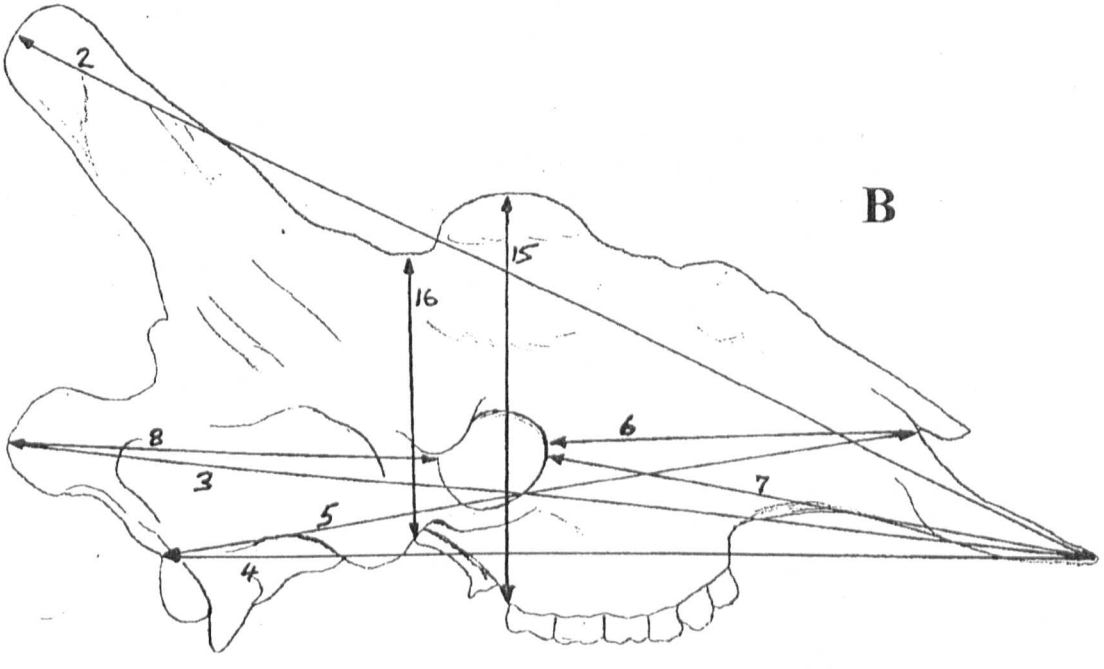
Range 0 to 15kg. Demarcated in 0.2kg increments. Therefore accurate to approximately 0.05 to 0.1kg.

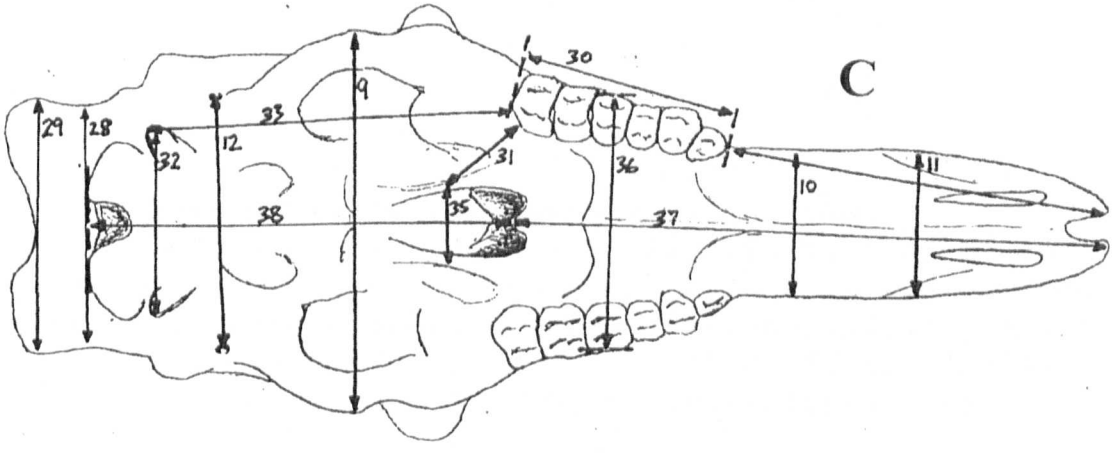
Used for: **MASS.**

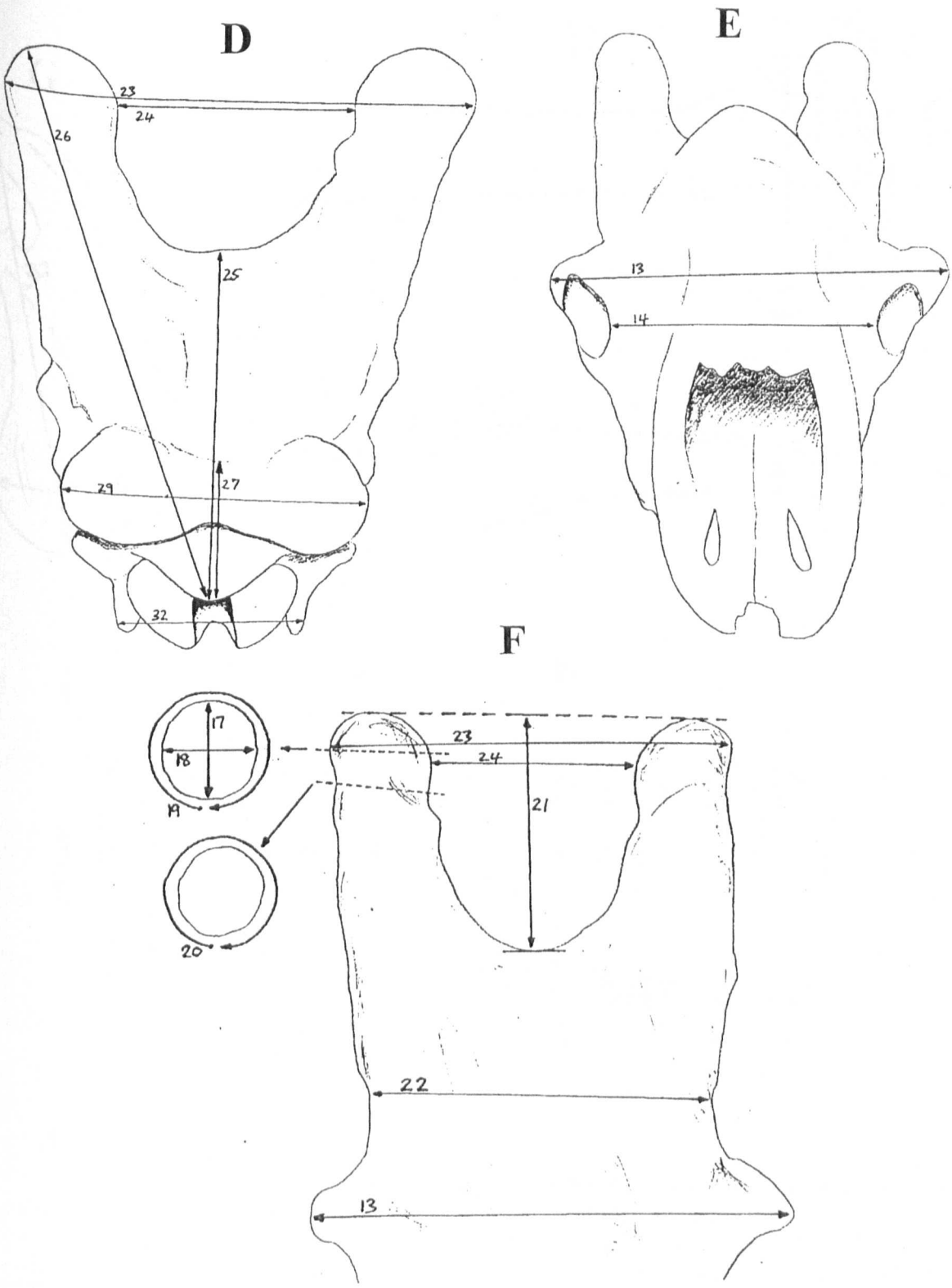
APPENDIX 2.3.6:
COMPLETE SET OF MEASUREMENTS TAKEN

A total of 59 measurements (with some taken bilaterally) were taken from crania, mandibles and forelimbs. The measurements (referenced by the relevant numbers) are described in Appendix 2.3.3.



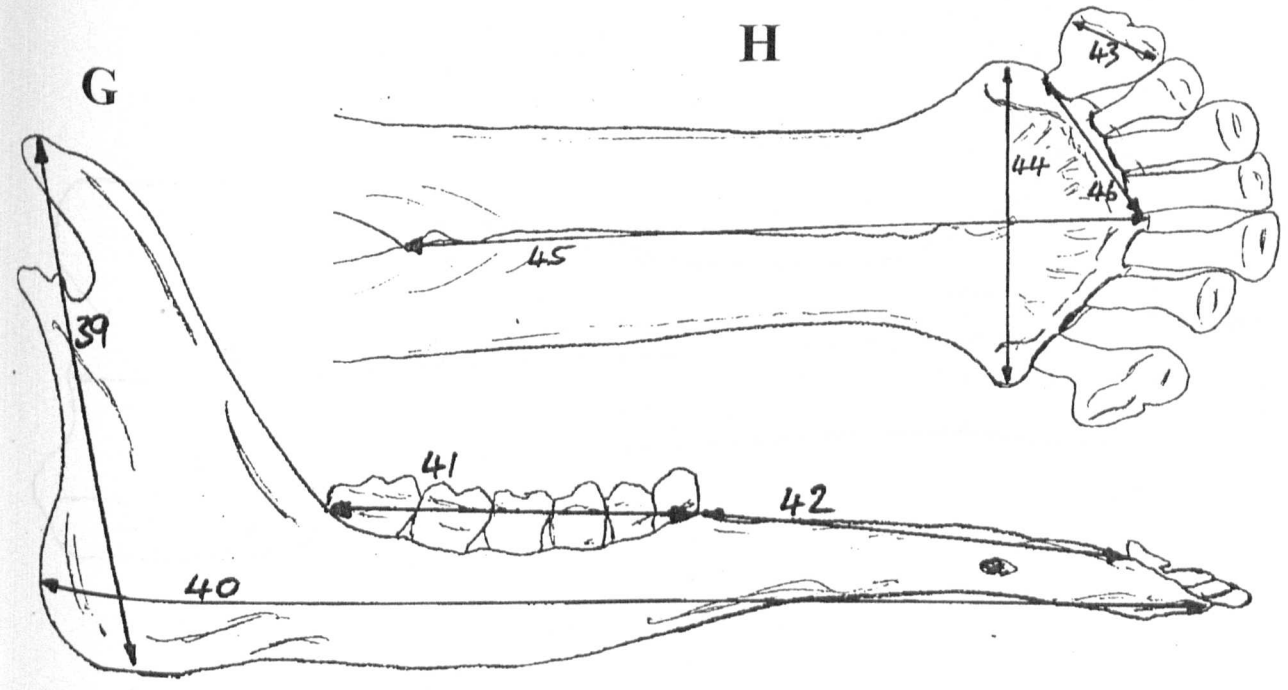


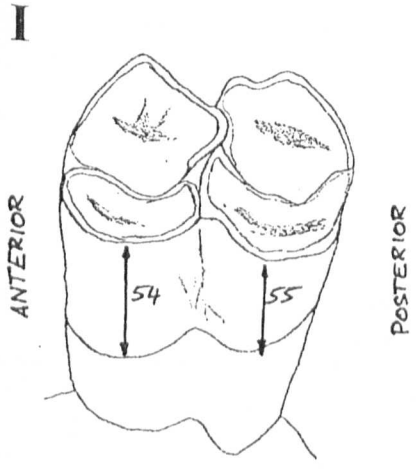


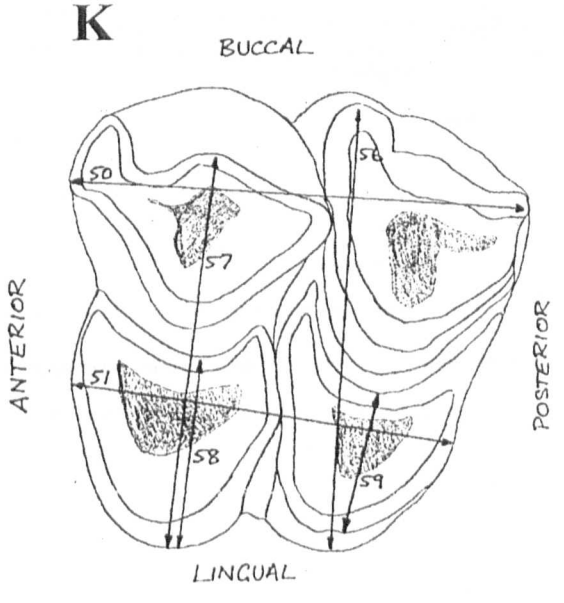


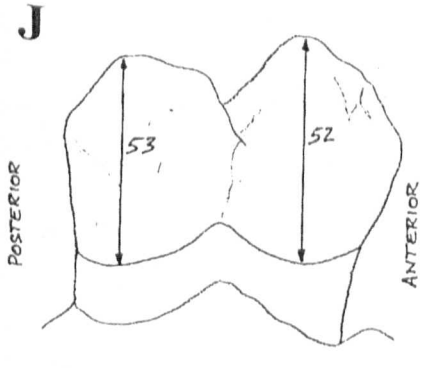
Appendix 2.3.6 continued: Skull measurements taken.

Previous Page: A - *Top view of skull;* **B** - *Side view of skull;* **C** - *Bottom view of skull.*
This page: D - *Back view of skull;* **E** - *Front view of skull;* **F** - *Front view of parietal horns.*
Long dashed lines indicate an extrapolated measurement point. Short dashed lines indicate a section through a structure, referring to the insets as indicated. Diagrams based on specimen number BMNH1986.1604. All numbers refer to Appendix 2.3.3.



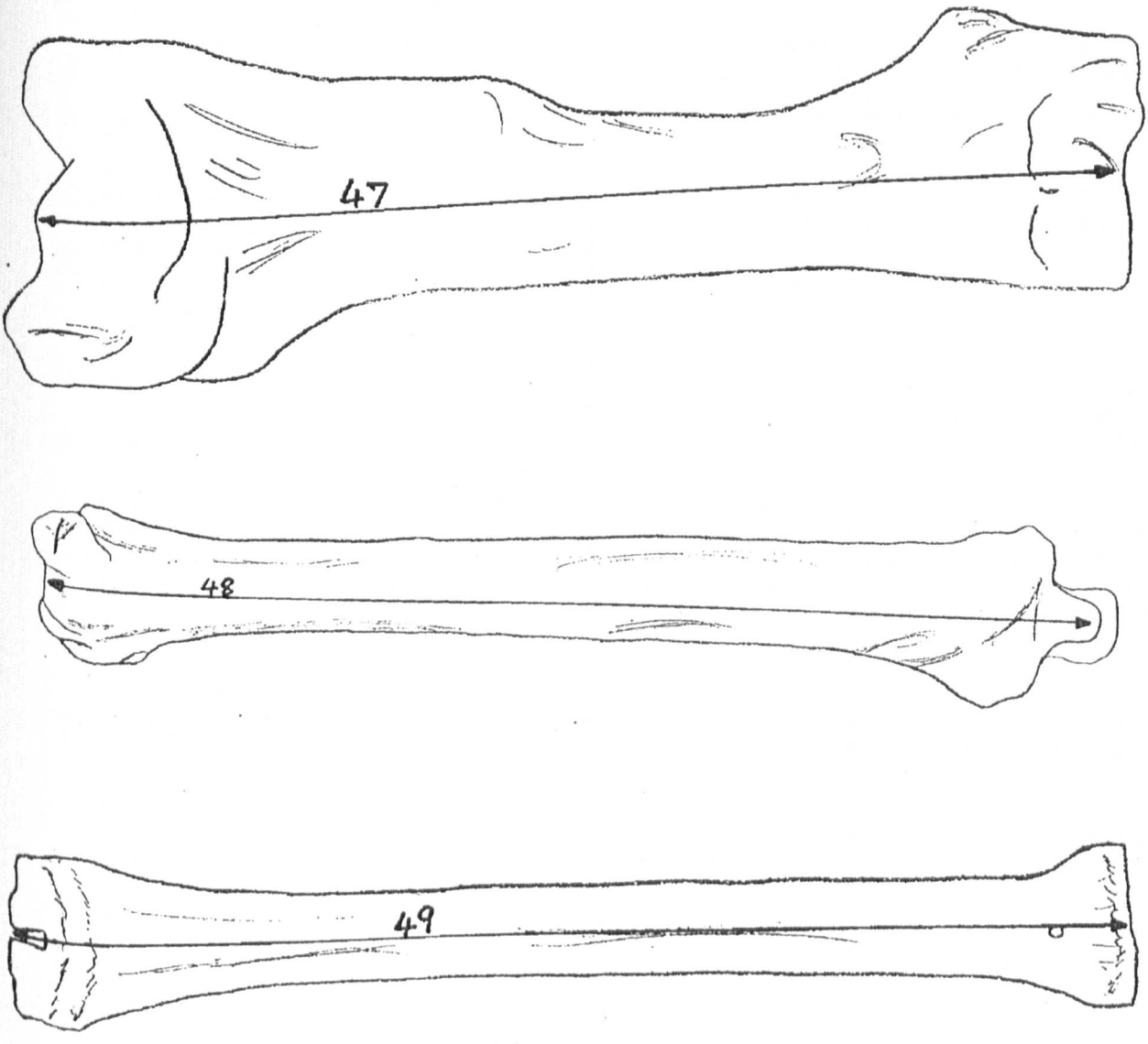






Appendix 2.3.6 continued: Mandible and tooth measurements taken.

G - *Side view of dentary (mandible);* **H** - *Top view of the anterior of the mandible;* **I** - *Inside view of the first maxillary molar;* **J** - *Outside view of the first maxillary molar;* **K** - *Top view of the occlusal surfaces of the first maxillary molar. Diagrams based on specimen number BMNH1986.1604. Not drawn to the same scale. All numbers refer to Appendix 2.3.3.*



Appendix 2.3.6 continued: Forelimb bones measurements taken.
L - *Humerus;* **M** - *Radius / Ulna;* **N** - *Metacarpus. Diagrams based on specimen number BMNH1986.1604. Not drawn to the same scale. All numbers refer to Appendix 2.3.3.*

APPENDIX 2.4.1:

AGE ESTIMATES, BASED ON TOOTH ERUPTION STAGE, OF SUB-ADULT SPECIMENS EXAMINED.

Two specimens were of known age (*).

Museum Identification Number	Age Class After Hall-Martin (1975, 1976)	Estimated Age (up to x)
NMZB29121f	1	Foetus
NMZB29118	1	1 day*
FMNH127884	1	4 weeks
BMNH1923.10.20.19	1	4 weeks
FMNH32904	1	4 weeks
AMNH82003	2	10 months
AMNH83459	2	10 months
BMNH1909.11.27.1	2	10 months
FMNH127879	2	10 months
FMNH34428	2	10 months
USNM163112	2	10 months
BMNH1966.429	4	15 months
NMZB26177	5	18 months
USNM162989	5	18 months
AMNH53548	6	2.5 years
BMNH1906.2.12.1	7	3 years
BMNH1938.7.8.21	7	3 years
PCSWA11	7	3 years
EEI6	7	3 years
NMZB11544	7	3 years
AMNH165052	7	3 years
NMZB22959	7	3 years
AMNH54122	8	3.5 years
FMNH29515	8	3.5 years
NMZB60806	8	3.5 years
NMZB60807	8	3.5 years
NMZB60811	8	3.5 years
NMZB60812	8	3.5 years
AMNH83605	8	3.5 years
BMNH1898.4.28.1	8	3.5 years
BMNH1899.12.10.1	9	4 years
BMNH1899.12.10.2	10	5 years
AMNH27753	10	5 years
FMNH27475	10	5 years
ZM39692	10	5 years
AMNH165051	11	5.5 years
FMNH34427	11	5.5 years
PCCAMII138	11	5.5 years
ZM37058	11	5.5 years
NMZB60813	11	5.5 years*
AMNH83458	12	6 years
BMNH1907.2.4.15	12	6 years
BMNH1923.1.17.8	12	6 years
FMNH32905	12	6 years

APPENDIX 2.4.2:
AGE ESTIMATES OF ADULT SPECIMENS

Age estimates for adult specimens derived from lingual occlusal surface width and lingual height of the first permanent maxillary molar (M^1) using Hall-Martin's (1975, 1976) equations. All measurements are given in millimetres. Both height and width values are the mean of up to four measurements from the proximal and distal lingual cusps of both left and right maxillary M^1. Age estimates for each data set are given according to the following equations:

Lingual occlusal surface width: $y = 1.3648x - 0.9404$

Lingual height: $y = -0.9558x + 20.7986$

where y = age estimate and x = width or height measurement. The two data sets provide significantly different age estimates. Age estimates from the lingual occlusal surface width will be used in subsequent analyses. Shaded cells indicate missing data.

Accession Number	Lingual Occlusal Surface		Lingual Height		Difference Between Age Estimates
	Mean Width	Age Estimate	Mean Height	Age Estimate	
	mm.	*Years.*	*mm.*	*Years.*	*Years.*
AMNH24290	8.70	10.93	16.38	5.15	5.79
AMNH24291	8.05	10.05	14.00	7.42	2.63
AMNH24292	8.37	10.48	14.50	6.94	3.54
AMNH24293	10.64	13.58	12.00	9.33	4.25
AMNH27752	11.86	15.25	8.50	12.67	2.58
AMNH53543	7.89	9.83	16.50	5.03	4.80
AMNH53546	11.24	14.41	7.50	13.63	0.78
AMNH53549	9.77	12.39	13.00	8.37	4.02
AMNH53550	8.89	11.19	13.75	7.66	3.54
AMNH54123	8.89	11.19	11.50	9.81	1.39
AMNH82001	8.86	11.16	10.00	11.24	-0.08
AMNH82002	6.81	8.35	16.75	4.79	3.56
AMNH83460	9.29	11.74	11.88	9.45	2.29
BMNH1842.2.6.16	11.70	15.03	12.63	8.73	6.30
BMNH1873.8.29.7	6.50	7.93	16.13	5.39	2.54
BMNH1897.1.30.1	8.25	10.31	14.75	6.70	3.61
BMNH1898.2.18.1	6.00	7.25	18.25	3.36	3.89
BMNH1898.7.2.5	11.00	14.07	7.33	13.79	0.28
BMNH1898.7.2.6	7.98	9.94	14.25	7.18	2.77
BMNH1899.7.8.5	10.00	12.71	9.50	11.72	0.99
BMNH1900.3.18.3	14.13	18.35	2.25	18.65	-0.30
BMNH1901.5.14.1	6.28	7.62	18.75	2.88	4.75
BMNH1901.8.9.47	12.28	15.81	8.50	12.67	3.14
BMNH1901.8.9.49	10.90	13.94	11.50	9.81	4.13
BMNH1901.8.9.50	12.60	16.26	7.67	13.47	2.79
BMNH1902.11.12.1	5.55	6.63	20.13	1.56	5.07
BMNH1903.4.16.1	11.67	14.98	7.75	13.39	1.59
BMNH1903.11.17.1					
BMNH1904.11.2.2	9.90	12.57	12.75	8.61	3.96
BMNH1906.2.12.2	7.58	9.40	14.50	6.94	2.46
BMNH1906.10.26.1	10.13	12.88	12.25	9.09	3.79
BMNH1907.7.8.255	7.45	9.23	17.13	4.43	4.80
BMNH1912.2.24.1	8.80	11.07	13.88	7.54	3.53
BMNH1912.2.24.2	7.70	9.57	11.63	9.69	-0.12
BMNH1923.10.20.8	8.78	11.04	12.50	8.85	2.18
BMNH1931.2.1.48	12.30	15.85	10.25	11.00	4.84
BMNH1931.2.1.49			16.50	5.03	
BMNH1933.4.2.2	11.78	15.13	10.38	10.88	4.25
BMNH1938.7.8.22	11.50	14.75	10.00	11.24	3.51
BMNH1939.4329A	10.28	13.08	10.75	10.52	2.56
BMNH1962.220	15.23	19.84	3.63	17.33	2.50
BMNH1964.225			8.50	12.67	

Accession Number	Lingual Occlusal Surface		Lingual Height		Difference Between Age Estimates
	Mean Width	Age Estimate	Mean Height	Age Estimate	
	mm.	*Years.*	*mm.*	*Years.*	*Years.*
BMNH1986.1604	11.70	15.03	7.75	13.39	1.64
BMNH671a	11.73	15.06	8.63	12.55	2.51
BMNH671c	10.33	13.15	12.75	8.61	4.54
BMNHALButler	8.70	10.93	16.25	5.27	5.67
EEI1	10.98	14.04			
EEI2	10.45	13.32	15.88	5.63	7.69
EEI3	13.52	17.51	12.00	9.33	8.18
EEI4	13.46	17.43	10.50	10.76	6.67
EEI5					
EEI7	10.22	13.00	18.00	3.59	9.41
EEI8	13.85	17.96	14.67	6.78	11.18
EEI9			8.00	13.15	
EEIGc94.11.25.6MA			8.33	12.83	
Etosha1	12.62	16.28	12.00	9.33	6.95
Etosha2	11.41	14.63	10.50	10.76	3.87
FMNH32901	10.54	13.45	12.00	9.33	4.12
FMNH32902	11.13	14.25	11.00	10.28	3.97
FMNH34422	13.70	17.75	10.75	10.52	7.23
FMNH34423	9.18	11.58	13.25	8.13	3.45
FMNH34424	13.15	17.00	9.00	12.20	4.81
FMNH34425	11.54	14.81	9.00	12.20	2.62
FMNH34429	7.65	9.50	14.25	7.18	2.32
FMNH34930	10.14	12.90	12.00	9.33	3.57
FMNH127878	16.02	20.92	8.00	13.15	7.77
FMNH127880	10.94	14.00	13.00	8.37	5.62
FMNH127881	10.55	13.46	13.00	8.37	5.09
FMNH127882	11.53	14.80	11.75	9.57	5.23
FMNH127883	12.74	16.45	7.25	13.87	2.58
FMNH127885			6.25	14.82	
FMNH127886	12.21	15.73	11.50	9.81	5.92
KNP6					
KNP7					
KNP8			10.00	11.24	
NMZB11525					
NMZB11533					
NMZB20221					
NMZB20382	10.40	13.25	12.63	8.73	4.52
NMZB22635					
NMZB22862					
NMZB23978	12.68	16.36	8.63	12.55	3.80
NMZB23979	10.37	13.21	11.13	10.17	3.04
NMZB23983	12.75	16.46	10.38	10.88	5.58
NMZB23984	12.45	16.05	9.63	11.60	4.45
NMZB26178	12.90	16.67	11.00	10.28	6.38
NMZB26179	14.00	18.17	9.25	11.96	6.21
NMZB26180	9.48	11.99	13.13	8.25	3.74
NMZB26184	13.48	17.45	6.88	14.23	3.22
NMZB26185	13.30	17.21	9.75	11.48	5.73
NMZB26186	10.53	13.42	12.50	8.85	4.57
NMZB26187	10.95	14.00	12.38	8.97	5.03
NMZB26188	11.13	14.24	12.75	8.61	5.63
NMZB26189	13.75	17.83	10.63	10.64	7.18
NMZB26200	13.25	17.14	9.00	12.20	4.95
NMZB27144	12.25	15.78	11.63	9.69	6.09
NMZB29099					
NMZB29100	8.90	11.21	15.00	6.46	4.74
NMZB29101	9.40	11.89	13.25	8.13	3.75
NMZB29102	5.20	6.16	11.50	9.81	-3.65

Accession Number	Lingual Occlusal Surface		Lingual Height		Difference Between Age Estimates
	Mean Width	Age Estimate	Mean Height	Age Estimate	
	mm.	*Years.*	*mm.*	*Years.*	*Years.*
NMZB29111	15.33	19.98	8.50	12.67	7.30
NMZB29112	12.95	16.73	6.50	14.59	2.15
NMZB29113	10.95	14.00	9.13	12.08	1.93
NMZB58342			7.25	13.87	
NMZB60800					
NMZB60801			9.00	12.20	
NMZB60802	10.83	13.84	9.75	11.48	2.37
NMZB60803	14.29	18.56	6.50	14.59	3.97
NMZB60804	10.95	14.00	14.38	7.06	6.95
NMZB60805	10.18	12.95	11.50	9.81	3.14
NMZB60809	9.03	11.38	16.13	5.39	5.99
NMZB60810	11.98	15.40	8.88	12.32	3.09
PCCAMII139	9.25	11.68	12.63	8.73	2.95
PCCongoNoNum1	12.50	16.12	7.25	13.87	2.25
PCCongoNoNum2			15.00	6.46	
PCJ39	10.13	12.88	10.75	10.52	2.35
PCJ66	7.00	8.61	15.00	6.46	2.15
PCMN275	11.38	14.58	9.50	11.72	2.87
PCMN276	9.00	11.34	10.75	10.52	0.82
PCMN277	12.38	15.95	10.63	10.64	5.31
PCMN278	9.00	11.34	13.13	8.25	3.09
PCMN280	11.17	14.30	9.17	12.04	2.26
PCMN579			6.50	14.59	
PCNNChad138	13.38	17.31	8.25	12.91	4.40
PCSudan16					
PCSWA9	11.25	14.41	10.00	11.24	3.17
PCTAN76	12.38	15.95	7.33	13.79	2.16
PCTAN89	10.25	13.05	12.25	9.09	3.96
PCTAN102	11.75	15.10	6.00	15.06	0.03
TM12141	12.32	15.87	8.50	12.67	3.20
USNM121010	11.49	14.74	10.25	11.00	3.74
USNM154033	7.88	9.81	16.38	5.15	4.66
USNM155438	10.29	13.10	11.25	10.05	3.05
USNM162016	9.66	12.24	14.50	6.94	5.30
USNM162017	6.92	8.51	15.25	6.22	2.29
USNM162018	9.48	12.00	12.25	9.09	2.91
USNM162988	11.76	15.11	7.38	13.75	1.36
USNM163113	8.43	10.57	15.00	6.46	4.10
USNM163312	9.34	11.81	14.38	7.06	4.75
USNM163324	10.82	13.83	13.25	8.13	5.69
USNM182124			5.00	16.02	
USNM182125			10.00	11.24	
USNM182192	10.81	13.82	9.00	12.20	1.62
USNM200151	8.56	10.75	15.00	6.46	4.29
USNM251797	11.73	15.07	7.25	13.87	1.20
USNM251798	11.85	15.23	7.88	13.27	1.96
USNM251799	9.08	11.45	10.88	10.40	1.05
USNM251800	9.19	11.60	10.75	10.52	1.08
USNM296145					
ZM17176	9.03	11.38	15.63	5.86	5.52

APPENDIX 2.4.3:
CORRELATION OF MEASURED VARIABLES TO AGE ESTIMATE.

Product-moment correlation coefficients (r) for each variable against estimated age. Female data are for all specimens for which data are available. Male data are presented separately for all specimens and for those specimens over 12 years old. Degrees of freedom equal n-1.
* = 0.05 > p > 0.01; ** = 0.01 > p > 0.001; *** = p < 0.001.

All Female	r	n	p
MASS	0.016	38	NS
PPHL	0.028	31	NS
POL	-0.015	31	NS
PCL	-0.068	29	NS
NCL	0.248	36	NS
NOL	0.318	36	NS
PAOL	-0.042	31	NS
OOL	0.193	40	NS
ZGW	0.440	40	**
MMW	0.139	33	NS
MPW	0.260	25	NS
EAMW	0.200	37	NS
MOW	0.268	40	NS
LAW	0.157	39	NS
MMH	0.065	40	NS
BPH	-0.090	38	NS
APD	-0.148	38	NS
LAD	-0.067	38	NS
TIC	-0.199	36	NS
NAC	-0.204	36	NS
HSP	-0.104	40	NS
EWB	0.177	40	NS
EWT	0.089	39	NS
IWT	0.118	39	NS
FMP	0.122	37	NS
EWO	0.246	40	NS
MWO	0.246	40	NS
MXTL	-0.220	40	NS
PPM	0.281	33	NS
PGW	0.107	35	NS
PGM	0.162	36	NS
TMP	0.056	29	NS
IPW	0.283	31	NS
SWM	0.598	40	***
PPL	0.069	29	NS
PACL	-0.055	38	NS
MAL	0.171	34	NS
MDTL	-0.053	35	NS
DIL	0.245	35	NS
CAW	-0.296	25	NS
WMS	-0.081	30	NS
LMS	0.237	34	NS
ITL	-0.174	34	NS
HUL	0.359	10	NS
RUL	0.371	11	NS
MEL	0.536	7	NS

All Male	r	n	p
MASS	0.397	56	**
PPHL	0.341	50	*
POL	0.436	50	**
PCL	0.442	47	**
NCL	0.507	53	***
NOL	0.462	56	***
PAOL	0.541	50	***
OOL	-0.008	56	NS
ZGW	0.377	56	**
MMW	0.463	56	***
MPW	0.305	47	*
EAMW	0.516	53	***
MOW	0.540	56	***
LAW	0.450	56	***
MMH	0.096	56	NS
BPH	0.323	56	*
APD	0.351	56	**
LAD	0.251	56	NS
TIC	0.327	56	**
NAC	0.385	56	**
HSP	0.223	56	**
EWB	0.402	56	**
EWT	0.321	56	*
IWT	0.165	56	NS
FMP	0.400	53	**
EWO	0.270	56	*
MWO	0.318	56	*
MXTL	0.276	56	*
PPM	0.272	49	NS
PGW	0.342	46	*
PGM	0.391	49	**
TMP	0.367	50	**
IPW	0.400	48	**
SWM	0.488	56	***
PPL	0.424	49	**
PACL	0.283	53	*
MAL	0.384	46	**
MDTL	0.436	47	**
DIL	0.370	46	**
CAW	-0.082	21	NS
WMS	0.426	42	**
LMS	0.215	45	NS
ITL	-0.011	44	NS
HUL	0.183	6	NS
RUL	-0.351	5	NS
MEL	-0.058	4	NS

Male >12yrs	r	n	p
MASS	0.208	41	NS
PPHL	0.2	38	NS
POL	0.275	38	NS
PCL	0.352	36	*
NCL	0.409	39	**
NOL	0.334	41	*
PAOL	0.25	38	NS
OOL	0.226	41	NS
ZGW	0.195	41	NS
MMW	0.32	41	*
MPW	0.181	37	NS
EAMW	0.31	38	NS
MOW	0.283	41	NS
LAW	0.23	41	NS
MMH	0.087	41	NS
BPH	0.275	41	NS
APD	0.208	41	NS
LAD	0.139	41	NS
TIC	0.2	41	NS
NAC	0.253	41	NS
HSP	0.186	41	NS
EWB	0.324	41	*
EWT	0.263	41	NS
IWT	0.145	41	NS
FMP	0.41	39	**
EWO	0.166	41	NS
MWO	0.198	41	NS
MXTL	0.261	41	NS
PPM	0.013	36	NS
PGW	0.015	32	NS
PGM	0.257	35	NS
TMP	0.193	38	NS
IPW	0.485	35	**
SWM	0.399	41	**
PPL	0.24	37	NS
PACL	0.284	39	NS
MAL	0.101	32	NS
MDTL	0.32	32	NS
DIL	-0.015	32	NS
CAW	-0.117	11	NS
WMS	0.214	30	NS
LMS	0.009	32	NS
ITL	0.06	32	NS
HUL	0.177	4	NS
RUL	-0.672	3	NS
MEL	-1	2	-

APPENDIX 2.5.1:

T-TEST RESULTS COMPARING VARIABLES BETWEEN MALE AND FEMALE SPECIMENS WITH THE SEX RECORDED.

Notes: The *t* values reported are for separate variance *t*-tests where no assumption of the equality of the two sample variances is made. In no case did the *t* value from a pooled variance analysis give a conflicting result.

Variable	Sex	n	Mean	SD	t	p
MASS	Female	24	2.190	0.420	-18.835	<0.001
	Male	45	8.738	2.260		***
PPHL	Female	20	632.3	26.78	-13.817	<0.001
	Male	36	762.0	43.39		***
POL	Female	21	624.8	24.23	-10.316	<0.001
	Male	36	699.8	29.91		***
PCL	Female	20	582.5	26.05	-7.847	<0.001
	Male	36	637.6	26.95		***
NCL	Female	25	454.1	22.15	-8.422	<0.001
	Male	40	507.6	28.72		***
NOL	Female	24	221.4	10.50	-9.318	<0.001
	Male	41	252.9	16.73		***
PAOL	Female	20	360.4	13.64	-8.815	<0.001
	Male	37	398.9	19.01		***
OOL	Female	26	232.7	10.80	-10.055	<0.001
	Male	48	263.4	15.23		***
ZGW	Female	27	223.8	8.626	-6.418	<0.001
	Male	49	239.0	11.86		***
MMW	Female	22	78.50	5.705	-11.083	<0.001
	Male	47	97.11	7.932		***
MPW	Female	15	83.00	5.305	-10.702	<0.001
	Male	33	103.5	7.657		***
EAMW	Female	26	153.6	7.684	-7.289	<0.001
	Male	47	169.9	11.36		***
MOW	Female	27	262.3	13.84	-9.106	<0.001
	Male	49	296.8	18.87		***
LAW	Female	27	174.3	10.96	-5.719	<0.001
	Male	50	190.1	12.77		***
MMH	Female	26	226.2	20.05	-10.587	<0.001
	Male	50	294.8	36.37		***
BPH	Female	24	163.3	12.89	-10.330	<0.001
	Male	49	199.1	15.79		***
APD	Female	26	22.95	4.468	-23.556	<0.001
	Male	49	53.11	6.535		***
LAD	Female	26	20.07	2.905	-22.011	<0.001
	Male	49	54.13	10.07		***
TIC	Female	25	68.28	9.600	-25.819	<0.001
	Male	49	168.2	23.30		***
NAC	Female	25	67.74	9.289	-26.754	<0.001
	Male	49	156.3	19.19		***
HSP	Female	26	93.96	15.33	-14.998	<0.001
	Male	49	156.1	19.97		***
EWB	Female	26	171.5	15.35	-12.849	<0.001
	Male	50	223.4	18.97		***
EWT	Female	26	140.3	25.83	-12.484	<0.001
	Male	49	219.5	26.72		***
IWT	Female	26	101.8	26.88	-1.706	0.094
	Male	49	112.8	25.62		NS
FMS	Female	25	176.0	13.37	-8.675	<0.001
	Male	46	206.4	15.25		***
FMP	Female	25	241.8	23.91	-14.995	<0.001
	Male	46	332.9	25.46		***
FMO	Female	25	86.20	6.758	-10.073	<0.001
	Male	47	108.5	12.01		***

Variable	Sex	n	Mean	SD	t	p
EWO	Female	26	120.9	7.990	-11.905	<0.001
	Male	50	147.7	11.39		***
MWO	Female	26	120.9	7.990	-11.847	<0.001
	Male	50	148.2	11.93		***
MXTL	Female	27	143.3	5.476	-3.747	<0.001
	Male	50	148.1	5.283		***
PPM	Female	21	35.26	6.182	-2.181	0.035
	Male	36	38.83	5.570		*
PGW	Female	23	77.91	6.646	-4.191	<0.001
	Male	44	85.91	8.699		***
PGM	Female	24	160.2	13.70	-5.804	<0.001
	Male	41	179.9	12.19		***
TMP	Female	19	240.9	10.96	-7.229	<0.001
	Male	37	264.8	13.12		***
IPW	Female	18	37.00	3.290	-2.113	0.041
	Male	35	43.89	18.72		*
SWM	Female	26	149.1	5.741	-3.581	0.001
	Male	49	154.4	6.940		***
PPL	Female	19	344.7	15.146	-6.380	<0.001
	Male	35	377.7	22.68		***
PACL	Female	25	216.1	13.45	-5.402	<0.001
	Male	45	233.5	11.86		***
MAL	Female	26	495.5	19.35	-6.546	<0.001
	Male	38	531.6	24.59		***
MDTL	Female	27	171.1	5.782	-1.674	0.099
	Male	41	173.8	7.475		NS
DIL	Female	27	183.3	11.49	-5.224	<0.001
	Male	39	200.8	15.60		***
CAW	Female	21	25.91	3.057	-2.221	0.033
	Male	18	27.78	2.188		*
WMS	Female	23	55.65	5.382	-2.843	0.007
	Male	32	59.78	5.216		***
LMS	Female	25	139.4	9.840	-2.888	0.005
	Male	37	148.7	15.69		***
ITL	Female	25	41.32	3.537	-0.844	0.403
	Male	37	42.05	3.077		NS
HUL	Female	8	437.9	15.29	-6.615	<0.001
	Male	7	501.7	21.12		***
RUL	Female	9	697.2	35.52	-7.052	<0.001
	Male	7	812.4	29.78		***
MEL	Female	6	662.7	18.42	-5.800	<0.001
	Male	4	741.0	22.44		***

APPENDIX 2.5.2:

RECORDED AND ASSIGNED SEXES OF ADULT GIRAFFE SPECIMENS USED IN THIS STUDY.

Specimen	Initial Sex Status	Assigned Sex	Specimen	Initial Sex Status	Assigned Sex
AMNH24290	GNR Male	Male	NMZB26178	Male	Male
AMNH24291	GNR Male	Male	NMZB26179	GNR Male	Male
AMNH24292	GNR Male	Male	NMZB26180	GNR Male	Male
AMNH24293	GNR Male	Male	NMZB26185	GNR Male	Male
AMNH27752	Male	Male	NMZB26186	GNR Male	Male
AMNH53550	Male	Male	NMZB26187	GNR Male	Male
AMNH54123	GNR Male	Male	NMZB26188	GNR Male	Male
AMNH82001	GNR Male	Male	NMZB26189	GNR Male	Male
AMNH83460	Male	Male	NMZB26200	Male	Male
BMNH1842.2.6.16	Male	Male	NMZB27144	Male	Male
BMNH1897.1.30.1	GNR Male	Male	NMZB29099	GNR Male	Male
BMNH1898.7.2.5	GNR Male	Male	NMZB29100	GNR Male	Male
BMNH1899.7.8.5	GNR Male	Male	NMZB29102	GNR Male	Male
BMNH1901.8.9.47	Male	Male	NMZB29111	Male	Male
BMNH1903.11.17.1	Male	Male	NMZB29112	Male	Male
BMNH1904.11.2.2	GNR Male	Male	NMZB29113	GNR Male	Male
BMNH1906.10.26.1	Male	Male	NMZB58342	Male	Male
BMNH1906.2.12.2	GNR Male	Male	NMZB60800	GNR Male	Male
BMNH1923.10.20.8	Male	Male	NMZB60801	Male	Male
BMNH1931.2.1.48	Male	Male	NMZB60802	Male	Male
BMNH1933.4.2.2	GNR Male	Male	PCCAMII139	Male	Male
BMNH1938.7.8.22	Male	Male	PCCongoNoNum1	GNR Male	Male
BMNH1962.220	Male	Male	PCCongoNoNum2	GNR Male	Male
BMNH1964.225	GNR Male	Male	PCJ39	Male	Male
BMNH1986.1604	Male	Male	PCJ66	Male	Male
BMNHALButler	GNR Male	Male	PCMN276	Male	Male
EEI1	GNR Male	Male	PCMN278	Male	Male
EEI3	GNR Male	Male	PCMN280	Male	Male
EEI4	GNR Male	Male	PCMN579	Male	Male
EEI5	GNR Male	Male	PCNNChad138	GNR Male	Male
EEI8	GNR Male	Male	PCSudan16	Male	Male
EEIGc94.11.25.6MA	GNR Male	Male	PCTAN102	GNR Male	Male
FMNH127878	Male	Male	PCTAN76	Male	Male
FMNH127881	Male	Male	TM12141	Male	Male
FMNH127882	Male	Male	USNM121010	Male	Male
FMNH127883	Male	Male	USNM154033	GNR Male	Male
FMNH34422	Male	Male	USNM155438	Male	Male
FMNH34425	Male	Male	USNM162016	GNR Male	Male
FMNH34930	GNR Male	Male	USNM162017	Male	Male
KNP6	GNR Male	Male	USNM162018	Male	Male
KNP7	GNR Male	Male	USNM163113	Male	Male
KNP8	GNR Male	Male	USNM163312	Male	Male
NMZB11525	Male	Male	USNM182124	GNR Male	Male
NMZB20221	Male	Male	USNM182125	GNR Male	Male
NMZB20382	Male	Male	USNM182192	GNR Male	Male
NMZB22862	Male	Male	USNM200151	GNR Male	Male
NMZB23978	Male	Male	USNM251799	GNR Male	Male
NMZB23979	GNR Male	Male	USNM296145	GNR Male	Male
NMZB23983	Male	Male	ZM17176	GNR Male	Male
NMZB23984	Male	Male			

Specimen	Initial Gender Status	Assigned Gender	Specimen	Initial Gender Status	Assigned Gender
AMNH53543	Female	Female	FMNH127885	Female	Female
AMNH53546	Female	Female	FMNH127886	Female	Female
AMNH53549	Female	Female	FMNH32901	Female	Female
AMNH82002	GNR Female	Female	FMNH32902	Female	Female
BMNH1873.8.29.7	GNR Female	Female	FMNH34423	GNR Female	Female
BMNH1898.2.18.1	Female	Female	FMNH34424	Female	Female
BMNH1898.7.2.6	GNR Female	Female	FMNH34429	GNR Female	Female
BMNH1900.3.18.3	Female	Female	NMZB26184	GNR Female	Female
BMNH1901.8.9.49	GNR Female	Female	NMZB60803	Female	Female
BMNH1901.8.9.50	Female	Female	NMZB60804	Female	Female
BMNH1902.11.12.1	Female	Female	NMZB60805	Female	Female
BMNH1903.4.16.1	Female	Female	NMZB60809	Female	Female
BMNH1912.2.24.1	GNR Female	Female	PCMN275	Female	Female
BMNH1912.2.24.2	GNR Female	Female	PCMN277	Female	Female
BMNH1931.2.1.49	Female	Female	PCSWA9	Female	Female
BMNH1939.4329A	GNR Female	Female	PCTAN89	Female	Female
EEI2	GNR Female	Female	USNM162988	Female	Female
EEI7	GNR Female	Female	USNM163324	Female	Female
EEI9	GNR Female	Female	USNM251797	Female	Female
Etosha1	GNR Female	Female	USNM251798	GNR Female	Female
Etosha2	GNR Female	Female	USNM251800	GNR Female	Female
FMNH127880	Female	Female			

APPENDIX 2.6.1:

GIRAFFE SPECIMEN LOCATIONS AND GEOGRAPHICAL GROUPINGS

The provenance of each specimen, with the corresponding population group is given. Latitude and longitude co-ordinates are given where available. 'Unas' in the population group column indicates that the specimen could not be unequivocally assigned to a population group, usually due to a vague locality or the locality could not be found in the available maps and gazetteers. Some given locations, though vague, did allow the specimen to be assigned to a population group.

Accession Number	Provenance	Northing	Easting	Popn
AMNH24290	BSA - Bechuanaland.			SWC
AMNH24291	BSA, Bechuanaland, Tuley.			Unas
AMNH24292	BSA, Bechuanaland, Tuley.			Unas
AMNH24293	BSA, Bechuanaland, Tuley.			Unas
AMNH27752	Kenya, Naungu.			Unas
AMNH27753	Kenya, Komarock.	1°18'S	37°13'E	ESK
AMNH53543	Belgian Congo, Oriental Faradje.	3°44'N	29°43'E	ECC
AMNH53544	Belgian Congo, Garamba	3°37'N	28°35'E	ECC
AMNH53546	Belgian Congo, Garamba	3°37'N	28°35'E	ECC
AMNH53548	Belgian Congo, Garamba	3°37'N	28°35'E	ECC
AMNH53549	Belgian Congo, Garamba	3°37'N	28°35'E	ECC
AMNH53550	Belgian Congo, Garamba	3°37'N	28°35'E	ECC
AMNH54122	British East Africa, Sultan Hamad.	2°1'S	37°22'E	ESK
AMNH54123	British East Africa, Northern Guasanyra.	1°10'N	39°0'E	EEK
AMNH54326	Faradje, Dist Nele, NE Belgian Congo.	3°44'N	29°43'E	ECC
AMNH82001	British East Africa, Kenya Colony, Northern Uaso nyiro	1°10'N	39°0'E	EEK
AMNH82002	British East Africa, Kenya Colony, Northern Uaso nyiro	1°10'N	39°0'E	EEK
AMNH82003	British East Africa, Kenya Colony.			Unas
AMNH83458	Bechuanaland Protectorate, Mababe Flats.	19°S	24°E	SWC
AMNH83459	Bechuanaland, Mabebe Flats.	19°S	24°E	SWC
AMNH83460	Bechuanaland, Mabebe Flats.	19°S	24°E	SWC
AMNH83605	Southern Rhodesia, East of Ngamo Station.	19°5'S	27°28'E	SWC
AMNH165051	South West Africa, Etosha Pan. Farm Lombart, Camp 2.	19°10'S	15°55'E	SWC
AMNH165052	South West Africa, Etosha Pan. Farm Lombart, Camp 2.	19°10'S	15°55'E	SWC
AZ635	Langjan Nature Reserve (?RSA?) spent final year in a zoo.			Unas
AZ1948	Lyndberg District (?RSA?)			Unas
BMNHA. L. Butler	Soudan			Unas
BMNH671a	Koraqua, Klipfontein.	33°10'S	25°32'E	SWC
BMNH671b	Chue Spring, Maadji Mountains, South Africa.			Unas
BMNH671c	North Africa			Unas
BMNH1842.12.6.16	"Cape of Good Hope", South Africa.			Unas
BMNH1873.8.29.7	Dembelas, Abyssinia.	13°46'N	39°1'E	ENE
BMNH1896.2.29.1	Khanan Country, Northern Kalahari, Botswana.			SWC
BMNH1897.1.30.1	East of Loroghi Mountains, Kenya.			EEK
BMNH1898.2.18.1	South East of junction of Benue and Niger Rivers	8°0'N	7°0'E	WSN
BMNH1898.4.28.1	North East Africa			Unas
BMNH1898.7.2.4	Up country from Mombasa	3°59'S	39°40'E	ESK
BMNH1898.7.2.5	Up country from Mombasa, 100 miles up the railway	3°59'S	39°40'E	ESK
BMNH1898.7.2.6	60 miles north east by east of Baringo, Kenya.	0°50'N	36°50'E	EEK
BMNH1899.12.10.1	Rhombo River, Kilimanjaro District. 25 miles NE of Kilimanjaro.	3°16'S	37°38'E	ESK

Accession Number	Provenance	Northing	Easting	Popn
BMNH1899.12.10.2	Rhombo River, Kilimanjaro District. 25 miles NE of Kilimanjaro. British East Africa.	3°16'S	37°38'E	ESK
BMNH1899.7.8.5	100 miles east of Loroghi			EEK
BMNH1900.1.3.3	Helogale, British East Africa			Unas
BMNH1900.3.18.3	Athi Plains, British East Africa	2°57'S	38°31'E	ESK
BMNH1900.4.3.1	East bank of Great Loangwa River, Northern Rhodesia	13°30'S	31°30'E	SZT
BMNH1901.5.14.1	White Nile, within 100 miles of the junction of the Bahr-el Jebel and the White Nile.	8°N	32°E	ECU
BMNH1901.8.9.47	Guas' Ngishu Plateau.	1°0'N	35°0'E	ECU
BMNH1901.8.9.48	Guas Ngishu Plateau.	1°0'N	35°0'E	ECU
BMNH1901.8.9.49	Guas Ngishu Plateau.	1°0'N	35°0'E	ECU
BMNH1901.8.9.50	Guas' Ngishu Plateau.	1°0'N	35°0'E	ECU
BMNH1902.11.12.1	Kodok, Fashoda, Sudan. West of the White Nile.	9°53'N	32°4'E	ENA
BMNH1902.11.13.1	Mongalla, White Nile	5°10'N	31°46'E	ECU
BMNH1903.11.17.1	Northern Transvaal			SEW
BMNH1903.11.18.1	Kilimanjaro	3°3'S	37°21'E	ESK
BMNH1903.4.15.1	Guasonggishu Plateau, Kenya.	1°0'N	35°0'E	ECU
BMNH1903.4.16.1	Lake Baringo, Kenya.	0°38'N	36°5'E	ECU
BMNH1903.8.13.1	Egyptian Sudan			ENA
BMNH1904.1.21.1	South of Lado	5°2'N	31°41'E	ECU
BMNH1904.11.2.1	Kenya			Unas
BMNH1904.11.2.2	East of Sharua, northern Nigeria. CHECK THIS			WSN
BMNH1906.10.26.1	Angola			SWA
BMNH1906.2.12.1	Northern Nigeria, 30 miles north of Yola.	9°11'N	12°30'E	WSN
BMNH1906.2.12.2	Northern Nigeria, 25 miles north of Yola.	9°11'N	12°30'E	WSN
BMNH1907.12.15.2	South Abyssinia			EEK
BMNH1907.2.4.15	Loanwa River	13°30'S	31°30'E	SZT
BMNH1907.7.8.255	Shari (now Chari) River, Cameroon / Chad border.	12°48'N	14°34'E	WCP
BMNH1908.7.5.1	Kenya			Unas
BMNH1908.8.12.2	Nigeria			WSN
BMNH1909.11.27.1	Sullam Hamud St., Uganda Railway, British East Africa.	2°1'S	37°22'E	ESK
BMNH1910.10.17.1	Petauke, northern Rhodesia (Zambia).	14°16'S	31°22'E	SZT
BMNH1910.5.16.2	East of Shoozi (?) Valley, French Congo.			ECC
BMNH1912.2.24.1	Archer's Post, Eusso Nyiro, British East Africa.	0°39'N	37°41'E	EEK
BMNH1912.2.24.2	Archer's Post, Eusso Nyiro, British East Africa.	0°39'N	37°41'E	EEK
BMNH1912.2.24.3	Archer's Post, N. Eusso Nyiro, British East Africa.	0°39'N	37°41'E	EEK
BMNH1912.2.24.4	Archer's Post, N. Eusso Nyiro, British East Africa.	0°39'N	37°41'E	EEK
BMNH1912.2.24.5	Archer's Post, N. Eusso Nyiro, British East Africa.	0°39'N	37°41'E	EEK
BMNH1919.7.15.445	Guas Ngishu Plateau, British East Africa.	1°0'N	35°0'E	ECU
BMNH1923.10.20.8	Jubaland			EEK
BMNH1925.2.20.16	River Umzinguane, Matabeleland, Zimbabwe.	22°11'S	29°55'E	SEW
BMNH1925.2.20.17	River Umzinguane, Matabeleland, Zimbabwe.	22°11'S	29°55'E	SEW
BMNH1925.2.20.18	River Umzinguane, Matabeleland, Zimbabwe.	22°11'S	29°55'E	SEW
BMNH1926.12.13.15	Lado, Sudan	5°2'N	31°41'E	ECU
BMNH1928.11.11.24	Bedadi, south east of Lake Chad	10°45'N	18°18'E	WCP
BMNH1931.2.1.48	Mabebe Falls, Mogogilo River, Kalahari.	19°S	24°E	SWC
BMNH1933.4.2.2	Tanganyika Territory, HM Eastern African Dependencies			Unas
BMNH1938.7.8.21	Balahuti, Angola.	16°30'S	16°55'E	SWA
BMNH1938.7.8.22	Bi-Indu near French Cameroons, 40km south east of Rei Bouba	8°25'N	14°30'E	WCP
BMNH1939.4328	Bahr el Ghazal	7°46'N	27°40'E	ENA
BMNH1939.4329A	N'Gamiland, Botswana			SWC
BMNH1939.4804	Cunene River, 150 miles south west of Humbe, Angola.	16°42'S	14°55'E	SWA
BMNH1962.220	Langata, near Nairobi, Kenya.	1°24'S	36°46'E	ESK
BMNH1964.225	South of Karamoja, Uganda.	2°45'N	34°15'E	ECU

Accession Number	Provenance	Northing	Easting	Popn
BMNH1966.429	Doddieburn Ranch, Matabeleland, Rhodesia.	21°10'S	29°22'E	SCZ
BMNH1986.1604	West Nicholson, Zimbabwe.	21°3'S	29°20'E	SCZ
BMNH1986.2500	Matabeleland, Zimbabwe			SCZ
EEI1	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI2	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI3	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI4	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI5	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI6	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI7	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI8	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEI9	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
EEIGc94.11.25.6MA	Etosha National Park, Namibia.	19°10'S	15°55'E	SWC
Etosha1	Leeubos, Etosha National Park, Namibia.	19°2'S	15°49'E	SWC
Etosha2	Leeubos, Etosha National Park, Namibia.	19°2'S	15°49'E	SWC
FMNH15576	Abyssinia			Unas
FMNH27475	Northern Uganda. / Uganda, northern part of the country.			ECU
FMNH29515	Kenya Colony, Athis River. / Kenya, Eastern Province, Machakos District, Athis River.	2°8'S	38°0'E	ESK
FMNH32901	Ethiopia, Sidamo, Boram Border. / Ethiopia, Sidamo, Boran, Burgi, 15 mi S	5°23'N	37°56'E	EEK
FMNH32902	Abyssinia, Sidamo, Boram Border. / Ethiopia, Sidamo, Boran, Burgi, 15 mi S.	5°23'N	37°56'E	EEK
FMNH32904	Ethiopia, Sidamo, Boram Border. / Ethiopia, Sidamo, Boran, Burgi, 15 mi S	5°23'N	37°56'E	EEK
FMNH32905	Abyssinia, Sidamo, Boram Border. / Ethiopia, Sidamo, Boran, Burgi, 15 mi S.	5°23'N	37°56'E	EEK
FMNH34422	Bechuanaland, Kalahari Desert, Mabebe Flats./ Botswana, Ngamiland, Tsotsoroga Pan, Mababe Flats.	19°S	24°E	SWC
FMNH34423	Bechuanaland, Mabebe Flats. / Botswana, Ngamiland, Tsotsoroga Pan, Mabebe Flats.	19°S	24°E	SWC
FMNH34424	Bechuanaland, Mabebe Flats. / Botswana, Ngamiland, Mabebe Flats.	19°S	24°E	SWC
FMNH34425	Bechuanaland, Kalahari Desert, Kwaai, Mochaba River. / Botswana, Ngamiland, Kwaai, Mochaba River.	19°S	24°E	SWC
FMNH34426	Botswana, Ngamiland, Tsotsoroga Pan, Mabebe Flats.	19°S	24°E	SWC
FMNH34427	Bechuanaland, Mabebe Flats. / Botswana, Ngamiland, Tsotsoroga Pan, Mabebe Flats.	19°S	24°E	SWC
FMNH34428	Bechuanaland, Mabebe Flats / Botswana, Ngamiland, Tsotsoroga Pan, Mabebe Flats.	19°S	24°E	SWC
FMNH34429	Bechaunaland, Mabebe Flats. / Botswana, Ngamiland, Mabebe Flats.	19°S	24°E	SWC
FMNH34930	Kenya Colony, Voi. / Kenya, Coastal Province, Taita District, Voi, Sagalla.	3°24'S	38°35'E	ESK
FMNH54251	Tanganyika (Zoo specimen) / Zoo, Tanzania, East slope of Mount Meru.			Unas
FMNH127878	Saronia, Tanganyika Territory, Africa. / Tanzania, Mara Province, Serengeti Plains, Seronera.	2°25'S	34°5'E	ESK
FMNH127879	Tanzania, Mara Province, Serengeti Plains, Seronera.	2°25'S	34°5'E	ESK
FMNH127880	Tanzania, Mara Province, Serengeti Plains, Seronera.	2°25'S	34°5'E	ESK
FMNH127881	North of Lake Manyara at Seteti, Tanganyika Territory, Africa. / Tanzania, Arusha Province, Lake Manyara, Seteti.	3°20'S	35°50'E	ESK
FMNH127882	Cherangani Hills, Kenya Colony, Africa. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU

Accession Number	Provenance	Northing	Easting	Popn
FMNH127883	Cherangani Hills, Kenya Colony, Africa. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU
FMNH127884	Cherangani Hills. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU
FMNH127885	Cherangani Hills, Kenya Colony, Africa. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU
FMNH127886	Cherangani Hills, Kenya Colony, Africa. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU
FMNH127887	Cherangani Hills, Kenya Colony, Africa. / Kenya, Rift Valley Province, Cherangany Hills.	1°10'N	35°20'E	ECU
KNP1	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP2	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP3	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP4	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP5	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP6	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP7	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
KNP8	Kruger National Park, South Africa.	24°59'S	31°36'E	SEW
NMZ11525	Chilongozi Game Reserve, Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ11533	Chilongozi Game Reserve, Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ11544	Two miles from Kalumbi Pontoon, down Fusingazi (?) Road (Left bank), Luangwa Valley.	13°30'S	31°30'E	SZT
NMZ20221	Five miles south west of Kalumbi Pontoon, Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ20382	Left Bank of Luangwa River, Zambia.	13°30'S	31°30'E	SZT
NMZ22635	Kasungo dambo, Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ22862	Luangwa Valley, south, Game Reserve, Zambia.	13°30'S	31°30'E	SZT
NMZ22947	Luangwa Valley Game Reserve, Zambia.	13°30'S	31°30'E	SZT
NMZ22957	Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ22958	Luangwa Valley, south, Game Reserve, Zambia.	13°30'S	31°30'E	SZT
NMZ22959	Luangwa Valley, south, Game Reserve, Zambia.	13°30'S	31°30'E	SZT
NMZ23977	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23978	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23979	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23980	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23981	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23982	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23983	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23984	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23985	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23986	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ23987	Towla, Zimbabwe.	21°26'S	29°51'E	SCZ
NMZ26177	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26178	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26179	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26180	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26181	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26182	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26183	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26184	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26185	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26186	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26187	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26188	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26189	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26190	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26191	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ

Accession Number	Provenance	Northing	Easting	Popn
NMZ26200	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ26865	Makalamabedi Botletle River, 3 miles west of Thamafupi Game Camp, Botswana.			SWC
NMZ27144	Chipinda Pools, Gulweni area where road to Nyala cuts southern game fence.	21°23'S	32°15'E	SCZ
NMZ27451	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ29099	Luangwa Valley, Zambia.	13°30'S	31°30'E	SZT
NMZ29100	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ29101	Originally from Wankie NP (Ngamo). Released in Matopos when 18months old. Lived to 9.5 years.	19°5'S	27°28'E	Unas
NMZ29102	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ29103	Tamafupi, Botswana.			SWC
NMZ29104	Gubatsa Hills.	19°S	24°E	SWC
NMZ29105	Savuti 'Swamp', Botswana.	18°43'S	24°7'E	SWC
NMZ29106	Gubatsa Hills, West Mabebe, Botswana.	19°S	24°E	SWC
NMZ29107	Gubatsa Hills, West Mabebe, Botswana.	19°S	24°E	SWC
NMZ29108	Savuti 'Swamp', Botswana.	18°43'S	24°7'E	SWC
NMZ29109	Savuti 'Swamp', Botswana.	18°43'S	24°7'E	SWC
NMZ29110	Gubatsa Hills, West Mabebe, Botswana.	19°S	24°E	SWC
NMZ29111	1.5 miles noth west of Kwikamba Pan on the main road, Chobe Game Reserve, Botswana.	18°30'S	24°30'E	SWC
NMZ29112	Ngwezumba Bridge, Chobe Game Reserve, Botswana.	18°26'S	24°51'E	SWC
NMZ29113	Ngezumba, Botswana.	18°26'S	24°51'E	SWC
NMZ29114	55 miles west and 6 miles south of Mohembo.	18°20'S	21°0'E	SWC
NMZ29118	Matopos NP, Zimbabwe, parents from Wankie NP.			Unas
NMZ29121	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ58342	East of Fishons, Zimbabwe.	21°26'S	32°0'E	SCZ
NMZ58343	Kundani Hill, Chipinda Pools, Zimbabwe(?)	21°23'S	32°15'E	SCZ
NMZ59379	Whitewaters Game Park, Zimbabwe.	19°20'S	29°59'E	SCZ
NMZ60800	Nuanetsi.	22°18'S	31°26'E	SCZ
NMZ60801	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60802	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60803	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60804	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60805	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60806	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60807	Chobe, Botswana.	18°30'S	24°30'E	SWC
NMZ60808	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ60809	Doddieburn Ranch, Zimbabwe.	21°10'S	29°22'E	SCZ
NMZ60810	Kyle Recreational Park, Zimbabwe.	20°10'S	31°0'E	Unas
NMZ60811	Setache PO, Mozambique.	21°41'S	33°30'E	SZT
NMZ60812	Lake McIlwaine NP, Salisbury, Rhodesia.	17°54'S	30°48'E	Unas
NMZ60813	Matopos NP, Zimbabwe.	20°30'S	28°30'E	Unas
PCCAMII138	Near Bi-Indu, 40km South East of Rei Bouda, Northern Cameroon.	8°25'N	14°30'E	WCP
PCCAMII139	Near Bi-Indu, 40km South East of Rei Bouda, Northern Cameroon.	8°25'N	14°30'E	WCP
PCCongoNoNum1	5 miles from Gondokoro	4°55'N	31°50'E	ECU
PCCongoNoNum2	4 hours from Nimule	3°30'N	32°10'E	ECU
PCJ39	Kotice, Jubaland	0°9'N	41°55'E	EEK
PCJ66	Muti, Jubaland			EEK
PCMN275	Yanga, South of Lake Iro, Ubangi-Shari	9°30'N	19°3'E	WCP
PCMN277	Kijnon, by Lado	5°10'N	31°30'E	ENA
PCMN278	Kijnon, by Lado	5°10'N	31°30'E	ENA
PCMN280	15 miles north west of Sirgoi Rock, Gwashengeshu.	1°N	35°20'E	ECU
PCMN579	West of Lado	4°55'N	31°30'E	ENA
PCNNChad138	Maio, south of lake Iro, Ubangi-Chari	9°35'N	19°8'E	WCP
PCNNChad209	66km south of Fort Archambault, Ubangi-Shari	8°25'N	18°35'E	WCP

Accession Number	Provenance	Northing	Easting	Popn
PCSudan16	Mongalla, southern Sudan	5°19'N	31°49'E	ECU
PCSWA11	Balahuti, 5 miles East of Nambubi	16°30'S	16°55'E	SWA
PCSWA9	Balahuti, 5 miles East of Nambubi	16°30'S	16°55'E	SWA
PCTAN102	Engaruka, Northern Tanganyika	2°55'S	37°E	ESK
PCTAN76	Engaruka, Northern Tanganyika	2°55'S	35°E	ESK
PCTAN89	Engaruka, Northern Tanganyika	2°55'S	36°E	ESK
TM12141	Kaokaveld, South West Africa.	18°S	13°E	SWC
USNM121010	Lake Baringo, British East Africa.	0°38'N	36°5'E	ECU
USNM154033	Southern Rhodesia, Matabeleland.			SCZ
USNM155438	Kenya, Guas Ngishu Plateau.	1°0'N	35°0'E	ECU
USNM162016	British East Africa, Kilima Kui, Kapiti Plateau.	1°38'S	37°0'E	ESK
USNM162017	British East Africa, Kilima Kui, Kapiti Plateau.	1°38'S	37°0'E	ESK
USNM162018	Uln Station, British East Africa.			Unas
USNM162988	Kenya, Sotik, Guaso Nyiro.	1°0'N	35°0'E	ECU
USNM162989	British East Africa, Sotik, Guaso Nyiro.	1°0'N	35°0'E	ECU
USNM163112	British East Africa, Sotik, Guaso Nyiro.	1°0'N	35°0'E	ECU
USNM163113	British East Africa, Guas Nyiro River.	0°30'N	37°30'E	EEK
USNM163312	British East Africa, Guas Ngishu Plateau.	1°0'N	35°0'E	ECU
USNM163324	British East Africa, Guaso Nyiro.	0°30'N	37°30'E	EEK
USNM182124	British East Africa, Koga Water.	6°14'S	32°25'E	EST
USNM182125	British East Africa, Marsabit Road.	2°20'N	37°59'E	EEK
USNM182192	British East Africa, Guaso Nyiro.	0°30'N	37°30'E	EEK
USNM200151	British East Africa, Guas Ngishu Plateau.	1°0'N	35°0'E	ECU
USNM251797	Tanganyika Territory, Dodoma. / Savanda, Dodoma, Tanganyika Territory.	6°10'S	35°45'E	EST
USNM251798	Tanganyika Territory, Dodoma. / Mukurse, Dodoma, TT / Mkese, Dodoma, Tanzania.	5°19'S	34°26'E	EST
USNM251799	Tanganyika Territory, Mkata Plains.	7°0'S	37°30'E	EST
USNM251800	Tanzania.			Unas
USNM296145	South West Africa, Gaucha.	19°47'S	20°35'E	SWC
ZM17176	Northern Rhodesia.			Unas
ZM37058	Phalaborwa District, Transvaal, South Africa.	23°56'S	31°9'E	SZT
ZM39692	Witvlei area between Gobabis and Windhoek, South West Africa.	22°25'S	18°30'E	SWC

APPENDIX 3.7.1:

LIST OF MUSEUM SKINS EXAMINED

Accession Number	Population Group
AMNH53453	ECC
AMNH53547	ECC
AMNH53548	ECC
AMNH53549	ECC
BMNH1901.8.9.47	ECU
BMNH1901.8.9.48	ECU
BMNH1901.8.9.50	ECU
BMNH1903.4.15.1	ECU
BMNH1904.1.21.1	ECU
BMNH1926.12.13.15	ECU
FMNH127884	ECU
FMNH127887	ECU
USNM163312	ECU
BMNH1897.1.30.1	EEK
BMNH1898.7.2.6	EEK
BMNH1899.7.8.5	EEK
BMNH1912.2.24.2	EEK
BMNH1912.2.24.3	EEK
BMNH1912.2.24.4	EEK
BMNH1912.2.24.5	EEK
USNM163324	EEK
BMNH1903.8.13.1	ENA
BMNH1939.4328	ENA
AMNH54122	ESK
BMNH1898.7.2.5	ESK
BMNH1899.12.10.1	ESK
BMNH1904.11.12.1	ESK
BMNH1908.7.5.1	ESK
FMNH29515	ESK
FMNH127879	ESK
USNM162017	ESK
USNM162988	ESK
USNM162989	ESK
USNM163112	ESK
USNM251797	EST
NMZB27451	SCZ

Accession Number	Population Group
NMZB29121	SCZ
USNM154033	SCZ
BMNH1925.2.20.16	SEW
BMNH1925.2.20.17	SEW
BMNH1925.2.20.18	SEW
BMNH1939.4804	SWA
AMNH83458	SWC
AMNH83459	SWC
AMNH83460	SWC
BMNH1896.2.29.1	SWC
BMNH1982.2500	SWC
FMNH34422	SWC
FMNH34423	SWC
FMNH34424	SWC
FMNH34425	SWC
FMNH34426	SWC
FMNH34427	SWC
FMNH34428	SWC
FMNH34429	SWC
NMZB29104	SWC
NMZB29105	SWC
NMZB29106	SWC
NMZB29107	SWC
NMZB29108	SWC
NMZB29109	SWC
NMZB29114	SWC
BMNH1900.4.3.1	SZT
BMNH1910.10.17.1	SZT
NMZB11533	SZT
NMZB11544	SZT
NMZB20382	SZT
BMNH1907.7.8.255	WCP
BMNH1928.11.11.24	WCP
BMNH1938.7.8.22	WCP
BMNH1906.2.12.1	WSN
BMNH1908.8.12.2	WSN

APPENDIX 3.7.2:

DETAILED RESULTS OF CHARACTER CODING FOR EACH SPECIMEN.

Accession Number	AMNH53453	AMNH53547	AMNH53548	AMNH53549	BMNH1901.8.9.47	BMNH1901.8.9.48	BMNH1901.8.9.50	BMNH1903.4.15.1	BMNH1904.1.21.1	BMNH1926.12.13.15
Population Group	ECC	ECC	ECC	ECC	ECU	ECU	ECU	ECU	ECU	ECU
Body Spots										
1 Size distribution of fore flank spots.	1	1	1	1	-	-	-	0	-	-
2 General size of fore flank spots.	2	2	1	2	-	-	-	2	-	-
3 Shape of fore flank spots.	1	1	1	1	-	-	-	0	-	-
4 Width of fore flank lines.	1	1	1	1	-	-	-	1	-	-
5 Size distribution of rump spots.	1	0	1	1	-	-	-	0	-	-
6 General size of rump spots.	1	1	1	1	-	-	-	1	-	-
7 Shape of rump spots.	1	1	1	1	-	-	-	1	-	-
8 Width of rump lines.	1	0	0	0	-	-	-	1	-	-
9 Fine projections blurring spot edges.	1	1	1	1	-	-	-	1	1	-
10 Spots grouped into rosettes.	0	0	0	0	-	-	-	0	-	-
11 Major constrictions in spots.	0	0	0	0	-	-	-	0	0	-
12 Radiating marks within larger spots?	0	0	0	0	-	-	-	1	0	-
13 Darker centre to spots.	0	0	0	0	-	-	-	1	0	-
Head Spots										
14 Sides spotted (Below eye-mouth line).	1	1	1	1	1	1	1	1	-	1
15 Face spotted (Above eye-mouth line).	1	1	1	0	0	1	0	1	-	1
16 Spots between the eye and ear.	1	1	1	1	1	1	1	-	-	1
17 Spots between ear and horns.	1	1	1	1	1	1	1	-	-	1
18 Spots around parietals.	0	0	0	0	0	-	1	-	-	0
19 Size distribution of head spots.	0	0	1	0	1	1	0	0	-	1
20 General size of head spots.	1	1	1	1	0	0	0	0	-	1
21 White ear patch.	0	0	0	0	-	-	0	-	-	-
Limb Spots										
22 Fore limb spots below the hocks.	0	1	1	1	-	-	-	0	-	-
23 Hind limb spots below the hocks.	0	1	1	1	-	-	-	0	-	-
24 Fore limb spots between the legs.	-	-	-	-	-	-	-	1	-	-
25 Hind limb spots between the legs.	-	-	-	-	-	-	-	0	-	-
26 Size distribution of hind limb spots.	0	1	0	0	-	-	-	0	-	-
27 General size of hind limb spots.	0	1	1	1	-	-	-	1	-	-
28 Shape of hind limb spots.	1	1	1	1	-	-	-	1	-	-
29 Size distribution of fore limb spots.	0	1	1	1	-	-	-	0	-	-
30 General size of fore limb spots.	1	1	1	1	-	-	-	2	-	-
31 Shape of fore limb spots.	1	1/2	1	1	-	-	-	1	-	-
32 Change from body spots to limb spots.	1	1	1	1	-	-	-	1	-	-
Belly Spots										
33 Belly spots present or absent.	0	1	1	1	-	-	-	2	-	-
Neck Spots										
34 Continuous transverse bands.	0	0	0	0	0	-	-	0	-	0
35 Radiating lines within spots.	0	0	0	0	0	-	-	1	-	0
36 Size distribution of neck spots.	1	1	1	1	0	-	-	1	-	1
37 General size of neck spots.	2	1	2	1	2	-	-	2	-	2
38 Shape of neck spots.	1	1	1	1	0	-	-	1	-	0
39 Neck spots are elongated.	1	0	0	0	1	-	-	0	-	0
40 Width of neck lines.	2	2	2	2	2	-	-	2	-	2

Accession Number	FMNH127884	FMNH127887	USNM163312	BMNH1897.1.30.1	BMNH1898.7.2.6	BMNH1899.7.8.5	BMNH1912.2.24.2	BMNH1912.2.24.3	BMNH1912.2.24.4	BMNH1912.2.24.5	USNM163324
Population Group	ECU	ECU	ECU	EEK	EEK	EEK	EEK	EEK	EEK	EEK	EEK
Body Spots											
1 Size distribution of fore flank spots.	0	0	1	-	-	-	0	0	-	-	0
2 General size of fore flank spots.	2	2	2	-	-	-	2	2	-	-	2
3 Shape of fore flank spots.	1	0	0	-	-	-	0	0	-	-	0
4 Width of fore flank lines.	1	0	0	-	-	-	0	0	-	-	0
5 Size distribution of rump spots.	1	0	1	-	-	-	0	1	-	-	1
6 General size of rump spots.	1	2	1	-	-	-	2	2	-	-	2
7 Shape of rump spots.	1	0	0	-	-	-	0	0	-	-	0
8 Width of rump lines.	0	0	0	-	-	-	0	0	-	-	0
9 Fine projections blurring spot edges.	1	1	1	-	-	-	0	0	-	-	0
10 Spots grouped into rosettes.	0	0	0	-	-	-	0	0	-	-	0
11 Major constrictions in spots.	1	1	1	-	-	-	0	0	-	-	0
12 Radiating marks within larger spots?	1	0	0	-	-	-	0	0	-	-	0
13 Darker centre to spots.	1	0	0	-	-	-	0	0	-	-	0
Head Spots											
14 Sides spotted (Below eye-mouth line).	1	1	1	-	1	1	-	-	1	1	1
15 Face spotted (Above eye-mouth line).	0	0	1	-	1	0	-	-	1	1	1
16 Spots between the eye and ear.	1	1	1	-	1	0	-	-	-	1	1
17 Spots between ear and horns.	1	-	1	-	1	0	-	-	-	-	1
18 Spots around parietals.	0	0	0	1	1	0	-	-	-	0	1
19 Size distribution of head spots.	1	1	1	-	1	1	-	-	1	1	1
20 General size of head spots.	0/2	1	0/1	-	2	2	-	-	2	2	2
21 White ear patch.	0	0	0	-	0	0	0	-	-	0	0
Limb Spots											
22 Fore limb spots below the hocks.	0	0	0	-	-	-	0	1	-	-	1
23 Hind limb spots below the hocks.	1	1	0	-	-	-	1	1	-	-	1
24 Fore limb spots between the legs.	-	-	-	-	-	-	-	-	-	-	-
25 Hind limb spots between the legs.	-	-	-	-	-	-	-	-	-	-	-
26 Size distribution of hind limb spots.	0	0	0	-	-	-	-	-	-	-	1
27 General size of hind limb spots.	1	1	1	-	-	-	-	-	-	-	1
28 Shape of hind limb spots.	1/2	1	1	-	-	-	-	-	-	-	0/1
29 Size distribution of fore limb spots.	1	0	0	-	-	-	-	-	-	-	1
30 General size of fore limb spots.	1	1	1	-	-	-	-	-	-	-	1/2
31 Shape of fore limb spots.	2	1	1	-	-	-	-	-	-	-	0/1
32 Change from body spots to limb spots.	1	1	1	-	-	-	-	-	-	-	1
Belly Spots											
33 Belly spots present or absent.	2	0	0	-	-	-	-	-	-	-	1
Neck Spots											
34 Continuous transverse bands.	0	0	0	-	0	0	0	-	0	0	0
35 Radiating lines within spots.	0	0	0	-	0	0	0	-	0	0	0
36 Size distribution of neck spots.	1	0	0	-	1	0	0	-	1	1	0
37 General size of neck spots.	1	1	2	-	2	2	2	-	2	2	2
38 Shape of neck spots.	1	0	0(1)	-	0	0	0	-	0	0	0
39 Neck spots are elongated.	0	0	1	-	0	0	0	-	0	0	0
40 Width of neck lines.	2	1	1	-	0	0	0	-	0	0	0

Accession Number	BMNH1903.8.13.1	BMNH1939.4328	AMNH54122	BMNH1898.7.2.5	BMNH1899.12.10.1	BMNH1904.11.12.1	BMNH1908.7.5.1	FMNH29515	FMNH127879	USNM162017	USNM162988
Population Group	ENA	ENA	ESK	ESK	ESK	ESK	ESK	ESK	ESK	ESK	ESK
Body Spots											
1 Size distribution of fore flank spots.	-	-	1	-	0	1	-	0	0	-	1
2 General size of fore flank spots.	-	-	1	-	1	1	-	1(2)	2	-	1
3 Shape of fore flank spots.	-	-	2	-	2	2	-	2	2	-	2
4 Width of fore flank lines.	-	-	1	-	1	1	-	0	1	-	1
5 Size distribution of rump spots.	-	-	1	-	0	1	-	1	1	1	1
6 General size of rump spots.	-	-		-	1	1	-	1	1	0	1
7 Shape of rump spots.	-	-	2	-	2	2	-	2	2	2	2
8 Width of rump lines.	-	-	0	-	0	0	-	0	1	0	0
9 Fine projections blurring spot edges.	-	-	1	-	1	1	-	1	1	1	1
10 Spots grouped into rosettes.	-	-	0	-	0	0	-	0	0	0	0
11 Major constrictions in spots.	-	-	2	-	2	2	-	2	2	2	2
12 Radiating marks within larger spots?	-	-	0	-	0	0	-	0	0	0	0
13 Darker centre to spots.	-	-	0	-	0	0	-	0	0	0	0
Head Spots											
14 Sides spotted (Below eye-mouth line).	1	1	1	-	0	1	1	1	1	1	1
15 Face spotted (Above eye-mouth line).	0	0	0	-	0	1	1	0	0	0	0
16 Spots between the eye and ear.	0	1	1	-	1	1	1	1	1	1	1
17 Spots between ear and horns.	0	1	1	-	1	1	1	1	-	1	1
18 Spots around parietals.	0	0	1	-	1	1	0	0	-	1	1
19 Size distribution of head spots.	0	1	1	-	1	1	1	1	0	1	1
20 General size of head spots.	1	0/1	0/1	-	0/1	0/1	1	1	1	1	1
21 White ear patch.	0	-	-	-	-	-	-	-	1	0	0
Limb Spots											
22 Fore limb spots below the hocks.	-	-	1	-	-	2	-	-	2	1	0
23 Hind limb spots below the hocks.	-	-	1	-	-	2	-	-	2	1	0
24 Fore limb spots between the legs.	-	-	-	-	-	1	-	-	-	-	-
25 Hind limb spots between the legs.	-	-	-	-	-	1	-	-	-	-	-
26 Size distribution of hind limb spots.	-	-	1	-	0	1	-	1	1	1	0
27 General size of hind limb spots.	-	-	0	-	0	0	-	0	1	0	0
28 Shape of hind limb spots.	-	-	2	-	2	2	-	2	2	2	2
29 Size distribution of fore limb spots.	-	-	1	-	1	1	-	1	1	-	1
30 General size of fore limb spots.	-	-	1	-	1	1	-	1	1	-	1
31 Shape of fore limb spots.	-	-	2	-	2	2	-	2	2	-	2
32 Change from body spots to limb spots.	-	-	1	-	1	1	-	1	1	1	1
Belly Spots											
33 Belly spots present or absent.	-	-	1	-	1	0	-	-	0	1	2
Neck Spots											
34 Continuous transverse bands.	0	0	0	0	0	0	0	0	0	0	0
35 Radiating lines within spots.	0	0	0	0	0	0	0	0	0	0	0
36 Size distribution of neck spots.	0	1	1	1	1	1	1	1	1	1	1
37 General size of neck spots.	1	1	1	1	1	1	1	1	1	1	1
38 Shape of neck spots.	0/1	0/1	2	2	2	1/2	2	2	2	2	2
39 Neck spots are elongated.	0	0	0	0	1	0	0	0	0	0	1
40 Width of neck lines.	2	2	2	2	2	2	2	1(0)	2	1	1

Accession Number	USNM162989	USNM163112	USNM251797	NMZB27451	NMZB29121	USNM154033	BMNH1925.2.20.16	BMNH1925.2.20.17	BMNH1925.2.20.18	BMNH1939.4804
Population Group	ESK	ESK	EST	SCZ	SCZ	SCZ	SEW	SEW	SEW	SWA
Body Spots										
1 Size distribution of fore flank spots.	1	0	1	1	1	-	-	-	-	1
2 General size of fore flank spots.	1	2	1	1/2	1	-	-	-	-	2
3 Shape of fore flank spots.	2	2	2	1	1	-	-	-	-	1
4 Width of fore flank lines.	0	1	0	1	0	-	-	-	-	2
5 Size distribution of rump spots.	1	1	1	1	1	-	-	-	-	1
6 General size of rump spots.	1	1	0	1	1	-	-	-	-	1
7 Shape of rump spots.	2	2	2	1	1	-	-	-	-	1
8 Width of rump lines.	0	0	0	0	1	-	-	-	-	1
9 Fine projections blurring spot edges.	1	1	1	1	1	-	-	-	-	1
10 Spots grouped into rosettes.	0	0	0	0	0	-	-	-	-	0
11 Major constrictions in spots.	2	2	2	1	1	-	-	-	-	1
12 Radiating marks within larger spots?	0	0	0	0	0	-	-	-	-	0
13 Darker centre to spots.	0	0	0	0	0	-	-	-	-	0
Head Spots										
14 Sides spotted (Below eye-mouth line).	1	1	1	0	0	0	0	1	0	-
15 Face spotted (Above eye-mouth line).	1	1	1	0	0	0	0	0	0	-
16 Spots between the eye and ear.	1	1	1	0	0	0	0	1	0	-
17 Spots between ear and horns.	1	1	1	0	0	0	0	1	0	-
18 Spots around parietals.	1	-	1	0	0	0	0	0	0	-
19 Size distribution of head spots.	1	1	1	0	0	-	-	1	-	-
20 General size of head spots.	1	1	1	-	-	-	-	1	-	-
21 White ear patch.	0	-	1	-	1	0	0	0	0	-
Limb Spots										
22 Fore limb spots below the hocks.	1/2	1	-	-	1	0	-	-	-	1
23 Hind limb spots below the hocks.	1/2	1	-	-	1	0	-	-	-	1
24 Fore limb spots between the legs.	-	-	-	-	-	-	-	-	-	0
25 Hind limb spots between the legs.	-	-	-	-	-	-	-	-	-	0
26 Size distribution of hind limb spots.	0	0	0	1	1	-	-	-	-	1
27 General size of hind limb spots.	0	0	0	0	1	-	-	-	-	1
28 Shape of hind limb spots.	2	2	2	1/2	1	-	-	-	-	1
29 Size distribution of fore limb spots.	0	1	1	1	1	-	-	-	-	1
30 General size of fore limb spots.	0	1	0	0	1	-	-	-	-	1
31 Shape of fore limb spots.	2	2	2	1	1	-	-	-	-	1
32 Change from body spots to limb spots.	1	1	1	1	1	-	-	-	-	1
Belly Spots										
33 Belly spots present or absent.	1	2	1	1	1	-	-	-	-	1
Neck Spots										
34 Continuous transverse bands.	0	0	0	0	0	-	-	-	-	-
35 Radiating lines within spots.	0	0	0	0	0	0	-	-	-	-
36 Size distribution of neck spots.	1	1	1	0	1	0	-	-	-	-
37 General size of neck spots.	1	1	1	1	1	1	-	-	-	-
38 Shape of neck spots.	2	2	2	1	1	1	-	-	-	-
39 Neck spots are elongated.	0	0	0	0	0	0	-	-	-	-
40 Width of neck lines.	2	2	2	1	0	2	-	-	-	-

Accession Number	AMNH83458	AMNH83459	AMNH83460	BMNH1896.2.29.1	BMNH1982.2500	FMNH34422	FMNH34423	FMNH34424	FMNH34425	FMNH34426
Population Group	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC
Body Spots										
1 Size distribution of fore flank spots.	1	1	1	-	-	1	1	1	1	1
2 General size of fore flank spots.	1	1/2	2	-	-	1	1	1	1	1
3 Shape of fore flank spots.	1/2	1(2)	1	-	-	1(2)	1	1(2)	2(1)	1
4 Width of fore flank lines.	1	1	1	-	-	2	1	1	2	1
5 Size distribution of rump spots.	1	1	1	-	-	1	1	1	1	1
6 General size of rump spots.	0/1	0	1/2	-	-	1	1/0	1	1	1
7 Shape of rump spots.	1/2	1(2)	1(2)	-	-	1	1	1(2)	2(1)	1(2)
8 Width of rump lines.	0	0	1	-	-	2	1	1	2	1
9 Fine projections blurring spot edges.	1	1	1	-	-	1	1	1	1	1
10 Spots grouped into rosettes.	0	0	0	-	-	0	0	1	0	1
11 Major constrictions in spots.	1	1	1	-	-	1(2)	1	1	2(1)	1/2
12 Radiating marks within larger spots?	0	0	0	-	-	0	0	0	0	0
13 Darker centre to spots.	0	0	0	-	-	0	0	0	0	0
Head Spots										
14 Sides spotted (Below eye-mouth line).	0	1	1	1	1	1	1	0	1	1
15 Face spotted (Above eye-mouth line).	0	0	0	0	0	0	0	0	0	0
16 Spots between the eye and ear.	0	0	0	0	0	0	0	0	0	1
17 Spots between ear and horns.	0	0	0	0	0	0	0	0	0	1
18 Spots around parietals.	0	0	0	0	0	0	0	0	0	0
19 Size distribution of head spots.	-	-	-	-	-	-	-	-	-	0
20 General size of head spots.	-	-	-	-	-	-	-	-	-	0
21 White ear patch.	0	0	0	-	0	-	1	1	1	1
Limb Spots										
22 Fore limb spots below the hocks.	1	1	0	-	-	0	1	0	1	0
23 Hind limb spots below the hocks.	1	1	0	-	-	0	1	0	1	0
24 Fore limb spots between the legs.	-	-	-	-	-	-	-	-	-	-
25 Hind limb spots between the legs.	-	-	-	-	-	-	-	-	-	-
26 Size distribution of hind limb spots.	0	1	1	-	-	-	1	-	-	-
27 General size of hind limb spots.	0	0	0	-	-	-	1(0)	-	-	-
28 Shape of hind limb spots.	1	1	1	-	-	-	1	-	-	-
29 Size distribution of fore limb spots.	1	1	1	-	-	-	-	-	-	-
30 General size of fore limb spots.	1	1	1	-	-	-	-	-	-	-
31 Shape of fore limb spots.	1	1	1	-	-	-	-	-	-	-
32 Change from body spots to limb spots.	1	1	1	-	-	-	-	-	-	-
Belly Spots										
33 Belly spots present or absent.	1	1	1	-	-	0	0	0	1	-
Neck Spots										
34 Continuous transverse bands.	0	0	0	0	0	0	0	0	0	0
35 Radiating lines within spots.	0	0	0	0	0	0	0	0	0	0
36 Size distribution of neck spots.	1	1	1	1	1	0	1	0	1	1
37 General size of neck spots.	1	0/1	1	1	1	1	1	1	1	1
38 Shape of neck spots.	1	1	1	1	1	1	1	1	1	1
39 Neck spots are elongated.	0	0	0	0	0	0	0	0	0	0
40 Width of neck lines.	0	0	0	2	2	1	1	1	1	2

Accession Number	FMNH34427	FMNH34428	FMNH34429	NMZB29104	NMZB29105	NMZB29106	NMZB29107	NMZB29108	NMZB29109	NMZB29114
Population Group	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC	SWC
Body Spots										
1 Size distribution of fore flank spots.	1	1	1	1	1	1	1	1	1	1
2 General size of fore flank spots.	1	1	1(2)	1	1(2)	1(2)	1(2)	1(2)	1	2(1)
3 Shape of fore flank spots.	1(2)	1	1	1	1	1	1	1	1	1
4 Width of fore flank lines.	2	1	2	1	2	1	1	1(2)	2	2
5 Size distribution of rump spots.	1	1	0	1	1	1	1	1	1	1
6 General size of rump spots.	1	1	1	1	1(0)	1	1/0	1	1	1(2)
7 Shape of rump spots.	1	1	1(2)	1	1	1	1	1	1	1
8 Width of rump lines.	2	1	1	1	1	0	0	1	1	2
9 Fine projections blurring spot edges.	1	1	1	1	1	1	1	1	1	1
10 Spots grouped into rosettes.	0	0	0	0	0	0	0	1	0	0
11 Major constrictions in spots.	1	1	1	1	0	1	1	1(2)	1(2)	1
12 Radiating marks within larger spots?	0	0	0	0	0	0	0	0?	0	0
13 Darker centre to spots.	0	0	0	0	0	0	0	0	0	0
Head Spots										
14 Sides spotted (Below eye-mouth line).	1	1	0	1	1	1	1	0	-	1
15 Face spotted (Above eye-mouth line).	0	0	0	0	0	0	0	0	-	0
16 Spots between the eye and ear.	0	0	0	0	0	0	0	0	0	0
17 Spots between ear and horns.	0	0	0	0	0	0	0	0	0	0
18 Spots around parietals.	0	0	0	0	0	0	0	0	0	0
19 Size distribution of head spots.	-	-	-	-	-	-	-	-	-	-
20 General size of head spots.	-	-	-	-	-	-	-	-	-	-
21 White ear patch.	1	1	1	-	1	-	1	-	1	-
Limb Spots										
22 Fore limb spots below the hocks.	0	1	1	0	0	-	1	0	1	1
23 Hind limb spots below the hocks.	0	1	1	1	1	-	1	1	1	1
24 Fore limb spots between the legs.	-	-	-	-	-	-	-	-	-	-
25 Hind limb spots between the legs.	-	-	-	-	-	-	-	-	-	-
26 Size distribution of hind limb spots.	1	0	1	1	-	0	0	1	1	1
27 General size of hind limb spots.	0	0	0	0	-	1	0	0	0	0
28 Shape of hind limb spots.	1	1	1	1	-	1	1	1	1	1
29 Size distribution of fore limb spots.	-	1	-	1	-	1	1	-	1	1
30 General size of fore limb spots.	-	0	-	1	-	0	0	-	0	0
31 Shape of fore limb spots.	-	1	-	1	-	1	1	-	1	1
32 Change from body spots to limb spots.	1	1	1	1	-	1	1	1	1	1
Belly Spots										
33 Belly spots present or absent.	1	0	-	1	1	-	1	1	1	-
Neck Spots										
34 Continuous transverse bands.	0	0	0	0	0	0	0	0	0	0
35 Radiating lines within spots.	0	0	0	0	0	0	0	0	0	0
36 Size distribution of neck spots.	1	1	1	1	1	1	1	1	1	1
37 General size of neck spots.	0	1	0	1	1(2)	1(2)	1	1	1	1
38 Shape of neck spots.	1	1	1	1	1	1	1	1	1	1
39 Neck spots are elongated.	0	0	0	0	0	0	1	0	0	0
40 Width of neck lines.	2	1	1	1	1	1	0	1	1	2

Accession Number	BMNH1900.4.3.1	BMNH1910.10.17.1	NMZB11533	NMZB11544	NMZB20382	BMNH1906.2.12.1	BMNH1907.7.8.255	BMNH1908.8.12.2	BMNH1928.11.11.24	BMNH1938.7.8.22
Population Group	SZT	SZT	SZT	SZT	SZT	WSN	WCP	WSN	WCP	WCP
Body Spots										
1 Size distribution of fore flank spots.	-	1	-	1	1	-	-	1	1	1
2 General size of fore flank spots.	-	1	-	1(2)	1	-	-	1(0)	1	1
3 Shape of fore flank spots.	-	1	-	1	1	-	-	1	1	1
4 Width of fore flank lines.	-	2	-	2	1	-	-	1	1	2
5 Size distribution of rump spots.	1	1	-	1	1	-	-	1	1	1
6 General size of rump spots.	1(0)	1/0	-	1(0)	1	-	-	1	1	1(0)
7 Shape of rump spots.	1(2)	1	-	1	1	-	-	1	1	1
8 Width of rump lines.	1/2	2	-	1	0	-	-	1	1	2
9 Fine projections blurring spot edges.	0	0	-	0	0	-	-	0	0	0
10 Spots grouped into rosettes.	1	0	-	0	0	-	-	0	1	0
11 Major constrictions in spots.	1(2)	1	-	1(2)	1	-	-	1	1	1
12 Radiating marks within larger spots?	0	0	-	0	0	-	-	0	0	0
13 Darker centre to spots.	0	0	-	0	0	-	-	0	0	0
Head Spots										
14 Sides spotted (Below eye-mouth line).	-	1	-	1	1	0	1	0	1	1
15 Face spotted (Above eye-mouth line).	-	0	-	0	0	0	0	0	0	0
16 Spots between the eye and ear.	-	1	-	1	1	0	0	0	0	1
17 Spots between ear and horns.	-	1	-	1	1	0	0	1	0	-
18 Spots around parietals.	-	0	-	1	1	0	0	0	0	-
19 Size distribution of head spots.	-	1	-	1	1	-	-	1	-	1
20 General size of head spots.	-	1/0	-	0	0	-	-	0	-	1/0
21 White ear patch.	-	-	-	0	0	-	-	-	-	0
Limb Spots										
22 Fore limb spots below the hocks.	-	0	-	0	0	-	-	0	0	0
23 Hind limb spots below the hocks.	-	1	-	1	0	-	-	0	0	0
24 Fore limb spots between the legs.	-	0	-	-	-	-	-	-	-	-
25 Hind limb spots between the legs.	-	0	-	-	-	-	-	-	-	-
26 Size distribution of hind limb spots.	-	1	-	1	1	-	-	1	-	1
27 General size of hind limb spots.	-	0	-	0	0	-	-	0	-	0
28 Shape of hind limb spots.	-	1	-	1	1	-	-	1	-	1
29 Size distribution of fore limb spots.	-	1	-	1	1	-	-	-	-	-
30 General size of fore limb spots.	-	1/0	-	0	0	-	-	-	-	-
31 Shape of fore limb spots.	-	1	-	1	1	-	-	-	-	-
32 Change from body spots to limb spots.	-	1	-	1	1	-	-	1	-	1
Belly Spots										
33 Belly spots present or absent.	-	2	-	1	1	-	-	0	1	1
Neck Spots										
34 Continuous transverse bands.	-	0	0	0	0	0	0	0	0	0
35 Radiating lines within spots.	-	0	0	0	0	0	0	0	0	0
36 Size distribution of neck spots.	-	1	1	1	1	1	1	1	1	1
37 General size of neck spots.	-	1	1	1(0)	1	1(0)	1(0)	1	1	1
38 Shape of neck spots.	-	1	1	1	1	1	1	1	1	1
39 Neck spots are elongated.	-	0	0	0	0	0	0	0	0	0
40 Width of neck lines.	-	2	2	2	2	2	2	2	2	2

APPENDIX 4.8.1:

FREQUENCY OF RECORDING OF EACH VARIABLE FOR ADULT MALE AND FEMALE SPECIMENS.

Any missing data is typically due to damage to the specimen. Maximum n for males = 99, maximum n of females = 43.

Male Data	MASS	PPHL	POL	PCL	NCL	NOL	PAOL	OOL
Specimens Measured	90	76	75	73	83	87	76	96
% measured.	91%	77%	76%	74%	84%	88%	77%	97%
	ZGW	MMW	MPW	EAMW	MOW	LAW	MMH	BPH
Specimens Measured	96	93	71	92	98	99	98	95
% measured.	97%	94%	72%	93%	99%	100%	99%	96%
	APD	LAD	TIC	NAC	HSP	EWB	EWT	IWT
Specimens Measured	99	99	99	99	99	98	98	98
% measured.	100%	100%	100%	100%	100%	99%	99%	99%
	FMP	EWO	MWO	MXTL	PPM	PGW	PGM	TMP
Specimens Measured	91	99	99	98	72	73	77	74
% measured.	92%	100%	100%	99%	73%	74%	78%	75%
	IPW	SWM	PPL	PACL	MAL	MDTL	DIL	CAW
Specimens Measured	72	96	70	91	67	73	70	25
% measured.	73%	97%	71%	92%	68%	74%	71%	25%
	WMS	LMS	ITL	HUL	RUL	MEL		
Specimens Measured	59	68	67	11	10	7		
% measured.	60%	69%	68%	11%	10%	7%		

Female Data	MASS	PPHL	POL	PCL	NCL	NOL	PAOL	OOL
Specimens Measured	40	33	33	31	38	38	33	43
% measured.	93%	77%	77%	72%	88%	88%	77%	100%
	ZGW	MMW	MPW	EAMW	MOW	LAW	MMH	BPH
Specimens Measured	43	35	26	40	43	42	43	41
% measured.	100%	81%	60%	93%	100%	98%	100%	95%
	APD	LAD	TIC	NAC	HSP	EWB	EWT	IWT
Specimens Measured	41	41	39	39	43	43	42	42
% measured.	95%	95%	91%	91%	100%	100%	98%	98%
	FMP	EWO	MWO	MXTL	PPM	PGW	PGM	TMP
Specimens Measured	40	43	43	43	36	37	39	31
% measured.	93%	100%	100%	100%	84%	86%	91%	72%
	IPW	SWM	PPL	PACL	MAL	MDTL	DIL	CAW
Specimens Measured	33	43	31	41	36	37	37	27
% measured.	77%	100%	72%	95%	84%	86%	86%	63%
	WMS	LMS	ITL	HUL	RUL	MEL		
Specimens Measured	32	36	36	12	13	8		
% measured.	74%	84%	84%	28%	30%	19%		

APPENDIX 4.8.2:

SUMMARY STATISTICS FOR ALL MALE AND FEMALE DATA.

Variable	Gender	n	Min	Max	Mean	SD
MASS	Female	40	1.4	3.0	2.194	0.415
	Male	90	3.0	14.8	8.227	2.196
PPHL	Female	33	562.0	713.5	628.3	26.77
	Male	76	658.0	869.0	755.3	44.43
POL	Female	33	570.0	708.0	621.4	22.29
	Male	75	616.0	749.5	692.2	31.89
PCL	Female	31	542.0	665.5	581.3	23.24
	Male	73	570.0	688.0	632.2	27.32
NCL	Female	38	390.0	513.0	450.9	22.92
	Male	83	445.5	601.0	503.9	26.44
NOL	Female	38	195.0	244.0	220.5	9.867
	Male	87	218.0	290.0	250.1	15.53
PAOL	Female	33	318.0	410.5	358.2	13.52
	Male	76	348.5	440.5	394.2	19.85
OOL	Female	43	207.0	258.5	231.3	10.24
	Male	96	227.5	302.5	263.0	15.60
ZGW	Female	43	197.0	239.0	221.2	10.34
	Male	96	210.0	269.0	237.2	11.83
MMW	Female	35	60.0	163.0	79.74	15.62
	Male	93	76.0	111.0	94.41	8.040
MPW	Female	26	73.0	93.0	81.96	4.754
	Male	71	81.0	119.0	99.90	8.382
EAMW	Female	40	138.0	174.0	154.4	7.636
	Male	92	135.0	187.0	169.8	10.18
MOW	Female	43	227.0	290.0	258.4	14.97
	Male	98	249.0	337.0	291.6	20.03
LAW	Female	42	148.0	197.0	173.5	10.70
	Male	99	159.0	216.0	188.2	13.03
MMH	Female	43	193.5	288.0	221.9	18.67
	Male	98	212.0	402.5	288.0	36.96
BPH	Female	41	137.0	190.0	160.85	12.56
	Male	95	160.0	250.0	196.6	16.38
APD	Female	41	14.5	35.5	23.01	4.448
	Male	99	38.5	69.5	53.30	6.901
LAD	Female	41	14.0	36.5	20.14	3.719
	Male	99	36.0	78.0	54.25	9.636
TIC	Female	39	54.5	112.5	69.80	10.89
	Male	99	115.5	227.0	169.1	23.55
NAC	Female	39	54.5	104.5	69.44	9.914
	Male	99	114.5	217.0	156.9	20.95
HSP	Female	43	66.0	153.0	94.19	17.53
	Male	99	109.0	229.0	157.6	21.04
EWB	Female	43	140.0	202.0	170.1	15.16
	Male	98	170.0	275.0	222.5	19.73
EWT	Female	42	97.0	227.0	138.7	24.63
	Male	98	169.0	296.0	222.6	27.82
IWT	Female	42	64.0	194.0	100.0	24.84
	Male	98	57.0	174.0	115.2	26.82
FMS	Female	40	150.0	207.0	175.6	11.06
	Male	91	170.0	244.0	204.4	14.49
FMP	Female	40	201.0	315.5	240.1	21.93
	Male	91	278.0	421.0	334.2	26.21
FMO	Female	40	70.0	101.0	85.70	6.665
	Male	91	85.0	160.0	107.5	10.15
EWO	Female	43	103.0	135.0	120.7	8.142
	Male	99	113.0	173.0	145.3	11.16
MWO	Female	43	103.0	135.0	120.7	8.142
	Male	99	113.0	180.0	146.0	11.25

Variable	Gender	n	Min	Max	Mean	SD
MXTL	Female	43	131.5	154.0	142.7	4.862
	Male	98	130.5	163.0	147.3	6.149
PPM	Female	36	23.5	45.5	34.72	5.705
	Male	72	28.00	53.5	38.01	5.100
PGW	Female	37	61.0	91.0	76.51	7.263
	Male	73	62.00	107.0	85.73	8.252
PGM	Female	39	128.0	189.5	159.7	13.12
	Male	77	145.0	209.0	177.9	11.32
TMP	Female	31	201.0	275.5	238.9	11.85
	Male	74	225.5	295.0	261.9	14.25
IPW	Female	33	29.0	42.0	37.33	3.007
	Male	72	34.0	50.0	40.46	3.922
SWM	Female	43	131.0	160.0	147.7	6.937
	Male	96	137.0	169.0	152.7	7.267
PPL	Female	31	310.0	382.0	345.1	14.37
	Male	70	324.0	421.0	373.5	23.18
PACL	Female	41	179.0	250.0	213.8	12.74
	Male	91	196.0	256.0	232.5	11.20
MAL	Female	36	442.0	551.5	494.1	19.20
	Male	67	465.0	579.5	527.5	23.19
MDTL	Female	37	158.5	185.5	170.5	5.487
	Male	73	154.5	191.0	173.8	7.533
DIL	Female	37	161.0	217.5	183.2	10.68
	Male	70	158.0	233.5	200.3	13.87
CAW	Female	27	21.0	32.0	25.90	3.047
	Male	25	20.75	31.0	27.79	2.273
WMS	Female	32	45.0	67.0	55.78	4.851
	Male	59	44.0	68.0	58.42	5.118
LMS	Female	36	118.0	160.0	137.5	9.620
	Male	68	102.0	176.0	149.09	12.92
ITL	Female	36	34.0	49.0	41.02	3.492
	Male	67	35.0	49.0	41.62	3.380
HUL	Female	12	417.5	463.5	441.3	14.48
	Male	11	475.5	543.0	506.2	20.84
RUL	Female	13	632.0	758.5	702.3	39.13
	Male	10	780.5	888.0	824.9	36.34
MEL	Female	8	638.5	693.5	664.3	20.56
	Male	7	709.0	782.0	747.9	23.09

APPENDIX 4.8.3:

T-TEST AND F-TEST RESULTS COMPARING ALL VARIABLES FOR COMPLETE SPECIMENS AND SPECIMENS WITH MISSING DATA.

All t values are pooled variance t-values except for *, which have significantly different variances (as identified by the F-values).

Male Data

Variable	Group	n	t	p	F	p
PPHL	Complete	34	0.153	0.879	1.389	>0.05
	Missing	42		NS		NS
POL	Complete	34	-0.223	0.824	2.298	>0.05
	Missing	41		NS		NS
PCL	Complete	34	-0.518	0.606	1.701	>0.05
	Missing	39		NS		NS
NCL	Complete	34	-0.927*	0.357	1.792	0.040
	Missing	49		NS		*
NOL	Complete	34	0.685	0.495	1.097	>0.05
	Missing	53		NS		NS
PAOL	Complete	34	0.186	0.853	1.579	>0.05
	Missing	42		NS		NS
OOL	Complete	34	-0.429*	0.669	1.873	0.026
	Missing	62		NS		*
ZGW	Complete	34	0.321*	0.749	2.018	0.015
	Missing	62		NS		*
MMW	Complete	34	-0.237	0.813	1.477	>0.05
	Missing	59		NS		NS
MPW	Complete	34	0.659	0.512	1.397	>0.05
	Missing	37		NS		NS
EAMW	Complete	34	0.108	0.914	1.533	>0.05
	Missing	58		NS		NS
MOW	Complete	34	0.174	0.862	1.202	>0.05
	Missing	64		NS		NS
LAW	Complete	34	-0.441	0.660	1.302	>0.05
	Missing	65		NS		NS
MMH	Complete	34	1.837	0.069	1.073	>0.05
	Missing	64		NS		NS
BPH	Complete	34	0.633	0.528	1.428	>0.05
	Missing	61		NS		NS
APD	Complete	34	1.351	0.180	1.250	>0.05
	Missing	65		NS		NS
LAD	Complete	34	0.140	0.889	1.535	>0.05
	Missing	65		NS		NS
TIC	Complete	34	0.651	0.516	1.658	>0.05
	Missing	65		NS		NS
NAC	Complete	34	1.040	0.301	1.446	>0.05
	Missing	65		NS		NS
HSP	Complete	34	-0.854	0.395	1.150	>0.05
	Missing	65		NS		NS
EWB	Complete	34	-0.132	0.895	1.667	>0.05
	Missing	64		NS		NS
EWT	Complete	34	-0.195	0.846	1.548	>0.05
	Missing	64		NS		NS
IWT	Complete	34	-0.212	0.833	1.150	>0.05
	Missing	64		NS		NS
FMP	Complete	34	-0.571	0.569	1.629	>0.05
	Missing	57		NS		NS
EWO	Complete	34	-0.711	0.479	1.175	>0.05
	Missing	65		NS		NS
MWO	Complete	34	-0.606	0.546	1.518	>0.05
	Missing	65		NS		NS
MXTL	Complete	34	-0.046	0.963	1.448	>0.05
	Missing	64		NS		NS

Variable	Group	n	t	p	F	p
PPM	Complete	34	1.616	0.111	1.673	>0.05
	Missing	38		NS		NS
PGW	Complete	34	0.096*	0.924	2.094	0.017
	Missing	39		NS		*
PGM	Complete	34	-0.881	0.381	1.129	>0.05
	Missing	43		NS		NS
TMP	Complete	34	-0.174	0.863	1.413	>0.05
	Missing	40		NS		NS
IPW	Complete	34	0.988	0.327	1.078	>0.05
	Missing	38		NS		NS
SWM	Complete	34	-0.140	0.889	1.218	>0.05
	Missing	62		NS		NS
PPL	Complete	34	-0.596	0.553	1.745	>0.05
	Missing	36		NS		NS
PACL	Complete	34	-0.411	0.682	1.431	>0.05
	Missing	57		NS		NS

Female Data

Variable	Group	n	t	p	F	p
PPHL	Complete	13	3.193	0.003	1.324	>0.05
	Missing	20		**		NS
POL	Complete	13	2.684	0.012	2.298	>0.05
	Missing	20		*		NS
PCL	Complete	13	3.054	0.005	2.512	>0.05
	Missing	18		**		NS
NCL	Complete	13	3.008	0.005	1.044	>0.05
	Missing	25		**		NS
NOL	Complete	13	1.763	0.086	1.161	>0.05
	Missing	25				NS
PAOL	Complete	13	1.711	0.097	2.201	>0.05
	Missing	20				NS
OOL	Complete	13	4.305	<0.001	1.196	>0.05
	Missing	30		***		NS
ZGW	Complete	13	3.903*	<0.001	3.461	0.013
	Missing	30		***		*
MMW	Complete	13	-0.213*	0.833	13.134	<0.001
	Missing	22				***
MPW	Complete	13	2.166	0.041	1.191	>0.05
	Missing	13		*		NS
EAMW	Complete	13	2.045	0.048	1.880	>0.05
	Missing	27		*		NS
MOW	Complete	13	3.333	0.002	1.790	>0.05
	Missing	30		**		NS
LAW	Complete	13	2.790	0.008	1.480	>0.05
	Missing	29		**		NS
MMH	Complete	13	2.190	0.034	1.609	>0.05
	Missing	30		*		NS
BPH	Complete	13	1.721	0.093	1.230	>0.05
	Missing	28				NS
APD	Complete	13	0.517	0.608	1.754	>0.05
	Missing	28				NS
LAD	Complete	13	-1.209	0.234	2.048	>0.05
	Missing	28				NS
TIC	Complete	13	-0.010	0.992	1.178	>0.05
	Missing	26				NS
NAC	Complete	13	0.079	0.938	1.498	>0.05
	Missing	26				NS
HSP	Complete	13	2.481	0.017	1.275	>0.05
	Missing	30		*		NS
EWB	Complete	13	3.008	0.004	1.586	>0.05
	Missing	30		**		NS

Variable	Group	n	t	p	F	p
EWT	Complete	13	2.202	0.033	1.620	>0.05
	Missing	29		*		NS
IWT	Complete	13	2.659	0.011	2.239	>0.05
	Missing	29		*		NS
FMP	Complete	13	3.865	<0.001	1.737	>0.05
	Missing	27		***		NS
EWO	Complete	13	3.689	0.001	1.317	>0.05
	Missing	30		***		NS
MWO	Complete	13	3.689	0.001	1.317	>0.05
	Missing	30		***		NS
MXTL	Complete	13	1.428*	0.161	4.123	0.006
	Missing	30				**
PPM	Complete	13	0.518	0.608	1.371	>0.05
	Missing	23				NS
PGW	Complete	13	0.674	0.505	1.609	>0.05
	Missing	24				NS
PGM	Complete	13	1.960	0.058	1.020	>0.05
	Missing	26				NS
TMP	Complete	13	2.097	0.045	1.047	>0.05
	Missing	18		*		NS
IPW	Complete	13	0.086*	0.932	2.569	0.049
	Missing	20				*
SWM	Complete	13	2.720	0.010	1.188	>0.05
	Missing	30		**		NS
PPL	Complete	13	2.382	0.024	1.063	>0.05
	Missing	18		*		NS
PACL	Complete	13	1.811	0.078	1.097	>0.05
	Missing	28				NS

APPENDIX 4.8.4:

RESULTS OF TESTS FOR NORMALITY, SKEWNESS AND KURTOSIS FOR ALL VARIABLES FOR MALE AND FEMALE DATA SETS.

All tests were performed on standardised data (mean = 0, SD = 1). Probability values testing for Normality were derived from a Kolmogorov – Smirnov one sample goodness of fit test using a Normal distribution with mean of 0 and SD of 1 as implemented by Systat (version 8). No male or female variable departed significantly from the Normal distribution. Skewness (G1) and Kurtosis (G2) statistics are given. Skewness and Kurtosis values are considered significant when the ratio of these values to their standard errors (SES and SEK respectively) is greater than 2. Significant values are highlighted. For the male data **MMH** is slightly positively skewed. For the female data **NOL** is slightly positively kurtotic (more 'peaked' than a Normal distribution).

Male Data

Variable	n	Normality p	Skewness G1	G1/SES	Kurtosis G2	G2/SEK
PPHL	76	0.746	0.086	0.313	-0.263	-0.482
POL	75	0.978	-0.366	-1.32	-0.313	-0.570
PCL	73	0.934	-0.199	-0.707	-0.335	-0.603
NCL	83	0.743	0.160	0.602	0.011	0.022
NOL	86	0.204	0.304	1.171	-0.263	-0.512
PAOL	76	1.000	-0.034	-0.123	-0.313	-0.574
OOL	96	0.325	-0.069	-0.280	-0.220	-0.451
ZGW	96	0.246	-0.352	-1.430	-0.131	-0.268
MMW	93	0.773	-0.072	-0.287	-0.570	-1.15
MPW	71	0.727	0.058	0.205	-0.246	-0.437
EAMW	91	0.743	-0.207	-0.819	-0.517	-1.033
MOW	98	0.806	-0.206	-0.846	-0.651	-1.348
LAW	99	0.676	-0.139	-0.575	-0.504	-1.049
MMH	98	0.069	0.835	3.427	0.604	1.251
BPH	95	0.550	0.530	2.143	0.451	0.921
APD	99	0.903	0.108	0.445	-0.331	-0.688
LAD	99	0.553	0.225	0.928	-0.752	-1.564
TIC	99	0.828	0.098	0.403	-0.321	-0.668
NAC	99	0.540	0.066	0.271	-0.317	-0.660
HSP	99	0.855	0.304	1.251	0.381	0.794
EWB	98	0994	0.009	0.037	-0.156	-0.323
EWT	98	0.799	0.406	1.665	-0.497	-1.030
IWT	98	0.834	0.336	1.378	-0.266	-0.552
FMP	91	0.977	0.270	1.067	0.398	0.796
EWO	99	0.968	-0.002	-0.008	-0.301	-0.626
MWO	99	0.940	0.277	1.142	0.193	0.403
MXTL	98	0.757	-0.086	-0.354	0.232	0.480
PPM	72	0.798	0.278	0.981	0.150	0.268
PGW	73	0.754	-0.209	-0.745	0.372	0.669
PGM	77	0.866	0.085	0.309	0.957	1.768
TMP	74	0.926	-0.048	-0.172	-0.174	-0.316
IPW	71	0.181	0.391	1.371	-0.602	-1.070
SWM	97	0.982	-0.117	-0.478	-0.530	-1.091
PPL	70	0.793	-0.178	-0.620	-0.504	-0.890
PACL	90	0.599	0.225	0.885	-0.531	-1.056

Female Data

Variable	n	Normality p	Skewness G1	G1/SES	Kurtosis G2	G2/SEK
PPHL	31	0.640	-0.156	-0.372	-1.045	-1.273
POL	31	0.787	-0.107	-0.255	0.179	0.218
PCL	29	0.841	-0.080	-0.184	0.306	0.362
NCL	36	0.958	-0.581	-1.479	0.942	1.227
NOL	36	0.674	-0.394	-1.004	1.802	2.347
PAOL	31	0.993	0.334	0.793	-0.345	-0.421
OOL	41	0.798	-0.301	-0.815	0.499	0.689
ZGW	41	0.840	-0.603	-1.631	-0.176	-0.244
MMW	34	0.696	-0.435	-1.08	1.489	1.890
MPW	25	0.976	0.137	0.295	0.007	0.008
EAMW	39	0.996	0.176	0.466	0.005	0.007
MOW	41	0.928	-0.205	-0.554	0.003	0.004
LAW	40	0.994	-0.214	-0.573	0.031	0.042
MMH	40	0.965	0.344	0.919	-0.444	-0.606
BPH	39	0.930	0.394	1.042	0.383	0.517
APD	39	0.631	0.533	1.409	0.127	0.172
LAD	39	0.962	0.191	0.504	-0.086	-0.116
TIC	37	0.956	0.287	0.740	-0.236	-0.311
NAC	37	0.933	0.157	0.404	-0.501	-0.660
HSP	41	0.993	0.382	1.035	0.441	0.608
EWB	41	0.536	0.220	0.596	-0.709	-0.979
EWT	40	0.451	-0.265	-0.708	-1.099	-1.501
IWT	40	0.765	0.024	0.065	-0.970	-1.324
FMP	39	0.884	-0.127	-0.335	-0.392	-0.528
EWO	41	0.876	-0.135	-0.366	-0.460	-0.635
MWO	41	0.876	-0.135	-0.366	-0.460	-0.635
MXTL	41	0.860	-0.196	-0.530	0.270	0.372
PPM	35	0.840	-0.005	-0.013	-0.697	-0.896
PGW	36	0.981	0.033	0.084	-0.459	-0.598
PGM	38	0.975	-0.368	-0.961	0.222	0.296
TMP	29	0.807	-0.249	-0.574	-1.221	-1.444
IPW	32	0.551	-0.667	-1.608	0.376	0.465
SWM	41	0.678	-0.517	-1.398	0.112	-0.154
PPL	29	0.981	-0.304	-0.701	-0.664	-0.785
PACL	38	0.961	-0.521	-1.362	0.101	0.134

APPENDIX 4.8.5:

PAIRED T-TEST RESULTS FOR COMPARISON OF REPLICATED CRANIAL MEASUREMENTS FOR 14 MALE SKULL SPECIMENS.

Variable	Side	n	t	p	Variable	Side	n	t	p
PPHL	Left	14	1.000	0.336	**TIC**	Left	14	1.264	0.228
	Right	13	1.897	0.082		Right	14	1.192	0.254
POL	Left	14	1.490	0.160	**NAC**	Left	14	0.000	1.000
	Right	13	1.477	0.165		Right	14	-0.465	0.649
PCL	Left	14	1.472	0.165	**HSP**		14	1.102	0.291
	Right	13	0.693	0.502	**EWB**		14	-0.812	0.431
NCL	Left	14	2.148	0.051	**EWT**		13	-1.389	0.190
	Right	13	1.105	0.291	**IWT**		13	-0.822	0.427
NOL	Left	14	1.325	0.208	**FMP**	Left	14	2.543	0.025
	Right	13	-0.267	0.794		Right	14	-0.279	0.785
PAOL	Left	14	-0.913	0.378	**EWO**		14	-2.709	0.018
	Right	13	-1.070	0.306	**MXTL**	Left	14	0.000	1.000
OOL	Left	13	-1.148	0.273		Right	13	-0.365	0.721
	Right	14	0.000	1.000	**PPM**	Left	12	-0.804	0.438
ZGW		14	0.434	0.671		Right	10	-0.408	0.693
MMW		14	-1.472	0.165	**PGW**		13	0.457	0.656
MPW		14	0.000	1.000	**PGM**	Left	14	1.000	0.336
EAMW		13	-1.897	0.082		Right	12	-0.506	0.623
MOW		14	1.295	0.218	**TMP**	Left	13	1.760	0.104
LAW		14	1.989	0.068		Right	13	-0.562	0.584
MMH	Left	14	1.727	0.108	**IPW**		11	0.000	1.000
	Right	14	1.685	0.116	**SWM**		14	-0.154	0.880
BPH		14	0.000	1.000	**PPL**	Left	13	0.743	0.472
APD	Left	14	0.291	0.775		Right	13	1.171	0.264
	Right	14	-0.322	0.752	**PACL**		14	-1.883	0.082
LAD	Left	14	-0.763	0.459					
	Right	14	-0.249	0.807					

APPENDIX 4.8.6:

MEASUREMENT ERROR ASSESSED BY CALCULATING STANDARD MAXIMUM DIFFERENCE.

Specimen	BMNH1898.7.2.5					BMNH1899.7.8.5				
	1st	*2nd*	*3rd*	*MD*	*SMD %*	*1st*	*2nd*	*3rd*	*MD*	*SMD %*
MASS	9.3	9.2	9.2	0.1	1.08%	5.5	5.5	5.5	0.0	0.00%
PPHL	778.5	778.0	777.5	1.0	0.13%	705.5	705.0	705.5	0.5	0.07%
POL	701.5	701.0	701.0	0.5	0.07%	659.0	659.0	659.0	0.0	0.00%
PCL	630.5	630.0	630.0	0.5	0.08%	600.0	601.0	600.5	1.0	0.17%
NCL	506.0	505.5	504.5	1.5	0.30%	477.0	477.5	475.5	2.0	0.42%
NOL	261.5	262.5	263.0	1.5	0.57%	239.5	241.0	240.5	1.0	0.42%
PAOL	402.0	401.5	401.5	0.5	0.12%	377.0	378.0	378.0	1.0	0.26%
OOL	273.0	272.5	272.0	1.0	0.37%	243.0	243.0	243.5	0.5	0.21%
ZGW	239.0	239.0	239.0	0.0	0.00%	219.0	219.0	219.0	0.0	0.00%
MMW	96.0	96.0	96.0	0.0	0.00%	82.0	82.0	82.0	0.0	0.00%
MPW	99.0	99.0	99.0	0.0	0.00%	91.0	91.0	91.0	0.0	0.00%
EAMW	165.0	165.0	165.0	0.0	0.00%	157.0	157.0	157.0	0.0	0.00%
MOW	294.0	294.0	294.0	0.0	0.00%	283.0	284.0	283.0	1.0	0.35%
LAW	209.0	209.0	210.0	1.0	0.48%	184.0	182.0	182.0	2.0	1.09%
MMH	290.0	290.5	289.5	1.0	0.34%	280.0	280.5	280.5	0.0	0.00%
BPH	200.0	199.0	198.0	2.0	1.01%	178.0	179.0	179.0	1.0	0.56%
APD	60.0	59.5	59.0	1.0	1.68%	54.0	53.0	53.5	1.0	1.87%
LAD	64.0	64.0	64.0	0.0	0.00%	48.0	48.0	48.5	0.5	1.04%
TIC	200.5	193.5	192.5	8.0	4.09%	161.5	160.5	159.5	2.0	1.25%
NAC	174.5	175.0	174.0	1.0	0.57%	132.5	132.0	132.0	0.5	0.38%
HSP	147.0	145.0	147.0	2.0	1.37%	124.0	124.0	125.0	1.0	0.80%
EWB	231.0	231.0	231.0	0.0	0.00%	204.0	204.0	200.0	4.0	1.97%
EWT	215.0	214.0	215.0	1.0	0.47%	188.0	189.0	189.0	1.0	0.53%
IWT	88.0	88.0	88.0	0.0	0.00%	92.0	92.0	92.0	0.0	0.00%
FMS	199.0	196.0	197.0	2.0	1.01%	195.0	199.0	193.0	6.0	3.07%
FMP	330.5	328.0	328.0	2.5	0.76%	299.5	299.5	299.5	0.0	0.00%
FMO	111.0	110.0	112.0	1.0	0.90%	105.0	104.0	104.0	1.0	0.96%
EWO	134.0	141.0	152.0	18.0	12.65%	137.0	143.0	137.0	6.0	4.32%
MWO										
MXTL	149.0	148.5	150.0	1.5	1.01%	133.0	133.0	133.0	0.0	0.00%
PPM	39.0	40.0	39.0	1.0	2.54%	41.0	41.5	42.0	1.0	2.41%
PGW	89.0	90.0	89.0	1.0	1.12%	87.0	86.0	85.0	2.0	2.33%
PGM	173.0	172.5	173.0	0.5	0.29%	166.5	167.0	166.5	0.5	0.30%
TMP	263.0	263.5	265.5	2.5	0.95%	251.5	251.0	252.0	1.0	0.40%
IPW	38.0	39.0	38.0	1.0	2.61%	35.0	34.0	34.0	1.0	2.91%
SWM	156.0	156.0	155.0	1.0	0.64%	137.0	134.0	136.0	3.0	2.21%
PPL	362.0	364.0	367.0	5.0	1.37%	359.0	359.5	359.5	0.5	0.14%
PACL	242.0	243.0	242.0	1.0	0.41%	212.0	212.0	213.0	1.0	0.47%

Specimen	BMNH1938.7.8.22					BMNH1962.220				
	1st	*2nd*	*3rd*	*MD*	*SMD %*	*1st*	*2nd*	*3rd*	*MD*	*SMD %*
MASS	7.5	7.6	7.4	0.2	2.67%	6.9	7.0	6.8	0.2	2.90%
PPHL	737.0	736.0	736.0	1.0	0.14%	737.5	737.0	736.0	1.5	0.20%
POL	672.5	671.5	671.0	2.5	0.37%	696.5	695.5	696.5	1.0	0.14%
PCL	610.0	610.0	610.0	0.0	0.00%	640.5	639.5	639.5	1.0	0.16%
NCL	470.0	469.5	469.0	1.0	0.21%	508.0	508.5	508.0	0.5	0.10%
NOL	236.0	235.5	235.5	0.5	0.21%	242.0	244.5	247.0	5.0	2.04%
PAOL	389.0	388.0	388.0	1.0	0.26%	393.0	392.0	392.0	1.0	0.25%
OOL	249.5	249.5	249.5	0.0	0.00%	268.0	269.0	268.5	1.0	0.37%
ZGW	232.0	232.0	232.0	0.0	0.00%	241.0	241.0	241.0	0.0	0.00%
MMW	92.0	93.0	92.0	1.0	1.08%	104.0	104.0	104.0	0.0	0.00%
MPW	104.0	104.0	104.0	0.0	0.00%	107.0	107.0	107.0	0.0	0.00%
EAMW	158.0	158.0	158.0	0.0	0.00%	162.0	162.0	162.0	0.0	0.00%
MOW	284.0	284.0	285.0	1.0	0.35%	306.0	306.0	306.0	0.0	0.00%
LAW	166.0	165.0	165.0	1.0	0.60%	197.0	195.0	196.0	2.0	1.02%
MMH	311.0	308.0	309.5	3.0	0.97%	317.5	315.5	316.0	2.0	0.63%
BPH	188.0	187.0	187.0	1.0	0.53%	202.0	203.0	200.0	3.0	1.49%
APD	47.5	47.5	47.0	0.5	1.06%	54.0	54.0	53.5	0.5	0.93%
LAD	56.5	56.5	54.0	2.5	4.49%	53.5	53.5	54.0	0.5	0.93%
TIC	161.0	162.0	159.0	2.0	1.24%	171.5	169.0	170.5	2.5	1.47%
NAC	140.0	139.5	138.5	1.5	1.08%	165.5	162.5	165.0	3.0	1.83%
HSP	150.0	150.0	150.0	0.0	0.00%	140.0	139.0	139.0	1.0	0.72%
EWB	209.0	208.0	207.0	2.0	0.96%	235.0	238.0	237.0	2.0	0.85%
EWT	203.0	203.0	203.0	0.0	0.00%	256.0	256.0	256.0	0.0	0.00%
IWT	96.0	96.0	95.0	1.0	1.05%	151.0	153.0	157.0	6.0	3.90%
FMS	191.0	194.0	184.0	10.0	5.27%	205.0	207.0	206.0	2.0	0.97%
FMP	309.5	304.5	304.0	5.5	1.80%	335.0	336.5	333.5	3.0	0.90%
FMO	100.0	99.0	99.0	1.0	1.01%	110.0	110.0	110.0	0.0	0.00%
EWO	135.0	140.0	136.0	5.0	3.65%	148.0	148.0	148.0	0.0	0.00%
MWO	139.0	142.0	141.0	3.0	2.13%					
MXTL	150.5	150.5	150.0	0.5	0.33%	155.0	155.5	154.5	1.0	0.65%
PPM	28.0	28.5	28.0	0.5	1.78%	40.0	39.0	38.5	1.5	3.83%
PGW	75.0	75.0	81.0	6.0	7.79%	95.0	95.0	95.0	0.0	0.00%
PGM	165.5	165.5	166.5	0.0	0.00%	179.5	179.0	179.0	0.5	0.28%
TMP	259.0	258.5	259.0	0.5	0.19%	258.0	258.5	258.0	0.5	0.19%
IPW	35.0	34.0	36.0	2.0	5.71%	47.0	46.0	46.0	1.0	2.16%
SWM	149.0	149.0	149.0	0.0	0.00%	165.0	166.0	165.0	1.0	0.60%
PPL	359.0	357.0	358.0	2.0	0.56%	375.5	375.5	374.5	1.0	0.27%
PACL	225.0	227.0	226.0	2.0	0.88%	238.0	239.0	240.0	2.0	0.84%

Specimen	BMNH1986.1604							
	1st	*2nd*	*3rd*	*MD*	*SMD %*		*Mean MD*	*Mean SMD %*
MASS	14.8	14.8	14.8	0.0	0.00%		0.1	0.07%
PPHL	787.0	787.0	787.0	0.0	0.00%		0.8	0.11%
POL	742.0	742.0	741.5	0.5	0.07%		0.9	0.13%
PCL	653.5	653.0	653.0	0.5	0.08%		0.6	0.10%
NCL	525.0	524.0	525.0	1.0	0.19%		1.2	0.26%
NOL	264.0	264.0	264.5	0.5	0.19%		1.7	0.62%
PAOL	411.0	410.5	411.5	1.0	0.24%		0.9	0.24%
OOL	288.5	289.0	289.5	1.0	0.35%		0.7	0.27%
ZGW	257.0	257.0	254.0	3.0	1.17%		0.6	0.28%
MMW	101.0	101.0	101.0	0.0	0.00%		0.2	0.21%
MPW	106.0	105.0	105.0	1.0	0.95%		0.2	0.19%
EAMW			197.0				0.0	0.00%
MOW	316.0	314.0	316.0	2.0	0.63%		0.8	0.29%
LAW	193.0	192.0	193.0	1.0	0.52%		1.4	0.68%
MMH	282.0	276.5	282.5	6.0	2.14%		2.4	0.86%
BPH	193.0	192.0	192.0	1.0	0.52%		1.6	0.94%
APD	62.5	63.0	63.0	0.5	0.80%		0.7	1.21%
LAD	70.0	69.5	70.0	0.5	0.72%		0.8	0.96%
TIC	207.5	208.0	209.5	2.0	0.96%		3.3	1.82%
NAC	190.0	189.5	189.5	0.5	0.26%		1.3	0.81%
HSP	162.0	163.0	161.0	2.0	1.23%		1.2	0.74%
EWB	258.0	256.0	255.0	3.0	1.17%		2.2	0.94%
EWT	277.0	277.0	277.0	0.0	0.00%		0.4	0.19%
IWT	137.0	138.0	139.0	2.0	1.45%		1.8	1.37%
FMS	223.0	220.0	221.0	3.0	1.36%		4.6	2.00%
FMP	369.5	370.0	370.0	0.5	0.14%		2.3	0.79%
FMO	110.0	111.0	110.0	0.0	0.00%		0.6	0.51%
EWO	164.0	171.0	163.0	8.0	4.82%		7.4	4.87%
MWO	180.0	180.0	180.0	0.0	0.00%		1.5	0.97%
MXTL	144.0	144.0	143.0	1.0	0.70%		0.8	0.62%
PPM	39.0	39.5	40.5	1.5	3.78%		1.1	2.23%
PGW	107.0	108.0	108.0	1.0	0.93%		2.0	1.86%
PGM	190.0	190.5	190.0	0.5	0.26%		0.4	0.21%
TMP	273.5	274.0	274.0	0.5	0.18%		1.0	0.45%
IPW	41.0	41.0	41.0	0.0	0.00%		1.0	1.67%
SWM	166.0	165.0	165.0	1.0	0.60%		1.2	0.62%
PPL	394.5	394.0	393.5	1.0	0.25%		1.9	0.55%
PACL	227.0	228.0	227.0	1.0	0.44%		1.4	0.61%

APPENDIX 4.8.7:

SAMPLE REGRESSION COEFFICIENTS FOR MALE AND FEMALE DATA.

Sample regression coefficients (r) are in normal type. The sample size for each coefficient is given in italics.

Male Data

	PPHL							
PPHL	1.000 *76*	**POL**						
POL	0.866 *75*	1.000 *75*	**PCL**					
PCL	0.768 *73*	0.922 *72*	1.000 *73*	**NCL**				
NCL	0.760 *72*	0.853 *71*	0.915 *72*	1.000 *83*	**NOL**			
NOL	0.750 *75*	0.798 *74*	0.766 *72*	0.882 *83*	1.000 *87*	**PAOL**		
PAOL	0.805 *76*	0.913 *75*	0.877 *73*	0.795 *72*	0.853 *75*	1.000 *76*	**OOL**	
OOL	0.606 *74*	0.631 *74*	0.516 *71*	0.466 *81*	0.338 *85*	0.358 *74*	1.000 *96*	**ZGW**
ZGW	0.469 *74*	0.465 *73*	0.423 *71*	0.302 *80*	0.156 *84*	0.311 *74*	0.758 *94*	1.000 *96*
MMW	0.531 *75*	0.634 *74*	0.524 *72*	0.508 *82*	0.399 *86*	0.419 *75*	0.677 *91*	0.367 *90*
MPW	0.448 *68*	0.534 *67*	0.359 *65*	0.287 *67*	0.268 *70*	0.344 *68*	0.616 *69*	0.383 *68*
EAMW	0.424 *72*	0.441 *71*	0.438 *69*	0.373 *78*	0.313 *82*	0.375 *72*	0.470 *89*	0.547 *90*
MOW	0.382 *75*	0.575 *74*	0.411 *72*	0.378 *82*	0.346 *86*	0.354 *75*	0.703 *95*	0.533 *95*
LAW	0.402 *76*	0.601 *75*	0.522 *73*	0.531 *83*	0.445 *87*	0.372 *76*	0.788 *96*	0.543 *96*
MMH	0.218 *76*	0.212 *75*	0.041 *73*	-0.007 *83*	0.026 *87*	0.093 *76*	0.267 *95*	0.164 *95*
BPH	0.568 *74*	0.629 *73*	0.489 *71*	0.401 *80*	0.316 *84*	0.415 *74*	0.692 *92*	0.533 *92*
APD	0.389 *76*	0.324 *75*	0.195 *73*	0.257 *83*	0.316 *87*	0.248 *76*	0.319 *96*	0.064 *96*
LAD	0.285 *76*	0.136 *75*	-0.026 *73*	0.059 *83*	0.156 *87*	0.094 *76*	0.196 *96*	0.130 *96*
TIC	0.347 *76*	0.235 *75*	0.082 *73*	0.159 *83*	0.239 *87*	0.169 *76*	0.297 *96*	0.144 *96*
NAC	0.375 *76*	0.276 *75*	0.159 *73*	0.217 *83*	0.207 *87*	0.179 *76*	0.381 *96*	0.269 *96*
HSP	0.780 *76*	0.508 *75*	0.540 *73*	0.589 *83*	0.481 *87*	0.438 *76*	0.395 *96*	0.347 *96*
EWB	0.370 *75*	0.411 *74*	0.241 *72*	0.182 *82*	0.223 *86*	0.296 *75*	0.525 *95*	0.436 *95*
EWT	0.269 *75*	0.265 *74*	0.281 *72*	0.228 *82*	0.010 *86*	0.126 *75*	0.427 *95*	0.402 *95*
IWT	0.087 *75*	0.170 *74*	0.275 *72*	0.176 *82*	-0.069 *86*	0.078 *75*	0.248 *95*	0.263 *95*
FMP	0.832 *70*	0.641 *69*	0.592 *69*	0.649 *80*	0.520 *81*	0.482 *70*	0.631 *88*	0.466 *88*
EWO	0.254 *76*	0.372 *75*	0.334 *73*	0.231 *83*	0.225 *87*	0.350 *76*	0.251 *96*	0.129 *96*
MWO	0.193 *76*	0.291 *75*	0.262 *73*	0.153 *83*	0.172 *87*	0.297 *76*	0.135 *96*	0.076 *96*
MXTL	0.449 *76*	0.374 *75*	0.331 *73*	0.393 *83*	0.382 *87*	0.358 *76*	0.212 *95*	0.145 *95*
PPM	0.343 *60*	0.491 *60*	0.580 *57*	0.553 *63*	0.402 *67*	0.442 *60*	0.341 *72*	0.260 *70*
PGW	0.243 *60*	0.392 *59*	0.493 *60*	0.553 *67*	0.436 *67*	0.420 *60*	0.007 *71*	-0.127 *71*
PGM	0.468 *64*	0.580 *63*	0.742 *63*	0.630 *70*	0.309 *71*	0.487 *64*	0.418 *76*	0.469 *75*
TMP	0.706 *74*	0.887 *73*	0.886 *71*	0.781 *70*	0.774 *73*	0.912 *74*	0.364 *72*	0.263 *72*
IPW	0.360 *60*	0.357 *60*	0.388 *57*	0.352 *63*	0.157 *67*	0.257 *60*	0.463 *71*	0.478 *70*
SWM	0.253 *75*	0.375 *74*	0.385 *72*	0.402 *82*	0.311 *86*	0.300 *75*	0.504 *93*	0.401 *93*
PPL	0.623 *70*	0.818 *70*	0.855 *67*	0.810 *67*	0.766 *70*	0.828 *70*	0.391 *69*	0.271 *68*
PACL	0.451 *70*	0.449 *70*	0.525 *69*	0.427 *80*	0.223 *81*	0.356 *70*	0.343 *89*	0.285 *88*

	MMW						
MMW	1.000 *93*	**MPW**					
MPW	0.887 *71*	1.000 *71*	**EAMW**				
EAMW	0.250 *88*	0.181 *66*	1.000 *92*	**MOW**			
MOW	0.706 *92*	0.739 *70*	0.328 *91*	1.000 *98*	**LAW**		
LAW	0.618 *93*	0.518 *71*	0.396 *92*	0.780 *98*	1.000 *99*	**MMH**	
MMH	0.511 *93*	0.687 *71*	-0.270 *92*	0.564 *97*	0.240 *98*	1.000 *98*	**BPH**
BPH	0.725 *89*	0.789 *69*	0.165 *88*	0.785 *94*	0.610 *95*	0.790 *94*	1.000 *95*
APD	0.460 *93*	0.394 *71*	0.458 *92*	0.412 *98*	0.308 *99*	0.227 *98*	0.338 *95*
LAD	0.352 *93*	0.294 *71*	0.246 *92*	0.293 *98*	0.067 *99*	0.135 *98*	0.134 *95*
TIC	0.442 *93*	0.372 *71*	0.401 *92*	0.372 *98*	0.219 *99*	0.156 *98*	0.227 *95*
NAC	0.496 *93*	0.378 *71*	0.447 *92*	0.391 *98*	0.252 *99*	0.186 *98*	0.322 *95*
HSP	0.309 *93*	0.161 *71*	0.389 *92*	0.041 *98*	0.147 *99*	-0.036 *98*	0.259 *95*
EWB	0.653 *92*	0.694 *70*	0.196 *91*	0.664 *97*	0.526 *98*	0.628 *97*	0.683 *94*
EWT	0.401 *92*	0.223 *70*	0.280 *91*	0.130 *97*	0.212 *98*	-0.045 *97*	0.201 *94*
IWT	0.161 *92*	0.036 *70*	0.077 *91*	-0.051 *97*	0.151 *98*	-0.089 *97*	0.111 *94*
FMP	0.627 *85*	0.484 *65*	0.381 *84*	0.420 *90*	0.427 *91*	0.310 *90*	0.591 *89*
EWO	0.021 *93*	0.101 *71*	0.144 *92*	0.210 *98*	0.192 *99*	0.038 *98*	0.246 *95*
MWO	-0.019 *93*	0.072 *71*	0.210 *92*	0.175 *98*	0.097 *99*	0.059 *98*	0.211 *95*
MXTL	0.556 *93*	0.455 *71*	0.126 *92*	0.193 *97*	0.138 *98*	0.364 *98*	0.379 *94*
PPM	0.126 *70*	-0.073 *55*	0.492 *69*	0.099 *71*	0.395 *72*	-0.443 *72*	-0.069 *72*
PGW	0.072 *71*	-0.189 *55*	0.246 *72*	-0.048 *72*	0.151 *73*	-0.359 *73*	-0.141 *71*
PGM	0.294 *74*	0.066 *57*	0.403 *74*	0.190 *76*	0.355 *77*	-0.172 *77*	0.233 *74*
TMP	0.420 *73*	0.376 *67*	0.273 *70*	0.418 *73*	0.390 *74*	0.100 *74*	0.456 *72*
IPW	0.308 *70*	0.148 *55*	0.385 *68*	0.213 *72*	0.274 *72*	-0.041 *72*	0.221 *72*
SWM	0.576 *92*	0.445 *70*	0.407 *92*	0.478 *95*	0.530 *96*	0.160 *96*	0.324 *92*
PPL	0.350 *69*	0.210 *63*	0.488 *66*	0.361 *69*	0.406 *70*	-0.242 *70*	0.216 *69*
PACL	0.517 *85*	0.425 *65*	0.072 *84*	0.210 *90*	0.264 *91*	0.438 *90*	0.556 *89*

	APD						
APD	1.000 *99*	**LAD**					
LAD	0.590 *99*	1.000 *99*	**TIC**				
TIC	0.852 *99*	0.909 *99*	1.000 *99*	**NAC**			
NAC	0.761 *99*	0.835 *99*	0.900 *99*	1.000 *99*	**HSP**		
HSP	0.216 *99*	0.233 *99*	0.223 *99*	0.331 *99*	1.000 *99*	**EWB**	
EWB	0.416 *98*	0.397 *98*	0.450 *98*	0.569 *98*	0.097 *98*	1.000 *98*	**EWT**
EWT	0.198 *98*	0.149 *98*	0.229 *98*	0.436 *98*	0.275 *98*	0.463 *98*	1.000 *98*
IWT	-0.195 *98*	-0.490 *98*	-0.371 *98*	-0.141 *98*	0.112 *98*	0.184 *98*	0.781 *98*
FMP	0.437 *91*	0.370 *91*	0.417 *91*	0.545 *91*	0.840 *91*	0.467 *90*	0.445 *90*
EWO	-0.152 *99*	-0.405 *99*	-0.336 *99*	-0.274 *99*	-0.020 *99*	0.017 *98*	0.046 *98*
MWO	-0.042 *99*	-0.324 *99*	-0.223 *99*	-0.195 *99*	-0.043 *99*	0.016 *98*	0.033 *98*
MXTL	0.351 *98*	0.363 *98*	0.375 *98*	0.333 *98*	0.388 *98*	0.254 *97*	0.103 *97*
PPM	0.017 *72*	-0.114 *72*	-0.019 *72*	0.088 *72*	0.277 *72*	0.015 *71*	0.372 *71*
PGW	0.199 *73*	-0.176 *73*	-0.007 *73*	-0.056 *73*	0.079 *73*	-0.238 *72*	0.124 *72*
PGM	0.003 *77*	-0.128 *77*	-0.058 *77*	0.101 *77*	0.428 *77*	0.021 *76*	0.396 *76*
TMP	0.152 *74*	-0.016 *74*	0.049 *74*	0.042 *74*	0.345 *74*	0.236 *73*	0.124 *73*
IPW	0.207 *72*	0.146 *72*	0.201 *72*	0.311 *72*	0.367 *72*	0.162 *71*	0.431 *71*
SWM	0.421 *96*	0.277 *96*	0.402 *96*	0.401 *96*	0.037 *96*	0.429 *95*	0.340 *95*
PPL	0.173 *70*	0.027 *70*	0.094 *70*	0.107 *70*	0.377 *70*	0.068 *69*	0.160 *69*
PACL	0.208 *91*	0.034 *91*	0.149 *91*	0.288 *91*	0.394 *91*	0.370 *90*	0.379 *90*

	IWT						
IWT	1.000 *98*	**FMP**					
FMP	0.189 *90*	1.000 *91*	**EWO**				
EWO	0.302 *98*	-0.002 *91*	1.000 *99*	**MWO**			
MWO	0.240 *98*	-0.028 *91*	0.926 *99*	1.000 *99*	**MXTL**		
MXTL	-0.123 *97*	0.475 *90*	-0.167 *98*	-0.073 *98*	1.000 *98*	**PPM**	
PPM	0.394 *71*	0.265 *68*	0.270 *72*	0.165 *72*	-0.221 *72*	1.000 *72*	**PGW**
PGW	0.230 *72*	0.089 *70*	0.254 *73*	0.244 *73*	0.076 *73*	0.497 *56*	1.000 *73*
PGM	0.440 *76*	0.438 *73*	0.218 *77*	0.139 *77*	-0.020 *77*	0.691 *65*	0.427 *67*
TMP	0.137 *73*	0.397 *68*	0.463 *74*	0.375 *74*	0.205 *74*	0.391 *58*	0.434 *58*
IPW	0.275 *71*	0.440 *68*	0.036 *72*	-0.047 *72*	0.235 *72*	0.413 *66*	0.252 *55*
SWM	0.129 *95*	0.343 *88*	-0.086 *96*	-0.057 *96*	0.485 *96*	0.219 *72*	0.284 *73*
PPL	0.122 *69*	0.359 *66*	0.406 *70*	0.287 *70*	0.132 *70*	0.626 *57*	0.529 *56*
PACL	0.330 *90*	0.580 *89*	-0.103 *91*	-0.033 *91*	0.396 *90*	0.135 *69*	0.058 *70*

	PGM					
PGM	1.000 *77*	**TMP**				
TMP	0.506 *62*	1.000 *74*	**IPW**			
IPW	0.436 *60*	0.189 *58*	1.000 *72*	**SWM**		
SWM	0.306 *77*	0.218 *73*	0.494 *70*	1.000 *96*	**PPL**	
PPL	0.559 *60*	0.866 *70*	0.331 *57*	0.270 *69*	1.000 *70*	**PACL**
PACL	0.505 *74*	0.315 *68*	0.102 *69*	0.323 *88*	0.087 *67*	1.000 *91*

APPENDIX 4.9.1:

LIST OF MALE AND FEMALE SKULLS USED IN TRADITIONAL MORPHOMETRIC ANALYSES

Note that, due to missing values for some parameters, not all specimens were used in all analyses.

Male			
AMNH82001	EEK	NMZB26185	SCZ
AMNH83460	SWC	NMZB26186	SCZ
BMNH1897.1.30.1	EEK	NMZB26188	SCZ
BMNH1898.7.2.5	ESK	NMZB26189	SCZ
BMNH1899.7.8.5	EEK	NMZB26200	SCZ
BMNH1901.8.9.47	EEK	NMZB29099	SZT
BMNH1904.11.2.2	WSN	NMZB29100	SCZ
BMNH1906.10.26.1	SWA	NMZB29102	SCZ
BMNH1906.2.12.2	WSN	NMZB29111	SWC
BMNH1923.10.20.8	EEK	NMZB29113	SWC
BMNH1931.2.1.48	SWC	NMZB58342	SCZ
BMNH1938.7.8.22	WCP	NMZB60801	SWC
BMNH1962.220	ESK	NMZB60802	SWC
BMNH1964.225	ECU	PCCAMII139	WCP
BMNH1986.1604	SCZ	PCCongoNoNum1	ECU
EEI1	SWC	PCCongoNoNum2	ECU
EEI3	SWC	PCJ39	EEK
EEI4	SWC	PCMN276	WCP
EEI5	SWC	PCMN280	ECU
EEI8	SWC	PCMN579	ENA
FMNH127878	ESK	PCNNChad138	WCP
FMNH127881	ESK	PCTAN76	ESK
FMNH34422	SWC	TM12141	SWC
FMNH34425	SWC	USNM121010	ECU
FMNH34930	ESK	USNM155438	EEK
KNP8	SEW	USNM162016	ECU
NMZB11525	SZT	USNM162017	ECU
NMZB20221	SZT	USNM163113	EEK
NMZB22862	SZT	USNM163312	EEK
NMZB23978	SCZ	USNM182124	EST
NMZB23979	SCZ	USNM182125	EEK
NMZB23983	SCZ	USNM182192	EEK
NMZB23984	SCZ	USNM200151	EEK
NMZB26179	SCZ	USNM251799	EST
NMZB26180	SCZ	USNM296145	SWC

Female	
AMNH53546	ECC
BMNH1900.3.18.3	ESK
BMNH1901.8.9.50	ECU
BMNH1939.4329A	SWC
EEI7	SWC
EEI9	SWC
Etosha1	SWC
FMNH127880	ESK
FMNH127885	ECU
FMNH127886	ECU
FMNH32901	EEK
FMNH32902	EEK
FMNH34423	SWC
FMNH34424	SWC
FMNH34429	SWC
NMZB26184	SCZ
NMZB60803	SWC
NMZB60804	SWC
NMZB60805	SWC
PCMN277	ENA
PCTAN89	ESK
USNM162988	ECU
USNM163324	EEK
USNM251797	EST

APPENDIX 4.9.2:

COMPLETE RESULTS OF DISCRIMINANT ANALYSES

Comparisons Made

Comparison Made	Male			Female
	All Male	*Old Male*	*Male – No EEK*	*Both*
Regional				
E v. W. v. S	Yes	Yes	Yes	
N v. S	Yes	Yes	Yes	Yes
E v. W	Yes	Yes	Yes	
Local				
Within East: All groups.	Yes	Yes	Yes	Yes
Within East: ECU v. EEK.	Yes			
Within East: ESK v. EST.	Yes			
Within East: [ECU / EEK] v. [ESK / EST]	Yes			
Within West: WCP v. WSN.	Yes			
Within South: SCZ v. SWC v. SZT.	Yes			
Within South: SCZ v. SWC.	Yes	Yes		
Within South: [SCZ / SWC] v. SZT	Yes			
Between Regions. Local Comparison.				
[ESK / EST] v. SZT.	Yes			
[ECU / EEK] v. [WCP / WSN]	Yes			

All Male Skull Analysis

Regional Comparison: *East versus West versus South*

Group Frequencies:

East	28
West	6
South	36

Classification Matrices:

Classification Matrix				
	East	South	West	% Correct
East	23	3	2	*82*
South	1	35	0	*97*
West	0	0	6	*100*
Total	*24*	*38*	*8*	*91*

Jack-knifed Classification Matrix				
	East	South	West	% Correct
East	21	3	4	*75*
South	1	35	0	*97*
West	1	0	5	*83*
Total	*22*	*38*	*9*	*87*

Support Statistics: Wilks' $\lambda = 0.208$. $p < 0.001$.
Cohen's $\kappa = 77.5\%$

Model Variables and Classification Functions:

	East	*South*	*West*
Constant	-2.384	-2.307	-4.577
MMH	3.987	-3.591	2.937
MOW	1.638	1.699	-2.550
HSP	-0.581	0.804	-2.111
MXTL	-0.232	-0.113	1.762
OOL	0.277	0.241	-2.742
BPH	-1.329	0.746	1.725

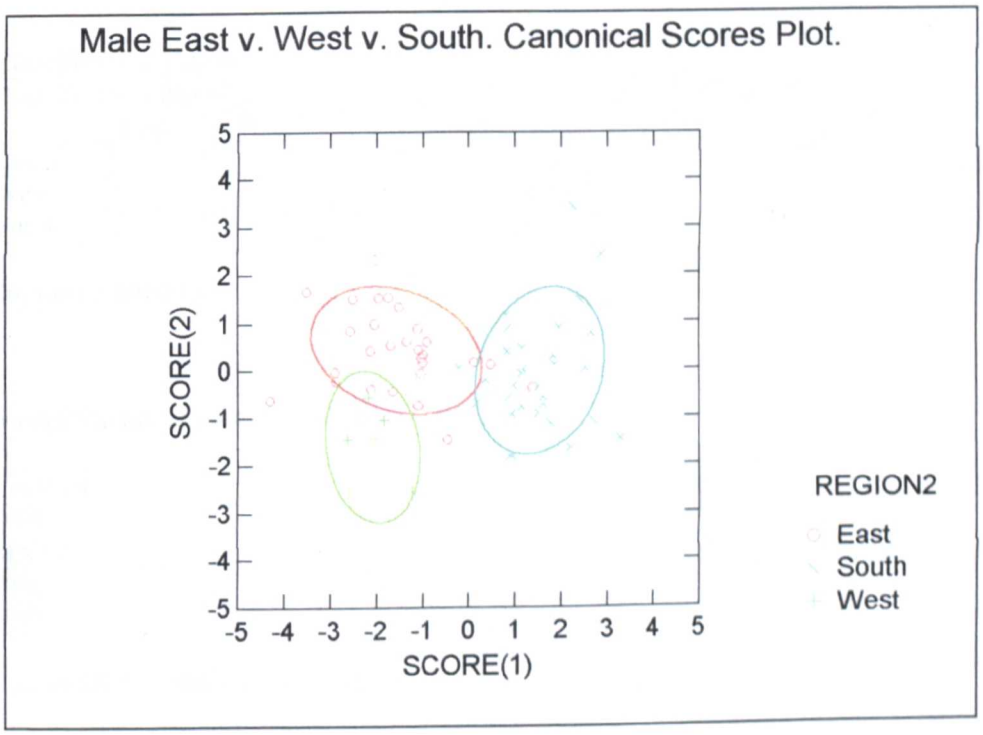
Canonical Discriminant Functions

	1	*2*
Constant	0.000	0.000
MMH	2.277	1.125
MOW	-1.098	0.161
HSP	-0.536	0.616
MXTL	0.101	-0.961
OOL	-0.198	1.443
BPH	-0.432	-1.628

Discriminant Function Data

	1	*2*
Eigenvalues	2.693	0.303
Canonical Correlations	0.854	0.482
Cumulative proportion of total dispersion	89.9%	100%

Canonical Plots:



Regional Comparison: *North versus South*

Group Frequencies: North 34
South 36

Classification Matrices:

Classification Matrix			
	North	**South**	**% Correct**
North	32	2	*94*
South	1	35	*97*
Total	*33*	*37*	*96*

Jack-knifed Classification Matrix			
	North	**South**	**% Correct**
North	32	2	*94*
South	1	35	*97*
Total	*33*	*37*	*96*

Support Statistics: Wilks' $\lambda = 0.3564$. $p < 0.001$.
$C_{pro} = 0.500$
Cohen's $\kappa = 92.0\%$

Model Variables and Classification Functions:

	North	*South*
Constant	-1.622	-1.522
MMH	2.582	-2.439
MOW	-1.909	1.803

Canonical Discriminant Functions

	1
Constant	0.000
MMH	1.895
MOW	-1.041

Discriminant Function Data

	1
Eigenvalues	1.806
Canonical Correlations	0.802
Cumulative proportion of total dispersion	100%

Regional Comparison: *East versus West*

Group Frequencies:

East	28
West	6

Classification Matrices:

Classification Matrix			
	East	**West**	**% Correct**
East	23	5	*82*
West	0	6	*100*
Total	*23*	*11*	*85*

Jack-knifed Classification Matrix			
	East	**West**	**% Correct**
East	23	5	*82*
West	0	6	*100*
Total	*23*	*11*	*85*

Support Statistics: Wilks' $\lambda = 0.4982$. $p < 0.001$.
$C_{pro} = 0.709$
Cohen's $\kappa = 61.1\%$

Model Variables and Classification Functions:

	East	*West*
Constant	-0.879	-4.716
OOL	-0.728	-5.880
MXTL	-0.080	3.082
BPH	1.070	4.266
HSP	-0.634	-2.907

Canonical Discriminant Functions

	1
Constant	0.676
OOL	2.017
MXTL	-1.238
BPH	-1.568
HSP	0.890

Discriminant Function Data

	1
Eigenvalues	1.007
Canonical Correlations	0.8708
Cumulative proportion of total dispersion	100%

Local Comparison: *Within the Eastern Region*

Group Frequencies:

ECU	7
EEK	12
ESK	6
EST	2

Classification Matrices:

Classification Matrix					
	ECU	**EEK**	**ESK**	**EST**	**%Correct**
ECU	6	1	0	0	*86*
EEK	1	11	0	0	*92*
ESK	0	0	6	0	*100*
EST	0	0	0	2	*100*
Total	*7*	*12*	*6*	*2*	*93*
Jack-knifed Classification Matrix					
	ECU	**EEK**	**ESK**	**EST**	**%Correct**
ECU	5	1	1	0	*71*
EEK	2	10	0	0	*83*
ESK	0	0	6	0	*100*
EST	0	0	0	2	*100*
Total	*7*	*11*	*7*	*2*	*85*

Support Statistics: Wilks' $\lambda = 0.0423$. $p < 0.001$.
Cohen's $\kappa = 78.2\%$

Model Variables and Classification Functions:

	ECU	*EEK*	*ESK*	*EST*
Constant	-5.889	-7.284	-3.391	-11.792
MMH	5.838	6.271	-0.847	-0.614
SWM	-5.962	-4.255	2.192	1.799
NOL	-2.542	-5.645	2.967	-7.881
HSP	-2.694	0.120	-3.540	9.410
EWT	0.691	-1.856	3.463	-10.019
MXTL	3.573	0.910	-1.629	-0.283

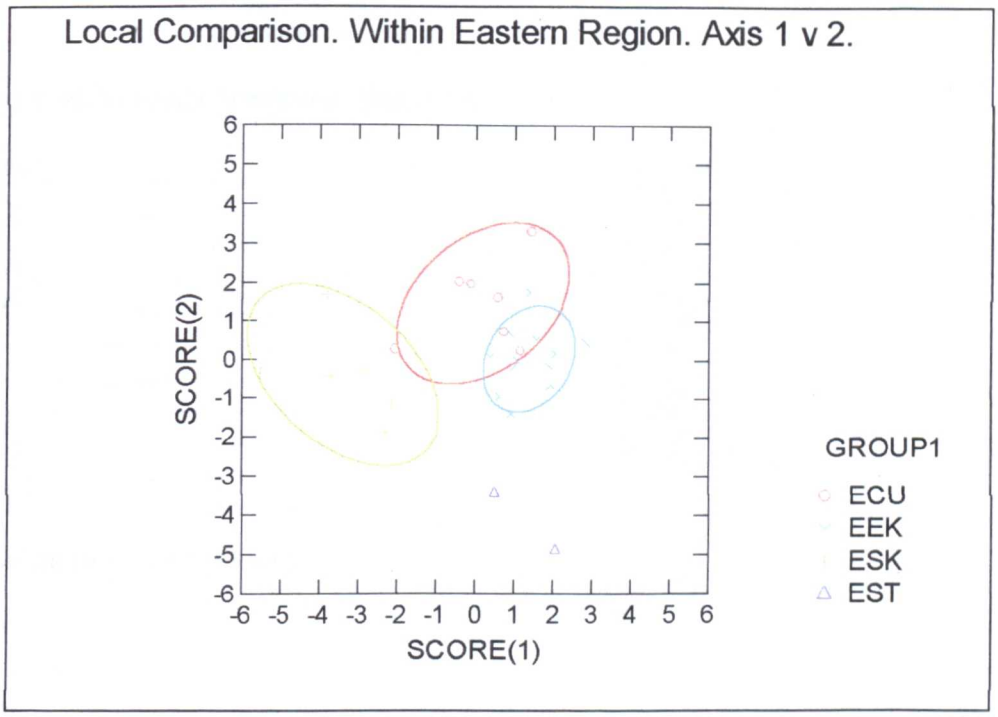
Canonical Discriminant Functions

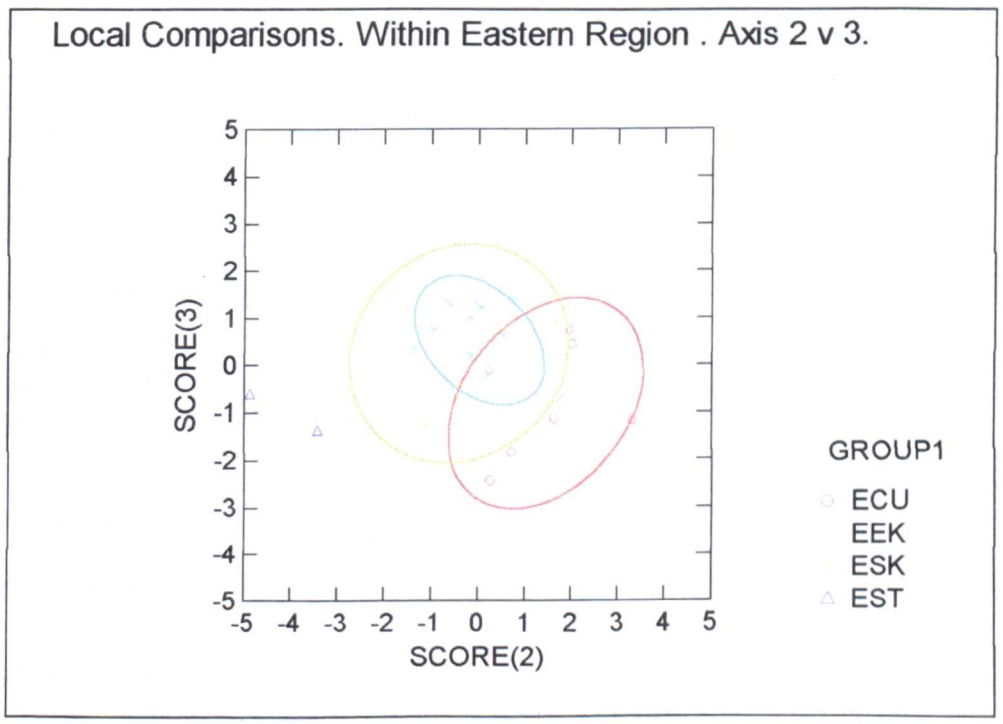
	1	*2*	*3*
Constant	-1.714	-0.785	-0.605
MMH	1.330	1.385	0.619
SWM	-1.239	-1.649	0.634
NOL	-1.858	0.592	-0.049
HSP	0.993	-1.931	-0.831
EWT	-1.321	1.613	0.986
MXTL	0.548	0.856	-1.588

Discriminant Function Data

	1	*2*	*3*
Eigenvalues	4.142	2.188	0.442
Canonical Correlations	0.898	0.828	0.554
Cumulative proportion of total dispersion	61.2%	93.5%	100%

Canonical Plots





Local Comparison: *Within Eastern Region: ECU versus EEK*

Group Frequencies: ECU 7
EEK 12

Classification Matrices:

Classification Matrix			
	ECU	**EEK**	**% Correct**
ECU	7	0	*100*
EEK	0	12	*100*
Total	*7*	*12*	*100*

Jack-knifed Classification Matrix			
	ECU	**EEK**	**% Correct**
ECU	6	1	*86*
EEK	1	11	*92*
Total	*7*	*12*	*89*

Support Statistics: Wilks' $\lambda = 0.0781$ $p = 0.002$
$C_{pro} = 0.535$
Cohen's $\kappa = 76.4\%$

Model Variables and Classification Functions:

	ECU	*EEK*
Constant	-11.502	-18.979
NOL	4.673	-21.026
EWT	12.795	-12.873
MMW	-10.877	10.133
MMH	8.276	23.416
BPH	12.533	-22.597
LAD	7.174	-10.676
HSP	-14.300	11.426
EWB	-12.179	9.382
MXTL	10.905	-8.898
SWM	-11.673	1.980

Canonical Discriminant Functions

	1
Constant	1.996
NOL	3.816
EWT	3.811
MMW	-3.119
MMH	-2.248
BPH	5.216
LAD	2.650
HSP	3.820
EWB	3.201
MXTL	2.940
SWM	-2.027

Discriminant Function Data:

	1
Eigenvalues	11.797
Canonical Correlations	0.960
Cumulative proportion of total dispersion	100%

Local Comparison: *Within Eastern Region. ESK versus EST*

Group Frequencies: ESK 6
EST 2

Classification Matrices:

Classification Matrix			
	ESK	**EST**	**% Correct**
ESK	6	0	*100*
EST	0	2	*100*
Total	*6*	*2*	*100*

Jack-knifed Classification Matrix			
	ESK	**EST**	**% Correct**
ESK	2	4	*33*
EST	0	2	*100*
Total	*2*	*6*	*50*

Support Statistics: Wilks' $\lambda = 0.2804$. $p = 0.0416$
$C_{pro} = 0.625$
Cohen's $\kappa = 1.00$

Model Variables and Classification Functions:

	ESK	*EST*
Constant	-0.812	-6.169
MMW	1.043	9.191
MMH	-0.897	-13.937

Canonical Discriminant Functions:

	1
Constant	-0.871
MMW	2.543
MMH	-3.110

Discriminant Function Data:

	1
Eigenvalues	2.566
Canonical Correlations	0.848
Cumulative proportion of total dispersion	100%

Local Comparison: *Within Eastern Region. [ECU and EEK] versus [ESK and EST]*

Group Frequencies: ECU / EEK 19
ESK / EST 8

Classification Matrices:

Classification Matrix			
	ECU/ EEK	**ESK/ EST**	**% Correct**
ECU/ EEK	19	0	*100*
ESK/ EST	0	8	*100*
Total	*19*	*8*	*100*

Jack-knifed Classification Matrix			
	ECU/ EEK	**ESK/ EST**	**% Correct**
ECU/ EEK	18	1	*95*
ESK/ EST	0	8	*100*
Total	*18*	*9*	*96*

Support Statistics: Wilks' $\lambda = 0.1931$. $p<0.001$.
$C_{pro} = 0.583$
Cohen's $\kappa = 0.907$

Model Variables and Classification Functions:

	ECU / EEK	*ESK / EST*
Constant	-6.479	-1.365
NOL	-3.959	0.428
MMH	8.822	-2.127
EWB	-3.696	0.918
MXTL	3.226	-1.593
SWM	-5.897	2.533

Canonical Discriminant Functions

	1
Constant	2.065
NOL	1.018
MMH	-2.542
EWB	1.071
MXTL	-1.119
SWM	1.957

Discriminant Function Data:

	1
Eigenvalues	4.178
Canonical Correlations	0.898
Cumulative proportion of total dispersion	100%

Local Comparison: *Within Western Region*

Group Frequencies: WCP 4
WSN 2

Classification Matrices:

Classification Matrix			
	WCP	WSN	% Correct
WCP	4	0	*100*
WSN	0	2	*100*
Total	*4*	*2*	*100*

Jack-knifed Classification Matrix			
	WCP	WSN	% Correct
WCP	2	2	*50*
WSN	2	0	*0*
Total	*4*	*2*	*33*

Support Statistics: Wilks' $\lambda = 0.1073$. $p = 0.1566$.
$C_{pro} = 0.556$.
Cohen's $\kappa = -0.5$.

Model Variables and Classification Functions:

	WCP	*WSN*
Constant	-30.585	-80.453
MOW	-127.614	-209.825
MMW	97.718	160.012
NOL	-38.186	-61.212

Canonical Discriminant Functions:

	1
Constant	9.151
MOW	16.459
MMW	-12.471
NOL	4.610

Discriminant Function Data:

	1
Eigenvalues	8.317
Canonical Correlations	0.945
Cumulative proportion of total dispersion	100%

Local Comparison: *Within Southern Region*

Group Frequencies: SCZ 15
SWC 15
SZT 4

Classification Matrices:

Classification Matrix				
	SCZ	SWC	SZT	% Correct
SCZ	13	2	0	*87*
SWC	3	12	0	*80*
SZT	0	0	4	*100*
Total	*16*	*14*	*4*	*85*

Jack-knifed Classification Matrix				
	SCZ	SWC	SZT	% Correct
SCZ	12	3	0	*80*
SWC	5	8	2	*53*
SZT	0	1	3	*75*
Total	*17*	*12*	*5*	*68*

Support Statistics: Wilks' $\lambda = 0.1668$. $p < 0.001$.
Cohen's $\kappa = 47.2\%$

Model Variables and Classification Functions:

	SCZ	*SWC*	*SZT*
Constant	-3.121	-4.559	-10.349
MMW	0.749	3.660	6.947
MMH	-6.496	-10.646	-13.859
LAD	0.248	-1.977	-2.189
HSP	-0.282	-2.544	-4.817
EWB	-0.181	2.490	2.622
MWO	1.524	0.573	-1.145
MXTL	0.824	1.426	4.210

Canonical Discriminant Functions:

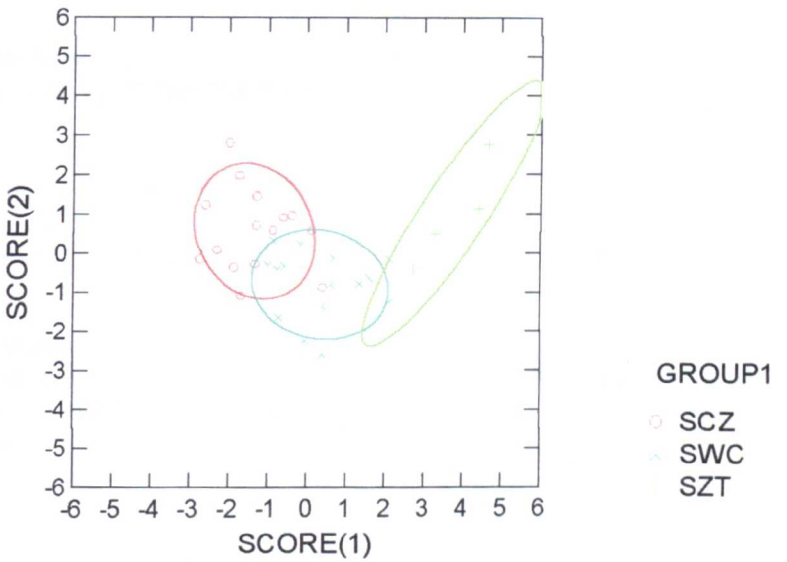
	1	*2*
Constant	-0.993	-0.214
MMW	1.580	0.135
MMH	-1.971	-0.715
LAD	-0.767	-0.919
HSP	-1.174	-0.205
EWB	0.898	1.147
MWO	-0.639	0.185
MXTL	0.729	-0.718

Discriminant Function Data:

	1	*2*
Eigenvalues	1.844	0.368
Canonical Correlations	0.805	0.519
Cumulative proportion of total dispersion	83.3%	100%

Canonical Plots:

Male. Within Southern Region Canonical Scores Plot.



Local Comparison: *SCZ versus SWC*

Group Frequencies: SCZ 15
SWC 15

Classification Matrices:

Classification Matrix			
	SCZ	**SWC**	**% Correct**
SCZ	13	2	*87*
SWC	3	12	*80*
Total	*16*	*14*	*83*

Jack-knifed Classification Matrix			
	SCZ	**SWC**	**% Correct**
SCZ	12	3	*80*
SWC	4	11	*73*
Total	*16*	*14*	*77*

Support Statistics: Wilks' $\lambda = 0.4887$. $p = 0.0068$
$C_{pro} = 0.500$
Cohen's $\kappa = 0.540$.

Model Variables and Classification Functions:

	SCZ	*SWC*
Constant	-1.923	-3.545
MMW	0.917	3.313
MMH	-3.736	-8.297
LAD	0.069	-1.796
HSP	0.035	-1.691
EWB	-0.016	2.878
EWT	0.532	-0.722

Canonical Discriminant Functions

	1
Constant	-0.821
MMW	1.212
MMH	-2.308
LAD	-0.944
HSP	-0.873
EWB	1.464
EWT	-0.635

Discriminant Function Data:

	1
Eigenvalues	1.046
Canonical Correlations	0.715
Cumulative proportion of total dispersion	100%

Local Comparison: *[SCZ and SWC] versus SZT*

Group Frequencies: SCZ and SWC 30
SZT 4

Classification Matrices:

Classification Matrix			
	SCZ/ SWC	**SZT**	**% Correct**
SCZ/ SWC	27	3	*90*
SZT	0	4	*100*
Total	*27*	*7*	*91*

Jack-knifed Classification Matrix			
	SCZ/ SWC	**SZT**	**% Correct**
SCZ/ SWC	27	3	*90*
SZT	0	4	*100*
Total	*27*	*7*	*91*

Support Statistics: Wilks' $\lambda = 0.5636$, $p = 0.0018$.
$C_{pro} = 0.792$.
Cohen's $\kappa = 67.3\%$.

Model Variables and Classification Functions:

	SCZ / SWC	*SZT*
Constant	-2.194	-7.914
MMW	1.983	4.891
MMH	-5.719	-11.127
HSP	-0.712	-3.179
MXTL	0.826	3.897

Canonical Discriminant Functions:

	1
Constant	-1.145
MMW	1.097
MMH	-2.041
HSP	-0.931
MXTL	1.159

Discriminant Function Data:

	1
Eigenvalues	0.774
Canonical Correlations	0.661
Cumulative proportion of total dispersion	100%

Between Regions, Local Comparison: *[ESK and EST] versus SZT*

Group Frequencies: ESK and EST 8
SZT 4

Classification Matrices:

Classification Matrix			
	ESK/ EST	**SZT**	**% Correct**
ESK/ EST	8	0	*100*
SZT	1	3	*75*
Total	*9*	*3*	*92*

Jack-knifed Classification Matrix			
	ESK/ EST	**SZT**	**% Correct**
ESK/ EST	7	1	*88*
SZT	1	3	*75*
Total	*8*	*4*	*83*

Support Statistics: Wilk's $\lambda = 0.2729$. $p = 0.0029$.
Cpro = 0.556.
Cohen's $\kappa = 61.8\%$

Model Variables and Classification Functions:

	ESK / EST	*SZT*
Constant	-0.781	-4.531
MOW	-0.986	6.636
MMH	0.659	-5.807

Canonical Discriminant Functions:

	1
Constant	-0.659
MOW	2.411
MMH	-2.045

Discriminant Function Data:

	1
Eigenvalues	2.665
Canonical Correlations	0.853
Cumulative proportion of total dispersion	100%

Between Regions, Local Comparison: *[ECU and EEK] versus [WCP and WSN]*

Group Frequencies: ECU and EEK 19
WCP and WSN 6

Classification Matrices:

Classification Matrix			
	ECU/ EEK	**WCP/WSN**	**% Correct**
ECU/ EEK	16	3	*84*
WCP/ WSN	0	6	*100*
Total	*16*	*9*	*88*

Jack-knifed Classification Matrix			
	ECU/ EEK	**WCP/WSN**	**% Correct**
ECU/ EEK	16	3	*84*
WCP/ WSN	1	5	*83*
Total	*17*	*8*	*84*

Support Statistics: Wilks' $\lambda = 0.5100$. $p = 0.0070$.
Cpro = 0.6352.
Cohen's $\kappa = 64.0\%$

Model Variables and Classification Functions:

	ECU / EEK	*WCP / WSN*
Constant	-2.302	-2.987
OOL	0.234	-4.254
MMH	4.502	1.004
BPH	-2.983	1.607
MXTL	-0.609	1.901

Canonical Discriminant Functions:

	1
Constant	-0.261
OOL	2.039
MMH	1.589
BPH	-2.085
MXTL	-1.140

Discriminant Function Data:

	1
Eigenvalues	0.961
Canonical Correlations	0.700
Cumulative proportion of total dispersion	100%

Alternative Analyses: 'Old' Male Skulls (>12 years)

Regional Comparison: *East versus West versus South*

Group Frequencies:

East	15
West	3
South	23

Classification Matrices:

Classification Matrix				
	East	South	West	% Correct
East	14	0	1	*93*
South	2	21	0	*91*
West	0	0	3	*100*
Total	*16*	*21*	*4*	*93*

Jack-knifed Classification Matrix				
	East	South	West	% Correct
East	14	0	1	*93*
South	2	21	0	*91*
West	0	0	3	*100*
Total	*16*	*21*	*4*	*93*

Support Statistics: Wilks' $\lambda = 0.2016$, $p < 0.001$.
Cohen's $\kappa = 87.6\%$

Model Variables and Classification Functions:

	East	*South*	*West*
Constant	-2.006	-3.101	-4.633
MOW	-1.883	4.094	-5.219
MMH	3.258	-5.082	5.504
HSP	-0.517	0.849	-1.488
MXTL	-0.220	-0.050	1.407

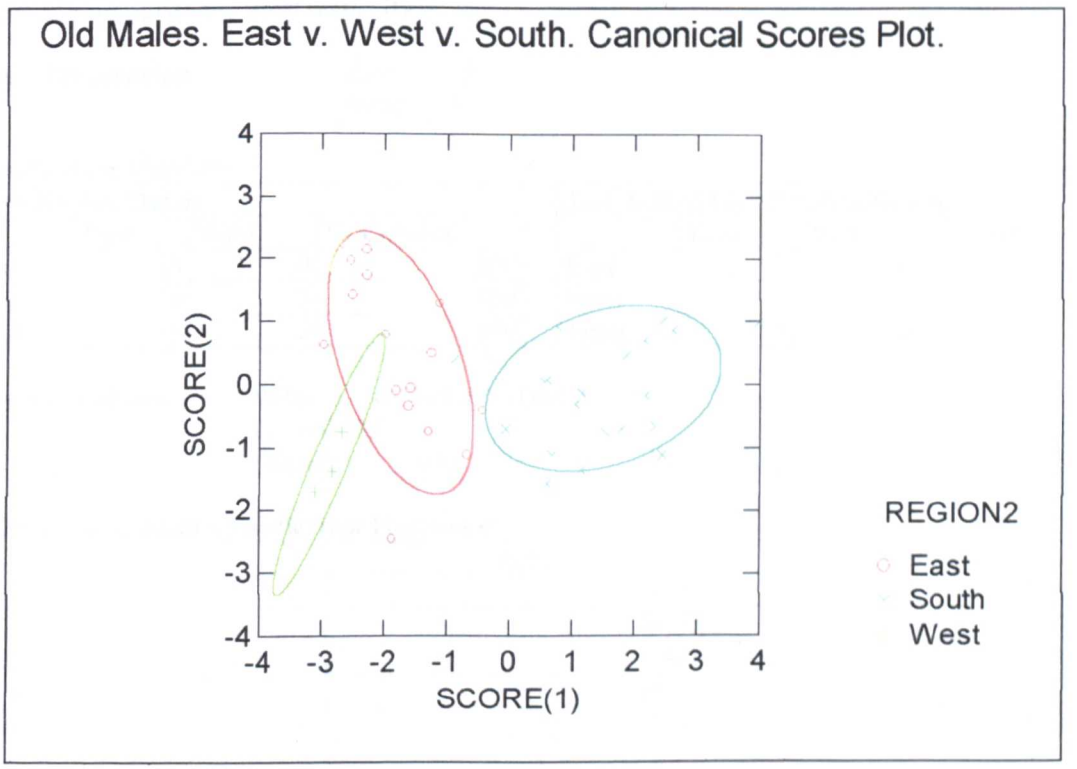
Canonical Discriminant Functions:

	1	*2*
Constant	-0.485	-0.117
MOW	1.932	0.706
MMH	-2.526	0.379
HSP	0.456	0.280
MXTL	-0.066	-0.956

Discriminant Function Data

	1	*2*
Eigenvalues	3.210	0.178
Canonical Correlations	0.873	0.389
Cumulative proportion of total dispersion	94.7%	100%

Canonical Plots:



Regional Comparison: *North versus South*

Group Frequencies: North 18
South 23

Classification Matrices:

Classification Matrix			
	North	**South**	**% Correct**
North	17	1	*94*
South	0	23	*100*
Total	*17*	*24*	*98*

Jack-knifed Classification Matrix			
	North	**South**	**% Correct**
North	17	1	*94*
South	0	23	*100*
Total	*17*	*24*	*98*

Support Statistics: Wilks' $\lambda = 0.2847$. $p < 0.001$.
$C_{pro} = 0.507$.
Cohen's $\kappa = 95.9\%$

Model Variables and Classification Functions:

	North	*South*
Constant	-1.584	-2.341
MOW	-2.280	3.783
MMH	3.444	-4.736

Canonical Discriminant Functions:

	1
Constant	-0.433
MOW	1.946
MMH	-2.626

Discriminant Function Data

	1
Eigenvalues	2.513
Canonical Correlations	0.846
Cumulative proportion of total dispersion	100%

Regional Comparison: *East versus West*

Group Frequencies:

East	15
West	3

Classification Matrices:

Classification Matrix			
	East	**West**	**% Correct**
East	15	0	*100*
West	0	3	*100*
Total	*15*	*3*	*100*

Jack-knifed Classification Matrix			
	East	**West**	**% Correct**
East	15	0	*100*
West	0	3	*100*
Total	*15*	*3*	*100*

Support Statistics: Wilks' $\lambda = 0.2099$. $p = 0.0002$.
$C_{pro} = 0.722$.
Cohen's $\kappa = 100\%$

Model Variables and Classification Functions:

	East	*West*
Constant	-0.815	-12.335
OOL	0.291	-13.179
MMW	0.489	10.641
EWT	-0.596	-11.630
MXTL	-0.247	8.832

Canonical Discriminant Functions:

	1
Constant	0.711
OOL	2.745
MMW	-2.068
EWT	2.248
MXTL	-1.850

Discriminant Function Data

	1
Eigenvalues	3.764
Canonical Correlations	0.889
Cumulative proportion of total dispersion	100%

Local Comparison: *Within the Eastern Region*

Group Frequencies:

ECU	4
EEK	5
ESK	6

Classification Matrices:

Classification Matrix				
	ECU	**EEK**	**ESK**	**%Correct**
ECU	3	1	0	*75*
EEK	0	5	0	*100*
ESK	1	0	5	*83*
Total	*4*	*6*	*5*	*87*
Jack-knifed Classification Matrix				
	ECU	**EEK**	**ESK**	**%Correct**
ECU	2	2	0	*50*
EEK	2	3	0	*60*
ESK	1	0	5	*83*
Total	*5*	*5*	*5*	*67*

Support Statistics: Wilks' $\lambda = 0.0871$. $p = 0.0354$.
Cohen's $\kappa = 50.5\%$

Model Variables and Classification Functions:

	ECU	*EEK*	*ESK*
Constant	-6.576	-10.406	-2.737
NOL	-7.322	-11.032	4.241
OOL	7.580	8.459	-2.017
MMH	19.304	22.934	-4.258
BPH	-19.337	-22.102	5.309
HSP	7.545	9.074	-6.654
EWT	-6.812	-9.221	4.297

Canonical Discriminant Functions:

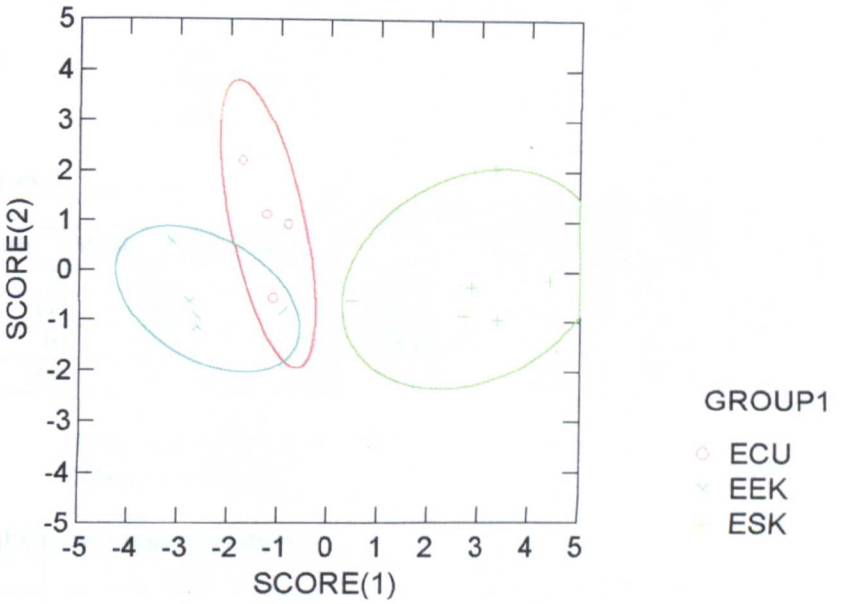
	1	*2*
Constant	1.637	-0.022
NOL	2.886	0.179
OOL	-2.077	1.060
MMH	-5.313	1.793
BPH	5.408	-2.443
HSP	-3.106	1.442
EWT	2.606	-0.462

Discriminant Function Data

	1	*2*
Eigenvalues	6.990	0.437
Canonical Correlations	0.935	0.551
Cumulative proportion of total dispersion	94.1%	100%

Canonical Plots

Old Males. Within East Comparison. Canonical Scores Plot.



Local Comparison: *Within Southern Region. SCZ versus SWC*

Group Frequencies: SCZ 11
SWC 11

Classification Matrices:

Classification Matrix			
	SCZ	**SWC**	**% Correct**
SCZ	9	2	*82*
SWC	3	8	*73*
Total	*12*	*10*	*77*

Jack-knifed Classification Matrix			
	SCZ	**SWC**	**% Correct**
SCZ	9	2	*82*
SWC	4	7	*64*
Total	*13*	*9*	*73*

Support Statistics: Wilks' $\lambda = 0.5748$. $p = 0.0417$.
$C_{pro} = 0.500$.
Cohen's $\kappa = 46.0\%$

Model Variables and Classification Functions:

	SCZ	*SWC*
Constant	-1.249	-1.047
NOL	1.246	-0.144
MMW	-1.813	1.015
EWB	-0.645	1.154
MWO	0.745	-0.740

Canonical Discriminant Functions:

	1
Constant	-0.123
NOL	0.847
MMW	-1.724
EWB	-1.097
MWO	0.905

Discriminant Function Data:

	1
Eigenvalues	0.740
Canonical Correlations	0.652
Cumulative proportion of total dispersion	100%

Male Skull without EEK Group Analysis

Regional Comparison: *East versus West versus South*

Group Frequencies:

East	16
West	36
South	6

Classification Matrices:

Classification Matrix				
	East	South	West	% Correct
East	12	1	3	*75*
South	2	34	0	*94*
West	1	0	5	*83*
Total	*15*	*35*	*8*	*88*

Jack-knifed Classification Matrix				
	East	South	West	% Correct
East	11	2	3	*69*
South	2	34	0	*94*
West	1	0	5	*83*
Total	*14*	*36*	*8*	*86*

Support Statistics: Wilks' $\lambda = 0.3304$. $p < 0.0001$.
Cohen's $\kappa = 73.8\%$

Model Variables and Classification Functions:

	East	*South*	*West*
Constant	-2.290	-1.646	-3.619
MOW	-2.261	1.645	-3.840
MMH	3.121	-2.097	4.259

Canonical Discriminant Function:

	1	*2*
Constant	0.000	0.000
MOW	1.586	0.786
MMH	-1.998	0.310

Discriminant Function Data

	1	*2*
Eigenvalues	1.926	0.035
Canonical Correlations	0.811	0.183
Cumulative proportion of total dispersion	98.2%	100%

Comments:
A classification with the addition of **NOL**, **OOL**, **HSP** and **MXTL** gave a slightly better classification function with 90% of specimen correctly allocated to their regional set (Wilks' $\lambda = 0.2098$). The jack-knifed classification matrix was identical in its classification efficiency. The difference being that three eastern skulls were assigned to the southern set and two to the western set (rather than the other way around).

Regional Comparison: *North versus South*

Group Frequencies:

North	22
South	36

Classification Matrices:

Classification Matrix			
	North	South	% Correct
North	20	2	*91*
South	2	34	*94*
Total	*22*	*36*	*93*

Jack-knifed Classification Matrix			
	North	South	% Correct
North	20	2	*91*
South	2	34	*94*
Total	*22*	*36*	*93*

Support Statistics: Wilks' $\lambda = 0.3534$. $p < 0.0001$.
$C_{pro} = 0.529$
Cohen's $\kappa = 85.1\%$

Model Variables and Classification Functions:

	North	*South*
Constant	-2.139	-1.233
MOW	-2.606	1.592
MMH	3.397	-2.076

Canonical Discriminant Function:

	1
Constant	0.000
MOW	1.533
MMH	-1.998

Discriminant Function Data

	1
Eigenvalues	1.830
Canonical Correlations	0.804
Cumulative proportion of total dispersion	100%

Comments:
Classification functions (adding **HSP** for three variables and **NOL** for four variables) gave the same overall classification efficiency. However, in each case the two remaining variables (**MOW** and **MMH**) dominated the classification function.

Regional Comparison: *East versus West*

Group Frequencies: East 16
West 6

Classification Matrices:

Classification Matrix			
	East	**West**	**% Correct**
East	15	1	*94*
West	1	5	*83*
Total	*16*	*6*	*91*

Jack-knifed Classification Matrix			
	East	**West**	**% Correct**
East	14	2	*88*
West	1	5	*83*
Total	*15*	*7*	*86*

Support Statistics: Wilks' $\lambda = 0.3722$.
$C_{pro} = 0.603$
Cohen's $\kappa = 66.5\%$

Model Variables and Classification Functions:

	East	*West*
Constant	-0.734	-2.758
OOL	0.435	-3.116
BPH	-0.229	2.096

Canonical Discriminant Functions:

	1
Constant	0.350
OOL	1.536
BPH	-1.006

Discriminant Function Data

	1
Eigenvalues	1.166
Canonical Correlations	0.734
Cumulative proportion of total dispersion	100%

Comments:
Addition of **MXTL** and **HSP** into the classification function allows all of the western specimens to be classified to the correct set in the jack-knifed classification matrix. However, the overall efficiency of the

classification remains the same as an additional eastern specimen is misclassified with the addition of these parameters.

Local Comparison: *Within the Eastern Region*

Group Frequencies:

ECU	7
ESK	6
EST	2

Classification Matrices:

Classification Matrix				
	ECU	**ESK**	**EST**	**%Correct**
ECU	6	1	0	*86*
ESK	0	6	0	*100*
EST	0	0	2	*100*
Total	*6*	*7*	*2*	*93*
Jack-knifed Classification Matrix				
	ECU	**ESK**	**EST**	**%Correct**
ECU	6	1	0	*86*
ESK	2	4	0	*67*
EST	1	0	1	*50*
Total	*9*	*5*	*1*	*73*

Support Statistics: Wilks' $\lambda = 0.0623$. $p = 0.0027$.
Cohen's $\kappa = 53.3\%$

Model Variables and Classification Functions:

	ECU	*ESK*	*EST*
Constant	-5.304	-2.471	-11.160
NOL	-2.306	1.628	-7.081
MMW	-4.505	-0.816	3.993
MMH	5.849	1.849	-2.150
HSP	-0.808	-4.218	7.047
EWT	1.089	2.387	-8.195

Canonical Discriminant Functions:

	1	*2*
Constant	-0.524	1.140
NOL	1.206	1.435
MMW	-1.242	1.348
MMH	1.125	-1.461
HSP	-1.725	-1.244
EWT	1.808	0.473

Discriminant Function Data

	1	*2*
Eigenvalues	4.320	2.019
Canonical Correlations	0.901	0.818
Cumulative proportion of total dispersion	68.1%	100%

Female Skull Analysis

The (relatively) delicate nature of the female skull meant that damage, resulting in missing values, occurred more frequently than in the male analyses. Also fewer female skulls were sampled. Two parallel analyses were carried out. Firstly only skulls with a complete complement of measured skull variables were included in the manual discriminant analysis. From this a classification function was derived. The set of skulls that had each of the selected variables available was then analysed to provide a larger sample size to test the efficacy of the classification function.
The results are presented together. The results of the second (larger sample size) analysis are given in square brackets following the first set of results.

Regional Comparison: *North versus South*

Group Frequencies:

North	13	[23]
South	11	[14]

Classification Matrices:

Classification Matrix			
	North	South	% Correct
North	12 [20]	1 [3]	*92 [87]*
South	1 [1]	10 [13]	*91 [93]*
Total	*13 [21]*	*11 [16]*	*92 [89]*

Jack-knifed Classification Matrix			
	North	South	% Correct
North	11 [20]	2 [3]	*85 [87]*
South	1 [2]	10 [12]	*91 [86]*
Total	*12 [22]*	*12 [15]*	*88 [86]*

Support Statistics:

Wilks' $\lambda = 0.4412$. $p = 0.0008$.	[Wilks' $\lambda = 0.4656$. $p < 0.0001$.]
$C_{pro} = 0.503$.	[0.530]
Cohen's $\kappa = 76.0\%$	[70.6%]

Model Variables and Classification Functions:

	North	*South*
Constant	-1.184 [-0.957]	-1.379 [-1.709]
BPH	1.306 [0.795]	-1.543 [-1.503]
LAD	0.853 [0.391]	-1.008 [-0.671]
EWB	-1.422 [-1.100]	1.680 [2.232]

Canonical Discriminant Functions:

	1
Constant	0.000 [0.089]
BPH	1.318 [1.070]
LAD	0.860 [0.494]
EWB	-1.434 [-1.551]

Discriminant Function Data

	1
Eigenvalues	1.267 [1.148]
Canonical Correlations	0.748 [0.731]
Cumulative proportion of total dispersion	100% [100%]

Local Comparison: *Within the Eastern Region*

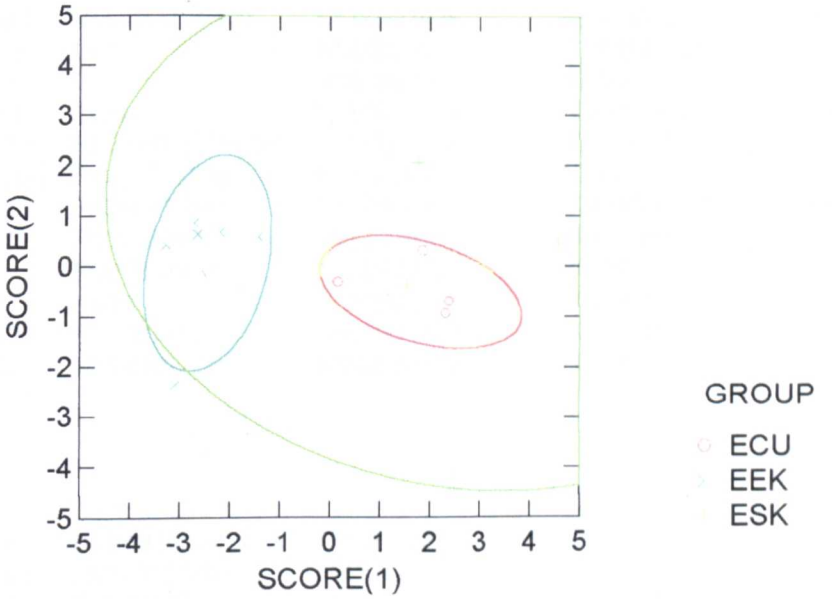
Group Frequencies:

ECU	4	[5]
EEK	3	[7]
ESK	3	[3]

Classification Matrices:

Classification Matrix				
	ECU	**EEK**	**ESK**	**%Correct**
ECU	3 [5]	0 [0]	1 [0]	*75 [100]*
EEK	0 [0]	3 [7]	0 [0]	*100 [100]*
ESK	1 [1]	0 [0]	2 [2]	*67 [67]*
Total	*4 [4]*	*3 [3]*	*3 [3]*	*80 [93]*
Jack-knifed Classification Matrix				

Females. Within Eastern Region Comparison.



	ECU	**EEK**	**ESK**	**%Correct**
ECU	3 [4]	0 [0]	1 [1]	*75 [80]*
EEK	0 [0]	3 [7]	0 [0]	*100 [100]*
ESK	1 [2]	0 [0]	2 [1]	*67 [33]*
Total	*4 [4]*	*3 [7]*	*3 [3]*	*80 80]*

Support Statistics: Wilks' $\lambda = 0.1336$. $p = 0.0115$. [Wilks' $\lambda = 0.1064$ $p < 0.0.0001$.]
Cohen's $\kappa = 71.9\%$ [67.9%]

Model Variables and Classification Functions:

	ECU	*EEK*	*ESK*
Constant	-1.370 [-1.767]	-5.519 [-6.455]	-1.990 [-2.898]
MOW	-0.187 [2.214]	-5.905 [-7.824]	2.436 [4.410]
BPH	0.893 [-0.280]	2.887 [3.094]	-2.022 [-2.313]

Canonical Discriminant Functions:

	1	*2*
Constant	0.554 [0.824]	0.017 [0.099]
MOW	2.008 [2.388]	0.167 [0.207]
BPH	-1.077 [0.933]	-1.166 [-1.033]

Discriminant Function Data

	1	*2*
Eigenvalues	3.896 [6.565]	0.528 [0.242]
Canonical Correlations	0.892 [0.932]	0.588 [0.441]
Cumulative proportion of total dispersion	88.1% [96.4%]	100% [100%]

Canonical Plots: The canonical scores plot presented is from the second, larger sample size, analysis.

APPENDIX 4.10.1:

SPECIMENS USED IN ANALYSIS OF SHAPE VARIATION.

Profiles.

Males.

AMNH27752	BMNH1964.225	NMZB20382	NMZB29099	PCNNChad138
AMNH54123	BMNH1986.1604	NMZB22862	NMZB29100	PCTAN76
BMNH1897.1.30.1	BMNHALButler	NMZB23978	NMZB29102	TM12141
BMNH1898.7.2.5	EEI3	NMZB23979	NMZB29111	USNM121010
BMNH1899.7.8.5	EEI5	NMZB23983	NMZB29113	USNM155438
BMNH1901.8.9.47	EEI8	NMZB23984	NMZB58342	USNM162017
BMNH1904.11.2.2	EEIGc94.11.25.6MA	NMZB26178	NMZB60801	USNM163312
BMNH1906.10.26.1	FMNH127878	NMZB26180	NMZB60802	USNM182124
BMNH1906.2.12.2	FMNH127881	NMZB26185	PCCAMII139	USNM182192
BMNH1923.10.20.8	FMNH34422	NMZB26186	PCCongoNoNum2	USNM200151
BMNH1931.2.1.48	FMNH34930	NMZB26187	PCJ39	USNM296145
BMNH1933.4.2.2	KNP7	NMZB26188	PCMN278	ZM17176
BMNH1938.7.8.22	NMZB11525	NMZB26189	PCMN280	
BMNH1962.220	NMZB20221	NMZB26200	PCMN579	

Females.

BMNH1873.8.29.7	BMNH1939.4329A	FMNH34423	NMZB60805	USNM162988
BMNH1898.2.18.1	FMNH127880	FMNH34424	NMZB60809	USNM251798
BMNH1898.7.2.6	FMNH127885	FMNH34429	PCMN275	
BMNH1900.3.18.3	FMNH127886	NMZB26184	PCMN277	
BMNH1901.8.9.50	FMNH32901	NMZB60803	PCSWA9	
BMNH1912.2.24.1	FMNH32902	NMZB60804	PCTAN89	

Parietal Horns.

Males.

AMNH24291	BMNH1931.2.1.48	KNP7	NMZB27144	PCNNChad138
AMNH24292	BMNH1933.4.2.2	KNP8	NMZB29099	PCTAN102
AMNH24293	BMNH1938.7.8.22	NMZB11525	NMZB29100	PCTAN76
AMNH27752	BMNH1962.220	NMZB20221	NMZB29102	USNM121010
AMNH53550	BMNH1986.1604	NMZB20382	NMZB29111	USNM155438
AMNH54123	BMNHALButler	NMZB22862	NMZB29112	USNM162016
AMNH82001	EEI3	NMZB23978	NMZB29113	USNM162017
AMNH83460	EEI5	NMZB23979	NMZB58342	USNM162018
BMNH1842.2.6.16	EEI8	NMZB23983	NMZB60800	USNM163113
BMNH1897.1.30.1	FMNH127878	NMZB23984	NMZB60801	USNM163312
BMNH1898.7.2.5	FMNH127881	NMZB26180	NMZB60802	USNM182125
BMNH1899.7.8.5	FMNH127882	NMZB26185	PCCAMII139	USNM182192
BMNH1901.8.9.47	FMNH127883	NMZB26186	PCCongoNoNum2	USNM200151
BMNH1903.11.17.1	FMNH34422	NMZB26187	PCMN276	USNM296145
BMNH1904.11.2.2	FMNH34425	NMZB26188	PCMN278	ZM17176
BMNH1906.10.26.1	FMNH34930	NMZB26189	PCMN280	
BMNH1906.2.12.2	KNP6	NMZB26200	PCMN579	

Females.

AMNH53543	BMNH1902.11.12.1	FMNH127880	NMZB11533*	PCSWA9
AMNH53546	BMNH1903.4.16.1	FMNH127885	NMZB26184	PCTAN89
AMNH53549	BMNH1912.2.24.1	FMNH127886	NMZB60803	USNM162988
BMNH1873.8.29.7	BMNH1912.2.24.2	FMNH32901	NMZB60804	USNM163324
BMNH1898.2.18.1	BMNH1931.2.1.49	FMNH32902	NMZB60805	USNM251800
BMNH1901.5.14.1*	BMNH1939.4329A	FMNH34423	NMZB60809	
BMNH1901.8.9.49	EEI7	FMNH34424	PCMN275	
BMNH1901.8.9.50	EEI9	FMNH34429	PCMN277	

* Note that neither BMNH1901.5.14.1 nor NMZB11533 were included in the gender assignment analysis. However, by inspection, both are clearly adult females and will be included in this analysis to increase sample size.

Face and Muzzle.

Males.

AMNH24290	BMNH1938.7.8.22	NMZB20221	NMZB26200	PCMN280
AMNH27752	BMNH1962.220	NMZB20382	NMZB29099	PCMN579
BMNH1897.1.30.1	BMNH1964.225	NMZB22862	NMZB29100	PCTAN76
BMNH1898.7.2.5	BMNH1986.1604	NMZB23978	NMZB29102	USNM121010
BMNH1899.7.8.5	EEI1	NMZB23979	NMZB29111	USNM155438
BMNH1901.8.9.47	EEI3	NMZB23983	NMZB29112	USNM182124
BMNH1903.11.17.1	EEI4	NMZB23984	NMZB29113	USNM182192
BMNH1904.11.2.2	EEI5	NMZB26180	NMZB58342	USNM200151
BMNH1906.10.26.1	EEI8	NMZB26185	NMZB60801	USNM296145
BMNH1906.2.12.2	EEIGc94.11.25.6MA	NMZB26186	NMZB60802	
BMNH1923.10.20.8	FMNH127878	NMZB26187	PCCongoNoNum1	
BMNH1931.2.1.48	FMNH127881	NMZB26188	PCCongoNoNum2	
BMNH1933.4.2.2	FMNH34930	NMZB26189	PCMN278	

Females.

BMNH1873.8.29.7	EEI9	FMNH34424	NMZB60809	USNM162988
BMNH1898.2.18.1	FMNH127880	FMNH34429	NMZB60810*	USNM251797
BMNH1900.3.18.3	FMNH127886	NMZB26184	PCMN275	USNM251798
BMNH1903.4.16.1	FMNH32901	NMZB60803	PCSWA9	USNM251800
BMNH1939.4329A	FMNH34423	NMZB60805	PCTAN89	

* Note that NMZB60810 was not included in the gender assignment analysis. However, by inspection, it is clearly the skull of an adult female and will be included in this analysis to increase sample size.

APPENDIX 4.10.2:

SHAPE ANALYSIS RESULTS.

The results presented describe the modelled shape variation first and then examine the geographic structure of the eigenscores with respect to the geographic grouping of the skull specimens.
Comparisons testing the null hypotheses that there is no geographically structured variation to the eigenscores (covariation coefficients of each specimen with the modelled eigenshape shape trends) were made. Test statistics for non-parametric Mann-Whitney U-tests (for paired comparisons) and Kruskal-Wallis tests (for multigroup comparisons) are presented where these were statistically significant and the null hypothesis rejected, inferring geographic structure to the eigenscores.

Male Skull Variation

Complete Skull Outline

General Shape Variation: All Male Data

Number of objects: 68
Number of input co-ordinates: 400
Number of interpolated points: 132
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 5, 7, 9, 25, 31, 24, 31.
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	275.061	98.140	98.140
2	1.076	0.384	98.524
3	0.591	0.211	98.735
4	0.462	0.165	98.900
5	0.372	0.133	99.033

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
North v South	ES2	Mann-Whitney	52.500	1	<0.001
North v South	ES5	Mann-Whitney	272.500	1	0.002

Within Regions Variation: Northern Region

Number of objects: 29
Number of input co-ordinates: 400
Number of interpolated points: 118
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 3, 6, 9, 14, 31, 24, 31.
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	117.190	98.260	98.260
2	0.443	0.372	98.631
3	0.285	0.239	98.870
4	0.201	0.168	99.038
5	0.150	0.126	99.164

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Eastern Groups	ES2	Kruskal-Wallis	13.838	4	0.008

Within Regions Variation: Southern Region

Number of objects: 35
Number of input co-ordinates: 400
Number of interpolated points: 111
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 5, 7, 9, 25, 14, 23, 28.

Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	155.010	98.554	98.554
2	0.447	0.284	98.838
3	0.427	0.271	99.109
4	0.180	0.114	99.224
5	0.166	0.106	99.329

Variation in the Median Horn Only

The segment describing the profile of the median horn was analysed separately. The 26 points interpolated for the analysis of all specimens were extracted from the data and analysed using standard eigenshape analysis for all data and for northern and southern regions separately.

General Shape Variation: All Male Data

Number of objects: 68
Number of input co-ordinates: 26
Number of output co-ordinates: 20
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	142.898	97.391	97.391
2	1.947	1.327	98.718
3	0.866	0.590	99.308
4	0.193	0.131	99.440
5	0.181	0.123	99.563

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
North v South	ES1	Mann-Whitney	1010.00	1	<0.001
North v South	ES2	Mann-Whitney	26.500	1	<0.001

Within Regions Variation: Northern Region

Number of objects: 29
Number of input co-ordinates: 26
Number of output co-ordinates: 20
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	65.389	97.815	97.815
2	0.636	0.952	98.767
3	0.400	0.598	99.365
4	0.126	0.188	99.553
5	0.076	0.114	99.667

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Northern Groups	ES1	Kruskal-Wallis	17.821	6	0.007
Northern Groups	ES3	Kruskal-Wallis	19.257	6	0.004
Northern Groups	ES4	Kruskal-Wallis	13.350	6	0.038
Eastern Groups	ES1	Kruskal-Wallis	14.732	4	0.005
Eastern Groups	ES3	Kruskal-Wallis	15.479	4	0.004
Eastern Groups	ES4	Kruskal-Wallis	10.318	4	0.035

Within Regions Variation: Southern Region

Number of objects: 35
Number of input co-ordinates: 26
Number of output co-ordinates: 20
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	70.279	98.813	98.813
2	0.283	0.397	99.210
3	0.125	0.176	99.385
4	0.104	0.146	99.531
5	0.082	0.115	99.646

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Southern Groups	ES3	Kruskal-Wallis	11.092	4	0.026

Parietal Horn Variation

General Shape Variation: All Male Data

Number of objects: 83
Number of input co-ordinates: 200
Number of interpolated points: 135
Number of internal landmarks: 1
Tolerance criterion: 98%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	144.261	98.613	98.613
2	0.752	0.514	99.127
3	0.224	0.153	99.280
4	0.186	0.127	99.407
5	0.168	0.115	99.522

Within Region Variation: Northern Region

Number of objects: 34
Number of input co-ordinates: 200
Number of interpolated points: 142
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	58.847	97.742	97.742
2	0.380	0.632	98.374
3	0.184	0.306	98.680
4	0.097	0.161	98.841
5	0.091	0.151	98.992

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Northern Groups	ES5	Kruskal-Wallis	13.048	6	0.042
Eastern Groups	ES5	Kruskal-Wallis	9.807	4	0.044

Within Regions Variation: Southern Region

Number of objects: 40
Number of input co-ordinates: 200
Number of interpolated points: 174
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	144.658	97.891	97.891
2	0.827	0.560	98.451
3	0.287	0.195	98.646
4	0.214	0.145	98.790
5	0.199	0.135	98.925

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Southern Groups	ES1	Kruskal-Wallis	16.058	4	0.003
Southern Groups	ES2	Kruskal-Wallis	14.170	4	0.007

Face and Muzzle Shape Variation

General Shape Variation: All Male Data

Number of objects: 61
Number of input co-ordinates: 200
Number of interpolated points: 110
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	99.677	99.357	99.357
2	0.110	0.112	99.469
3	0.073	0.074	99.543
4	0.040	0.041	99.584
5	0.036	0.036	99.620

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
North v South	ES3	Mann-Whitney	638.000	1	0.001

Within Regions Variation: Northern Region

Number of objects: 24
Number of input co-ordinates: 200
Number of interpolated points: 59
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	36.790	99.536	99.536
2	0.045	0.122	99.659
3	0.023	0.063	99.722
4	0.016	0.044	99.766
5	0.015	0.040	99.805

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
East v West	ES1	Mann-Whitney	8.000	1	0.040
Northern Groups	ES1	Kruskal-Wallis	15.759	6	0.015
Northern Groups	ES3	Kruskal-Wallis	16.838	6	0.010
Northern Groups	ES5	Kruskal-Wallis	13.660	6	0.034
Eastern Groups	ES1	Kruskal-Wallis	12.443	4	0.014
Eastern Groups	ES3	Kruskal_Wallis	13.378	4	0.010
Eastern Groups	ES5	Kruskal-Wallis	12.633	4	0.013

Within Regions Variation: Southern Region

Number of objects: 35
Number of input co-ordinates: 200
Number of interpolated points: 110
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	56.199	99.348	99.348
2	0.076	0.134	99.482
3	0.038	0.067	99.549
4	0.030	0.054	99.603
5	0.024	0.043	99.646

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
Southern Groups	ES3	Kruskal-Wallis	11.317	4	0.023

Female Skull Variation

Complete Skull Outline

General Shape Variation: All Female Data

Number of objects: 25
Number of input co-ordinates: 400
Number of interpolated points: 65
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 3, 5, 6, 4, 13, 17, 17.
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	109.523	93.008	93.008
2	5.898	5.009	98.017
3	1.280	1.087	99.104
4	0.280	0.238	99.342
5	0.193	0.164	99.506

Within Regions Variation: Northern Region

Number of objects: 15
Number of input co-ordinates: 400
Number of interpolated points: 60
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 3, 2, 6, 4, 11, 17, 17.
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	68.632	98.607	98.607
2	0.308	0.443	99.049
3	0.184	0.265	99.314
4	0.124	0.178	99.492
5	0.110	0.157	99.649

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
East v West	ES2	Mann-Whitney	26.000	1	0.027

Within Regions Variation: Southern Region

Number of objects: 10
Number of input co-ordinates: 400
Number of interpolated points: 60
Number of internal landmarks: 6
Tolerance criterion: 95%
Points per line segment: 3, 5, 6, 2, 13, 15, 16.
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	46.400	99.199	99.199
2	0.114	0.244	99.443
3	0.079	0.170	99.612
4	0.048	0.103	99.715
5	0.042	0.089	99.805

Parietal Horn Variation

General Shape Variation: All Female Data

Number of objects: 37
Number of input co-ordinates: 200
Number of interpolated points: 105
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:

Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	55.380	97.427	97.427
2	0.807	1.420	98.846
3	0.139	0.244	99.091
4	0.115	0.202	99.293
5	0.084	0.147	99.440

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
North v South	ES2	Mann-Whitney	257.000	1	0.001
North v South	ES4	Mann-Whitney	92.000	1	0.044

Within Region Variation: Northern Region

Number of objects: 10
Number of input co-ordinates: 200
Number of interpolated points: 86
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	32.803	97.490	97.490
2	0.483	1.437	98.926
3	0.101	0.301	99.227
4	0.068	0.201	99.428
5	0.063	0.186	99.615

Within Regions Variation: Southern Region

Number of objects: 14
Number of input co-ordinates: 200
Number of interpolated points: 105
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	21.332	98.508	98.508
2	0.141	0.650	99.158
3	0.045	0.208	99.366
4	0.040	0.187	99.553
5	0.031	0.145	99.698

Face and Muzzle Shape Variation

General Shape Variation: All Female Data

Number of objects: 24
Number of input co-ordinates: 200
Number of interpolated points: 97
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	39.591	99.422	99.442
2	0.053	0.133	99.575
3	0.031	0.077	99.652
4	0.022	0.055	99.707
5	0.018	0.046	99.753

Statistics:

Comparison	Eigenshape	Test	Statistic	df	p
North v South	ES3	Mann-Whitney	94.00	1	0.025
North v South	ES4	Mann-Whitney	20.500	1	0.009

Within Regions Variation: Northern Region

Number of objects: 12
Number of input co-ordinates: 200
Number of interpolated points: 74
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	19.880	99.544	99.544
2	0.030	0.151	99.695
3	0.019	0.096	99.792
4	0.009	0.045	99.837
5	0.008	0.040	99.876

Within Regions Variation: Southern Region

Number of objects: 10
Number of input co-ordinates: 200
Number of interpolated points: 75
Number of internal landmarks: 1
Tolerance criterion: 99%
Points per line segment:
Eigenvalue Data:

Eigenvector	Eigenvalue	% of Total Variance	Cumulative Variance %
1	15.202	99.553	99.533
2	0.022	0.141	99.694
3	0.012	0.079	99.773
4	0.009	0.058	99.831
5	0.008	0.054	99.885

APPENDIX 4.10.3:

EIGENSCORE PLOTS FOR SHAPE ANALYSIS

This Appendix presents plots of eigenscore values for each specimen, for each analysis. The eigenscore value represents the covariation of the specimen shape with the eigenshapes generated from each analysis.

Specimen sets are coded according to their local or regional geographic provenance.

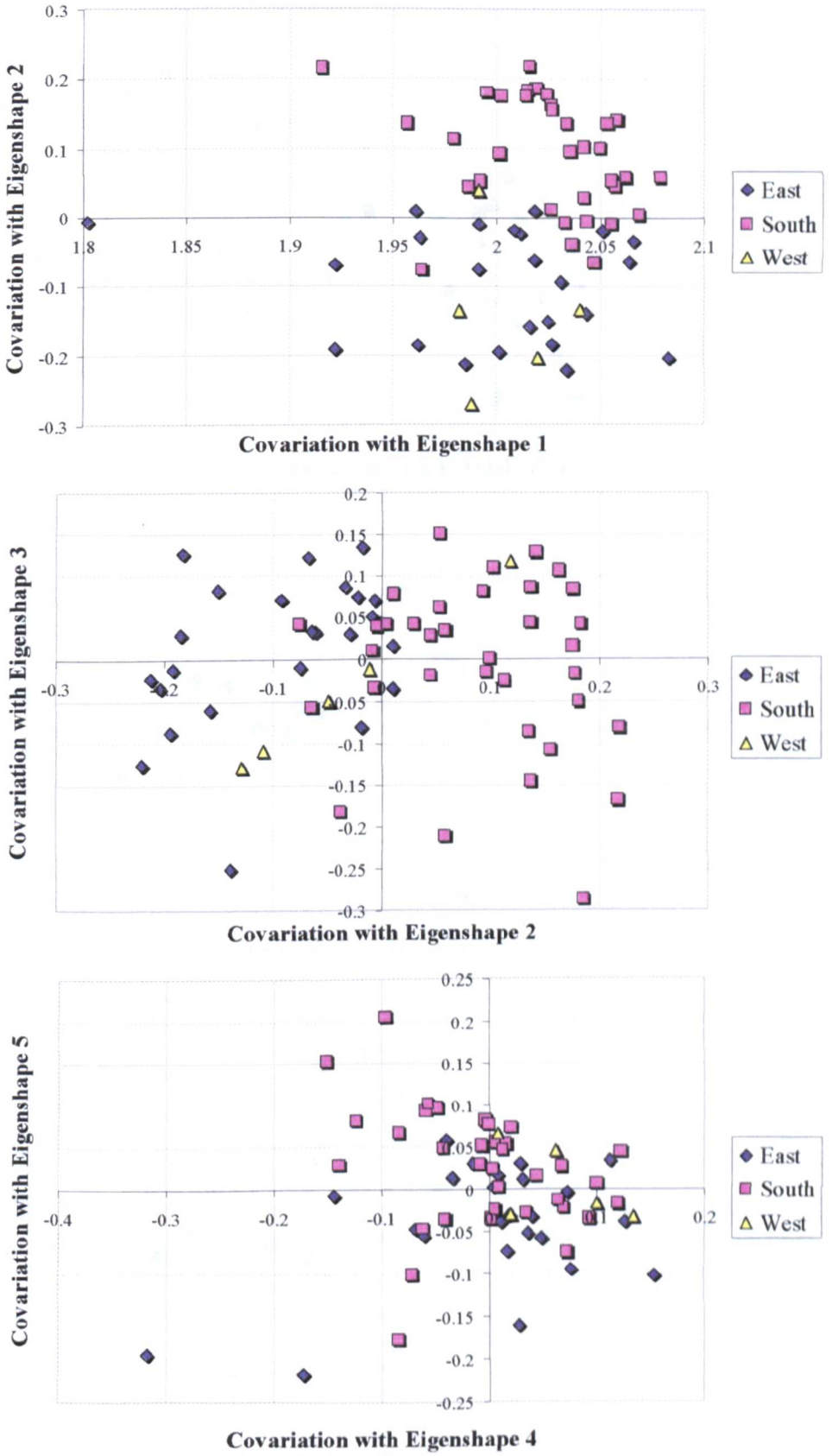


Figure A4.10.3.1: *General skull profile shape variation: Regional comparisons between all male skulls.*

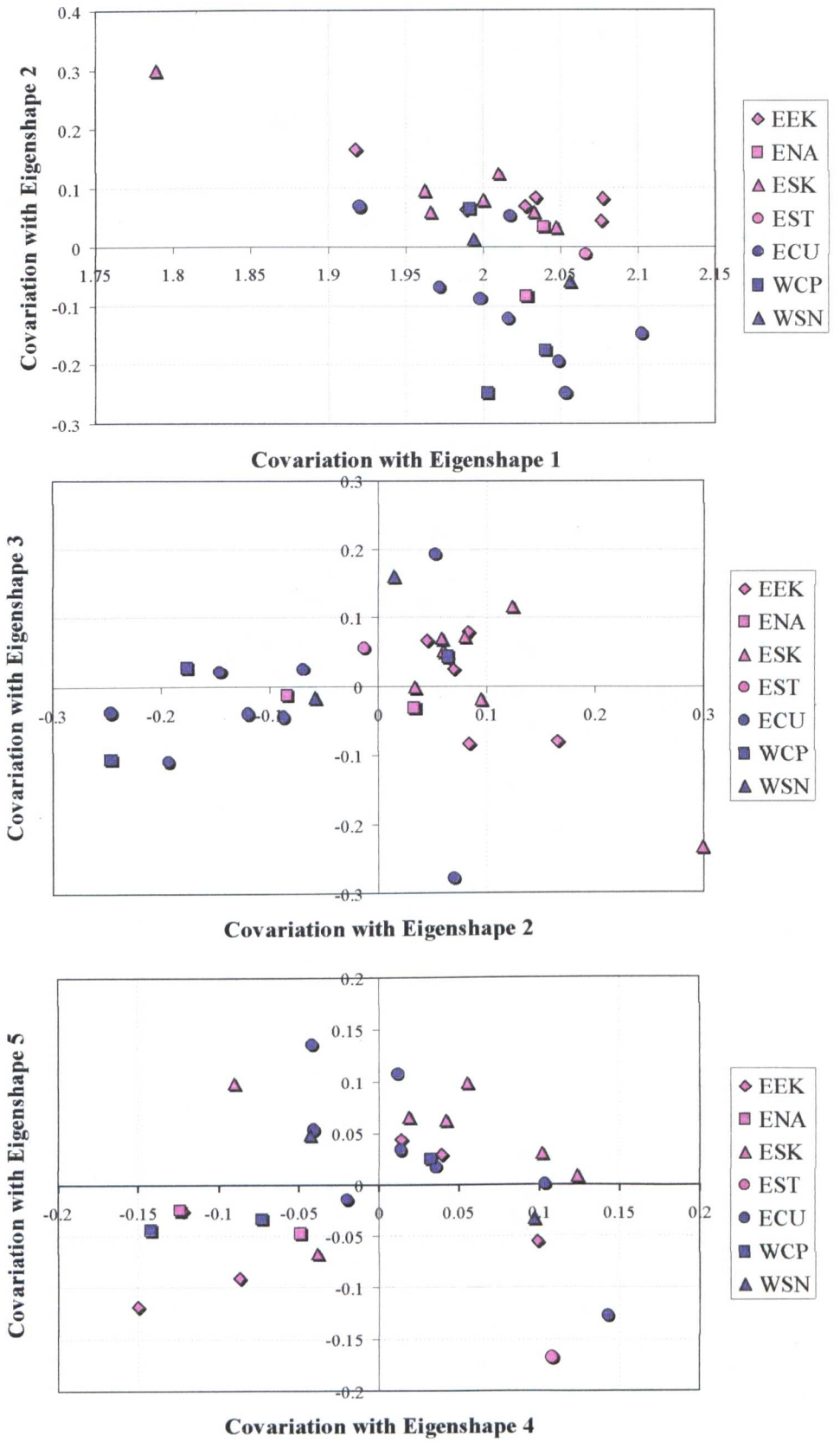


Figure A4.10.3.2: *Northern skull profile shape variation: Local comparisons between male skulls.*

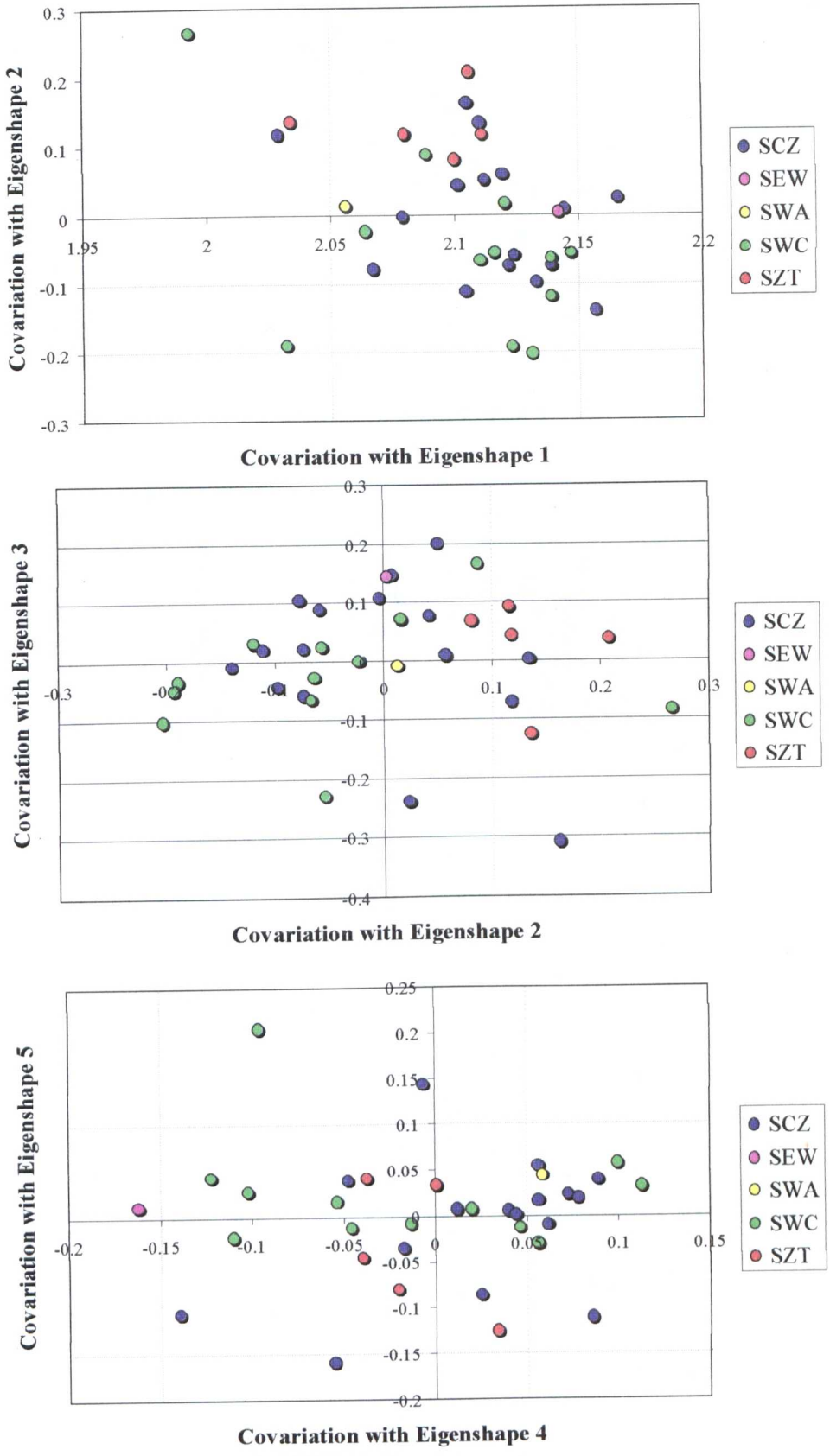


Figure A4.10.3.3: *Southern skull profile shape variation: Local comparisons between male skulls.*

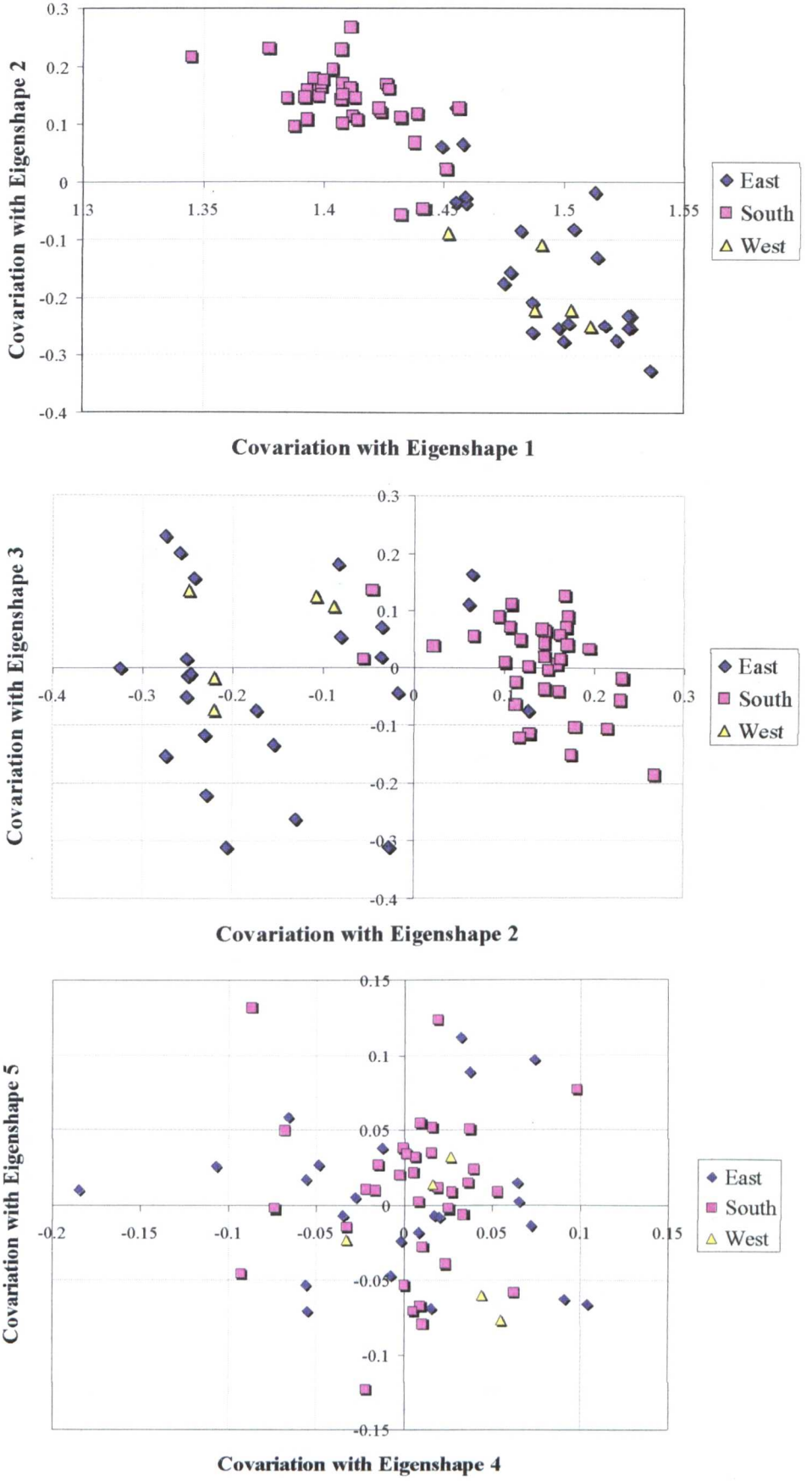


Figure A4.10.3.4: *General median horn profile shape variation: Regional comparisons between all male skulls.*

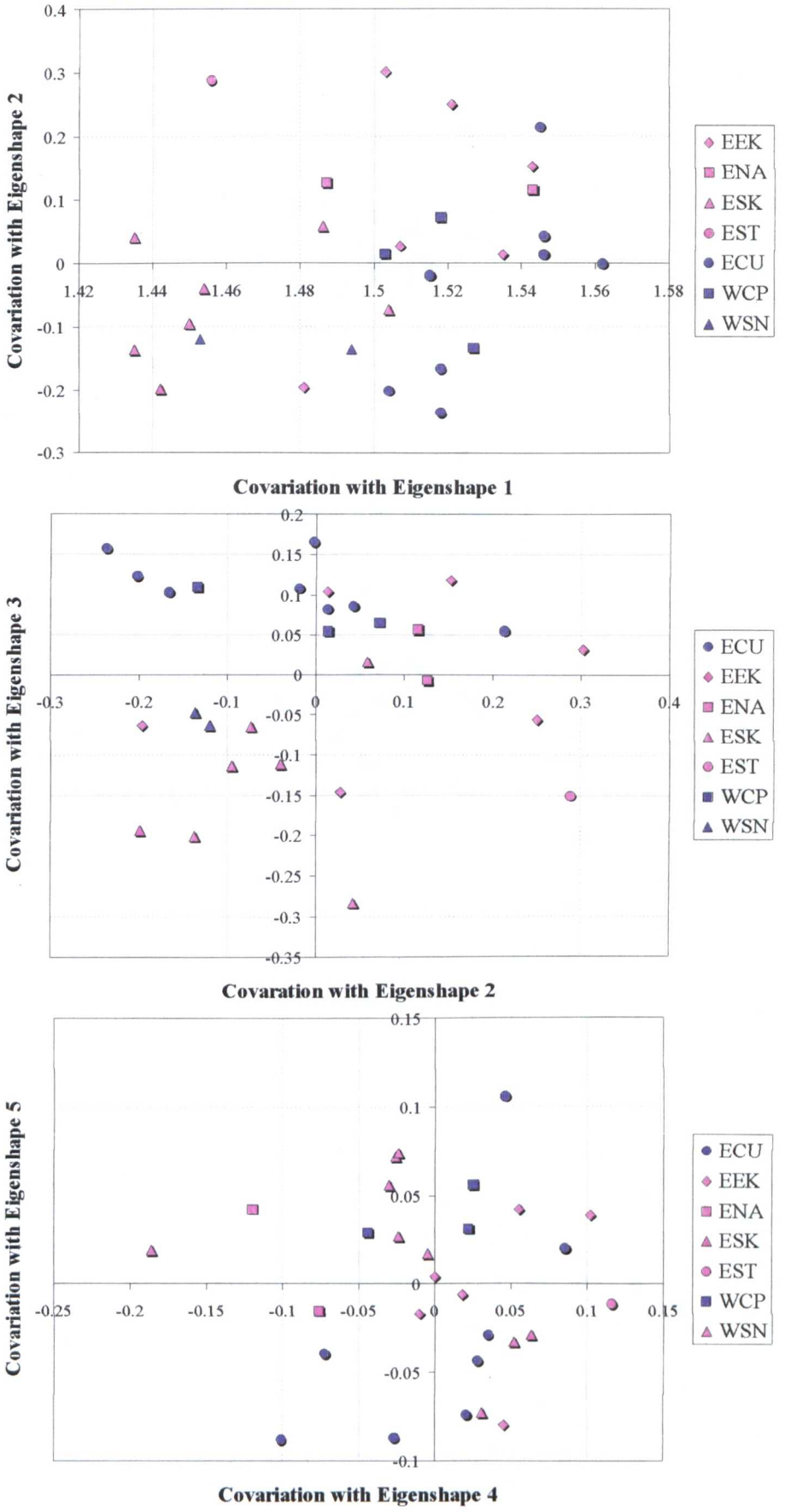


Figure A4.10.3.5: *Northern median horn profile shape variation: Local comparisons between male skulls.*

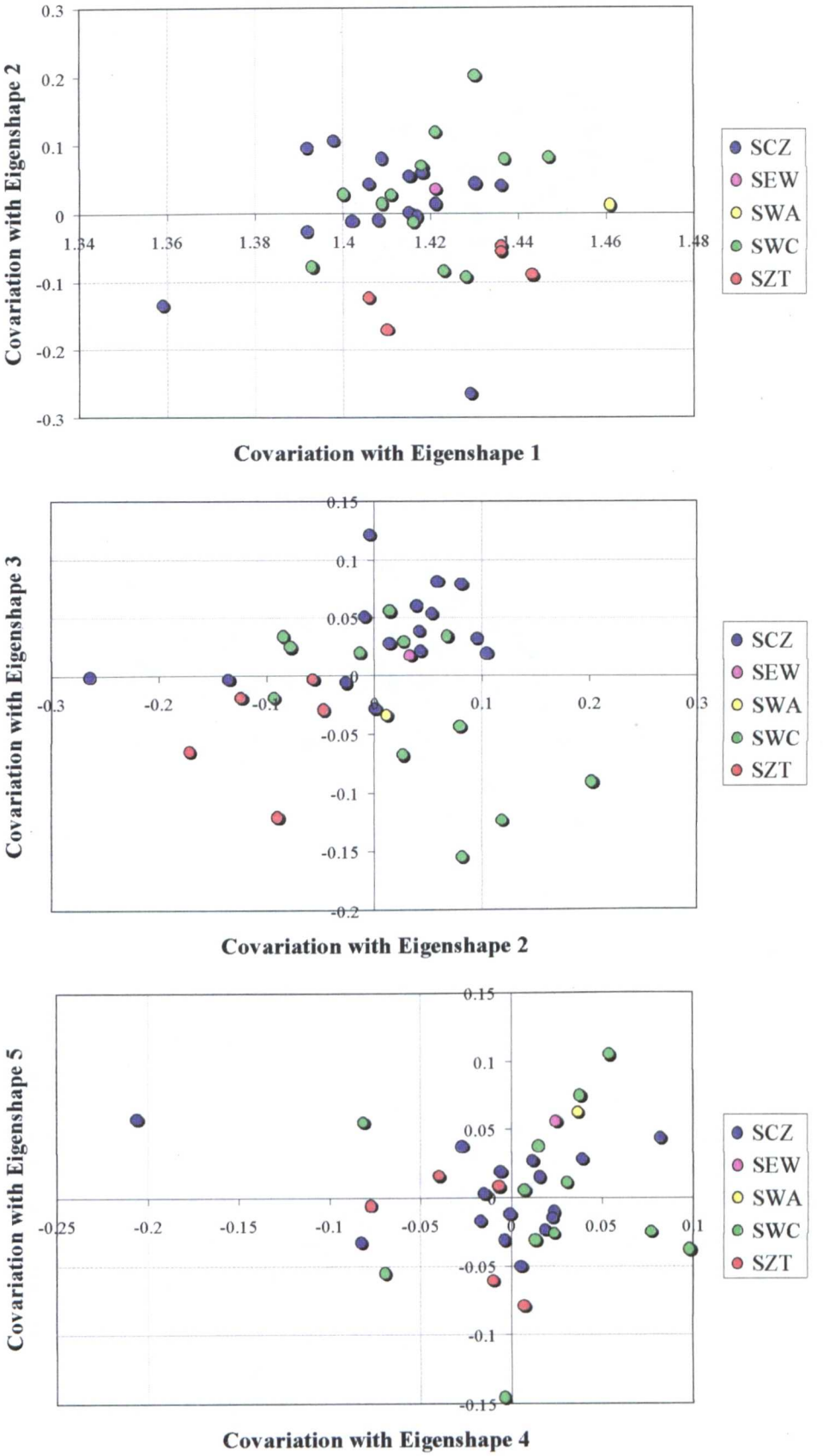


Figure A4.10.3.6: *Southern median horn profile shape variation: Local comparisons between male skulls.*

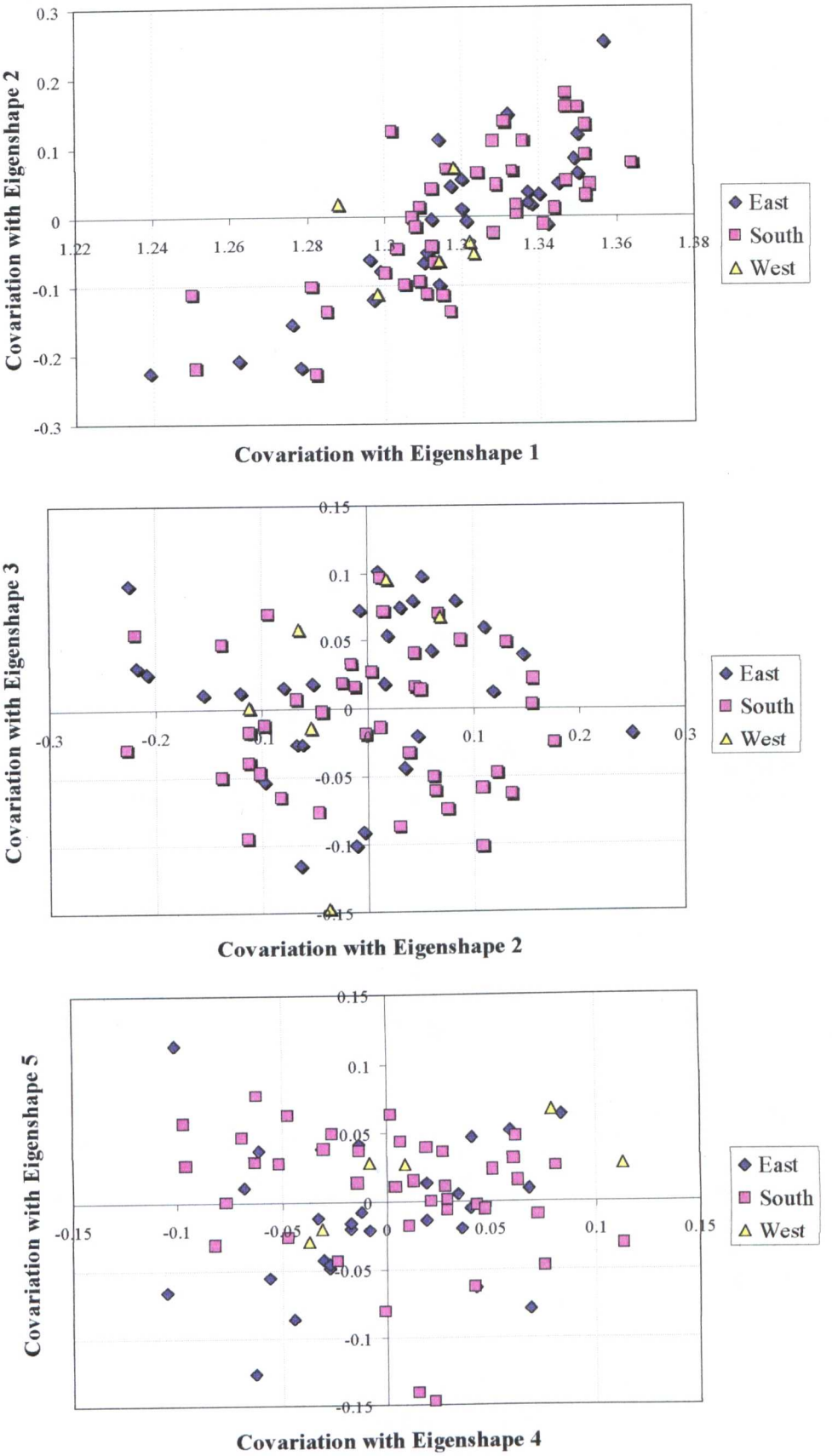


Figure A4.10.3.7: *General parietal horns shape variation: Regional comparisons between all male skulls.*

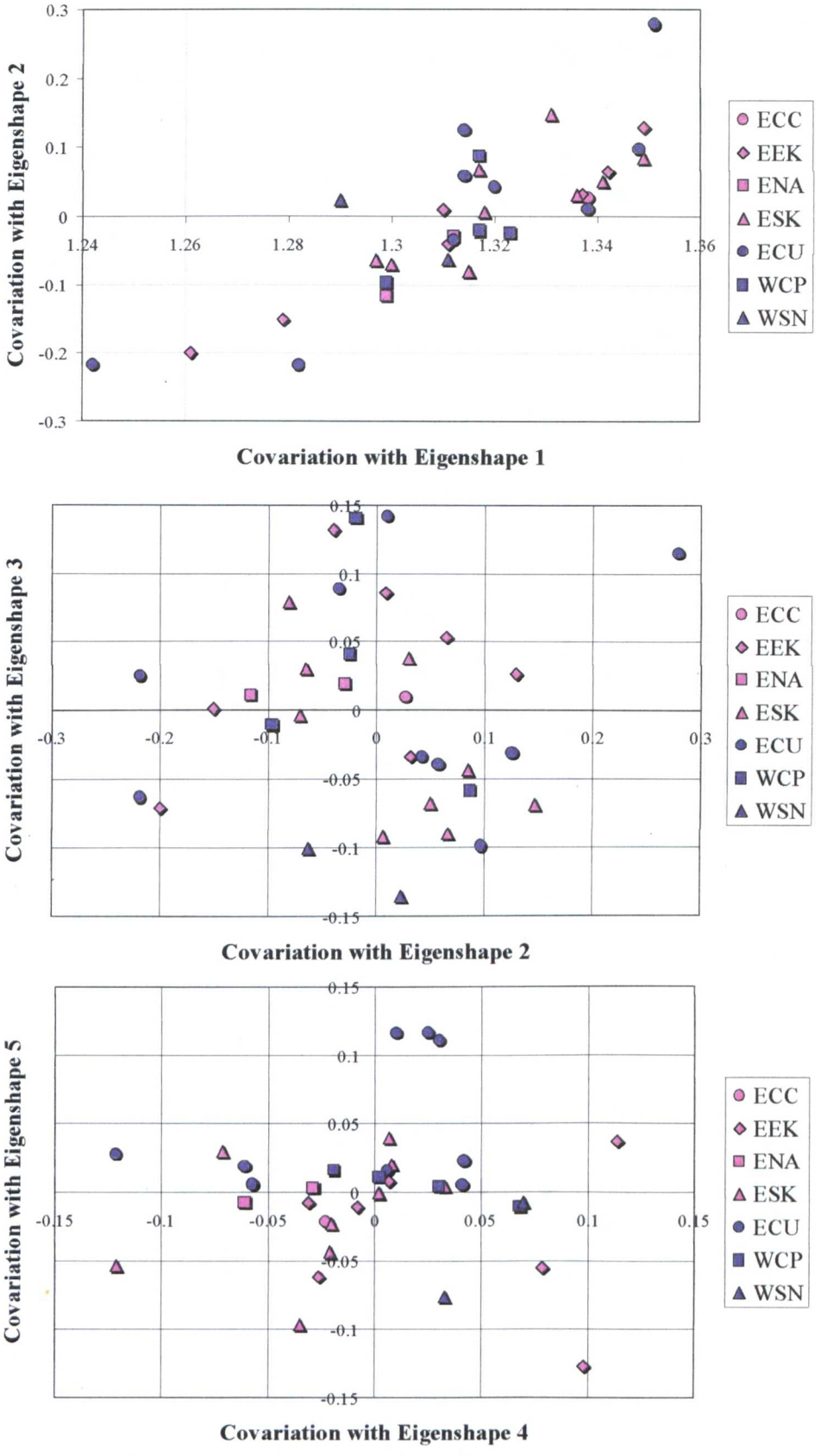


Figure A4.10.3.8: *Northern parietal horns shape variation: Local comparisons between male skulls.*

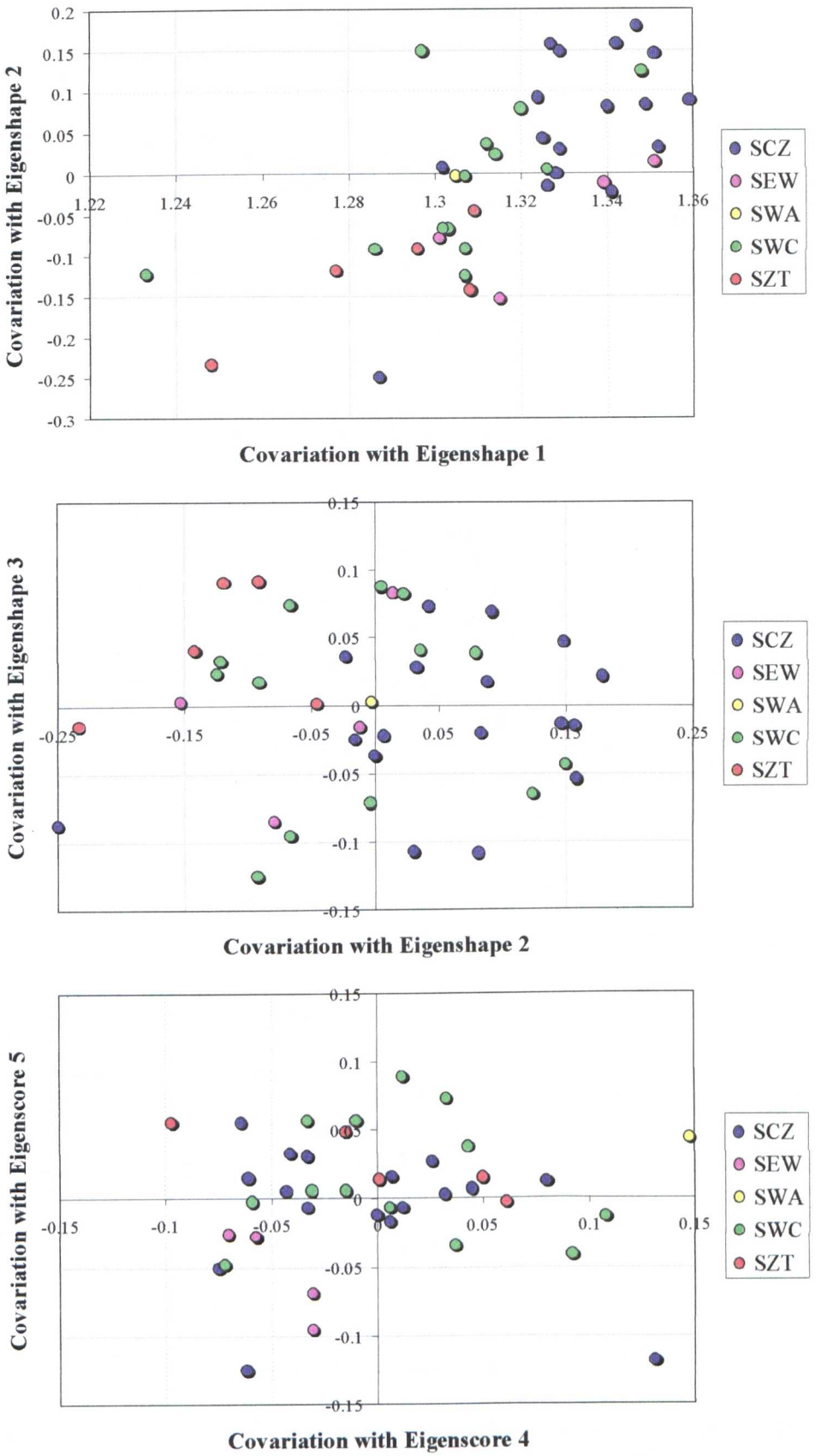


Figure A4.10.3.9: *Southern parietal horns shape variation: Local comparisons between male skulls.*

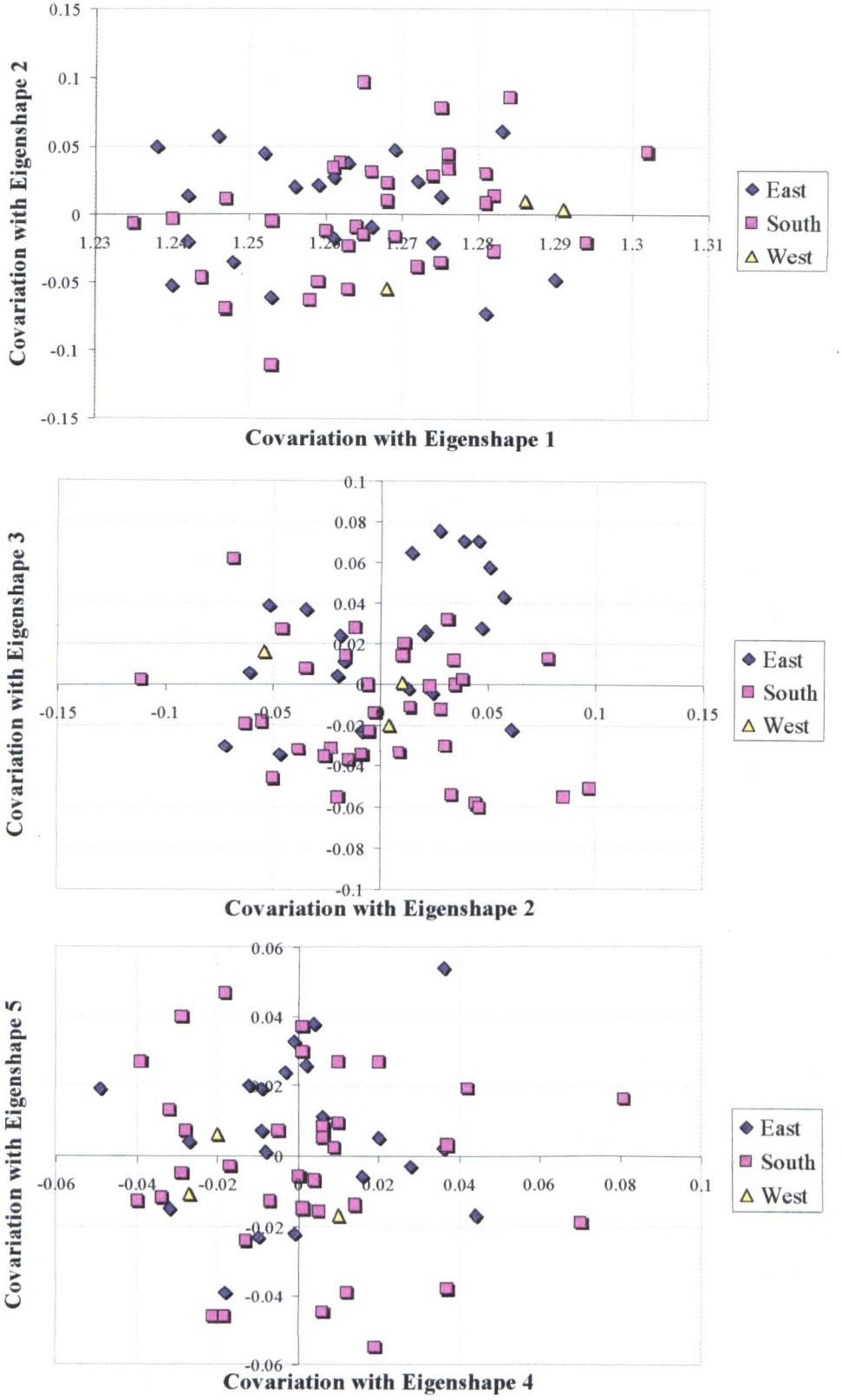


Figure A4.10.3.10: *General face and muzzle shape variation: Regional comparisons between all male skulls.*

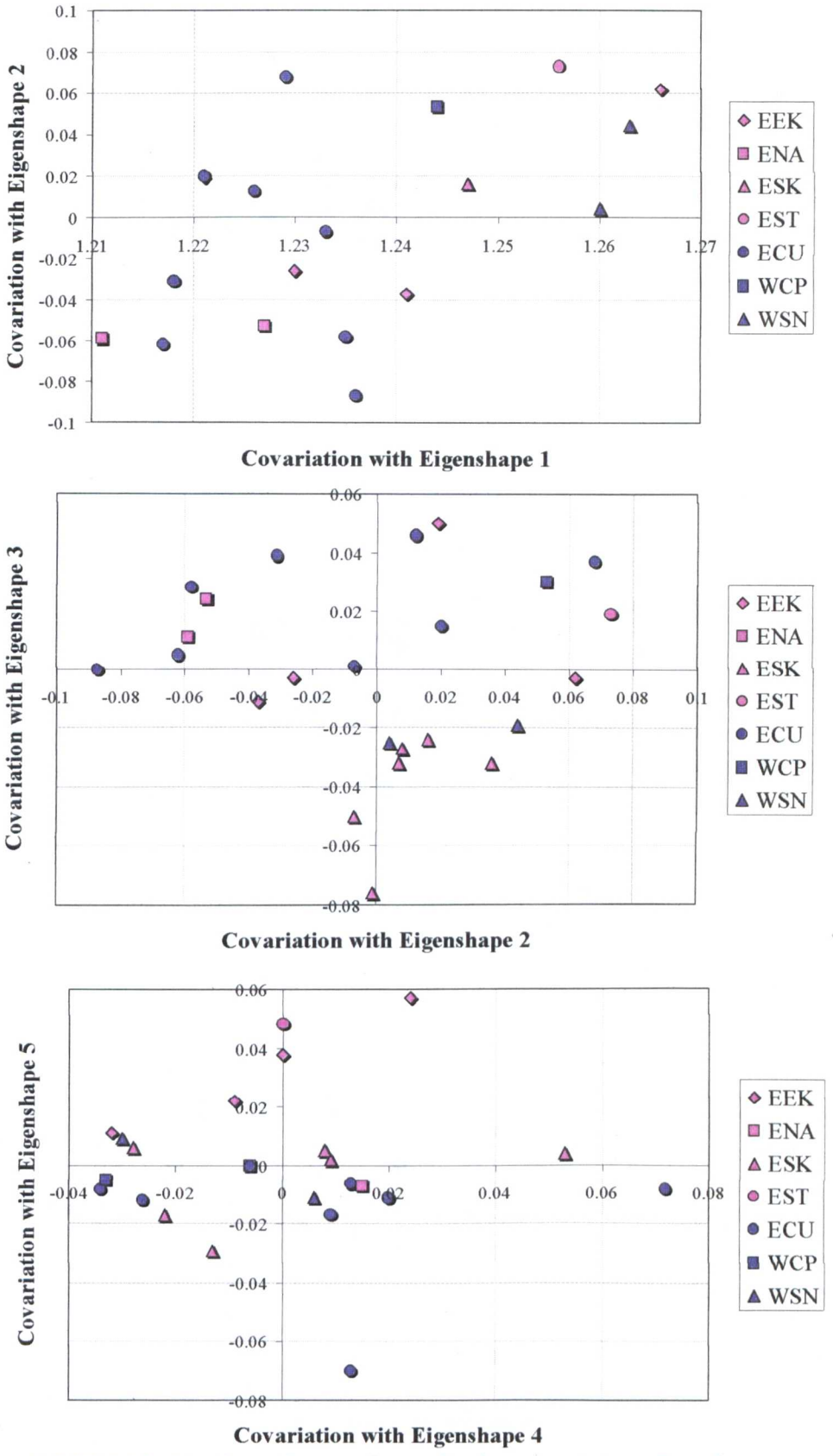


Figure A4.10.3.11: *Northern face and muzzle shape variation: Local comparisons between male skulls.*

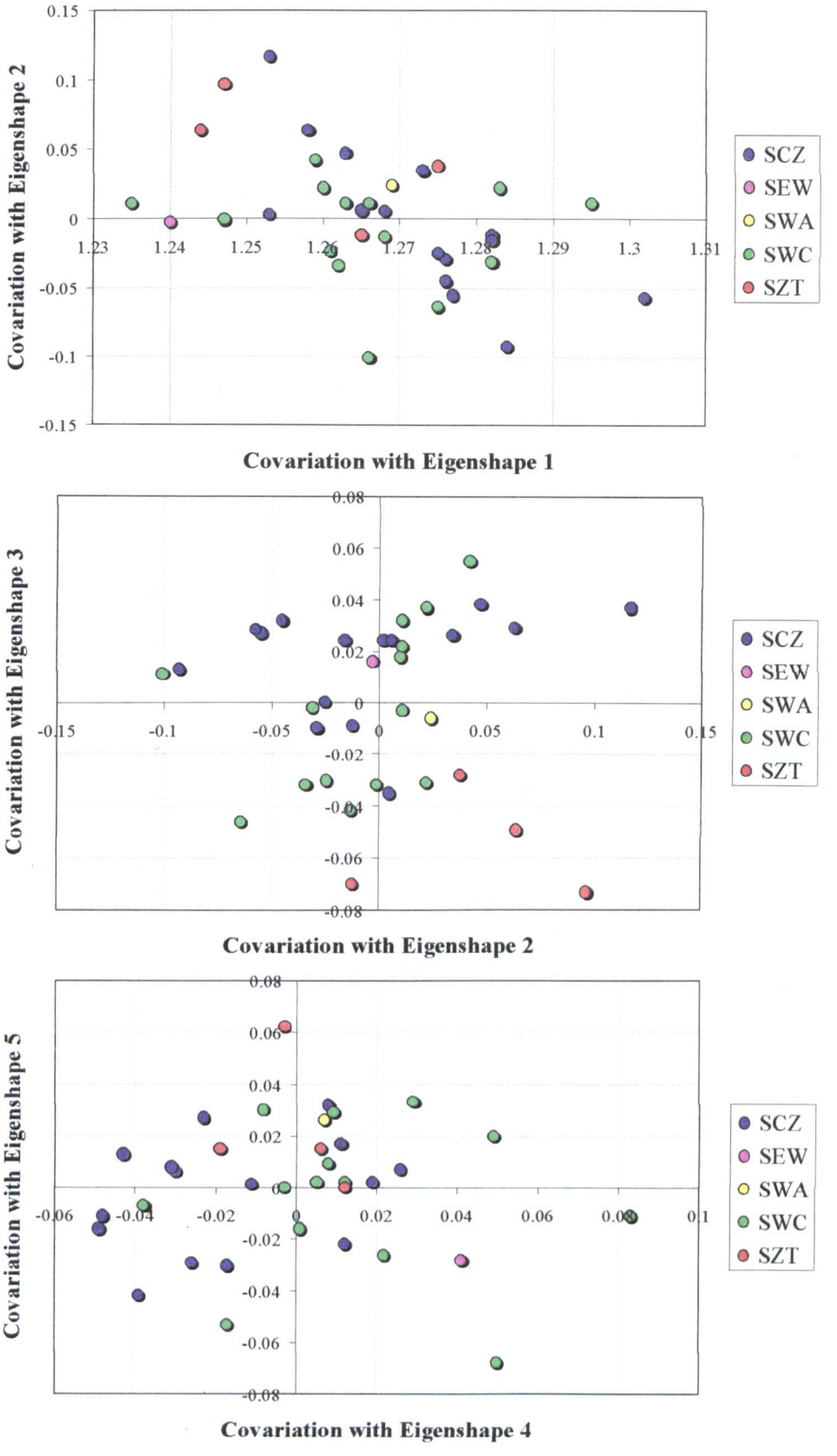


Figure A4.10.3.12: *Southern face and muzzle shape variation: Local comparisons between male skulls.*

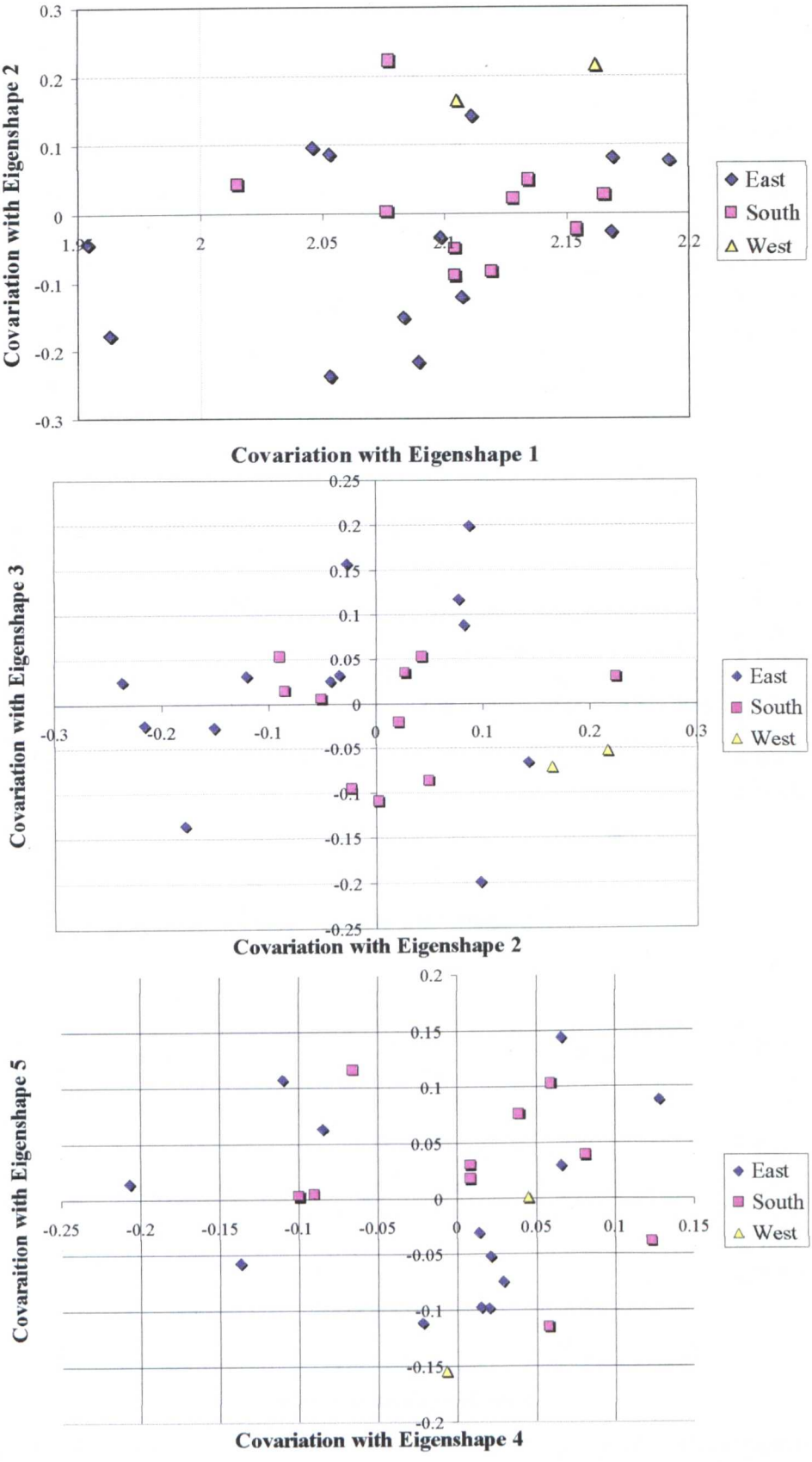


Figure A4.10.3.13: *General skull profile shape variation: Regional comparisons between all female skulls.*

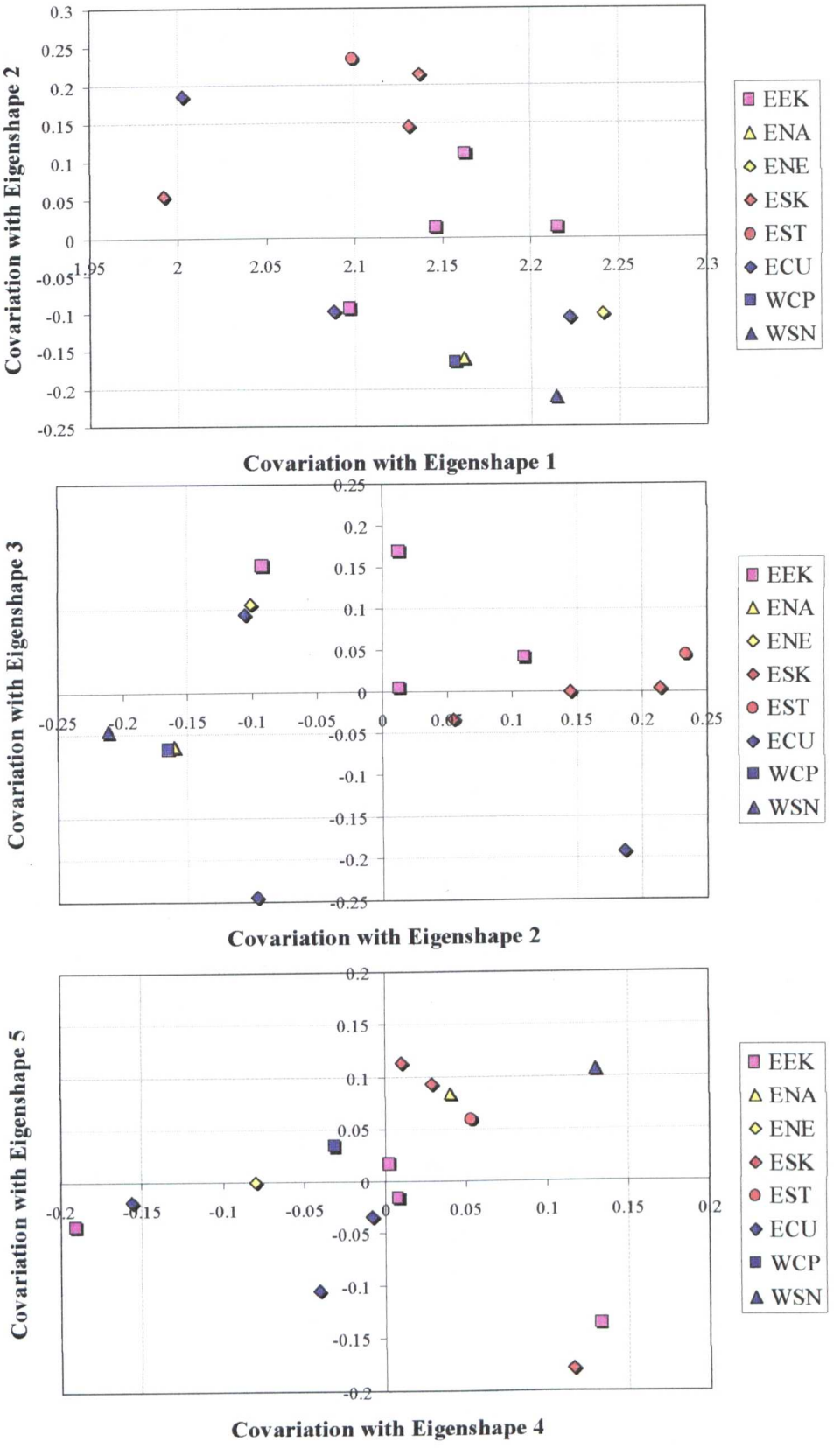


Figure A4.10.3.14: *Northern skull profile shape variation: Local comparisons between female skulls.*

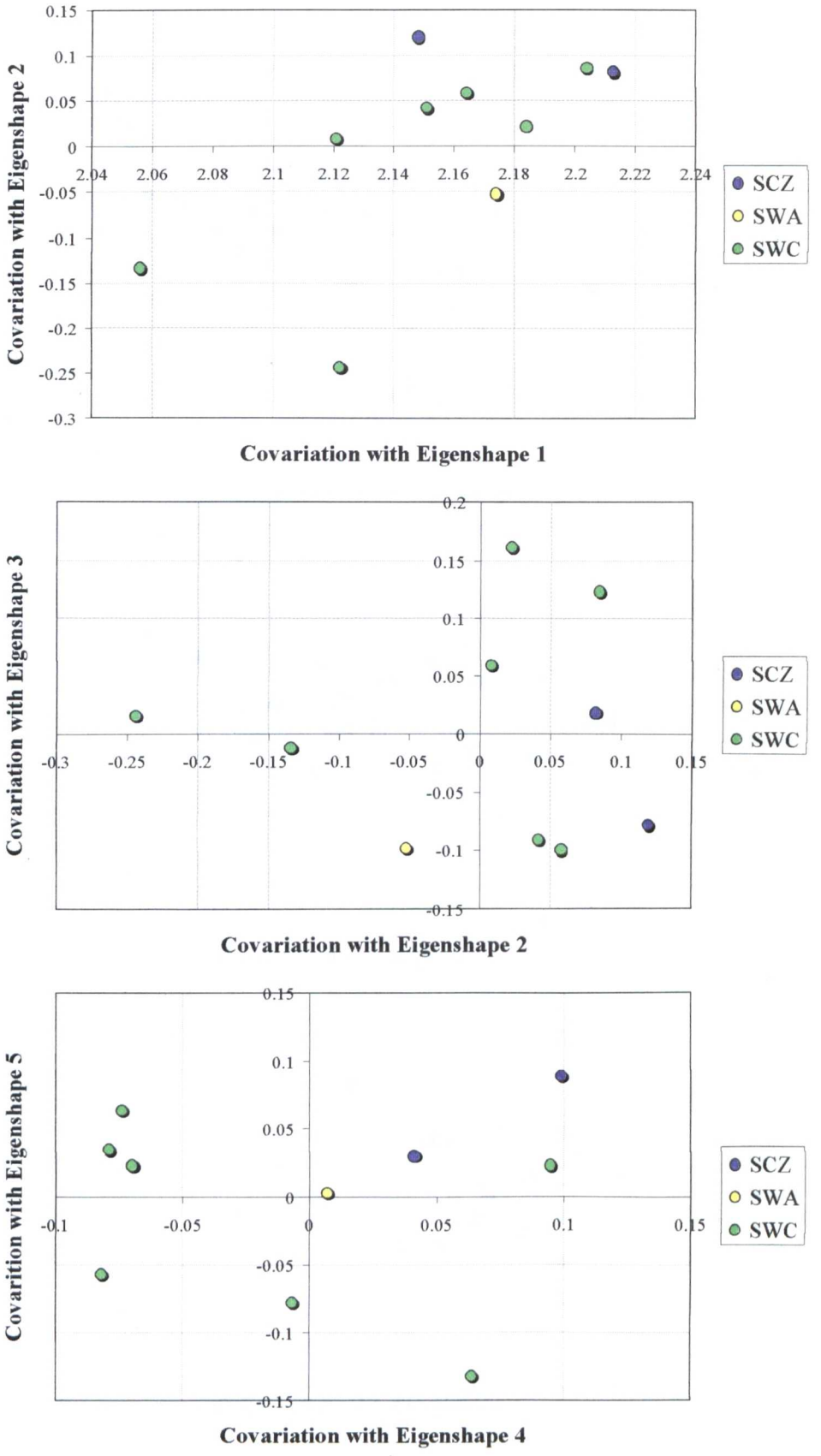


Figure A4.10.3.15: *Southern skull profile shape variation: Local comparisons between female skulls.*

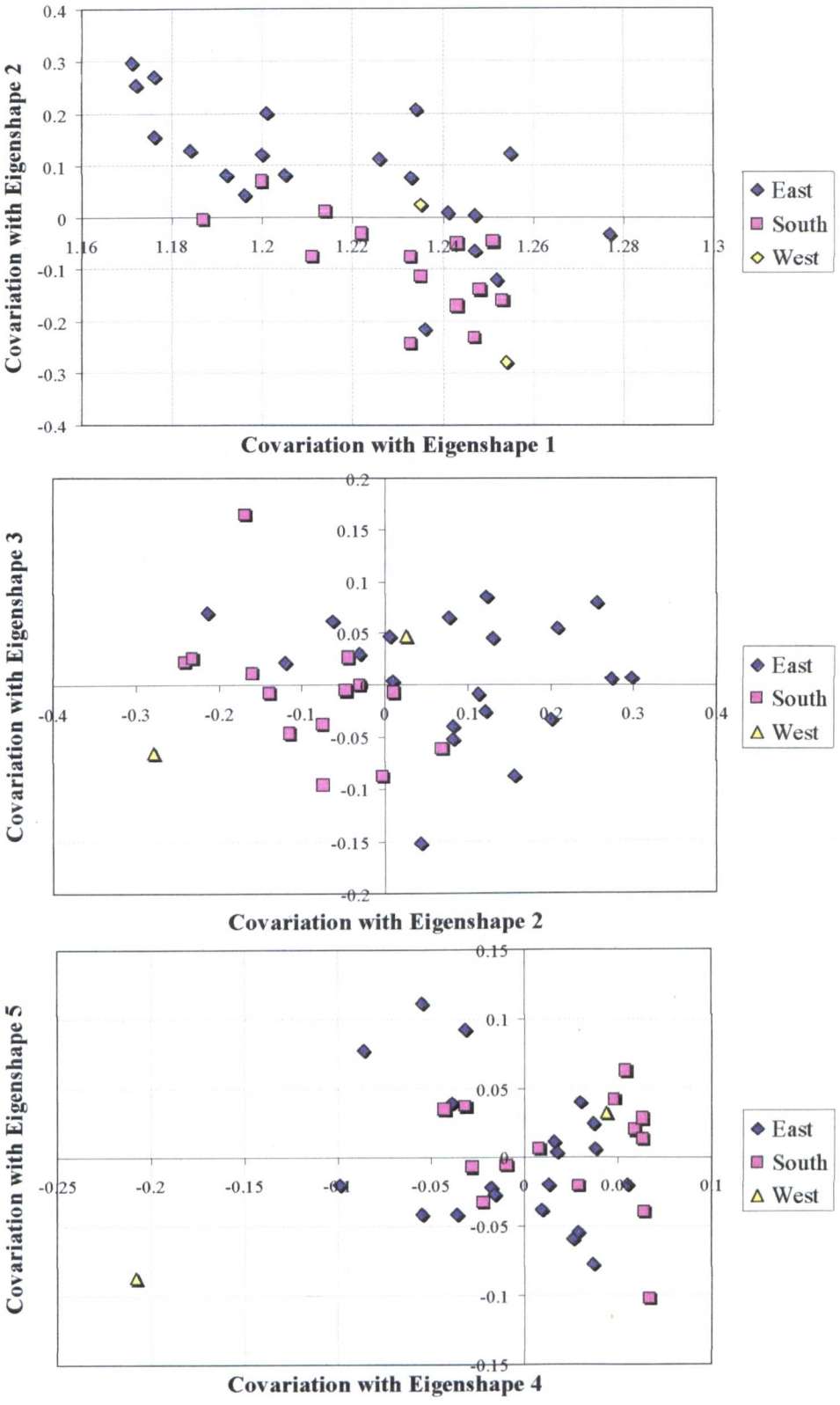


Figure A4.10.3.16: *General parietal horns shape variation: Regional comparisons between all female skulls.*

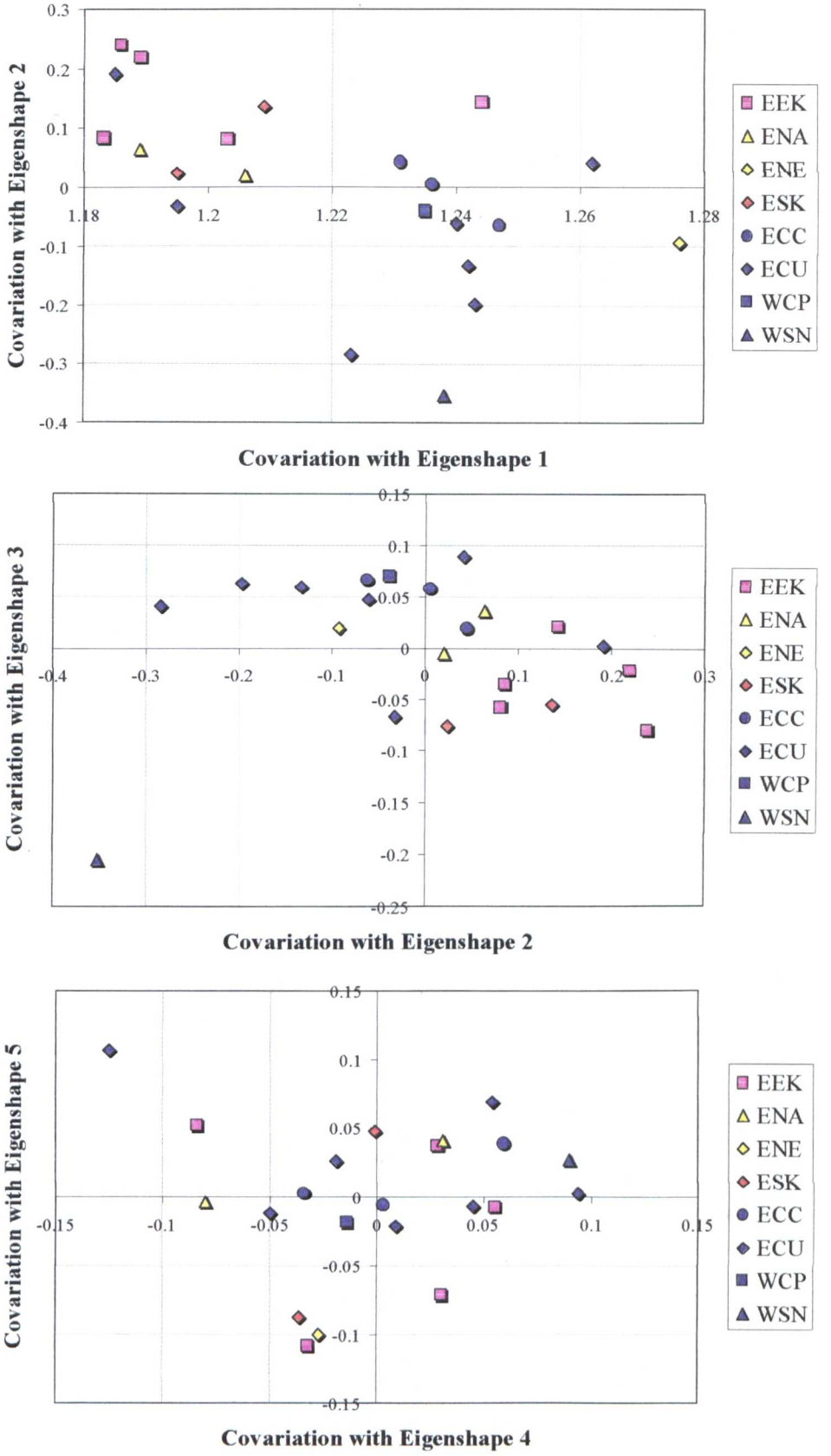


Figure A4.10.3.17: *Northern parietal horns shape variation: Local comparisons between female skulls.*

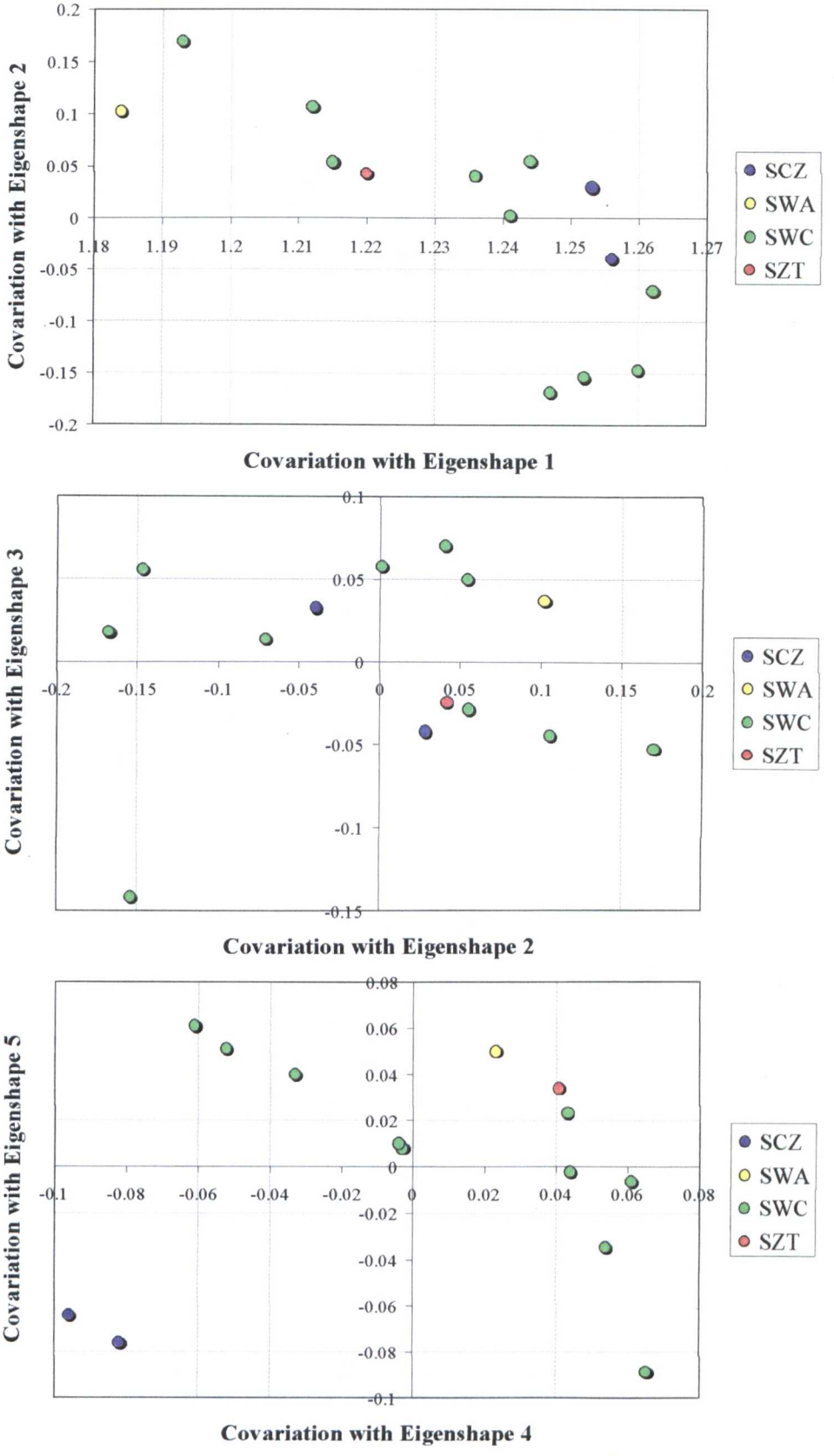


Figure A4.10.3.18: *Southern parietal horns shape variation: Local comparisons between female skulls.*

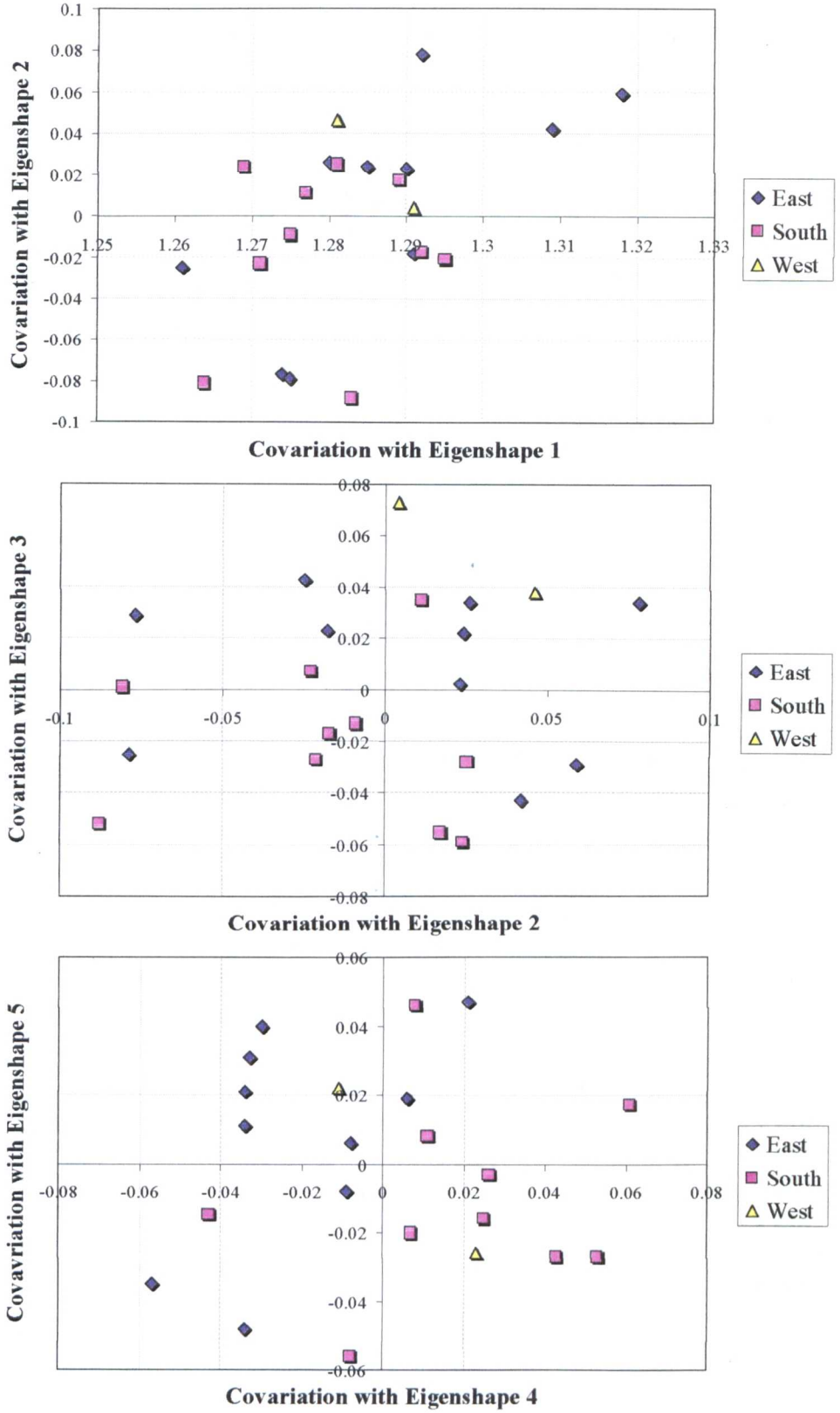


Figure A4.10.3.19: *General face and muzzle shape variation: Regional comparisons between all female skulls.*

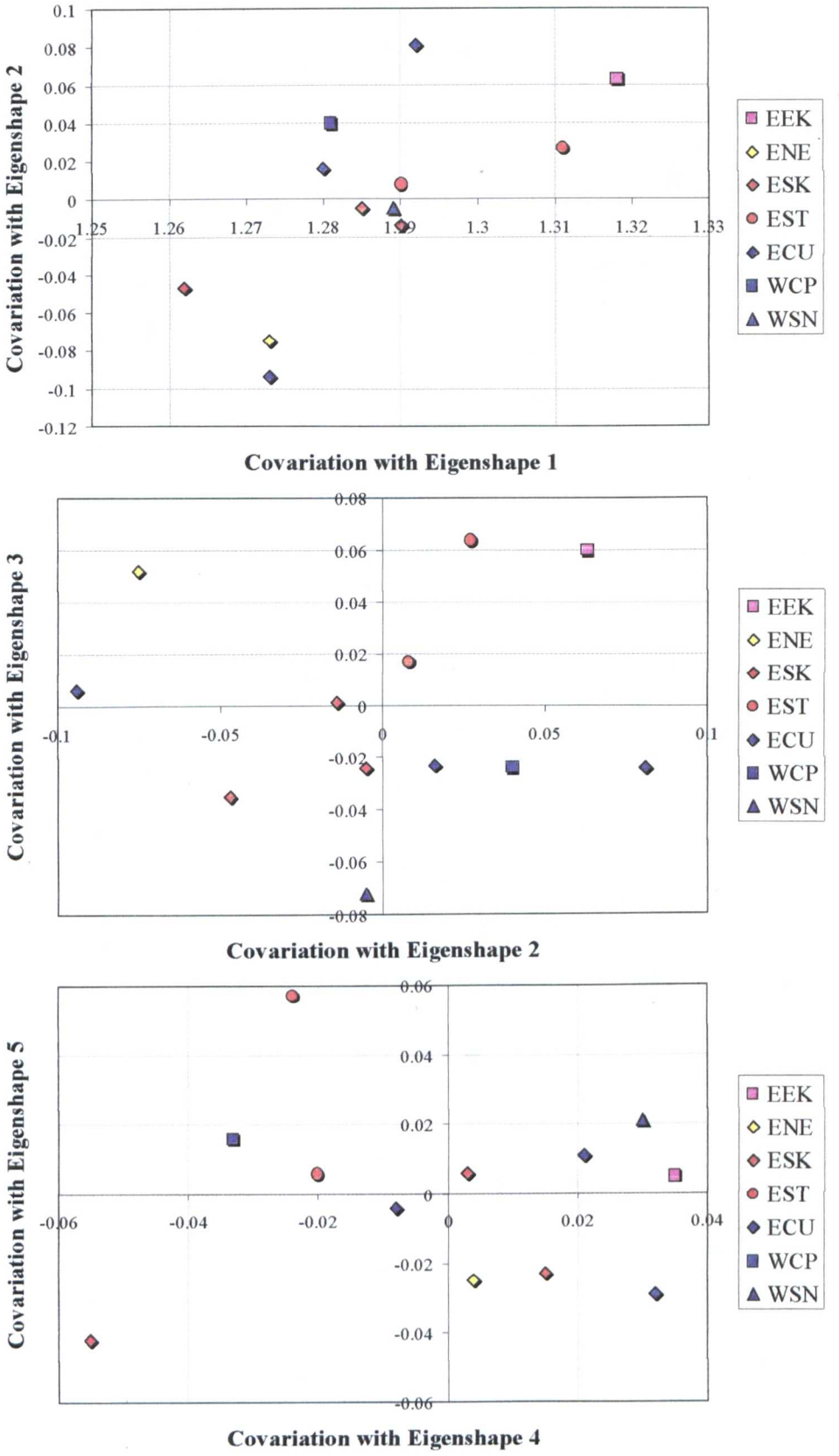


Figure A4.10.3.20: *Northern face and muzzle shape variation: Local comparisons between female skulls.*

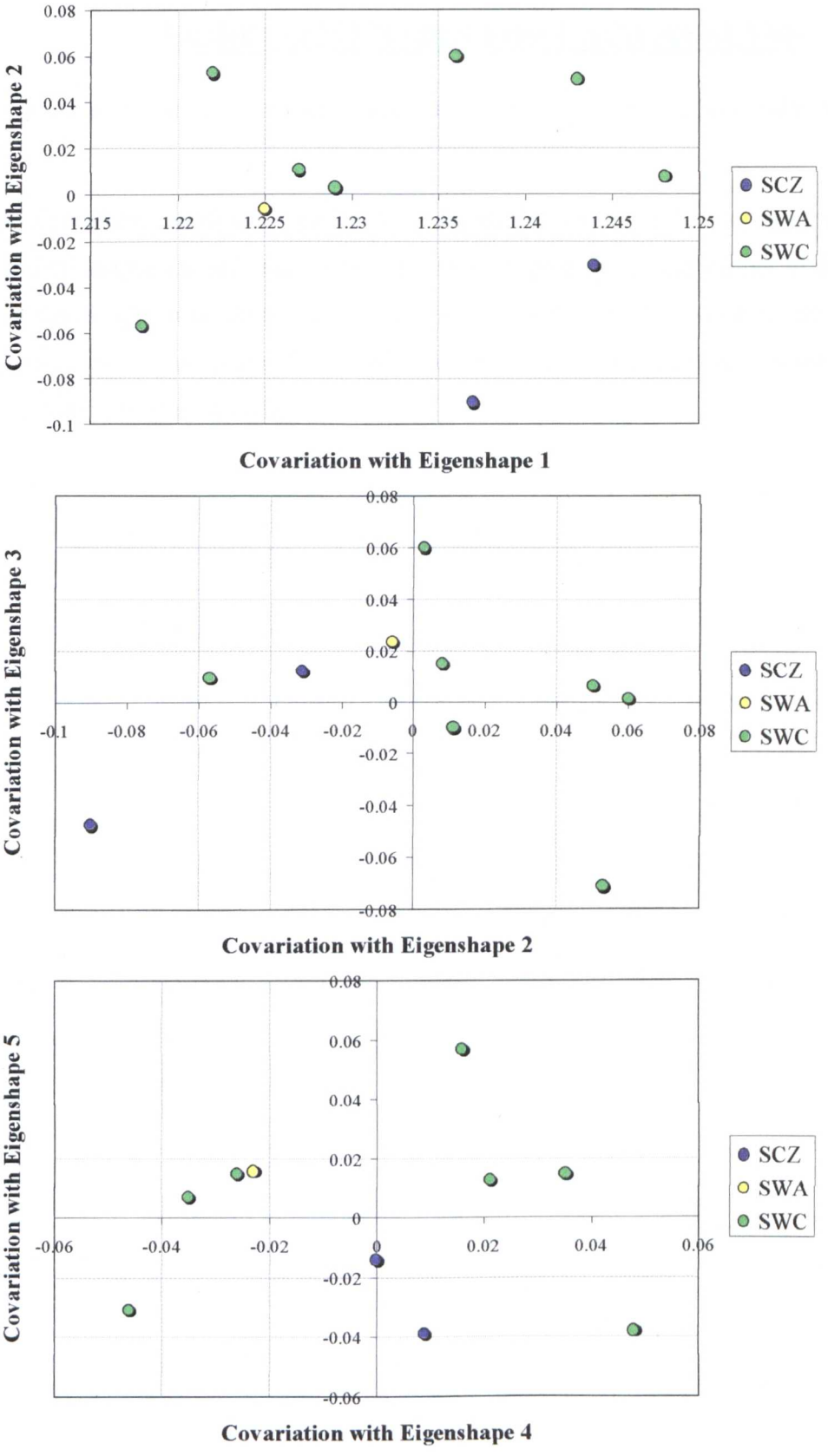


Figure A4.10.3.21: *Southern face and muzzle shape variation: Local comparisons between female skulls.*

APPENDIX 4.10.4:

MODELLED SHAPES FOR EACH ANALYSIS

The presented models demonstrate the shape trends and the variation indicated by each axis.

Five eigenshape trends are presented and numbered sequentially in each figure. Five modelled shapes for each eigenshape are superimposed upon each other to demonstrate the identified shape variation trends. The red outline indicates the lowest modelled value while the mauve represents the highest modelled value. Intervening colours represent intermediate modelled values.

Figure A4.10.4.1 *Male Skull Profile Variation - All Specimens.*

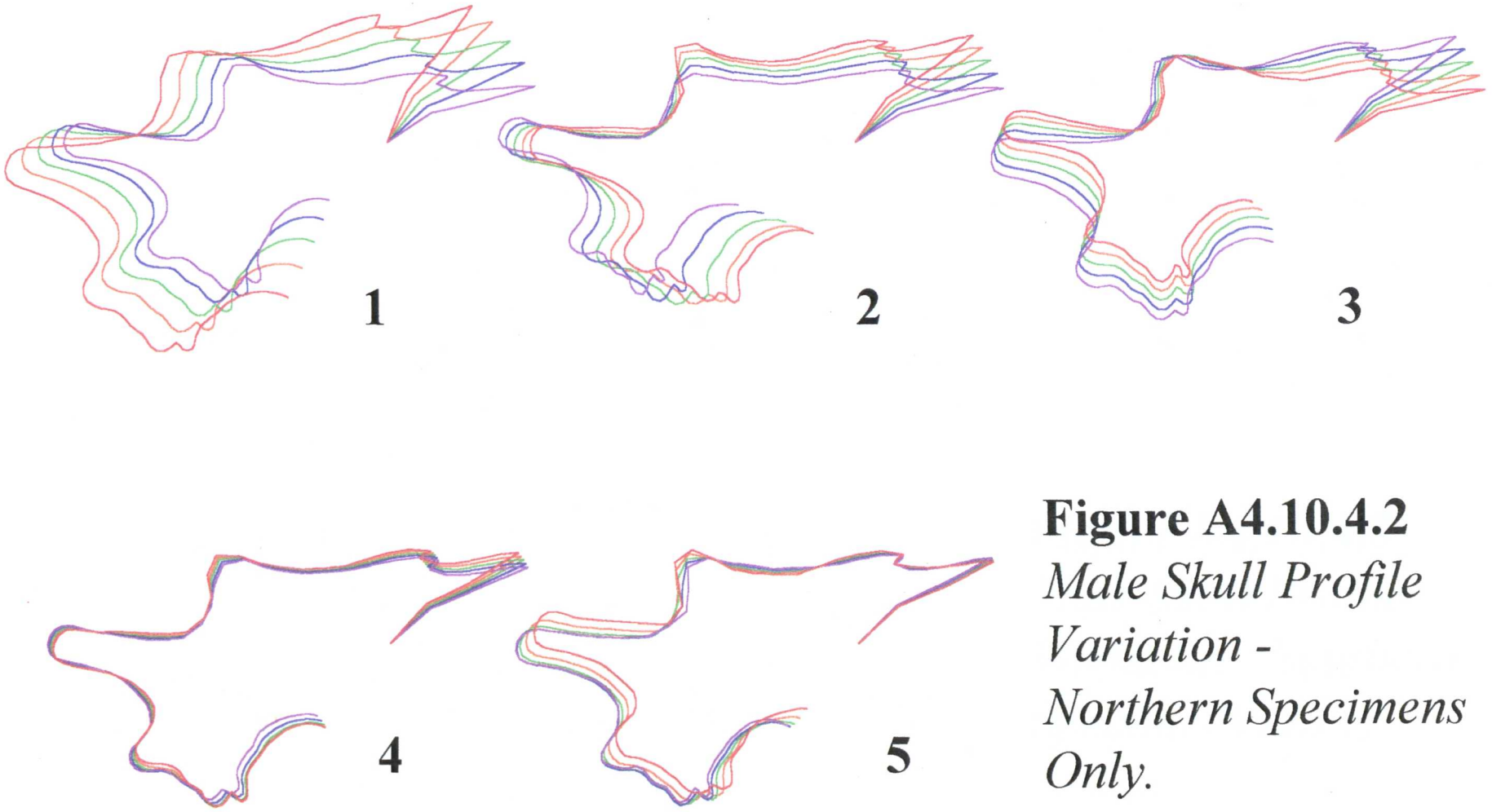


Figure A4.10.4.2
Male Skull Profile Variation - Northern Specimens Only.

Figure A4.10.4.3
Male Skull Profile Variation - Southern Specimens Only.

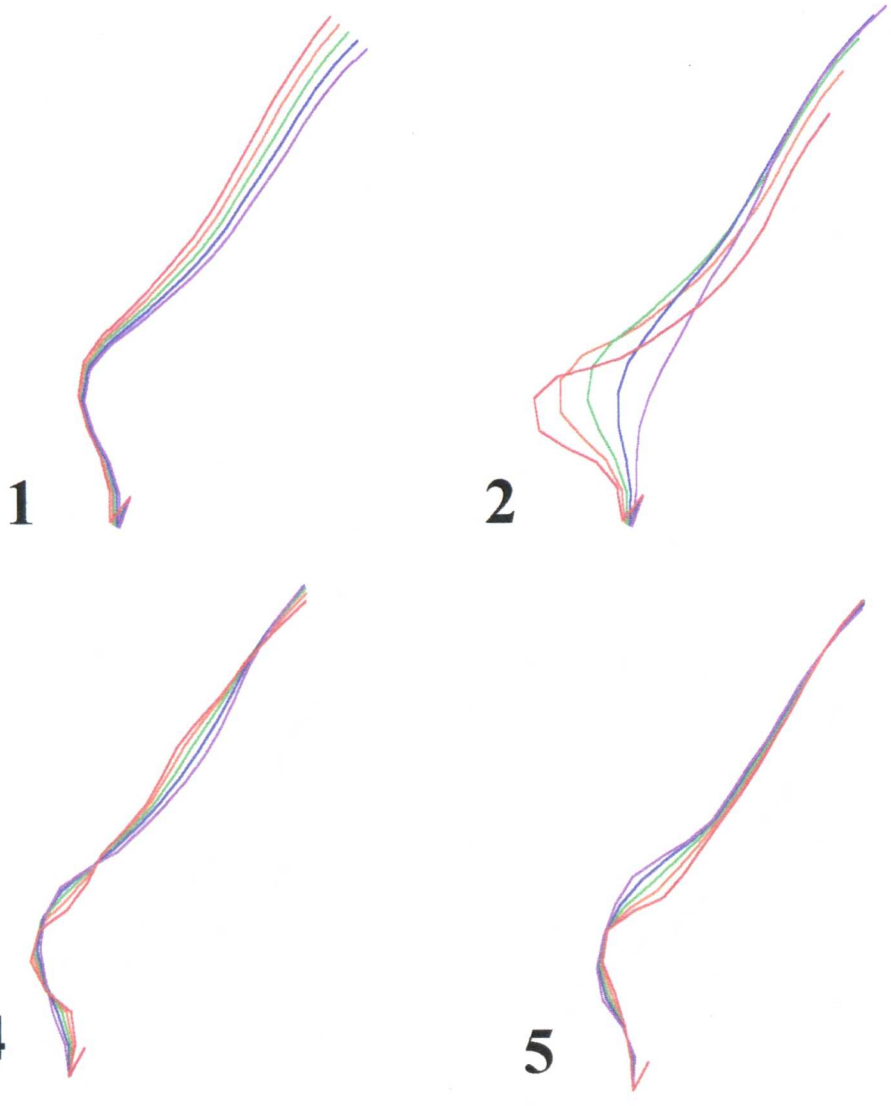
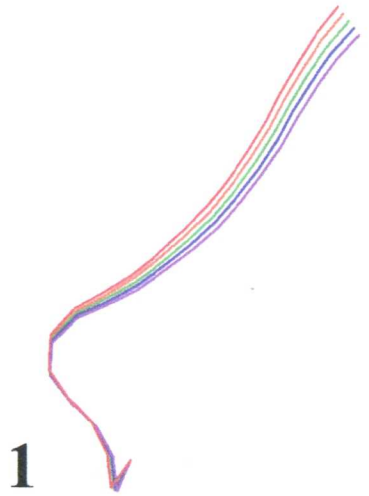
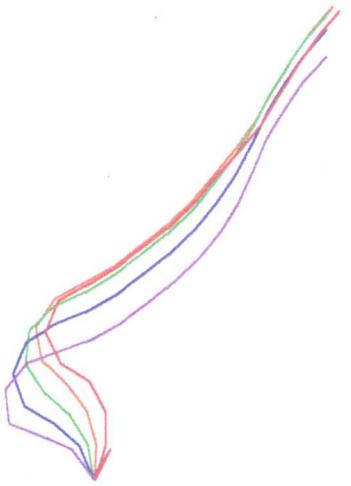
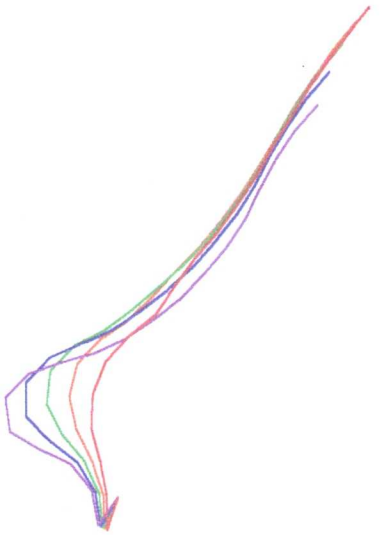
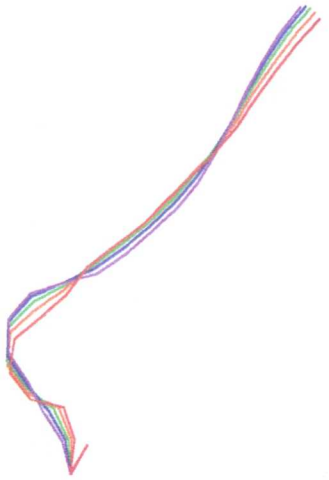


Figure A4.10.4.4
Male Median Horn Profile Variation - All Specimens.









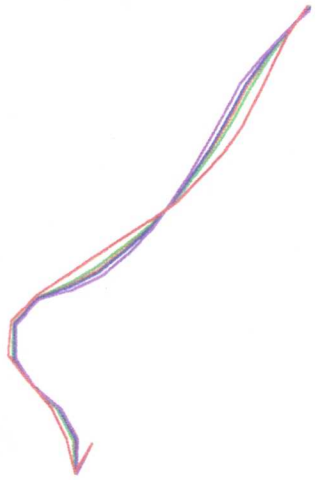
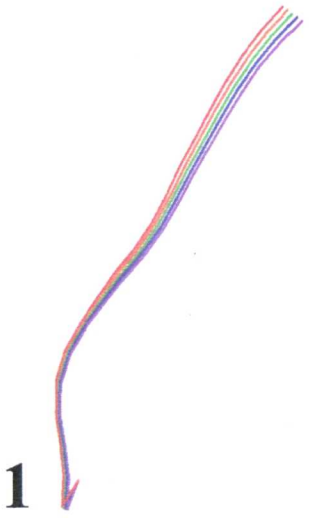
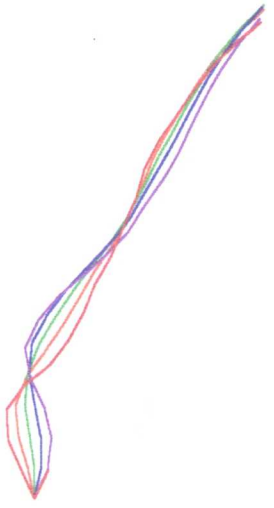
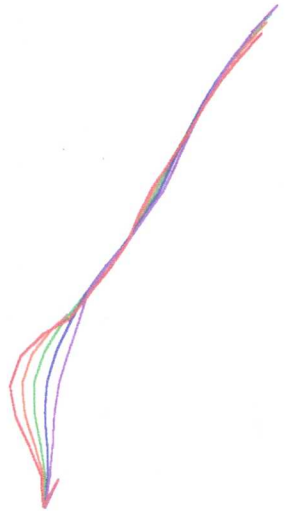
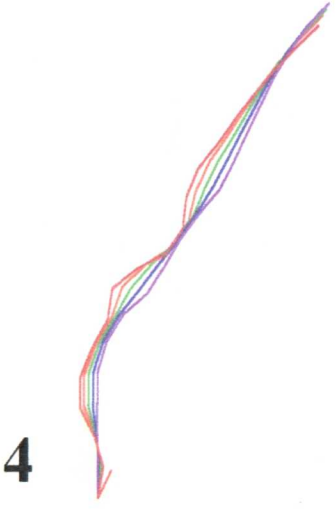


Figure A4.10.4.5
Male Median Horn Profile Variation - Northern Specimens Only.









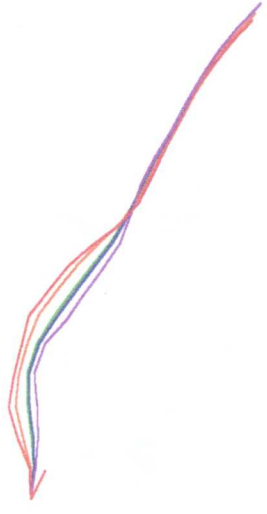


Figure A4.10.4.6
Male Median Horn Profile Variation - Southern Specimens Only.

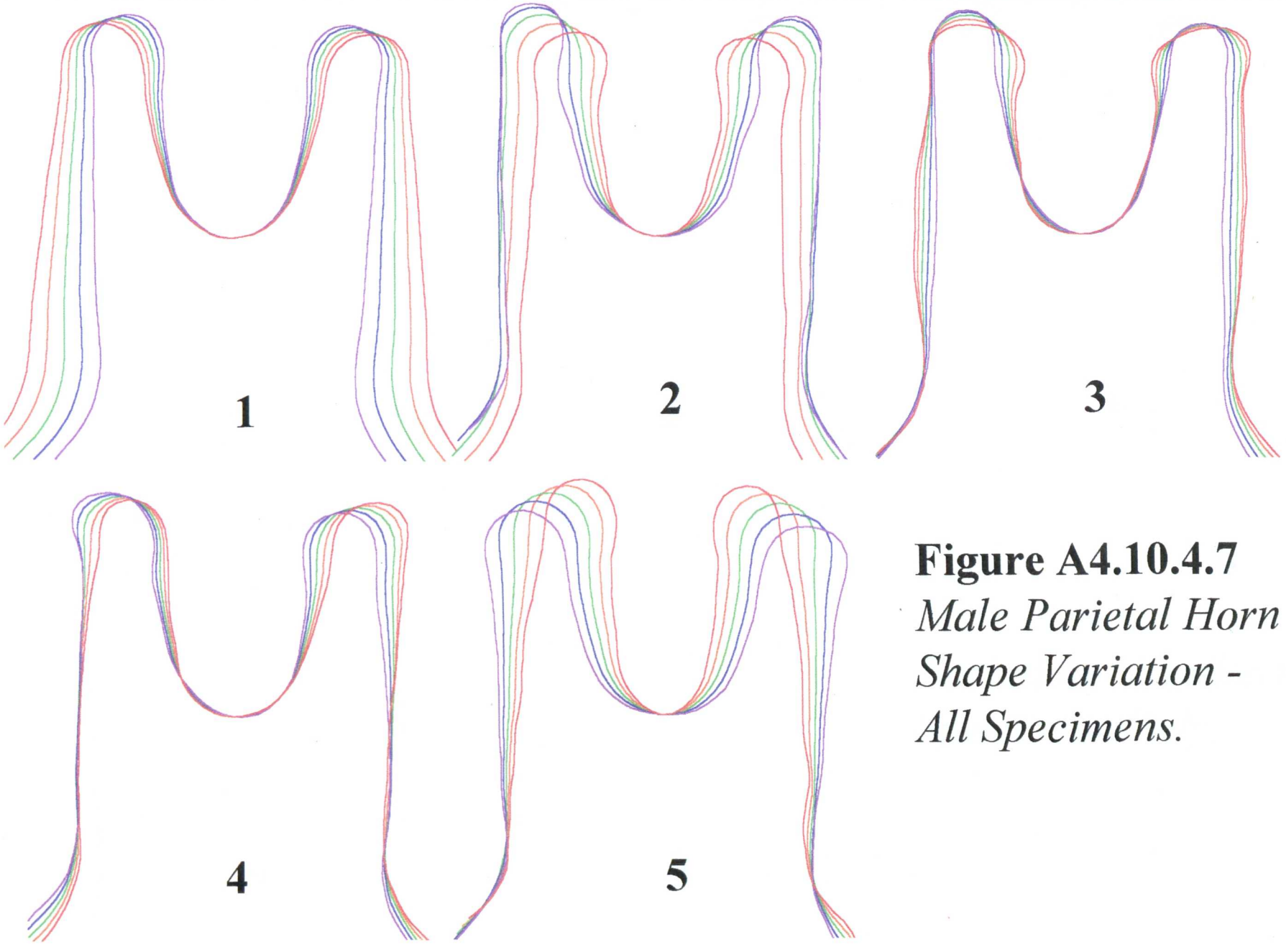


Figure A4.10.4.7
Male Parietal Horn Shape Variation - All Specimens.

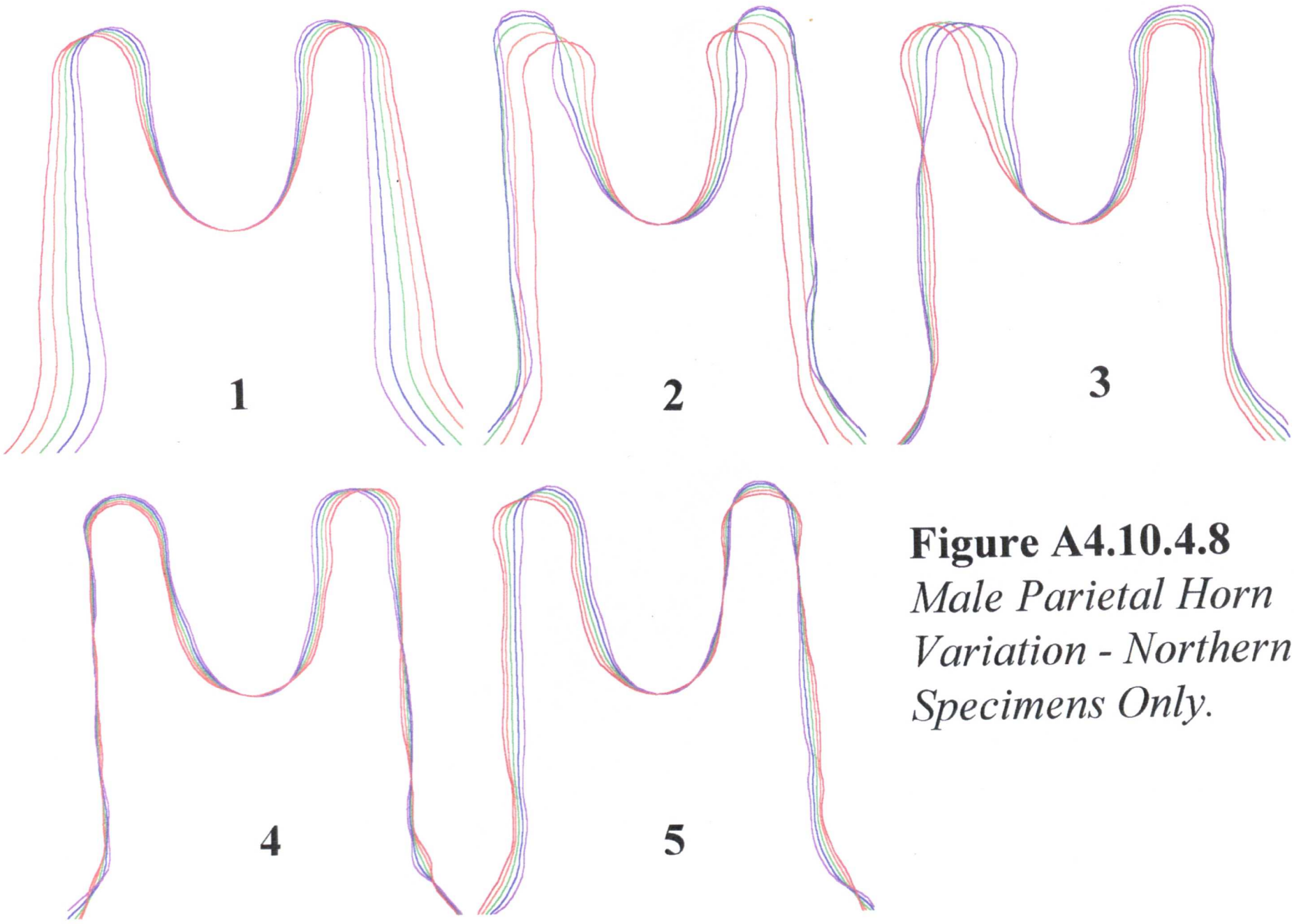


Figure A4.10.4.8
Male Parietal Horn Variation - Northern Specimens Only.

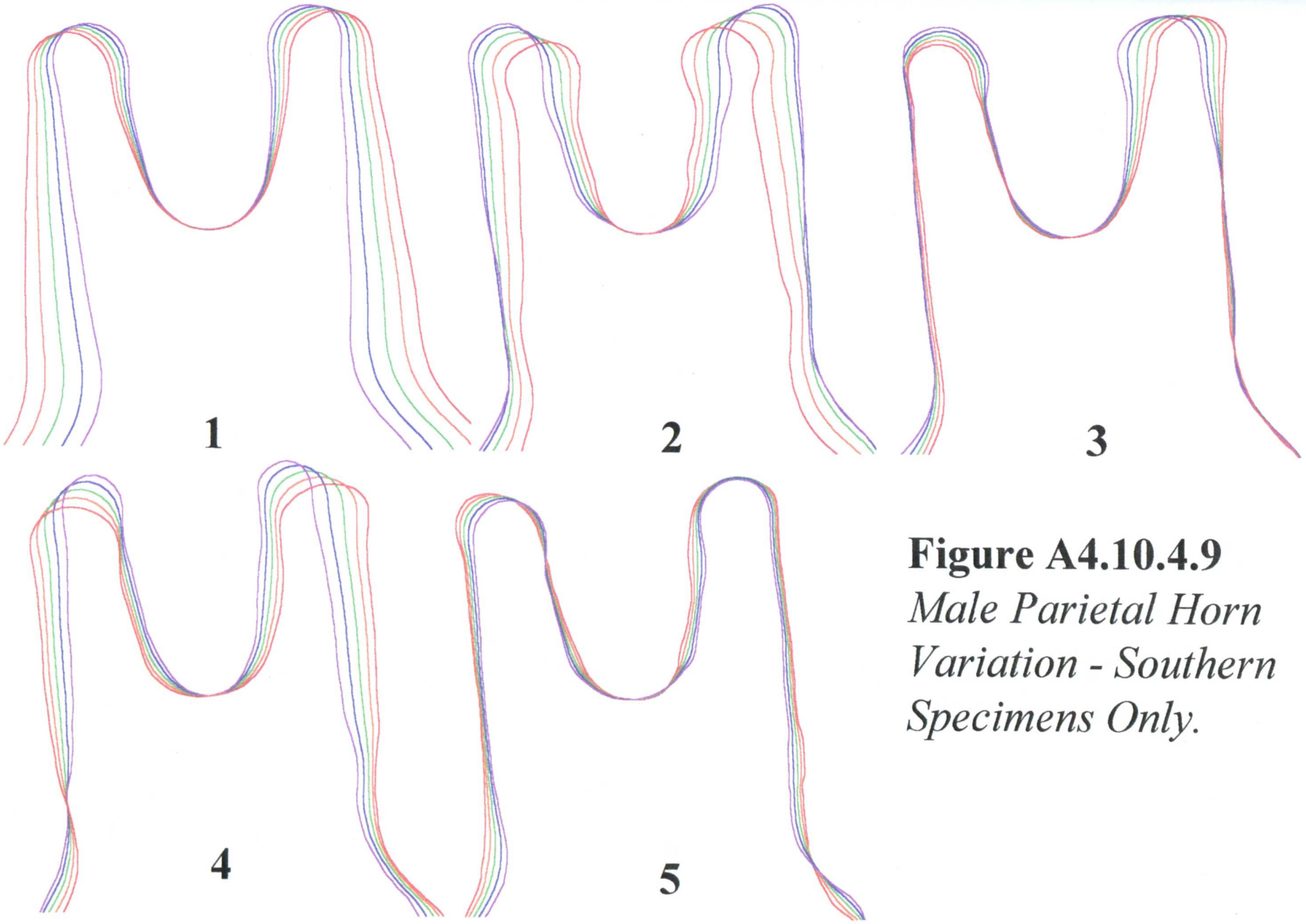


Figure A4.10.4.9
Male Parietal Horn Variation - Southern Specimens Only.

Figure A4.10.4.10
Male Face and Muzzle Variation - All Specimens.

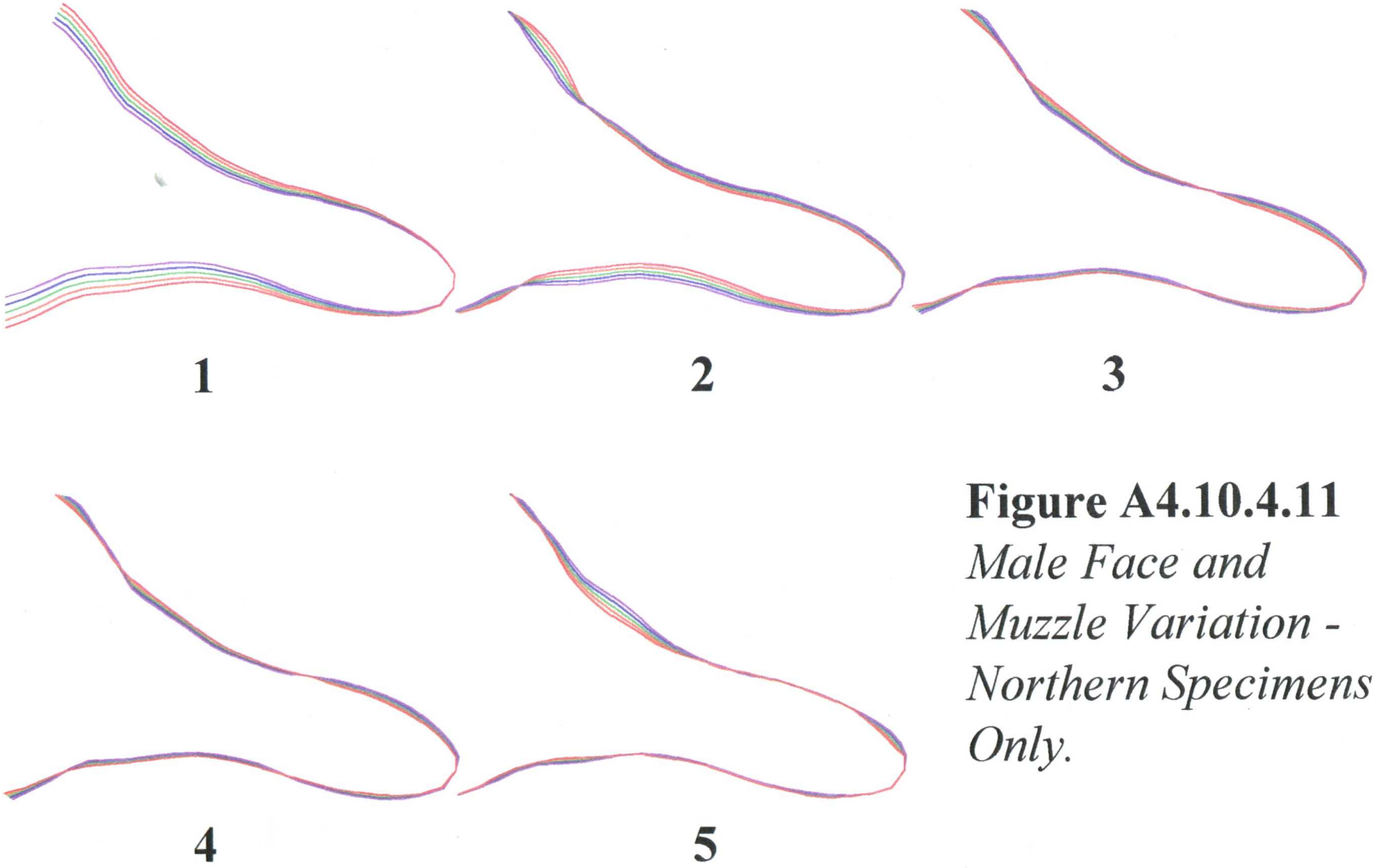


Figure A4.10.4.11
Male Face and Muzzle Variation - Northern Specimens Only.

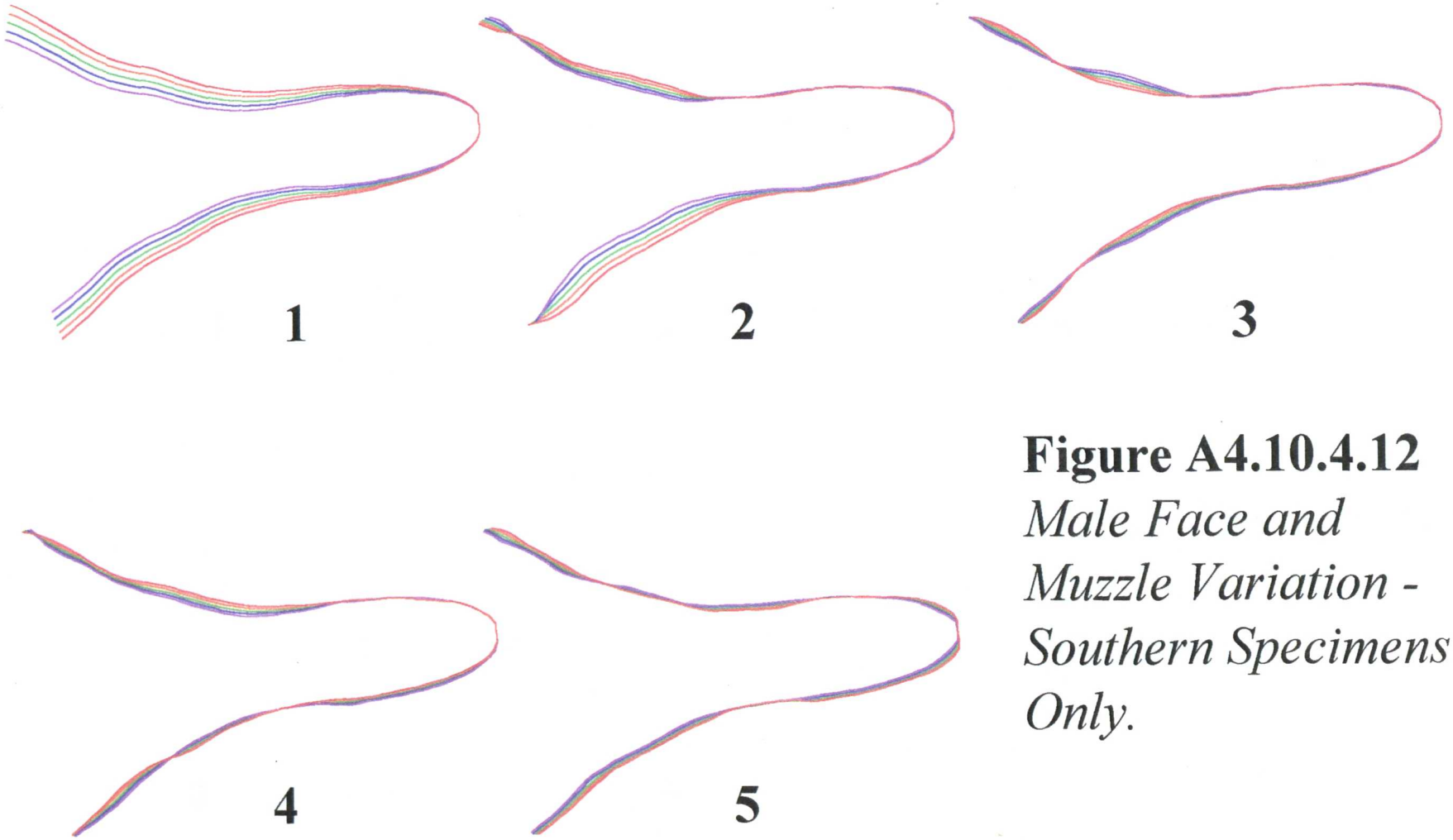


Figure A4.10.4.12
Male Face and Muzzle Variation - Southern Specimens Only.

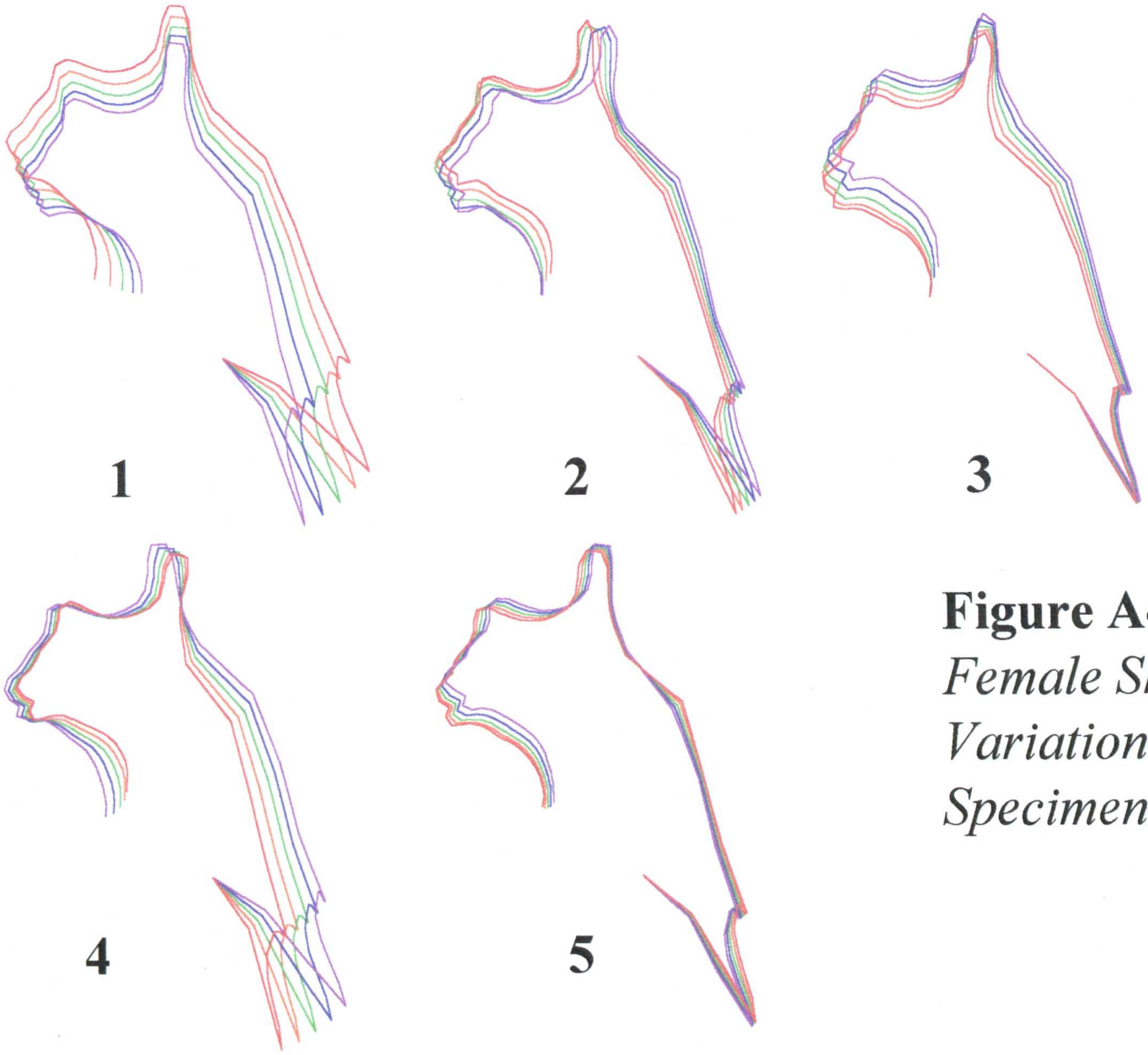


Figure A4.10.4.13
Female Skull Profile Variation - All Specimens.

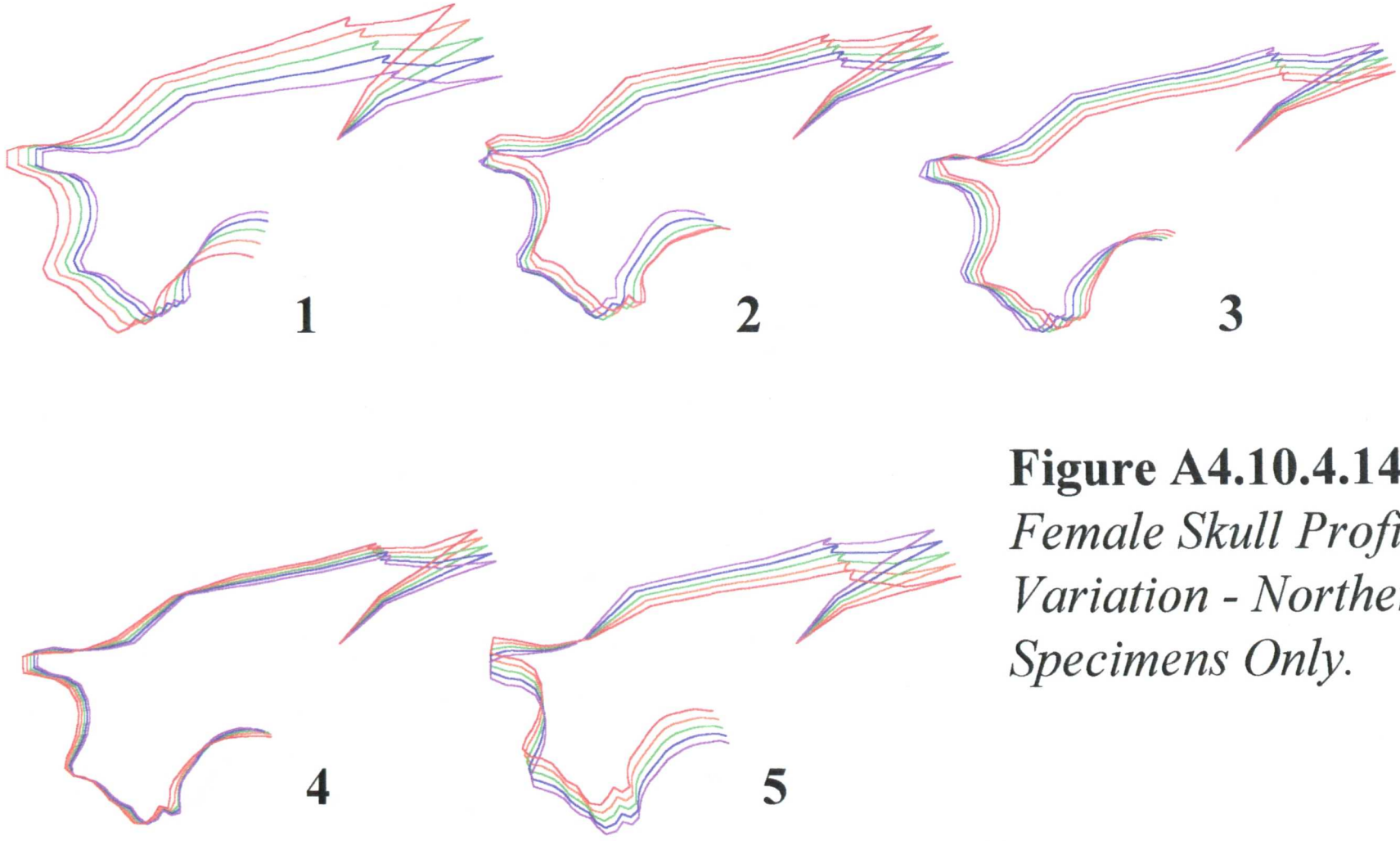


Figure A4.10.4.14
Female Skull Profile Variation - Northern Specimens Only.

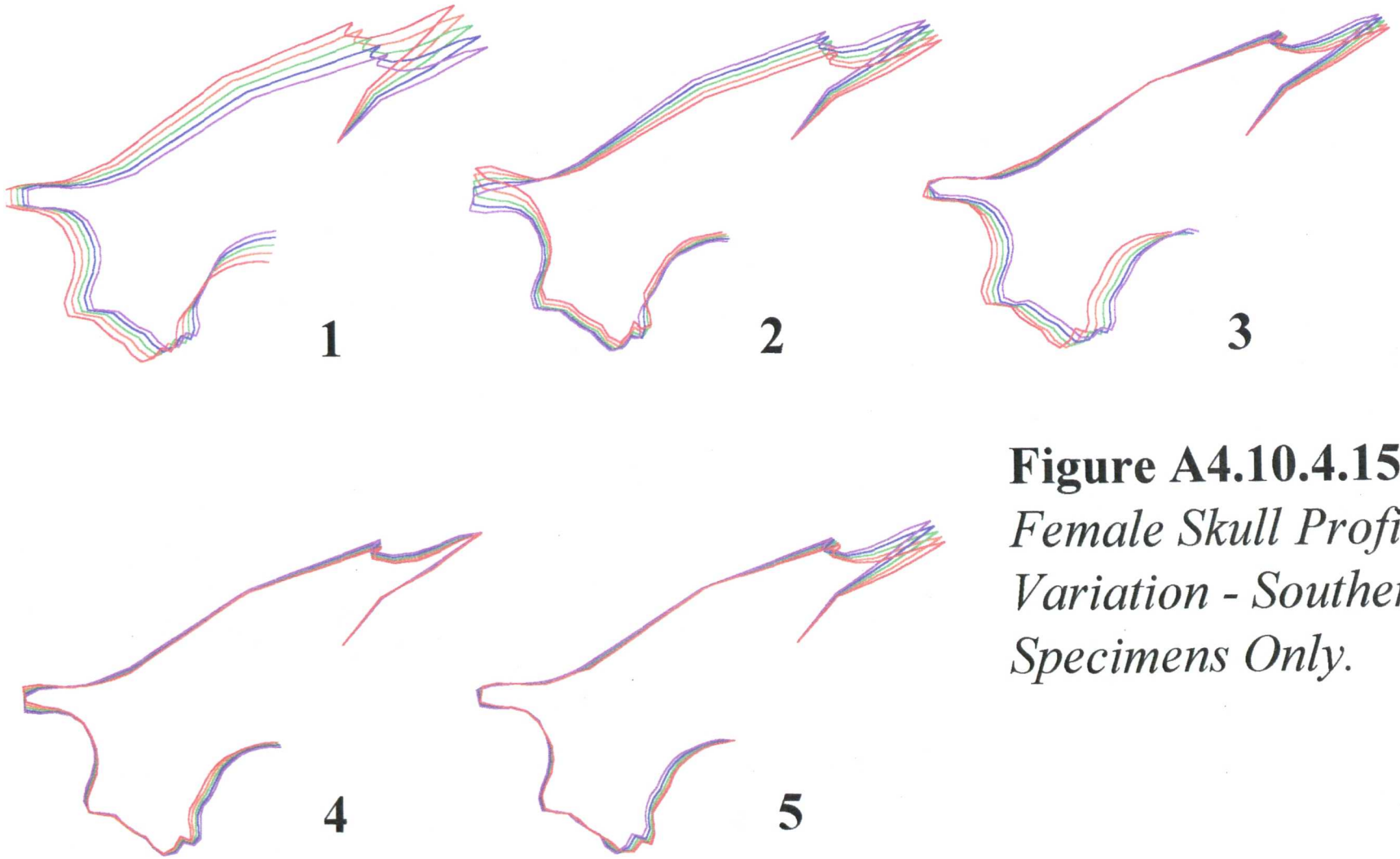


Figure A4.10.4.15
Female Skull Profile Variation - Southern Specimens Only.

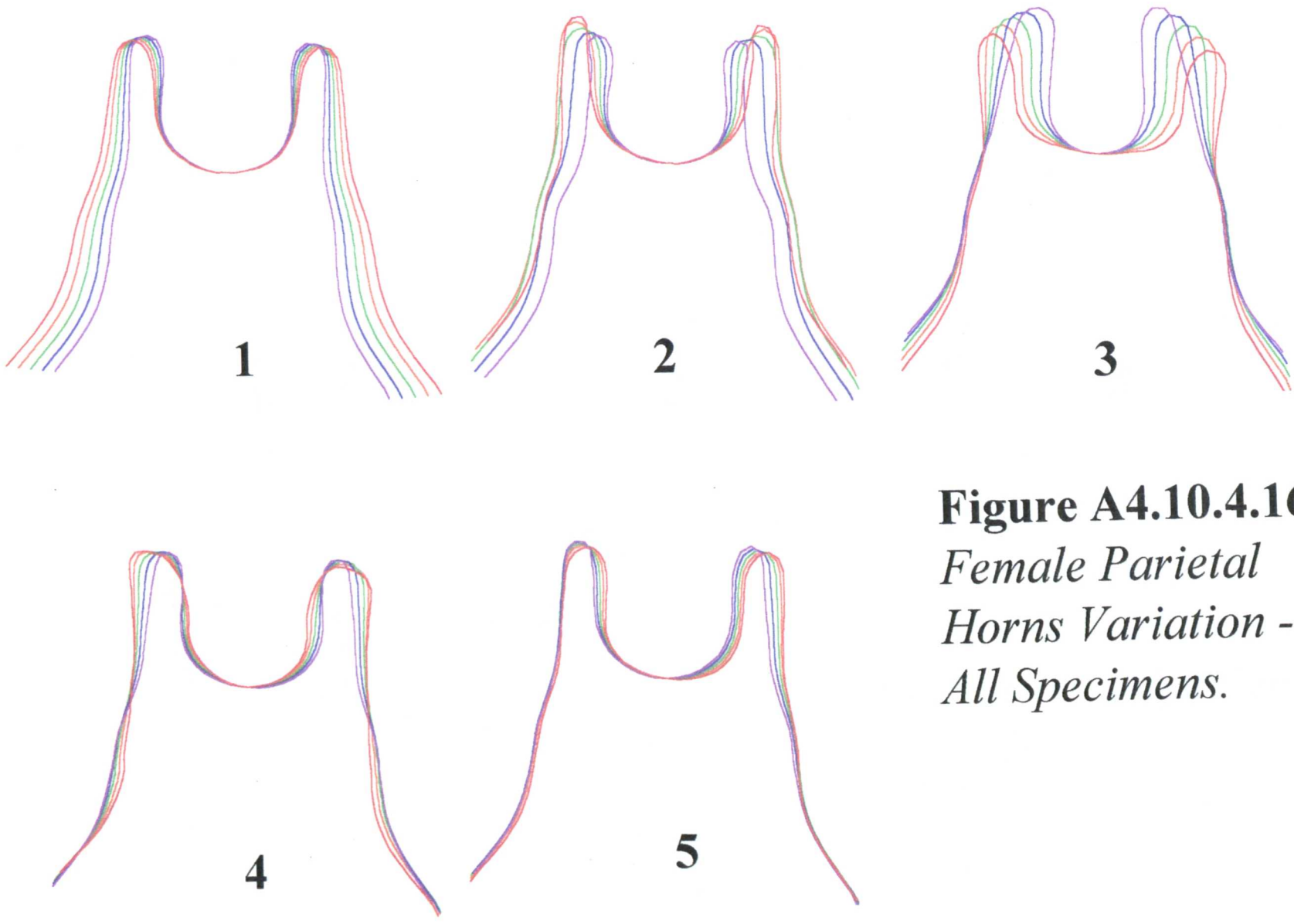


Figure A4.10.4.16
Female Parietal Horns Variation - All Specimens.

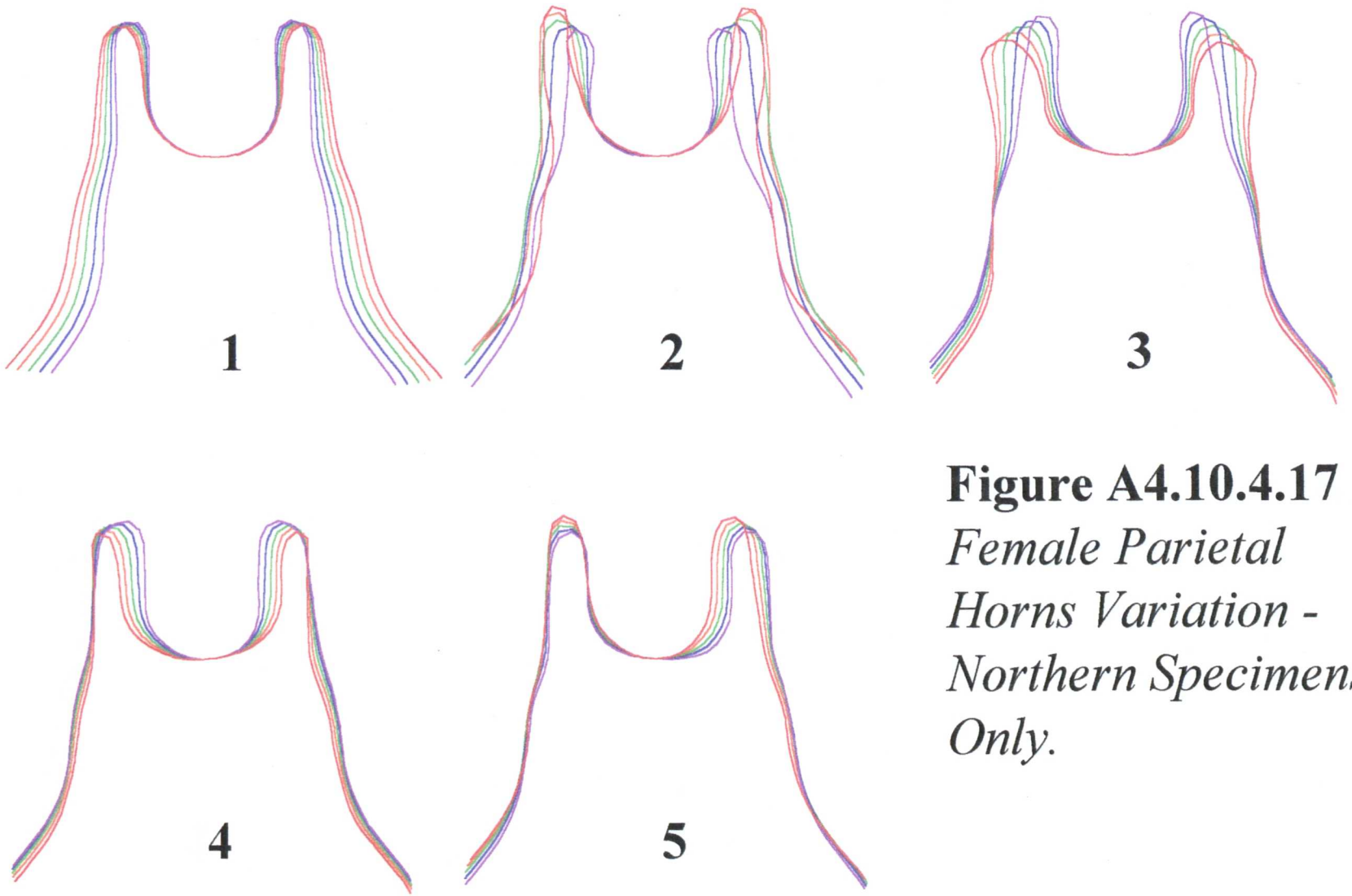


Figure A4.10.4.17
Female Parietal Horns Variation - Northern Specimens Only.

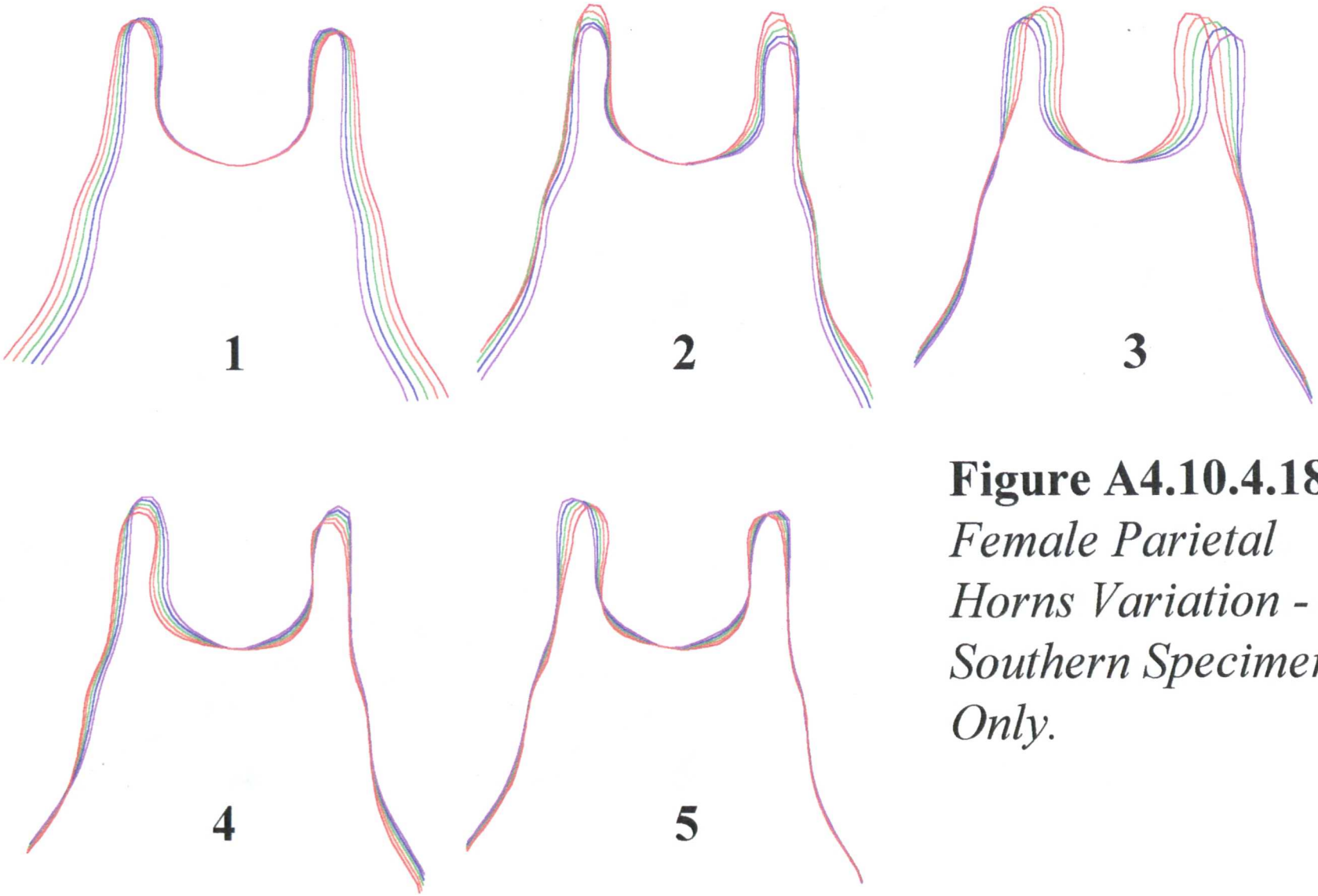


Figure A4.10.4.18
Female Parietal Horns Variation - Southern Specimens Only.

Figure A4.10.4.19
Female Face and Muzzle Variation - All Specimens.

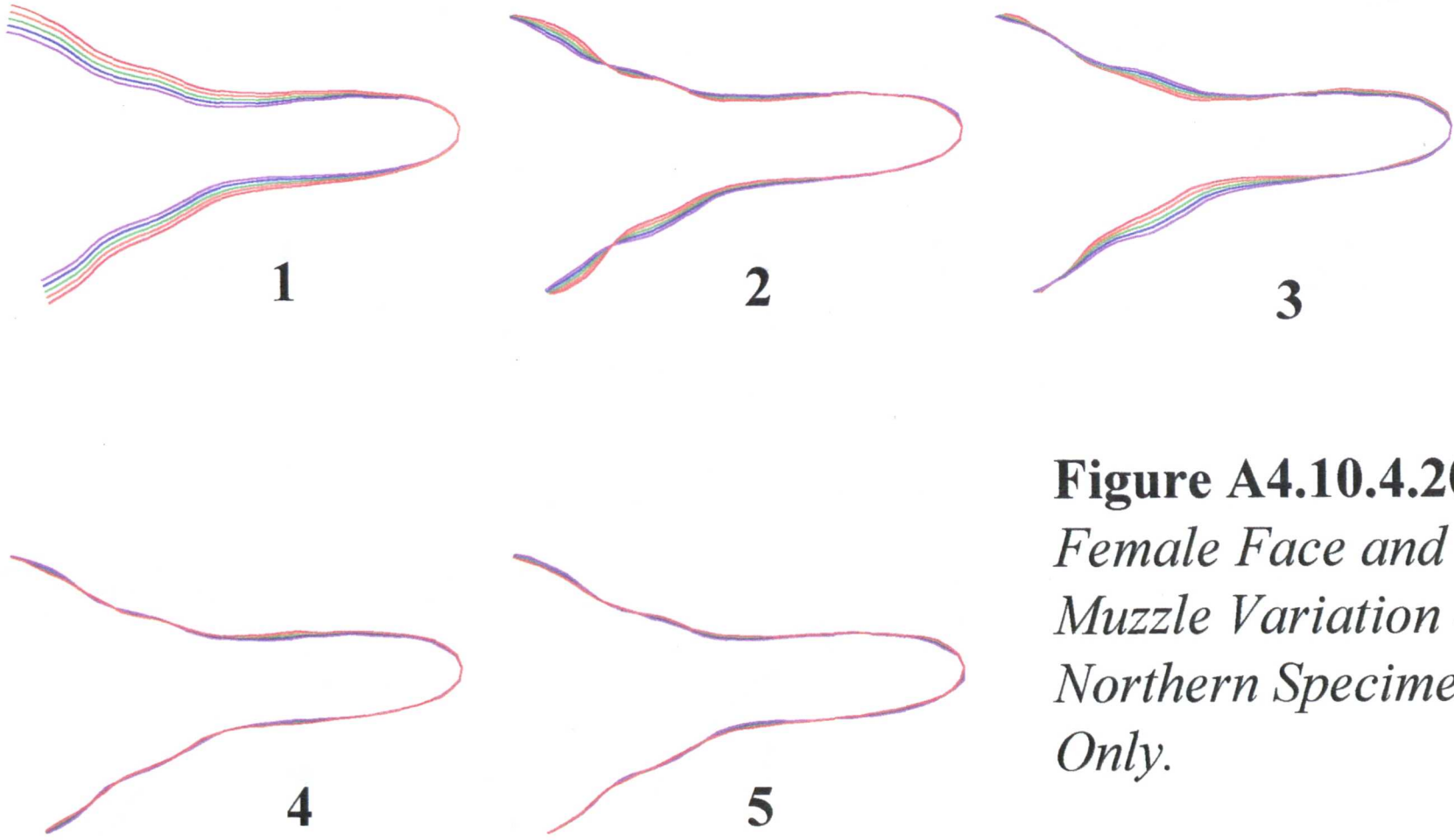


Figure A4.10.4.20
Female Face and Muzzle Variation - Northern Specimens Only.

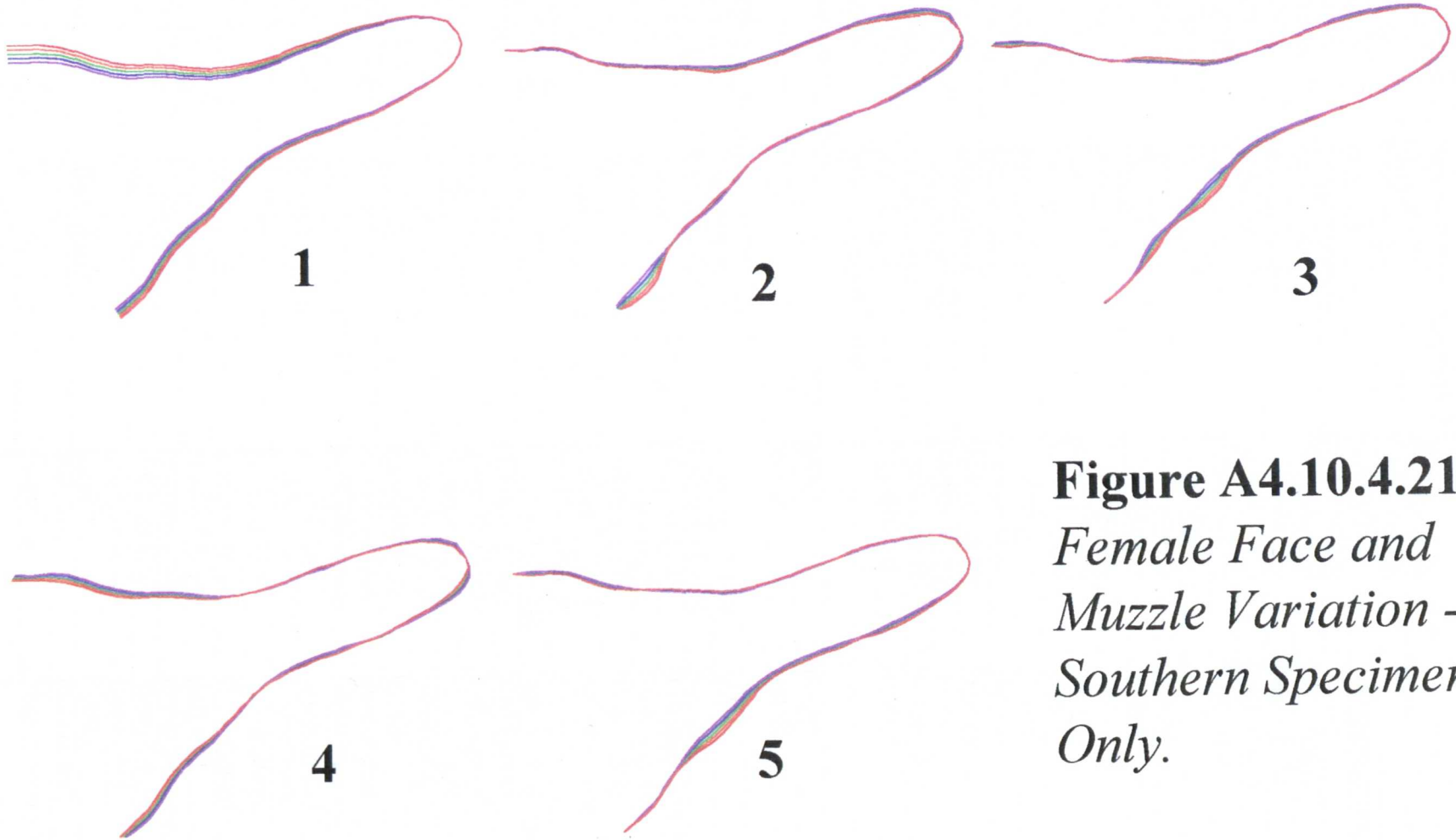


Figure A4.10.4.21
Female Face and Muzzle Variation - Southern Specimens Only.

APPENDIX 5.11.1:

TISSUE SAMPLES OBTAINED

Institution / Specimen	Source	Institution / Specimen	Source
American Museum of Natural History, New York, USA.			
AMNH24290	Base of skull	AMNH53550a	?
AMNH24292	Left parietal horn	AMNH53550b	?
AMNH24293	Base of skull	AMNH54122a	Left zygomatic arch
AMNH27752a	End of premaxillae	AMNH54122b	Various
AMNH27752b	Head of Humerus	AMNH54122c	Off vertebrae
AMNH27752c	Left radius/ulna	AMNH54123a	Below EAM
AMNH27753a	Below left EAM	AMNH54123b	Back of skull
AMNH27753b	Below right EAM	AMNH54123c	Premaxillae
AMNH53543a	Below nasals	AMNH82001	?
AMNH53543b	Parietals	AMNH82002a	Sternum
AMNH53543c	Around EAMs	AMNH82002b	Sternum
AMNH53544a	Base of rib	AMNH82002c	Ribs
AMNH53544b	Ribs	AMNH82003a	Incisors
AMNH53546a	Parietals	AMNH82003b	Ribs
AMNH53546b	Below left EAM	AMNH82003c	Sternum
AMNH53546c	Below nasals	AMNH83458a	Sternum
AMNH53548a	Base of braincase	AMNH83458b	Sternum
AMNH53548b	Inside nasal cavity	AMNH83460a	Incisors
AMNH53549a	Inside braincase	AMNH83460b	Below Eams
AMNH53549b	Base of skull	AMNH83605	Incisors
AMNH53549c	Off ethmoturbinal bone		
Bushmanland, Namibia.			
BML1	Skull?	BML3	Skull?
BML2	Skull?	BML4	Skull?
Natural History Muscum, London, UK.			
BM1898.4.28.1a	Parietal horns	BM1907.7.8.225b	EAMs
BM1898.4.28.1b	Maxillary teeth	BM1907.7.8.255a	Left orbit
BM1898.7.2.5a	Premax / maxillary suture	BM1912.2.24.2	Left maxillary toothrow
BM1898.7.2.5b	EAMs	BM1919.11.27.1	Back of skull
BM1898.7.2.6a	Orbits	BM1923.1.17.8	Under EAMs
BM1898.7.2.6b	Maxillary toothrow	BM1923.10.20.8	Incisiform teeth
BM1899.12.10.2a	Maxillary teeth	BM1923.10.20.19	General
BM1899.12.10.2b	Base of skull	BM1928.11.11.24a	Base of skull / suture
BM1899.7.8.5a	Right orbit	BM1931.2.1.48a	Under EAMs
BM1899.7.8.5b	Maxillary teeth	BM1931.2.1.48b	Left zygoma
BM1900.3.18.3	Back of skull	BM1938.7.8.21a	Base of skull
BM1901.5.14.1	Right EAM	BM1938.7.8.21b	Nasal cavity
BM1901.8.9.47a	Left orbit	BM1938.7.8.22	EAMs
BM1901.8.9.47b	EAMs	BM1939.4329Aa	Base of skull
BM1901.8.9.49	Base of skulls / EAMs	BM1939.4329Ab	Inside braincase
BM1901.8.9.50a	Inside braincase	BM1962.220	Inside right M^3
BM1901.8.9.50b	EAMs	BM1964.225a	Back of skull
BM1902.11.12.1a	Base of skull	BM1964.225b	Premaxillaries
BM1902.11.12.1b	Maxillary teeth	BM1964.225c	Right zygoma
BM1903.4.16.1a	Premaxillaries	BM1966.429a	Left orbit
BM1903.4.16.1b	Zygomatic arch	BM1966.429b	Inside braincase
BM1903.4.16.1c	Back of skull	BM1966.429c	Base of skull
BM1904.11.2.2	EAMs	BM1986.1604	Top of skull
BM1906.2.12.1	Back of skull	BM671a	EAMs
BM1906.10.26.1	EAMs	BM671ca	Base of skull
BM1907.2.4.15a	Base of skull / EAMs	BM671cb	skin - median horn
BM1907.2.4.15b	Left maxillary toothrow		

Etosha Ecological Institute, Namibia.			
EEI1b	Nasal cavity	EEI7b	Base of skull
EEI1c	Skin - parietal horns	EEI7c	Left maxilla
EEI2a	Left maxilla	EEI8a	Soft palate
EEI2b	Palate	EEI8b	Base of skull
EEI2c	Nasal cavity	EEI8c	Inside condyles
EEI3a	Base of skull	EEI9a	Right pterygoid
EEI3b	Left orbit	EEI9b	Brain case
EEI3c	Skin - face	EEI9c	Left maxillary
EEI4a	Base of skull	EEIGc94.11.25.6MAa	Gum
EEI4b	Back of skull	EEIGcb	Mandible - left ramus
EEI4c	Skin - back of skull	EEIGcc	Left premaxilla
EEI5a	Brain case	Etosha1a	Gum
EEI5b	Back of skull	Etosha1b	Left EAM
EEI5c	Right orbit	Etosha1c	Skin
EEI5d	Skin - right orbit	Etosha1d	Humerus
EEI6a	Gum	Etosha2a	Mandible
EEI6b	Base of skull	Etosha2b	Gum
EEI6c	Nasal cavity and septum	Etosha2c	Base of skull
EEI6d	Below left orbit	Etosha2d	Left orbit
EEI7a	Gum		
Field Museum of Natural History, Chicago, USA.			
FMNH27475	Base of skull	FMNH53765b	Radius/ulna
FMNH29515a	Inside braincase	FMNH54251	Below parietal horn
FMNH32901a	Inside braincase	FMNH127878a	Inside right PM^3
FMNH32901b	Base of skull	FMNH127878b	Base of skull
FMNH32902a	?	FMNH127878c	Top of skull
FMNH32904a	Inside braincase	FMNH127878d	Humerus
FMNH32904b	Incisiform teeth	FMNH127879a	Tip of left premaxilla
FMNH32905a	Base of skull	FMNH127879b	Inside canines
FMNH34422a	Inside hoof	FMNH127880a	In and around right EAM
FMNH34422b	Inside hoof	FMNH127880b	Inside nasal cavity
FMNH34423a	Inside braincase	FMNH127880c	Mandibular symphysis
FMNH34423b	Inside hoof	FMNH127880d	Inside incisors
FMNH34424a	Inside braincase	FMNH127881a	Inside right PM^3
FMNH34424b	Unknown origin	FMNH127881b	Top of skull
FMNH34424c	Inside hoof	FMNH127882a	Base of skull
FMNH34425a	Inside hoof	FMNH127882b	Top of skull
FMNH34425b	Inside hoof	FMNH127882c	Left orbit
FMNH34426	Sternum	FMNH127883a	Top of foramen magnum
FMNH34427	Inside braincase	FMNH127883b	Humerus
FMNH34428a	Inside braincase	FMNH127884	Premaxillae
FMNH34428b	Side of skull	FMNH127885a	Inside braincase
FMNH34428c	Right EAM	FMNH127885b	Right dentary
FMNH34428d	Right orbit	FMNH127885c	Base of skull
FMNH34429	Inside hoof	FMNH127886a	Top of skull
FMNH34930a	Cartilage(?)	FMNH127886b	Base of skull
FMNH34930b	Cartilage(?)	FMNH127886c	Gum tissue
FMNH34930c	Cartilage(?)	FMNH127886d	Inside teeth
FMNH53765a	Right femur	FMNH127887	Ribs
Kruger National Park, South Africa.			
KNP1a	Back of skull	KNP3b	Inside cervical vertebra
KNP1b	Palate	KNP3c	Base of Skull
KNP1c	Right maxilla	KNP4a	Base of Skull
KNP1d	Skin	KNP4b	Inside cervical vertebra
KNP2a	Back of skull	KNP4c	Right zygoma
KNP2b	Palate	KNP5a	Inside cervical vertebra
KNP2c	Left orbit	KNP5b	Occipital ridge
KNP3a	Base of skull	KNP5c	Left occipital condyle

National Museum of Natural History, Bulawayo, Zimbabwe.			
NMZB11525a	Back of skull	NMZB26200a	Base of skull
NMZB11525b	Back of skull / parietals	NMZB26200b	Inside brain case
NMZB11525c	Below premaxillae	NMZB26200c	Top and back of skull
NMZB11533a	Base and back of skull	NMZB27144a	Back of skull
NMZB11533b	Nasal cavity	NMZB27144b	Nasal cavity
NMZB11533c	Left M^3	NMZB27144c	Mandibular nerve foramen
NMZB11544a	Inside braincase	NMZB29099a	Base and back of skull
NMZB11544b	Inside brain case	NMZB29099b	Below premaxillae
NMZB11544c	Base of skull	NMZB29100a	Base of skull
NMZB20221a	EAMs	NMZB29101a	Inside brain case
NMZB20221b	Base of skull and jaw	NMZB29101b	Back and base of skull
NMZB20221c	Inside right M^3	NMZB29102a	Back of skull
NMZB20382a	Inside braincase	NMZB29102b	Base of skull
NMZB20382b	Inside brain case	NMZB29103a	Between teeth
NMZB20382c	Base of skull	NMZB29103b	Nerve foramen on mandible
NMZB22635a	Back of skull	NMZB29103c	Around incisiform teeth
NMZB22635b	Back of skull	NMZB29111a	Inside brain case
NMZB22635c	Back of skull	NMZB29111b	Incisiform teeth
NMZB22862a	Inside brain case	NMZB29111c	Base of skull
NMZB22862b	Back of skull	NMZB29112a	Base of skull
NMZB22947	Between teeth	NMZB29112b	Back of skull
NMZB22957a	Inside brain case	NMZB29113a	Base of skull
NMZB22957b	Base of skull	NMZB29114	?
NMZB22958	Around teeth	NMZB29118	EAMs
NMZB22959a	Around left orbit	NMZB29121Fa	Base of skull
NMZB22959b	Back of parietals	NMZB29121Fb	Inside brain case
NMZB23179b	Base of skull	NMZB58342	Top of skull
NMZB23179c	Base of skull	NMZB58343	Skull
NMZB23978a	Inside brain case	NMZB60801a	Inside brain case
NMZB23978b	Back of skull	NMZB60801b	Base of skull
NMZB23979a	Inside braincase	NMZB60802a	Inside occipital condyles
NMZB23979b	Back of skull	NMZB60802b	Occipital ridge
NMZB23980a	Mandibular symphysis	NMZB60803a	On skull
NMZB23983b	Inside brain case	NMZB60803b	Inside brain case
NMZB23983c	Back of skull	NMZB60803c	Base and back of skull
NMZB23984a	Blood from bullet hole	NMZB60803d	Nasal cavity
NMZB23984b	Base of skull	NMZB60804a	Around foramen magnum
NMZB23984c	Inside brain case	NMZB60804b	Base and back of skull
NMZB26177	Base of skull	NMZB60805a	Inside brain case
NMZB26178a	Nerve foramen and base of skull	NMZB60805b	Base of skull
NMZB26178b	Base of skull	NMZB60805c	Brain case / nasal cavity
NMZB26179a	Nasal cavity	NMZB60806a	Inside brain case
NMZB26180a	Around incisiform teeth	NMZB60806b	Back of skull
NMZB26180b	Back of skull	NMZB60807a	Back of skull
NMZB26184a	Back and base of skull	NMZB60807b	EAM
NMZB26184b	Base of skull	NMZB60807c	Nasal cavity
NMZB26185a	Inide occipital condyles	NMZB60809a	Inside brain case
NMZB26185b	Base of skull	NMZB60809b	Base of skull
NMZB26185c	Incisiform teeth	NMZB60810a	Inside brain case
NMZB26186a	Base of skull	NMZB60810b	Base of skull
NMZB26186b	Back of skull	NMZB60811a	Base of skull
NMZB26187	Back of skull	NMZB60811b	Base of skull
NMZB26188a	Back and base of skull	NMZB60812	Inside brain case
NMZB26188b	Parietal horns	NMZB60813a	Right zygoma
NMZB26189a	Back and base of skull	NMZB60813b	Base of skull
NMZB26189b	Right premaxilla	NMZB60813c	Left parietal

Powell-Cotton Museum, Birchington, Kent, UK.			
PCCongoNoNuma	Left orbit	PCMN579b	Base of skull
PCCongoNoNumb	Back and base of skull	PCMN579c	Inside braincase
PCCongoNoNumc	Right EAM	PCMN579d	Base of skull
PCCongoNoNumd	Nasal septum	PCNNChad138a	Below right EAM
PCCongoNoNume	Base of skull	PCNNChad138b	Below left EAM
PCMN278	Side of skull	PCNNChad138c	Back of skull
PCMN280	Foramen magnum	PCTan76	Above left orbit
PCMN579a	Inside braincase		
Transvaal Museum, Pretoria, South Africa.			
TM12141a	Side of skull	TM12141b	Occipital ridge
United States National Museum, Washington D.C.			
USNM121010a	Back of skull	USNM182124b	EAM
USNM121010b	Base of skull	USNM182124c	Right parietal
USNM154033a	Nasal sinus	USNM182125	Occipital ridge
USNM154033b	Parietals	USNM182192	All over skull
USNM155438a	Inside braincase	USNM200151a	Inside braincase
USNM155438b	Back of skull	USNM200151b	Inside left PM^4
USNM162016	Right pterygoid	USNM200151c	Horns and back of skull
USNM162017	Base of skull	USNM251797a	Inside braincase
USNM162018	Mandibular symphysis	USNM251797b	Base of skull
USNM162988	Inside braincase	USNM251797c	Back of skull
USNM162989	Back of skull	USNM251798a	Back of skull
USNM163112	Right zygoma	USNM251798b	Base of skull
USNM163113a	Sternum	USNM251798c	Mandible
USNM163113b	Sternum	USNM251799a	Inside braincase
USNM163312a	Left EAM	USNM251799b	Base of skull
USNM163312b	Right frontal	USNM251799c	Palatine
USNM163312c	Median horn	USNM251800a	Back of skull
USNM163324a	Palatine suture	USNM251800b	Base of skull
USNM163324b	Tip of right nasal	USNM251800c	Mandible
USNM182124a	Palatine / basisphenoid suture		
South African Museum, Cape Town, South Africa.			
ZM17176a	Skull	ZM39692a	EAM
ZM17176b	Iliac crest	ZM39692b	Inside brain case
ZM37058	Mandibular nerve foramen	ZM39692c	Nerve foramen

APPENDIX 5.11.2:

HAPLOTYPE GROUP COMPOSITION AND GEOGRAPHICAL PROVENANCE OF SPECIMENS.

Haplotype Group	n	Specimens	Provenance	Group	Lat.	Long.
Haplotype 1	25	A575	Kanyu, Botswana.	SWC	24°59's	25°20'E
		A1558	Nuanetsi, Zimbabwe.	SWC	21°25's	30°43'E
		A1559	Nuanetsi, Zimbabwe.	SWC	21°25's	30°43'E
		AMNH83548	Mabebe Flats, Botswana.	SWC	19° s	24° E
		AMNH83460	Mabebe Flats, Botswana.	SWC	19° s	24° E
		AMNH83605	Ngamo Station, Zimbabwe.	SWC	19° 5' s	27° 28' E
		BMNH1931.2.1.48	Mabebe Flats, Botswana.	SWC	19° s	24° E
		BMNH1939.4329A	Ngamiland, Botswana.	SWC	N/A	N/A
		FMNH34426	Mabebe Flats, Botswana.	SWC	19° s	24° E
		KNP1	Kruger N.P., South Africa.	SEW	24° 59' s	31° 36' E
		KNP2	Kruger N.P., South Africa.	SEW	24° 59' s	31° 36' E
		KNP4	Kruger N.P., South Africa.	SEW	24° 59' s	31° 36' E
		NMZB29101	Wankie N.P., Zimbabwe.	SWC	19° 5' s	27° 28' E
		NMZB29103	Tamafupi, Botswana.	SWC	N/A	N/A
		NMZB29111	Chobe G. R., Botswana.	SWC	18° 30' s	24° 30' E
		NMZB29112	Chobe G. R., Botswana.	SWC	18° 26' s	24° 51' E
		NMZB29114	Mohembo, Botswana.	SWC	18° 20' s	21° 0' E
		NMZB29118	Wankie / Matopos N. P. Zimbabwe,	SWC	N/A	N/A
		NMZB60801	Chobe G. R., Botswana.	SWC	18° 30' s	24° 30' E
		NMZB60805	Chobe G. R., Botswana.	SWC	18° 30' s	24° 30' E
		NMZB60806	Chobe G. R., Botswana.	SWC	18° 30' s	24° 30' E
		NMZB60807	Chobe G. R., Botswana.	SWC	18° 30' s	24° 30' E
		NMZB60812	Lake McIlwaine N. P., Zimbabwe.	Unass.	17° 54' s	30° 48' E
		USNM154033	Matabeleland, Zimbabwe.	SCZ	N/A	N/A
		ZM37058	Transvaal, South Africa.	SEW	23° 56' s	31°9' E
Haplotype 2	2	A2253	Okavango, Botswana.	SWC	19°24's	22°42'E
		BML3	Bushmanland, Namibia.	SWC	20°s	21°E
Haplotype 3	20	A2318	Kamanjab, Namibia.	SWC	19°37's	14°50'E
		BMNH1966.429	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		EEI1	Etosha N. P., Namibia.	SWC	19° 10' s	15° 55' E
		EEI4	Etosha N. P., Namibia.	SWC	19° 10' s	15° 55' E
		EEI6	Etosha N. P., Namibia.	SWC	19° 10' s	15° 55' E
		EEI9	Etosha N. P., Namibia.	SWC	19° 10' s	15° 55' E
		Etosha1	Leeubos, Etosha N. P., Namibia.	SWC	19° 2' s	15° 49' E
		NMZB26177	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26179	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26180	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26184	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26185	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26186	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26187	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26188	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26189	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB26200	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB29100	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB29102	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
		NMZB60809	Doddieburn Ranch, Zimbabwe.	SCZ	21° 10' s	29°22' E
Haplotype 4	9	A3369	Lake Rukwa, Tanzania.	EST	8°s	32°E
		FMNH127878	Serengeti, Tanzania.	ESK	2° 25' s	34° 5' E
		FMNH127879	Serengeti, Tanzania.	ESK	2° 25' s	34° 5' E
		NMZB11533	Luangwa Valley, Zambia	SZT	13° 30' s	31° 30' E
		NMZB20221	Luangwa Valley, Zambia	SZT	13° 30' s	31° 30' E

Haplotype Group	n	Specimens	Provenance	Group	Lat.	Long.
		NMZB22862	Luangwa Valley, Zambia	SZT	13° 30' S	31° 30' E
		NMZB22957	Luangwa Valley, Zambia	SZT	13° 30' S	31° 30' E
		NMZB22959	Luangwa Valley, Zambia	SZT	13° 30' S	31° 30' E
		NMZB29099	Luangwa Valley, Zambia	SZT	13° 30' S	31° 30' E
Haplotype 5	4	AMNH24292	Tuley, Zimbabwe.	SWC	21° 55' S	29° 12' E
		BMNH1938.7.8.21	Balahuti, Angola.	SWA	16° 30' S	16° 55' E
		BMNH1986.1604	West Nicholson, Zimbabwe.	SCZ	21° 3' S	29° 20' E
		NMZB29113	Ngezumba, Botswana.	SWC	18° 26' S	24° 51' E
Haplotype 6	3	AMNH27752	Naungu, Kenya.	Unass.	N/A	N/A
		AMNH27753	Komarock, Kenya.	ESK	1°18' S	37° 13' E
		USNM162016	Kapiti Plateau, Kenya.	ESK	1° 38' S	37° 0' E
Haplotype 7	9	AMNH53543	Oriental Faradje, DRC.	ECC	3° 44' N	29° 43' E
		AMNH53548	Garamba, DRC.	ECC	3°37' N	28° 35' E
		AMNH53550	Garamba, DRC.	ECC	3°37' N	28° 35' E
		BMNH1902.11.12.1	Kodok, Sudan.	ENA	9° 53' N	32° 4' E
		BMNH1906.2.12.1	Yola, Nigeria.	WSN	9° 11' N	12° 30' E
		BMNH1907.7.8.255	Chari River, Cameroon.	WCP	12° 48' N	14° 34' E
		BMNH1938.7.8.22	Bi-Indu, Cameroon.	WCP	8° 25' N	14° 30' E
		PCMN579	Lado, Sudan.	ENA	4° 55' N	31° 30' E
Haplotype 8	8	AMNH54123	Northern Guasanyra, Kenya.	EEK	1° 10' N	39° 0' E
		BM1898.4.28.1	North East Africa	Unass.	N/A	N/A
		BMNH1912.2.24.2	Archer's Post, Kenya.	EEK	0° 39' N	37° 41' E
		BMNH1923.10.20.8	Jubaland.	EEK	N/A	N/A
		BMNH1923.10.20.19	Jubaland.	EEK	N/A	N/A
		FMNH32902	Sidamo, Ethiopia.	EEK	5° 23' N	37° 56' E
		FMNH32904	Sidamo, Ethiopia.	EEK	5° 23' N	37° 56' E
		USNM163324	Guaso Nyiro, Kenya.	EEK	0° 30' N	37° 30' E
Haplotype 9	2	BMNH1897.1.30.1	Loroghi, Kenya.	EEK	N/A	N/A
		BMNH1899.7.8.5	Loroghi, Kenya.	EEK	N/A	N/A
Haplotype 10	3	FMNH127884	Cherangani Hills, Kenya.	ECU	1° 10' N	35° 20' E
		PCMN280	Sirgoi Rock, Kenya.	ECU	1° 0' N	35° 20' E
		USNM155438	Guas Ngishu Plateau, Kenya.	ECU	1° N	35° E
Haplotype 11	3	PCTan76	Engaruka, Tanzania.	ESK	2° 55' S	35° 0'E
		USNM162018	Uln Station, Kenya.	Unass.	N/A	N/A
		USNM251799	Mkata Plains, Tanzania.	EST	7° 0' N	37° 30' E
Unique Haplotypes	11	A1236	Kidepo Valley National Park, Uganda.	ECU	3°50'N	33°45'E
		AMNH53546	Garamba, DRC.	ECC	3° 37' N	28° 35' E
		BMNH1898.7.2.5	Mombasa, Kenya.	ESK	3° 59' S	39° 40' E
		EEI2	Etosha N. P., Namibia.	SWC	19° 10' S	15° 55' E
		EEIGc94.11.25.6MA	Etosha N. P., Namibia.	SWC	19° 10' S	15° 55' E
		FMNH27475	Northern Uganda.	ECU	N/A	N/A
		FMNH127880	Serengeti, Tanzania.	ESK	2° 25' S	34° 5' E
		PCNNChad138	Ubangi-Chari, Chad.	WCP	9° 35' N	19° 8' E
		USNM162017	Kapiti Plateau, Kenya.	ESK	1° 38' S	37° 0' E
		USNM251797	Savanda, Tanzania.	EST	6° 10' S	35° 45' E
		USNM251798	Mkese, Tanzania.	EST	5° 19' S	34° 26' E

APPENDIX 5.11.3:

GIRAFFE CONTROL REGION SEQUENCES

Giraffe Control Region sequences for the eleven haplotypes and eleven unique sequences. The members of each haplotype are listed in Appendix 5.11.2.

In the first list all sequences are referenced to the sequence for Haplotype01. Identical bases at the respective positions are indicated by a full stop (.).

In the second list complete sequences are given for all haplotypes with bases colour coded (A, C, G,) and variable sites shaded with yellow highlighting indicating a parsimoniously informative site and grey indicating an autapomorphic change. (Note that where a change is unique to one of the haplotype groups it is autapomorphic in terms of the analysis but synapomorphic, and characteristic, for the haplotype group.)

In both lists bases are numbered from 1 to 263, simply indicating the position in the sequenced segment. These numbers are used in the text. The sequenced segment corresponds to bases 434 to 696 of the 1002bp d-loop sequence of the giraffe. (As compared to Genbank sequence AF151090).

Each list provides the same information, formatted in a different way.

Sequence List 1:

```
                1         10         20         30         40         50    55
                |         |          |          |          |          |     |
Haplotype01     AACGCTATTAATATACTCCCACAAACACCAAGAACCCCATCAGTATTAAATTTTC
Haplotype02     ..........................................................
Haplotype03     ..........................................................T
Haplotype04     ....................................G.....................
Haplotype05     ..........................................................T
Haplotype06     ....................................T.....................T
Haplotype07     ..................A.....................T.....................T
Haplotype08     ....................................T.....................T
Haplotype09     ....................................T.....................T
Haplotype10     ....................................T.....................T
Haplotype11     ..........................................................
A1236           ....................................T.....................T
AM53546         ..................A.....................T.....................T
BM1898.7.2.5    ..........................................................
EEI2            ...................................T.....................T
EEIGc           ..........................................................T
FM127880        ................C.........................................
FM27475         ....................................T.....................T
PCNNChad138     ..................A.....................T.....................T
USNM162017      ..........................................................
USNM251797      ....................................G.....................
USNM251798      ....................................G.....................
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                    60         70         80         90        100        110
                     |          |          |          |          |          |
Haplotype01    CAAAAACCTACAACGACCAACACAGACTTCACACCCCACAGCCTAACATGTAATA
Haplotype02    ....................................................
Haplotype03    ......T.....................T..............G.A.....
Haplotype04    ....................................................
Haplotype05    ............................T..............G.A.....
Haplotype06    ..............T............T......T....C...G.A.....
Haplotype07    ..............T............T..............G.A.....
Haplotype08    ..............T............T......T.........AC....
Haplotype09    ............T...T.............................G.A.....
Haplotype10    .........T......T............T..............G.A.....
Haplotype11    ....................................................
A1236          ..............T............T..............G.A.....
AM53546        ..............T............T..............G.A.....
BM1898.7.2.5   ....................................................
EEI2           ............................T..............G.A.....
EEIGc          ......T.....................T..............G.A.....
FM127880       ..................................G.................
FM27475        ..............T............T......T........G.A.....
PCNNChad138    ..............T............T.....G........G.A.....
USNM162017     ....................................................
USNM251797     ........C...........................................
USNM251798     ....................................................

                111    120        130        140        150        160  165
                 |      |          |          |          |          |    |
Haplotype01    AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype02    ....................................................
Haplotype03    .....G............T.................................
Haplotype04    ....................................................
Haplotype05    .....G............T.................................
Haplotype06    ..C.................................................
Haplotype07    ..C..G........A.....................................
Haplotype08    ..C..G.........................C....................
Haplotype09    ..C..G..............................................
Haplotype10    ..C..G........A...C.............C....................
Haplotype11    ....................................................
A1236          ..C..G........A...C.............C....................
AM53546        ..C..G........A.....................................
BM1898.7.2.5   ....................................................
EEI2           .....G.....,.....T.................................
EEIGc          .....G............T.................................
FM127880       ....................................................
FM27475        ..C.....................................C...........
PCNNChad138    ..C..G........A.....................................
USNM162017     ....................................................
USNM251797     ....................................................
USNM251798     ....................................................
```

```
                  170        180        190        200        210        220
                   |          |          |          |          |          |
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Haplotype02       ........CT.....................................................
Haplotype03       ....G..GCT....T....C.....................................G.....
Haplotype04       .......GCT....T....C.....................................G.....
Haplotype05       ....G..GCT....T....C.....................................G.....
Haplotype06       .......GTT....T.T..C.....................................G.....
Haplotype07       .......GCT....T.............C.....................................
Haplotype08       .......GTT.........C.....................................G.....
Haplotype09       .......GCT....T....C........C.....................................
Haplotype10       .......GCT....T....C.....................................G.....
Haplotype11       .......GCT....TG...C........C.....................................
A1236             .......GCT....T.............C.....................................
AM53546           .......GCC....T.............C.....................................
BM1898.7.2.5      .......GCT....T....C........C.....................................
EEI2              ....G..GCT....T....C.....................................G.....
EEIGc             ....G..GCT....TG...C.....................................G.....
FM127880          .......GCT....TG...C.....................................G.....
FM27475           .......GTT.........C.....................C.....................
PCNNChad138       .......GCT....T.............C.....................................
USNM162017        .......GCT....TG...C........C.......C.....................
USNM251797        .......GCT....T....C.....................................
USNM251798        .......GCT....T....C.....................C.....................
```

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                  221    230        240        250        260  263
                   |      |          |          |          |   |
Haplotype01       ATAATAGTACATTATATTACATGCCCCATGCATATAAGCATGT
Haplotype02       ....................T.....................
Haplotype03       ....................T.....................
Haplotype04       ....................T.....................
Haplotype05       ....................T.....................
Haplotype06       ....................T.....................
Haplotype07       ....................T.....................
Haplotype08       ....................T.....................
Haplotype09       ....................T.....................
Haplotype10       ..............G.....................
Haplotype11       ....................T.....................
A1236             ..........................................
AM53546           ....................T.....................
BM1898.7.2.5      ....................T.....................
EEI2              ....................T.....................
EEIGc             ....................T.....................
FM127880          ..............C..T.....................
FM27475           ....................T.....................
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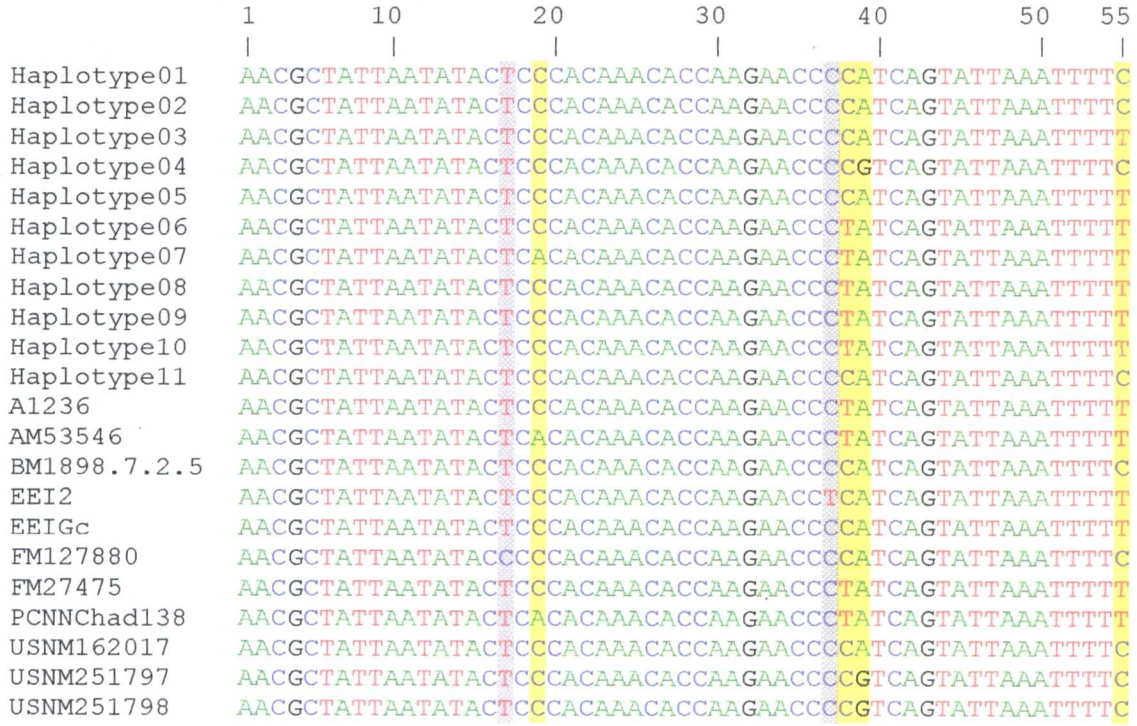

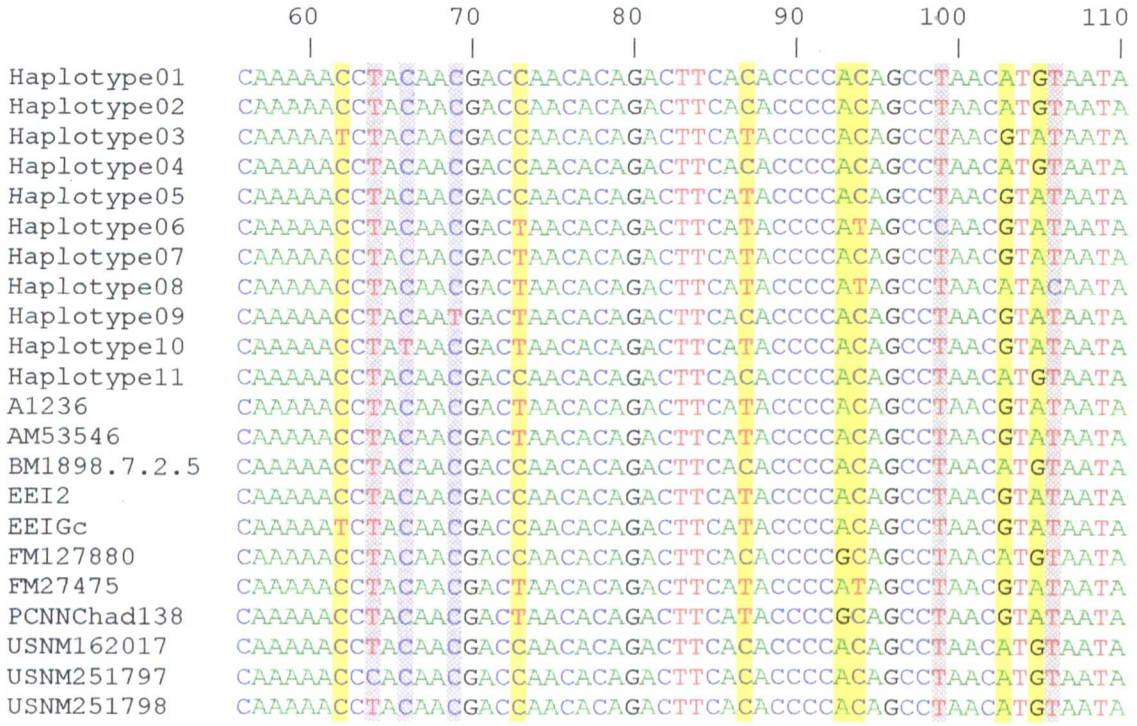

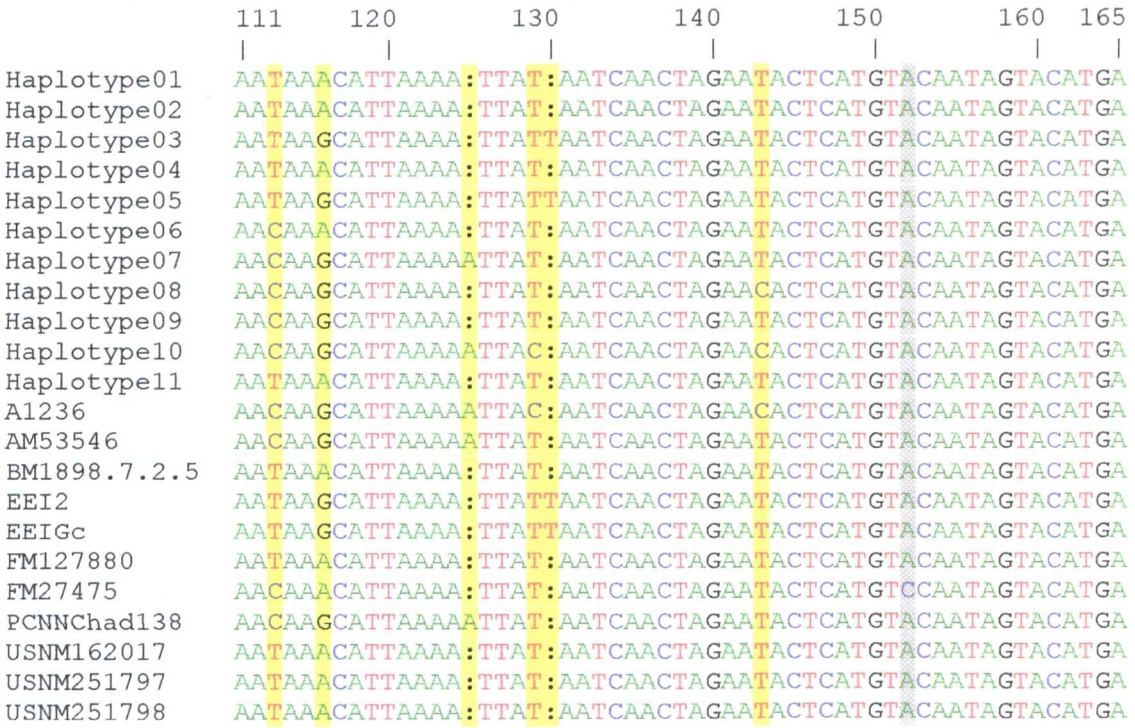
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Haplotype04 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype05 AATAAGCATTAAAA:TTATTAATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype06 AACAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype07 AACAAGCATTAAAAATTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype08 AACAAGCATTAAAA:TTAT:AATCAACTAGAACACTCATGTACAATAGTACATGA
Haplotype09 AACAAGCATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
Haplotype10 AACAAGCATTAAAAATTAC:AATCAACTAGAACACTCATGTACAATAGTACATGA
Haplotype11 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
A1236 AACAAGCATTAAAAATTAC:AATCAACTAGAACACTCATGTACAATAGTACATGA
AM53546 AACAAGCATTAAAAATTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
BM1898.7.2.5 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
EEI2 AATAAGCATTAAAA:TTATTAATCAACTAGAATACTCATGTACAATAGTACATGA
EEIGc AATAAGCATTAAAA:TTATTAATCAACTAGAATACTCATGTACAATAGTACATGA
FM127880 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
FM27475 AACAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTCCAATAGTACATGA
PCNNChad138 AACAAGCATTAAAAATTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
USNM162017 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
USNM251797 AATAAACATTAAAA:TTAT:AATCAACTAGAATACTCATGTACAATAGTACATGA
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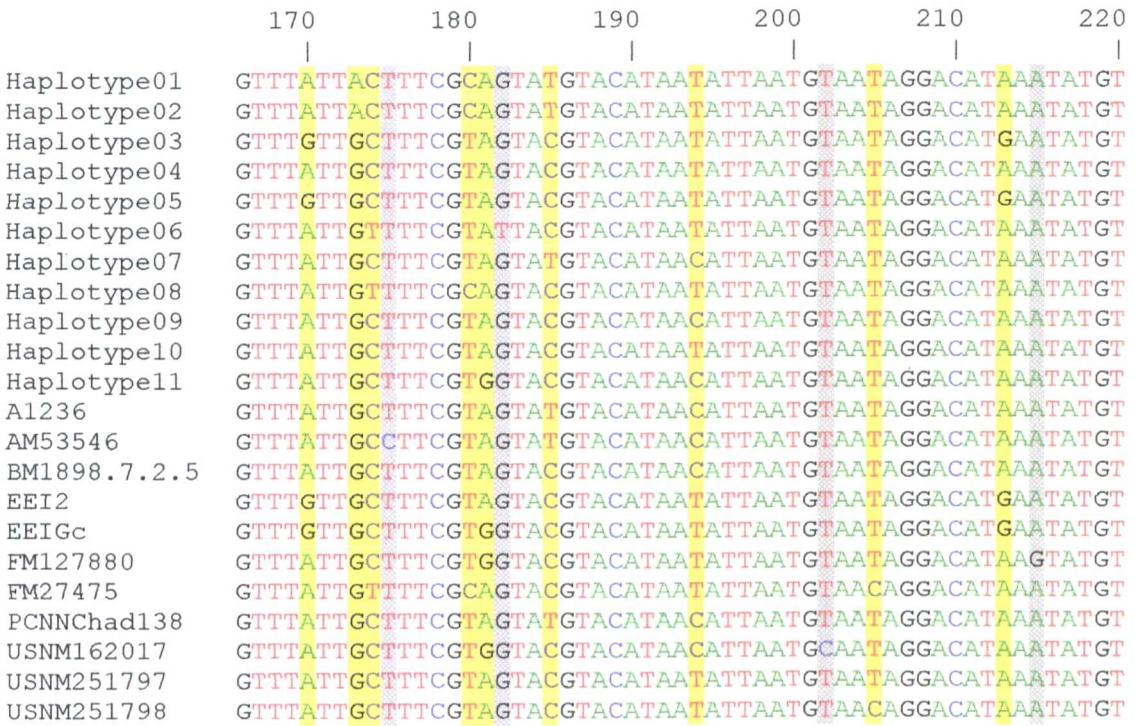
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Haplotype11 GTTTATTGCTTTCGTGGTACGTACATAACATTAATGTAATAGGACATAAATATGT
A1236 GTTTATTGCTTTCGTAGTATGTACATAACATTAATGTAATAGGACATAAATATGT
AM53546 GTTTATTGCCTTCGTAGTATGTACATAACATTAATGTAATAGGACATAAATATGT
BM1898.7.2.5 GTTTATTGCTTTCGTAGTACGTACATAACATTAATGTAATAGGACATAAATATGT
EEI2 GTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGT
EEIGc GTTTGTTGCTTTCGTGGTACGTACATAATATTAATGTAATAGGACATGAATATGT
FM127880 GTTTATTGCTTTCGTGGTACGTACATAATATTAATGTAATAGGACATAAGTATGT
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PCNNChad138 GTTTATTGCTTTCGTAGTATGTACATAACATTAATGTAATAGGACATAAATATGT
USNM162017 GTTTATTGCTTTCGTGGTACGTACATAACATTAATGCAATAGGACATAAATATGT
USNM251797 GTTTATTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATAAATATGT
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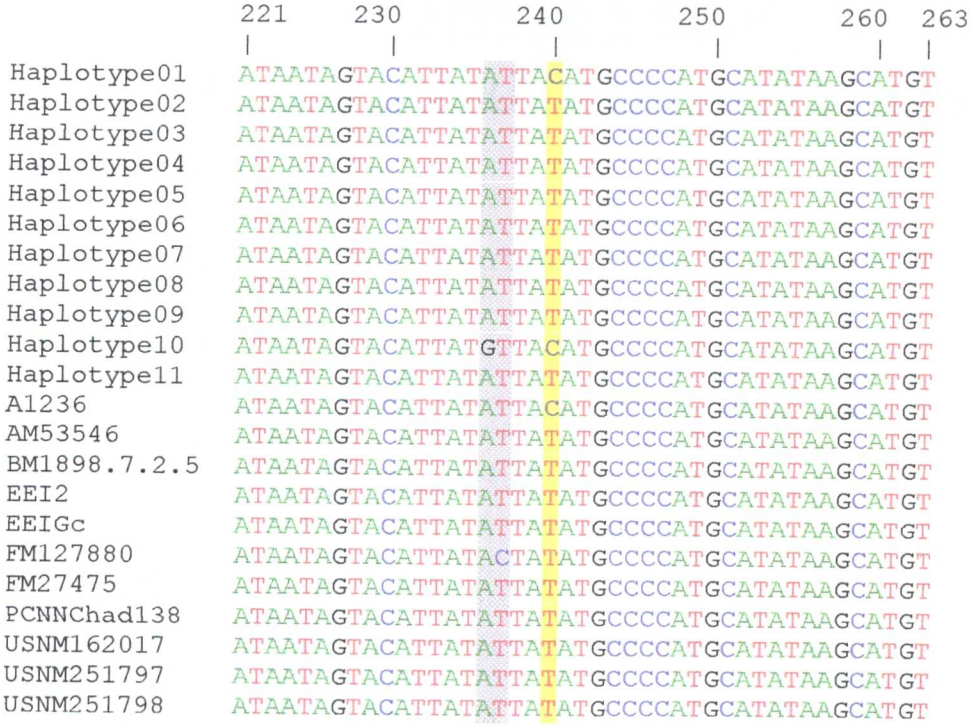
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230
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250
260
263
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Haplotype03 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
Haplotype04 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
Haplotype05 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
Haplotype06 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
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Haplotype08 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
Haplotype09 ATAATAGTACATTATATTATATGCCCCATGCATATAAGCATGT
Haplotype10 ATAATAGTACATTATGTTACATGCCCCATGCATATAAGCATGT
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APPENDIX 5.12.1:

INPUT DATA FOR NESTED CLADE ANALYSIS

```
    Giraffe Control Region mtDNA
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1 ECC
4 3.65 28.87
2 ECU
5 1.75 34.85
3 EEK 2.62 38.01
4 ENA
2 7.40 31.78
5 ESK
8 -2.34 36.02
6 EST
-6.62 34.92
7 SCZ
16 -21.16 29.36
8 SEW
4 -24.72 31.49
9 SWA
1 -16.50 16.92
10 SWC
31 -19.45 22.50
11 SZT
6 -13.50 31.50
12 WCP
3.10.27 16.07
13 WSN
1 9.18 12.50
15
Clade 1-1
3
VII XIII XIX
0 1 1
4
1 4 12 13
3 2 2 1
1 0 0 0
0 0 1 0
Clade 1-8
2
III XVI
0 1
2
7 10
14 6
0 1
Clade 1-9
2
V XV
0 1
3
7 9 10
1 1 2
0 0 1
Clade 1-10
3
IV XXI XXII
0 1 1
3
5 6 11
2 1 6
0 1 0
0 1 0
Clade 1-11
2
I II
1 0
3
7 8 10
1 4 19
0 0 2
Clade 1-13
2
XI XX
0 1
2
5 6
1 1
1 0
Clade 2-5
2
1-8 1-9
1 0
3
7 9 10
14 0 7
1 1 3
Clade 2-6
2
1-12 1-13
1 0
3
2 5 6
1 0 0
0 2 1
Clade 2-8
2
1-10 1-14
1 0
3
5 6 11
2 3 6
1 0 0
Clade 3-1
2
2-1 2-2
0 1
5
1 2 4 12 13
4 0 2 3 1
0 4 0 0 0
Clade 3-2
2
2-3 2-4
0 1
3
2 3 5
1 0 2
0 7 0
Clade 3-5
3
2-6 2-7 2-8
1 1 0
7
2 5 6 7 8 10 11
1 2 1 0 0 0 0
0 0 0 1 4 21 0
0 3 3 0 0 0 6
Clade 4-1
3
3-1 3-2 3-4
1 1 0
7
1 2 3 4 5 12 13
4 4 0 2 0 3 1
0 1 7 0 2 0 0
0 0 2 0 0 0 0
Clade 4-2
2
3-3 3-5
0 1
8
2 5 6 7 8 9 10 11
0 0 0 15 0 1 10 0
1 5 4 1 4 0 21 6
Total Cladogram
2
4-1 4-2
1 1
13
1 2 3 4 5 6 7 8 9
10 11 12 13
4 5 9 2 2 0 0 0 0 0
0 3 1
0 0 0 0 6 4 16 4 1
31 6 0 0
END
```

APPENDIX 5.12.2:

GEODIS NESTED CLADE ANALYSIS RESULTS OUTPUT

Differentiating population structure from history - Geodis 2.0
(c) Copyright, 1999-2001 David Posada and Alan R. Templeton
Contact: David Posada
Department of Zoology, Brigham Young University
Provo, UT 84602-5255
david.posada@byu.edu

Giraffe Control Region mtDNA

PERMUTATION ANALYSIS OF Clade 1-1
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 4.0625
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.8730

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE VII (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	956.4296	1.0000	0.2770
NESTED CLADE	956.6351	0.9430	0.3340

CLADE XIII (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.9110	1.0000
NESTED CLADE	849.4239	0.7050	0.6820

CLADE XIX (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.9110	1.0000
NESTED CLADE	744.5715	0.2650	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	956.4296	1.0000	0.2770
NESTED CLADE	159.6374	0.9430	0.3340

PERMUTATION ANALYSIS OF Clade 1-8
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.1000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3260

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE III (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	219.0021	0.3260	1.0000
NESTED CLADE	230.2834	0.3260	1.0000

CLADE XVI (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.3260	1.0000
NESTED CLADE	588.4100	1.0000	0.3260

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	219.0021	0.3260	1.0000
NESTED CLADE	-358.1266	0.3260	1.0000

PERMUTATION ANALYSIS OF Clade 1-9
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 0.8333
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE V (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	207.5711	0.7940	0.7880
NESTED CLADE	219.5893	0.7940	0.7880

CLADE XV (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.5820	1.0000
NESTED CLADE	542.2273	0.7880	0.7940

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	207.5711	0.7940	0.7880
NESTED CLADE	-322.6380	0.7940	0.7880

PERMUTATION ANALYSIS OF Clade 1-10
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 6.5185
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1800

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE IV (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	427.0871	0.3800	0.6690
NESTED CLADE	427.0871	0.3800	0.6690

CLADE XXI (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	8142.2599	1.0000	0.2650

CLADE XXII (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	8142.2599	1.0000	0.2670

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	427.0871	0.3800	0.6690
NESTED CLADE	-7715.1729	0.0490	1.0000

PERMUTATION ANALYSIS OF Clade 1-11
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 0.5159
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE I (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	503.6638	0.6480	1.0000
NESTED CLADE	509.6105	0.6480	1.0000

CLADE II (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6690	1.0000
NESTED CLADE	657.9737	1.0000	0.6480

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-503.6638	0.6690	0.9790
NESTED CLADE	148.3632	1.0000	0.6480

PERMUTATION ANALYSIS OF Clade 1-13
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 0.7500
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE XI (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-0.0000	0.6720	1.0000
NESTED CLADE	-0.0000	0.6720	1.0000

CLADE XX (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	0.0000	0.6720	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-0.0000	0.6720	1.0000
NESTED CLADE	-0.0000	1.0000	0.6720

PERMUTATION ANALYSIS OF Clade 2-5
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 6.4711
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0630

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 1-8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	240.7779	0.0370	0.9970
NESTED CLADE	590.9275	0.1990	0.8350

CLADE 1-9 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	228.5670	0.3010	0.7330
NESTED CLADE	634.1600	0.5760	0.4580

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-12.2109	0.8350	0.1990
NESTED CLADE	43.2325	0.7930	0.2410

PERMUTATION ANALYSIS OF Clade 2-6
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 4.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.4990

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 1-12 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0001	1.0000	0.2290
NESTED CLADE	262.5428	0.7300	0.4990

CLADE 1-13 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-0.0000	0.2290	1.0000
NESTED CLADE	210.0305	0.2290	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-0.0001	0.2290	1.0000
NESTED CLADE	-52.5122	0.4990	0.7300

PERMUTATION ANALYSIS OF Clade 2-8
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 3.2727
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2460

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 1-10 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	427.0871	0.2460	1.0000
NESTED CLADE	485.3082	0.2460	1.0000

CLADE 1-14 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	971.8195	0.7440	0.5020

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-427.0871	1.0000	0.2460
NESTED CLADE	486.5113	0.7440	0.5020

PERMUTATION ANALYSIS OF Clade 3-1
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 14.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 2-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	932.2625	0.2880	0.7120
NESTED CLADE	938.2361	0.2370	0.7630

CLADE 2-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0001	0.0000	1.0000
NESTED CLADE	1279.2398	0.9930	0.0070

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	932.2624	1.0000	0.0000
NESTED CLADE	-341.0038	0.0370	0.9630

PERMUTATION ANALYSIS OF Clade 3-2
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 10.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0150

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 2-3 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	0.0000	1.0000	1.0000

CLADE 2-4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	0.0000	1.0000	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	0.0000	1.0000	1.0000

PERMUTATION ANALYSIS OF Clade 3-5
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 56.7000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 2-6 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	233.3693	0.1670	0.8350
NESTED CLADE	1706.7449	0.9970	0.0050

CLADE 2-7 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	515.1118	0.0000	1.0000
NESTED CLADE	1029.0006	0.8220	0.1780

CLADE 2-8 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	529.5365	0.0150	0.9850
NESTED CLADE	560.3116	0.0200	0.9800

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	51.9904	0.5770	0.4230
NESTED CLADE	-559.0550	0.0090	0.9910

PERMUTATION ANALYSIS OF Clade 4-1
BASED ON 1000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 24.1676
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0170

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PART II.  GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 3-1 (Tip)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000
CLADE 3-2 (Tip)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000
CLADE 3-4 (Interior)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000

PART III.  TEST OF INTERIOR VS. TIP CLADES:
 TYPE OF DISTANCE    I-T DISTANCE      PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000

PERMUTATION ANALYSIS OF Clade 4-2
   BASED ON 1000 RESAMPLES

PART I.  EXACT CONTINGENCY TEST:
  OBSERVED CHI-SQUARE STATISTIC =     35.3454
  THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE =      0.0000

PART II.  GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 3-3 (Interior)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE       613.1030        0.0320        0.9680
      NESTED CLADE       822.0960        0.1740        0.8260
CLADE 3-5 (Tip)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE       933.7776        0.6400        0.3600
      NESTED CLADE       998.8138        0.8810        0.1190

PART III.  TEST OF INTERIOR VS. TIP CLADES:
 TYPE OF DISTANCE    I-T DISTANCE      PROB.<=       PROB.>=
      WITHIN CLADE       -320.6746        0.1050        0.8950
      NESTED CLADE       -176.7177        0.1560        0.8440

PERMUTATION ANALYSIS OF Total Cladogram
   BASED ON 1000 RESAMPLES

PART I.  EXACT CONTINGENCY TEST:
  OBSERVED CHI-SQUARE STATISTIC =     86.5034
  THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE =      0.0000

PART II.  GEOGRAPHIC DISTANCE ANALYSIS:
CLADE 4-1 (Tip)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000
CLADE 4-2 (Tip)
 TYPE OF DISTANCE        DISTANCE       PROB.<=       PROB.>=
      WITHIN CLADE         0.0000        1.0000        1.0000
      NESTED CLADE         0.0000        1.0000        1.0000

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

** ANALYSIS FINISHED **
It took 55.2600 seconds.
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Knowing ignorance is strength.

Ignoring knowledge is sickness.

If one is sick of sickness, then one is not sick.

The sage is not sick because he is sick of sickness.

Therefore he is not sick.

Tao Te Ching. Lao Tsu.

(Translation by Gia-Fu Feng and Jane English, 1972.)

And I gave my heart to know wisdom, and to know madness and folly: I perceived that this also is vexation of the spirit.

For in much wisdom is much grief: and he that increaseth knowledge increaseth sorrow.

Ecclesiastes. 1: 17-18.

King James Bible.