Evolutionary conservation genetics of invasive and endemic parrots

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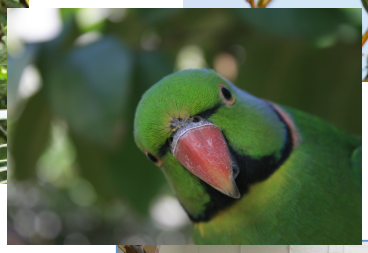

Declaration of Originality

Hazel Jackson wrote all the chapters within this thesis with editorial suggestions by PhD supervisor Jim Groombridge. Chapters 2-6 include collaborations with other researchers within and outside of the University of Kent, and contributions from other authors are acknowledged appropriately at the end of each chapter.

Chapter 2: Hazel Jackson conceived the idea, conducted the literature review, analysis and wrote the manuscript. Byron Morgan provided guidance for the statistical analysis, and all authors provided comments on the text.

Chapter 3: Hazel Jackson conceived the idea, conducted the genetic labwork, genetic analysis and wrote the manuscript. Diederik Strubbe provided statistical analysis on propagule pressure and the climatic niche hypothesis and all authors provided feedback and comments on the text. Researchers and volunteers within each country collected contemporary feather samples; Simon Tollington provided genetic data for the Mauritius ring-necked parakeets. Hazel Jackson collected native historical specimens from the NHM in Tring, UK.

Chapter 4: Hazel Jackson conceived the idea, conducted the labwork, genetic and GLM analysis and wrote the manuscript. Richard Nichols designed the modelling of genetic bottleneck effects, Diederik Strubbe provided collated population growth data for a number of European populations, climatic, niche and human demographic variables for GLM analysis, and Pim Edelaar provided data on Seville population growth data. All authors provided feedback and comments on the text. Researchers and volunteers within each country collected contemporary feather samples, while Hazel Jackson collected native historical specimens from the Natural History Museum in Tring, UK.

Chapter 5: The Seychelles Islands Foundation (SIF) and Hazel Jackson conceived the idea. SIF provided the contemporary black parrot samples, and historical specimens were obtained from the Muséum National d'Histoire Naturelle in Paris and the Natural History Museum in Tring. Hazel Jackson conducted the labwork on the museum specimens and Natalia Przelomska conducted the labwork on contemporary specimens. Jim Groombridge obtained morphometric data. Hazel Jackson conducted the analysis and wrote the manuscript while and all co-authors provided feedback and commented on the text.

Chapter 6: Hazel Jackson conceived the idea, conducted the labwork, analysis and wrote the manuscript. Paul Michael-Agapow provided additional R-script for phylogenetic diversity analysis. All co-authors provided feedback and commented on the text. Museum samples were

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Abstract

The world is now thought to have entered into a sixth mass extinction event, which unlike previous mass extinctions, is entirely driven by human impacts. The early colonisation of humans has led to as many as a thousand endemic bird species becoming extinct, while increasing human mediated transport of species around the world has led to invasive species becoming one of the largest global conservation challenges of today. Studies in molecular ecology can help us to unravel how evolutionary processes are important for informing conservation and invasion biology by understanding genetic mechanisms that enable populations to grow and adapt in a changing world. As genetic diversity is essential for the persistence of populations, this thesis aims to understand how species respond, at a genetic level, to human-driven events such as the reduction of a population to a small size, or the introduction of a species into a novel environment. The findings demonstrate the important use of genetic markers for phylogenetic reconstruction and understanding population structure. These phylogenetic reconstructions examine taxonomic distinctiveness and patterns of evolution, and allow the identification of ancestral origins for invasive ring-necked parakeets. Evidence from genetic phylogroups, trade data and drivers of population growth, highlight how multiple introductions and patterns of climate matching between the native and invasive ranges of ring-necked parakeets, are mechanisms for invasion success. In contrast to mild genetic bottleneck effects, high levels of diversity and the avoidance of problems associated with small population size within populations of invasive ring-necked parakeet, the endemic Seychelles black parrot exhibited a reduction in population size and reduced levels of genetic diversity over time. Moreover, the inclusion of new genetic data for a number of extinct parrot species enabled an examination of the loss of broader scale phylogenetic diversity, important for ecosystem function, as a result of extinctions of endemic species and invasions of ring-necked parakeets. The findings from this thesis have already been applied to conservation and invasion biology by contributing to the reclassification of the endemic Seychelles black parrot, and to improving the ability of ecological niche models to predict areas suitable for future invasions of ring-necked parakeets. Furthermore they provide a novel approach to identifying potential candidates as ecological replacements to restore ecosystem function and lost phylogenetic diversity.

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Chapter 1.

Introduction

The world is now thought to have entered into a sixth mass extinction event, which unlike previous mass extinctions, is entirely driven by human impacts (Höglund 2009). The early colonisation of humans has led to as many as a thousand endemic bird species becoming extinct (Höglund 2009), while increasing human mediated transport of species around the world has led to invasive species becoming one of the largest global conservation challenges of today (Sutherland *et al.* 2014).

The heart of this thesis is to deepen our understanding of evolutionary processes and how they can be used to inform conservation biology and invasion biology. Studies in molecular ecology can help us to unravel how rapid evolutionary forces enable populations to grow and adapt in a changing world. As genetic diversity is essential for the persistence of populations (Soule & Gilpin 1986), understanding how species respond at a genetic level to human-driven events such as the reduction of a population to a small size or the introduction of a species into a novel environment, has important applications to both endemic and invasive species. Indeed, there is substantial value in studying both invasive and endemic species together. Invasive species can be useful study systems to address a wide range of questions in ecology, evolution and biogeography (Sax *et al.* 2007). As they occur across wide spatial and temporal scales, they allow evolutionary and ecological processes such as adaptation and expansion, to be studied in 'real time' (Sax *et al.* 2007). While humans transport large numbers of species around the globe, only a small fraction of these become successfully established in environments outside of their native ranges. Insights into such species are invaluable towards informing conservation management of both invasive species and endangered endemic species.

1.1 Population genetics in invasion and conservation biology

Why is it important to study the genetics of populations, and what lessons can be learnt from studying invasive species and endemic populations together? Such species often exhibit different responses to demographic situations. For example, many populations suffer from a reduction to a small population size, and require intensive conservation efforts to avoid associated detrimental genetic impacts such as inbreeding and vulnerability to disease. In contrast, invasive species are interesting study systems as they appear to be able to avoid such detrimental impacts of a founding event, or small population size.

1.1.1 Evolutionary theory

The study of conservation genetics and its application in conservation and invasion biology is multifaceted. It can be used to identify evolutionary units for conservation efforts, and levels of genetic diversity in relation to the viability of a population (Frankham 1996; Bouzat 2010), for both endemic and invasive species. A fundamental principle in conservation genetics suggests that a loss in or reduced level of genetic diversity is related to individual or population fitness (Reed & Frankham 2001; Hansson & Westerberg 2002; Charpentier *et al.* 2005; Silva *et al.* 2006; Grueber *et al.* 2008). A decrease in fitness can occur through inbreeding depression or an increase in genetic load (Kirkpatrick & Jarne 2000; Fox *et al.* 2008; Charlesworth 2009), moreover, populations with low genetic diversity will also have a reduced evolutionary potential This body of evolutionary theory predicts that such a population will be less likely or able to adapt to environmental changes in the future (Franklin & Frankham 1998; Frankham *et al.* 1999).

1.1.2 Evolutionary mechanisms of invasion success

To begin to tackle problems associated with invasive species it is important to understand invasion mechanisms and identify determinants of invasion success and population growth in non-native environments. Studies of population genetics and evolutionary potential within invasive populations can provide insight into successful invasions (Walker *et al.* 2003; Lavergne & Molofsky 2007; Suarez & Holway 2008; Cameron *et al.* 2008). Contemporary research has focused on improving our understanding of invasive species and the evolutionary mechanisms that facilitate their successful establishment in novel environments by reconstructing taxonomic relationships, identifying ancestral source populations and routes of invasion, detecting hybridisation and introgression, and finally, by examining levels of genetic diversity and evolutionary adaptation (Le Roux & Wieczorek 2009).

1.1.3 Genetic impacts from a small population size

Population size is an important consideration in conservation (for example, population size is a key variable for the IUCN red list), however an additional measure, the effective population size (the number of individuals that genetically contribute to the next generation) is more relevant than the total census size of a population. Whilst ideally the total number of individuals within a population should be equal to the effective population size, in reality this is rarely the case. Thus, the effective population size is more informative for calculating rates at which genetic diversity will be lost within a small population following genetic drift (Freeland 2006). Small populations are vulnerable to inbreeding and loss of genetic diversity as the level of genetic diversity is related to population size (Frankham 1996). In theory levels of genetic variation should increase as population size increases (Frankham 1996). With this in mind, a small

population will therefore experience a reduced level of genetic diversity and a reduced evolutionary potential (Frankham 1996). Small endemic island populations are vulnerable to genetic factors that may increase their susceptibility to extinction, such as inbreeding depression and loss of genetic diversity, which are thought to contribute to the vulnerability of a species to extinction (Frankham 1996; 1997). As such, small populations that are increasingly prone to extinction are therefore often species of conservation concern (Frankham 2005). Isolated island populations are also highly prone to extinction in contrast to mainland populations, and it is argued that genetic factors contribute to this increased risk (Frankham 1997). A study of genetic variability among island populations found a significant majority of them had lower levels of variation in comparison to their mainland counterparts, and endemic species showed proportionately lower levels of genetic variation than non-endemic species (Frankham 1997).

As genetic systems, invasive species are intriguing because they appear able to avoid genetic difficulties associated with small population size at founding. Low genetic diversity, inbreeding and reduced fitness are likely to occur when invasive species colonise new areas with a small number of founders (Allendorf & Lundquist 2003). However, newly established invasive species may also increase levels of genetic diversity through hybridisation, mutation and migration (gene flow) (Blackburn & Lockwood 2009). Interestingly, the amount of genetic variability lost in a newly established invasive population varies linearly with the number of individuals in the founding group (Merilä & Björklund 1996).

Population bottlenecks (a reduction to a very small population size) have occurred in many species (Groombridge *et al.* 2001), which then often require intensive conservation management to help the population recover. For example, the Mauritius kestrel (*Falco punctatus)* was reduced to a single breeding pair and required 25 years of intensive management to enable a population recovery to 400-500 birds (Groombridge *et al.* 2001). Severe population bottlenecks can result in inbreeding depression (Heber & Briskie 2010), which in turn impacts traits related to reproductive fitness, such as number of eggs laid and juvenile survival (Crnokrak & Roff 1999). For example, a study of hatching failures across 51 threatened bird species found failure rates were higher than 10% in all populations which had passed through a bottleneck below 100-150 individuals (Heber & Briskie 2010).

While many such vulnerable populations require intensive conservation management to aid recovery, in some exceptional circumstances populations manage to increase their size unaided. One island endemic species, the Seychelles kestrel (*Falco araea*), experienced a severe bottleneck when its population was reduced to only eight individuals and was then considered critically endangered. Today, however, this kestrel is relatively common in the Seychelles

(Groombridge *et al.* 2009). It is thought that a number of ecological factors helped those birds recover with minimal conservation efforts. In contrast to its counterpart on Mauritius, the Seychelles kestrel did not suffer from reduced eggshell thickness and associated offspring production as a result of widespread DDT use. Moreover, levels of habitat loss were much lower on the Seychelles in comparison to Mauritius (Groombridge *et al.* 2009). In a similar way, invasive species often experience small population bottlenecks during founding events, and are therefore predicted to suffer inbreeding depression (Blackburn & Lockwood 2009). Invasive species should also experience significant losses of genetic diversity when at low population sizes (Dlugosch & Parker 2008), however, following a meta-analysis of aquatic invaders, just 37% (16 out of 43) showed evidence of a significant loss of genetic diversity in relation to their native source populations (Roman & Darling 2007). Occasionally levels of genetic diversity are increased through inter and intra-specific hybridisation despite a founding event (Le Roux & Wieczorek 2009).

1.1.4 Genetic rescue by multiple introductions

One mechanism of endemic population restoration, and also a suspected contributing factor of invasion success by invasive species, is '*genetic rescue*' by way of multiple introductions. Here, population fitness is enhanced by immigration of new alleles into a genetically impoverished population (Tallmon *et al.* 2004), subsequently replenishing genetic variation and reducing the effects of inbreeding depression (Ingvarsson 2001). Effects of genetic rescue (increased heterozygosity, outbreeding and population growth (Hogg *et al.* 2006; Pimm *et al.* 2006; Fredrickson *et al.* 2007)) can be dramatic. For example, addition of a single immigrant into an inbred population of *Drosophila* led to >50% increase in fitness in a single generation (Spielman & Frankham 1992), while introducing new genes into a threatened natural population of adders (*Vipera berus)* resulted in a three-fold increase in population size in just seven years (Madsen *et al.* 1999).

Genetic rescue is an effective tool for restoring endemic populations (Madsen *et al.* 1999; Ellstrand & Schierenbeck 2000; Facon *et al.* 2003; Frankham 2005; Lockwood *et al.* 2005; Bossdorf *et al.* 2005; Hogg *et al.* 2006). Isolated Scandinavian grey wolves (*Canis lupis*) suffered a severe population bottleneck, to only two individuals. The immigration of just one individual resulted in a increase in heterozygosity, significant outbreeding and a rise in population growth (Vila *et al.* 2003). Genetic rescue also successfully reduced inbreeding and increased genetic and reproductive fitness in a population of Mexican wolves (*Canis lupus baileyi*; Fredrickson *et al.* 2007). However, there is potential for genetic rescue programmes to facilitate an influx of genetic diversity into locally adapted populations, resulting in the target

population suffering a loss of certain elements of genetic diversity by replacement from the immigrant individuals (Bouzat *et al.* 2009).

The concept of multiple introductions has been identified as a potential explanation for invasive species avoidance of genetic problems and extraordinary invasive capacity (Collins *et al.* 2002; Kolbe *et al.* 2004; Lavergne & Molofsky 2007; Darling *et al.* 2008). Multiple introductions of different *Anolis* lizard species in non-native habitats led to admixture and increased genetic variation (Kolbe *et al.* 2007). Genetic microsatellite markers were used to indicate multiple introductions in the worldwide invasion history of common ragweed (*Ambrosia artemisiifolia;* Gaudeul *et al.* 2011), while mitochondrial DNA revealed multiple introductions occurred of the successfully invasive earthworm, *Dendrobaena octaedra* (Cameron *et al.* 2008). Such introductions from different source pools can result in hybridisation between genetically similar species or sub-species. Hybridisation between invasive species from multiple source pools can act as an impetus for the evolution of traits important for successful invasions (Ellstrad & Schierenbeck 2000), while hybridisation can inject genetic diversity into endemic populations for conservation management purposes. For example, as a result of a population bottleneck, isolated endemic panthers (*Puma concolor coryi*) in southern Florida experienced increased occurrences of genetic defects (Hedrick 2005). An introduction of eight female panthers into the population resulted in hybrid kittens with a much higher survival rate in contrast to 'purebred' kittens (Pimm *et al.* 2006).

1.1.5 Rapid adaptation in invasive species

Successful invasive species may also evolve and adapt during an invasion and range expansion in response to selection pressures from their new environments (Sakai *et al.* 2001). In response to ambient temperature experienced during growth, invasive European wild rabbits (*Oryctologus cuniculus*) in Australia genetically adapted to the warmer climate by growing longer ears and leaner bodies (Williams & Moore 1989). Following its introduction to North America from Europe, *Drospohila subobscura* experienced a rapid morphological change in response to the latitude of their new environment. A cline in wing length appeared within two decades after introduction (Huey *et al.* 2000).

1.1.6 Molecular tools

The two disciplines of conservation genetics and invasion biology utilise molecular tools for theoretical and practical application. Genetic studies are important in conservation for resolving taxonomic uncertainties, or determining evolutionary distinctiveness and units for conservation. Determining population structure and levels of genetic diversity within a population is important for numerous conservation management strategies such as translocations,

conservation management of endangered populations, and captive management (Frankham 2010). Such information is essential for retaining viable populations. Likewise the same genetic theory and techniques can be applied to invasion biology. Understanding evolution is integral to the study of invasion biology (Lee 2002) as determining population structure and levels of genetic diversity is important for understanding which mechanisms drive successful invasions (Frankham 2010). Importantly, molecular tools can be used to reveal genetic characteristics and adaptive responses that facilitate successful invasions (Lee 2002).

The selection of appropriate genes is important when inferring relationships between species or populations, as mutation rates differ in various genes (Sinclair & Pérez-Losada 2005). Mitochondrial DNA (mtDNA) is a small and simple haploid molecule that is maternally inherited (Baker 2000), and, owing to a lack of recombination, each unique mtDNA sequence is a haplotype transmitted intact through generations. Mitochondrial markers such as control region and cytochrome *b* are a reliable tool when reconstructing population phylogenies, and are sensitive to detecting population bottlenecks (Baker 2000, Freeland 2011), and for examining intraspecific relationships among closely related groups of individuals (Sinclair & Pérez-Losada 2005). The control region is a popular choice of marker among population geneticists, as it is non-coding and often the most variable part of mtDNA (Baker 2000). It is reasonably straightforward to amplify across different taxonomic groups because the control region is divided into three domains (I, II and III), which show differing degrees of variability and base composition (Baker & Marshall 1997). The central conserved region, domain II is flanked by two variable A-rich domains (I & II) which show higher levels of sequence variation (Ruokonen 2002). The central conserved region, domain II, has fewer indels and considerably lower among-species sequence divergence in comparison to its flanking hypervariable domains I & III. These hypervariable control region sequences are therefore useful for providing high resolution population analyses (Baker 2000).

Microsatellites are inherited through both parents and generally occur in non-coding regions of DNA. As they are highly polymorphic and abundant throughout the genome they are often utilised in population genetics (Baker 2000). Microsatellites are short DNA sequences composed of simple short repeat motifs, which can repeat about 5-100 times at each locus (Wan *et al.* 2004). In contrast to mtDNA, microsatellites experience much higher mutation rates (at 10^{-5} – 10^{-3} mutations per locus per generation). Mutations occur due to 'strand slippage' during DNA replication, resulting in changes in the number of repeat motifs. Characteristics of the repeat motif; length (ie, dinucleotide, trinucleotide, tetranucleotide etc), contiguity and type, influence the rate of mutation and allelic variation. For example, dinucleotide repeats have mutation rates 1.5 – 2.0 times higher than tetranucleotides (Baker 2000). The rapid mutation

rates and high variability of microsatellites make them suitable for inferring recent genetic events, and to discriminate genetically between individuals and populations (Freeland 2011).

Such microsatellite data is analysed under the assumption that populations are in Hardy-Weinberg Equilibrium (HWE). This model states that allele frequencies within a population will remain constant from one generation to the next in the absence of evolutionary influences such as mate choice, mutation, selection, genetic drift and gene flow (Hartl & Clark 2007). It is important to note that certain assumptions are made about the populations under analysis including: mating is random and population size is very large. Deviations from HWE may occur if an excess of heterozygotes or homozygotes are observed. Null alleles may be present leading to a false observation of excess homozygosity, whilst inbreeding may be prevalent within a population, as indicated by an excess of homozygotes. Finally, the presence of substructure within a population may result in Wahlund's effect, reducing the level of overall heterozygosity (Hartl & Clark 2007).

Usefully, such widely used molecular tools can have cross species utility and be applied to invasive and endemic species studies, for example a set of microsatellite loci originally designed on endemic endangered Mauritius parakeets (*Psittacula echo;* Raisin *et al.* 2009*),* were applied in this thesis to invasive ring-necked parakeets (*Psittacula krameri*)*,* and endemic Seychelles black parrots (*Coracopsis nigra barklyi*). More recent advances in genomic technologies have led to the use of next generation sequencing and the sequencing of complete genomes. Such techniques have transformed our ability to identify genes important for evolutionary adaptation, and to examine huge datasets with thousands of markers to improve the accuracy and power of research in conservation genetics (Allendorf *et al.* 2010; Stapley *et al.* 2010).

1.2 Invasive alien species

Humans have been trading species for millennia and contemporary global patterns of invasive species have been shaped by such human mediated transport of plant and animal species (Hulme 2009). However, not all species introduced to regions outside of their native range are considered invasive. Only species that establish, disperse and have a detrimental impact upon native biota are considered invasive. Improvements in global transport networks over time has increased connectivity of human populations and led to increasing frequencies of biological invasions (Mckinney & Lockwood 1999). Invasive alien species now represent an increasingly urgent economic, societal and environmental problem. Their rapid spread, competitive nature and transmission of infectious diseases poses a threat to global biodiversity and invasive species are one of the five main causes of global biodiversity loss, alongside climate change, pollution,

overexploitation and habitat loss (Millennium Ecosystem Assessment; 2005). Birds, mammals and amphibians are all experiencing changing IUCN categorisations, driven by the detrimental impacts of invasive species. In all cases invasive species have caused a deterioration of IUCN Red list category (McGeoch *et al.* 2010, Fig. 1.1).

Interestingly, climate change and accompanying land use changes have the potential to enhance species invasions. Predicted changes in climate may cause drastic species range shifts, as new regions become suitable for invasion. Forecasts using distribution models for the IUCN's top 100 worst invasive alien species, suggest that large areas will become suitable for invasion, although a number of areas could lose a significant number of invasive species paving the way for conservation restoration in these areas (Bellard *et al.* 2013). Ecological niche models are often applied as a method of predicting invasion risk by using environmental variables to determine geographic areas suitable for invasive species (Jiménez-Valverde *et al.* 2011; Strubbe *et al*. 2015). Ecological niche models characterise a species' fundamental (a range of environmental conditions in which a species can survive) and realised ecological niches (the range of environmental conditions in which a species is actually found) using occurence and spatial environmental data, which are then projected onto geographical regions outside a species native distribution (Strubbe *et al*. 2015). A species realised niche is usually only a part of its' fundamental niche owing to factors such as dispersal limitations. In the case of invasive species, the non-native niches they inhabit are often a subset of their native realised niche (Strubbe *et al.* 2015).

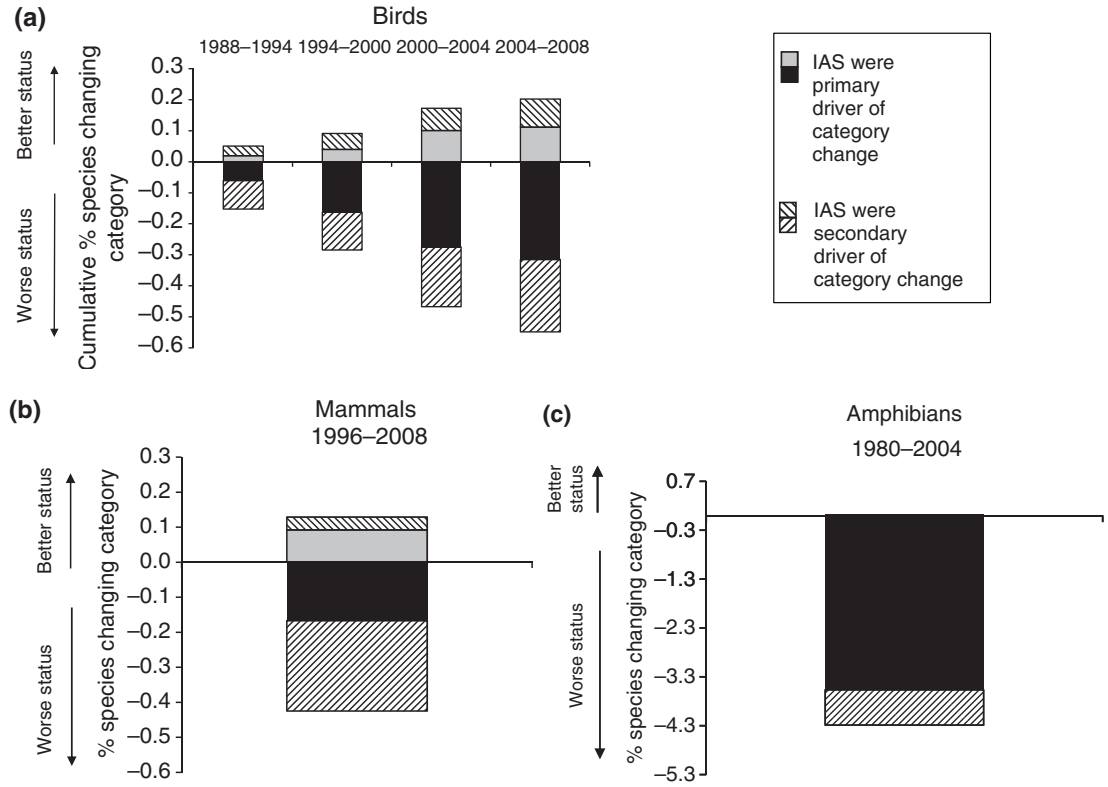

Fig. 1.1. Changes in IUCN categorisations over time for (a) birds, (b) mammals and (c) amphibians, as a result of invasive alien species (IAS) (taken from: McGeoch *et al.* 2010).

1.2.1 Ecological impacts

Invasive species have long been recognised as having detrimental impacts upon native species, ecosystems and communities (Sakai *et al.* 2001; Allendorf & Lundquist 2003; Gurevitch & Padilla 2004). Successful invasive species can drive wide-scale ecological changes as their establishment can result in habitat degradation, a loss of native biodiversity and ecosystem changes (Pyšek *et al.* 2012). Both single species and multiple species approaches provide evidence of the broad level of ecological impacts on native biota from invasive species, for example, invasive plants have been shown to have substantial impact upon native plant communities around the world. Such alien plant species reduce local plant diversity, increase plant productivity of the invasive community, and increase habitat heterogeneity. Invasive plant species have a bottom-up impact on taxa at higher trophic levels, depending on the degree of dependence on these plant species as a food source (Vilà *et al.* 2011). Introductions of predatory fish to lakes have caused extinctions of native fish and amphibians on a global scale (Vitule *et al.* 2009), while resource competition between invasive and native species frequently results in the displacement of native species. Invasive grey squirrels have displaced native red squirrels

through feeding competition, as the ability of grey squirrels to fully exploit good acorn crops in comparison to red squirrels has led to their successful establishment in the UK (Kenward & Holm 1993). Such resource competition can sometimes result in extirpation of native species. The South American fire ant (*Solenopsis invicta*) successfully invaded South-eastern areas of the USA with devastating consequences for endemic ant fauna. Species richness of ants in invaded areas dropped by 70%, and the total number of individuals dropped by 90%. The primary cause of the loss of anthropods was competition replacement (Porter & Savignano 1990). The deliberate introduction of cane toads as a biological control for crop pests has resulted in the depletion of a top-level predator, freshwater crocodiles, by 77% in tropical Australia. Crocodiles have no resistance to the cane toads toxic secretions, which, when ingested, cause mortality. The removal of these top-level predators from river systems may have major repercussions for the ecosystem community (Letnic *et al.* 2008).

A growing number of studies demonstrate the multi-faceted impacts on native community compositions and ecosystems. Introductions of nitrogen-fixers such as *Myrica faya*, a small evergreen shrub, have had multiple impacts by altering ecosystem functions (Vitousek & Walker 1989). Some invaders, such as *Spartina alternifora,* a grass originating from North America, can alter habitat suitability for native species by removing or adding physical structures, which in turn change erosion patterns (Simberloff 2011). Unseen, but nonetheless important are the ecosystem transformations occurring below ground. The predatory New Zealand flatworm, *Arthurdendyus traingulatus,* has dramatically reduced native lumbricid earthworm populations in the UK and Faroe Islands, reducing soil porosity and drainage, increasing waterlogging and reducing mole densities (Murchie & Gordon 2013). Invasive fungal pathogens such as chestnut blight (*Cryphonectria parasitica)* and white pine blister rust (*Cronartium ribicola),* have been responsible for massive tree death, with knock on ecological impacts including habitat degredation, loss of habitats, loss of tree species, and loss of food sources for wildlife such as grizzly bears and numerous bird species (Loo 2009).

Invasive species are often accompanied by the threat of novel pathogens and disease. Introduced diseases are a significant threat to many endemic species, especially small island populations (Frankham 1997), which are often ill equipped to cope with new pathogens (Wikelski *et al.* 2010). Exotic pathogens introduced to the Galapagos Islands are having a detrimental impact on wildlife populations (Gottdenker *et al.* 2008), while Hawaii has lost a considerable number of endemic bird species since the introduction of avian malaria at the end of the 20th Century (Wikelski *et al.* 2010). On a broader scale, the global trade and transport of parrots has led to the worldwide spread of Psittacine beak and feather disease (PBFD), a highly infectious and often fatal disease among psittacine birds (Heath *et al.* 2004). Originating from Australia (Heath *et al.*

2004), PBFD has now been reported in invasive parrot populations such as the recent report of PBFD in wild populations of invasive ring-necked parakeets in Great Britain (Sa *et al.* 2014), and endemic parrot species including the Mauritius parakeet, *Psittacula echo* (Kundu *et al.* 2012), Cape parrots, *Poicephalus robustus* (Regnard *et al.* 2015), and orange-bellied parrots, *Necophema chrysogaster* (Peters *et al.* 2014). Conservation management efforts for the critically endangered endemic Mauritius parakeet *Psittacula echo*, were compromised following an outbreak of PBFD which resulted in the loss of some 32 birds due for reintroduction (Tollington *et al.* 2013).

1.2.2 Evolutionary genetic impacts

Alongside ecological impacts, invasive species can prompt a change in the evolutionary trajectory of native species (Strauss *et al.* 2006; Hendry *et al.* 2008). The spread of invasive species in new environments can initiate rapid evolutionary processes such as selection, driving invasion success while altering genetic mechanisms at play in native populations. The evolutionary aspects of invasion biology have been examined in depth (Mooney & Cleeland 2001; Lee 2002; Sax *et al.* 2007; Westley 2011) and the understanding that invasive species can drive evolutionary change has led to further questions about the underlying genetic mechanisms and adaptive processes that drive such changes, and how rapidly and frequently they occur (Carroll, 2007; 2008).

Biological invasions can act as a dynamic form of novel selection (Carroll 2007). Exposure to invasive species can induce evolutionary adaptation in native species. Native predators at risk from invasive species can demonstrate behavioural and morphological changes that can reduce the risk of mortality (Carroll 2007). In Australia, two native snake species, *Psuedechis porphyiacus* and *Dendrelaphis punctualatus,* which are gape-limited predators, demonstrated morphological adaptation in response to exposure to invasive toxic cane toads. Both species showed a steady reduction in gape size and an increase in body length over a period of 80 years, as such reducing the risk of ingesting a cane toad large enough to kill it (Phillips & Shine 2004). Such rapid adaptations have been observed in different species, for example soapberry bugs in North America and Australia evolved rapidly in response to invasive weeds (Carroll 2007). In response to invasive lizards, *Anolis sagrei,* native Florida lizards, *Anolis carolinensis,* moved to higher perches and in just 20 generations, rapidly evolved larger toepads (Stuart *et al.* 2014). The ability of a native species to rapidly adapt in behaviour and morphology may enhance their ability to cope with biological invasions (Carroll 2007).

While native species have been observed to evolve in response to invaders, invasive species themselves can also undergo rapid evolution in response to their interactions with new

environmental conditions such as temperature and climate, and native species such as competitors, predators or prey, improving their chances of a successful invasion (Mooney & Cleland 2001; Lee 2002; Prentis 2008). Common adaptive responses to selective pressures include plasticity, phenology, physiology and morphology (Lee 2002). Hybridisation and introgression between invasive and native species is common across many taxa including fish, birds and mammals (Mooney & Cleland 2001). Encounters between native and invasive species can often dilute the native species gene-pool leaving no 'pure' natives (Huxel 1999), as seen in Scottish wildcats (*Felis silvestris*) that have hybridised with domestic cats (Beaumont *et al.* 2001). Interbreeding between invasive and native species can also increase the threat of extinction due to hybridisation introgression (Mooney & Cleland 2001). Small island populations are more vulnerable to extinction as a result of hybridisation as they are often less genetically diverse in contrast to more mainland species (Frankham 1997; Mooney & Cleland 2001). Introduced mallard ducks (*Anas platyrhynchos*) have hybridised with several closely related endemic duck species causing introgression, contributing to the population declines in New Zealand grey ducks (*Anas superciliosa*), and the endangered Hawaiian duck (*Anas wyvilliana*) (Rhymer & Simberloff 1996). Problems experienced by invasive species caused by loss of genetic diversity through founder events can be alleviated by hybridisation with native or other non-native species, injecting new 'native' genes into the population. Therefore, this mechanism can increase an invasive species chances of establishment in their new environment (Lee 2002).

1.2.3 Economic impacts and policy

In addition to their insidious threats to biodiversity, invasive species are significantly problematic and costly for global agriculture and the economy. On a global scale, estimated economic losses from invasive species are €1 trillion per year as a result of loss of productivity, damage to riverbanks and infrastructure, control and management (European Commission 2013). Europe experiences an annual economic impact of an estimated €12.5-20 billion from invasive species, of which €9.6 billion is from damage to infrastructure and health sectors. To reduce and mitigate problems posed by invasive species The EU's Biodiversity Strategy to 2020 contains a dedicated legislative instrument on invasive alien species. Recently in September 2014, the European Commission adopted a new Regulation on invasive alien species. Such regulations expect countries to take action towards dealing with problems posed by invasive species, for example, the United Nations (UN) Convention on Biological Diversity (CBD) expects countries to 'eradicate those alien species which threaten ecosystems, habitats or species', while the Bern Convention on the Conservation of European Wildlife and Natural Habitats requires the 'strict control of the introduction of non-indigenous species'.

1.3 Invasive and endemic parrots

The parrots (Psittaciformes) not only form one of the most distinctive groups of birds, but are also one of the most endangered groups of birds in the world with 95 (26.8%) of the 354 known parrot species currently threatened with extinction, and a further 36 species 'near threatened' (Jetz *et al.* 2014). Over the past 500 years approximately 163 avian extinctions have occurred across the globe, comprising some 20 parrot species (12%), half of which were island endemics (Collar 2000; Butchart *et al.* 2006). Oceanic island ecosystems are a particularly rich source of evolutionary diversity in the form of endemic populations and this diversity makes a substantial contribution to global biodiversity (Whittaker & Fernández-Palacios 2007). Unfortunately, endemic populations on small, isolated islands are affected by deterministic factors that include habitat loss and invasive species, as well as stochastic factors, making them acutely vulnerable to extinction (Shaffer 1981; Nott *et al.* 1995; Pimm *et al.* 1995; Frankham 1997, 2005). Major reasons for declining endemic parrot populations include invasive species, poaching, habitat loss and the pet trade (Cheke & Hume 2008; Perrin 2012).

Interestingly, while removal from their native habitat for the pet trade has caused the decline of many endemic parrots, the popularity of parrots as pets and their global transport has contributed to some 60 parrot species (16% of total living species) currently breeding outside of their native range, with the more widely-distributed species (*Agapornis, Amazona, Aratinga, Myiopsitta monachus, Psittacula)* being the most successful at establishing populations in non-native areas (Menchetti & Mori 2014). Parrots established in non-native environments are a result of numerous factors; in addition to their popularity as pets and high numbers being traded and bred, they are highly synanthropic, appearing to be adapted to surviving in a wide variety of environmental conditions (Duncan *et al.* 2003; Cassey *et al.* 2004).

Remarkably, a total of 13 alien species of parrot are established in Europe and one example is the monk parakeet *(Myiopsitta monachus)*. Having naturalised in Puerto Rico, Kenya, Japan, Spain, Italy, Belgium, Czech Republic, the UK and the USA (Butler 2005; Russello *et al.* 2008; Strubbe & Matthysen 2009), the monk parakeet has had severe ecological and economic impacts and is regarded as an agricultural pest in and out of its native range in South America (Russello *et al.* 2008). Having established self-sustaining populations in the USA in the 1960s, the species is continuing to expand its range and population size (Pruett-Jones *et al.* 2007; Russello *et al.* 2008). The parakeets' invasion of the USA seems to have been facilitated by the international pet bird trade, as their popularity as pets has allowed repeated release events in the USA resulting in successful establishment (Russello *et al.* 2008; Carrete & Tella 2008). Monk parakeets cause of severe economic damage in the USA, where electrical fires and power outages occur frequently as a result of parakeets nesting on utility structures (Pruett-Jones *et al.*

2007). Research indicates it would be necessary to remove 20% of the adult population or destroy 50% of the nests each year to reduce the population size monk parakeets in the USA (Pruett-Jones *et al.* 2007). A further suggested population control method for these invasive parakeets, to inhibit reproduction, is by using Diazacon (Avery *et al.* 2008). The species is also present in the UK, but is not regarded to be at a self-sustaining level, and measures to control the population to avoid successful establishment are currently underway (Fera 2012).

1.4 Study systems

This research presented a unique opportunity to study invasive and endemic parrots, with a geographical focus not only on the Asian and African continents and Europe, which represent the native and invasive distribution of the ring-necked parakeet (*Psittacula krameri*), but across the Indian Ocean islands where this species has also become invasive and is the former historic range of several evolutionarily important endangered and extinct island endemic species of parrots.

1.4.1 The globally invasive ring-necked parakeet

This research uses the ring-necked parakeet, *Psittacula krameri* (Scopoli 1769) as an invasive study system. The ring-necked parakeet is a global avian invader present in multiple locations across Europe. Owing to their widespread distribution and well-documented invasion history, ring-necked parakeets provide an ideal system to identify evolutionary and genetic mechanisms that allow a species to become successfully established in areas outside of its native range. Native to Asia and sub-Saharan Africa, there are four recognised subspecies of ring-necked parakeet (Forshaw 2010). Two subspecies are native to Asia (*P. k. borealis,* found in eastern Pakistan, throughout Northern India, Nepal and Burma, and *P. k. manillensis,* found in southern India and Sri-Lanka), and two native to Africa (*P. k. krameri,* found from Senegal to western Uganda and Southern Sudan, and *P. k. parvirostris,* found in Eastern Sudan to Northern Ethiopia; Fig. 1.2).

The ring-necked parakeet is one of the world's most successful invasive parrots and one of Europe's top 100 worst alien species **(**DAISIE European Invasive Alien Species Gateway, 2008**)**, with breeding populations established in over 35 countries across five continents (Butler 2003; Lever 2005). Large numbers of ring-necked parakeets have become established in a number of European countries since the late 1960s, including the UK, Germany, the Netherlands, France, Spain, Italy, Greece and Belgium (Lever 2005), as well as numerous other countries outside of Europe such as Mauritius and the Seychelles in the Indian Ocean. In their native range ring-necked parakeets are found in various woodland habitats, farmlands, and urban gardens and parks (Juniper & Parr 1998; Khan 2002), while in their invasive ranges, they

readily inhabit forests and parks that are within or surrounded by urban habitats (Strubbe & Matthysen 2007). The rapid spread of the ring-necked parakeet along with evidence of explosive population growth in Europe (Butler *et al.* 2013) is a major cause for agro-economic and environmental concern. For example, parakeets colonised the UK in the late 1960s from a few escaped pet birds; now they are the UKs fastest growing bird population, currently numbering >32,000 individuals (Butler *et al.* 2013; Peck *et al.* 2014).

In Europe ring-necked parakeets have been shown to compete with native species for nest cavities (Hernández-Brito *et al.* 2014; Strubbe & Matthysen 2007; 2009) and may have a detrimental impact upon the foraging behaviour of native birds (Peck *et al.* 2014). On Mauritius, invasive ring-necked parakeets compete with the endangered Mauritius parakeet (*Psittacula echo*) for nest sites and food resources (Tatayah *et al.* 2007; Jones *et al.* 2013), and are a suspected source of Psittacine beak and feather disease (PBFD), caused by the highly infectious Beak and Feather Disease Virus which threatens the population of the endangered endemic Mauritius parakeet (Kundu *et al.* 2012). Ring-necked parakeets also occur on the Seychelles where their recent establishment (Jones *et al.* 2013) presents a potential disease threat to the endemic Seychelles black parrot, *Coracopsis nigra barklyi* (Seychelles Islands Foundation, 2012).

Ring-necked parakeets are a severe crop pest across their native ranges and are known to decimate maize and fruit crops in India (Ramzan & Toor 1973; Forshaw 2010, Ahmad *et al.* 2012). Whilst crop damages by ring-necked parakeets have been widely recorded across Europe, few studies have yet to quantify the economic cost of such damage (Menchetti & Mori 2014). In the UK damages to vineyards in Surrey are estimated to cost around £5000 per year (Fletcher & Askew 2007). In addition to economic damage, invasive ring-necked parakeets may cause damage to human facilities. Three airplane birdstrikes were recorded in 2004 and 2005, from ring-necked parakeets at Heathrow Airport, UK, each costing an average of £20,000 (Fletcher & Askew 2007).

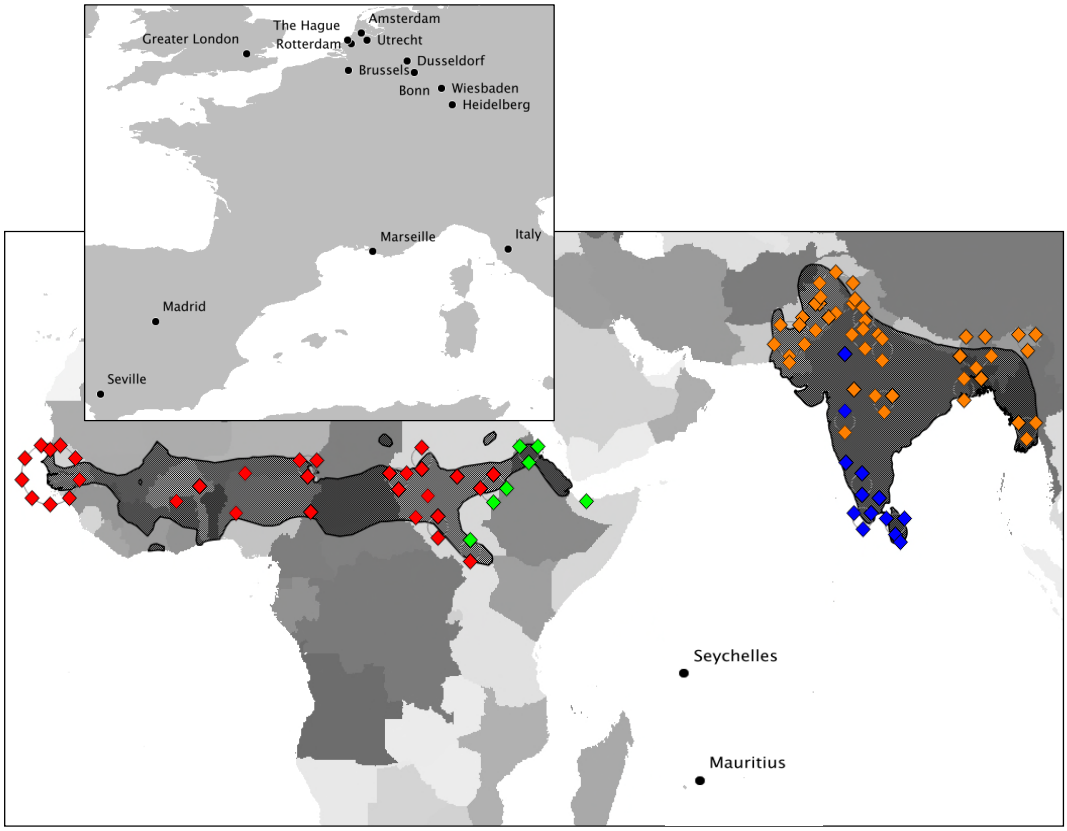

Fig. 1.2. Native range distribution of *Psittacula krameri* (black outlined area across Sub-Saharan Africa and Southern Asia (IUCN 2013)). Sample locations; diamonds = historical specimens from the native range. Colours refer to the subspecies designation given on the label of each museum specimen; *P. k. borealis* = orange, *P. k. manillensis* = blue, *P. k. krameri* = red, *P. k. parvirostris* = green; black dots = invasive populations sampled for this study from the invasive range. Locations of all museum specimens and sampled invasive populations are plotted; where necessary overlapping symbols have been displaced around the true co-ordinate point to display all sample information.

1.4.2 Endemic and extinct parrots of the Indian Ocean islands.

In addition to understanding invasive systems, examining the population genetics of endemic species is important for informing conservation management. The Western Indian Ocean islands are currently home to just two extant endemic parrot species, the Seychelles black parrot (*Coracopsis nigra barklyi)*, and the Mauritius parakeet (*Psittacula echo)*. However, these islands were formerly a rich source of parrot diversity, with at least five endemic island parrot species described prior to their extinction (Temple 1981; Hume 2007; Cheke & Hume 2009), making them an interesting study system (Fig. 1.3).

The Seychelles black parrot (*Coracopsis nigra barklyi*; Newton 1867) is a member of the *Coracopsis* genus comprising two species; the greater vasa parrot *C. vasa,* and the lesser vasa parrot, *C. nigra* (Forshaw 2010). An endangered species endemic to the Seychelles archipelago, the population of Seychelles black parrot, estimated at only 520–900 individuals (Reuleaux *et al.* 2013), is restricted to Praslin (Evans 1979; Watson 1984; Reuleaux *et al.* 2013), a small island of just 38km^2. Here the black parrots occur in their highest densities in the mature palm forest as found in the UNESCO World Heritage site of the Vallée de Mai. This area comprises a rare habitat containing all six of the palm species endemic to Seychelles (Usher 1993), which also form a substantial part of the parrot's diet and nesting habitat (Gaymer *et al.* 1969; Evans 1979; Reuleaux 2011; Reuleaux *et al.* in press). Previous records of the Seychelles black parrot occurring on Curieuse, Aride and Marianne islands, suggest a possible range contraction. In addition, field surveys over the last 40 years suggest that the population on Praslin may have declined between the 1960s and 1980s, prior to an estimate of 200–300 birds in 2001 and 520–900 by 2011 (Gaymer *et al.* 1969; Merritt *et al.* 1986; Rocamora & Skerrett 2001; Reuleaux *et al.* 2013). The history of possible range contraction and field observations raises concerns about whether this island subspecies has experienced a population bottleneck, and is now genetically impoverished, thereby compromising its evolutionary potential (Soulé 1972; Nunney 1993; Amos & Harwood 1998). As such the Seychelles black parrot is an important study system to understand the importance for conservation of such evolutionarily distinct endemic forms of parrot.

The islands of Mauritius, Seychelles, Madagascar, Reunion, Rodrigues and Grand Comoros remained largely pristine until the 16th century (Hume 2007). Human impacts including intense hunting and the introduction of predatory exotic mammals led to the extinction of many of the endemic parrots including the Reunion parakeet (*Psittacula eques*) by 1732, the Rodrigues parakeet (*Psittacula exsul*) by 1875, the Seychelles parakeet (*Psittacula wardi*) by 1906, and the Mascarene parrot (*Mascarinus mascarinus)* from Reunion by the end of the 19th century (Hume & Waters 2012). As invasive ring-necked parakeets are now present on these Indian Ocean islands, they present the ideal study system to examine the consequences of the extinctions of such evolutionarily distinct species and their replacement by invasive parrots.

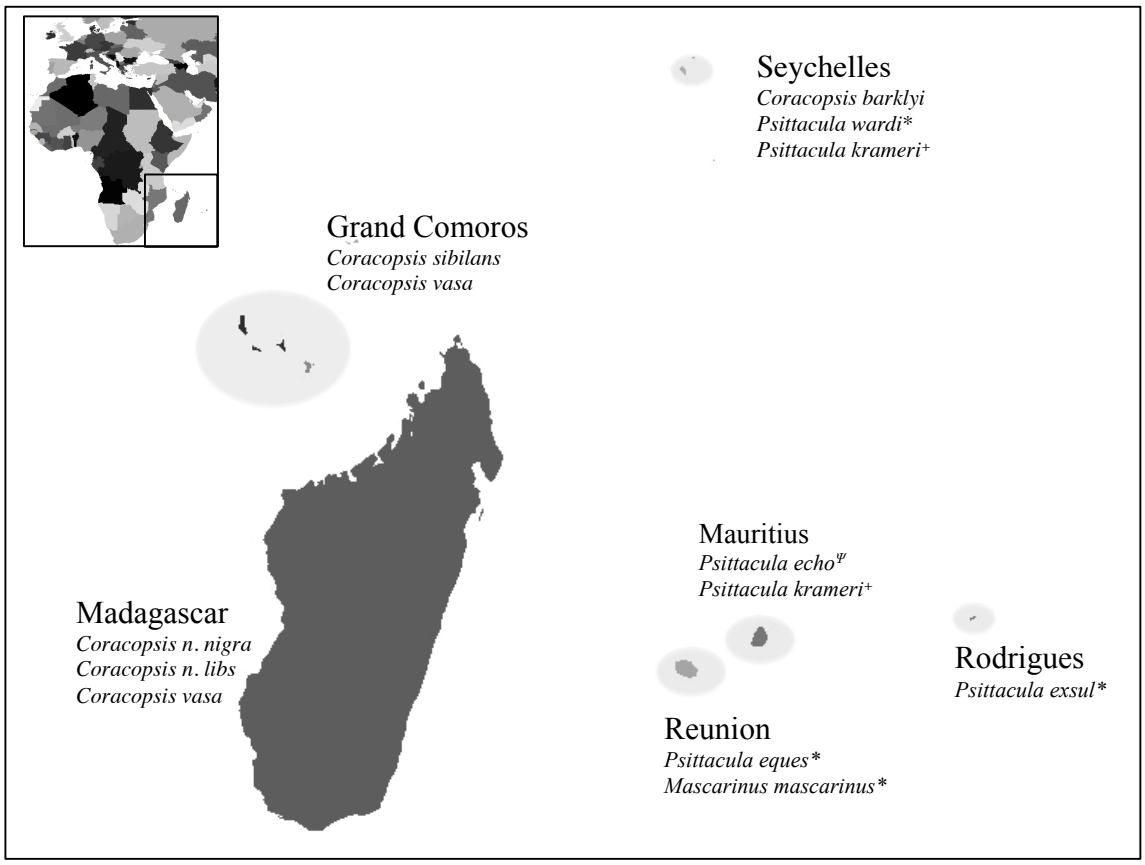

Fig. 1.3. Distribution of extinct*, invasive+, endangered endemic Ψ, and other endemic parrots across the Indian Ocean Islands. Small islands are highlighted with grey shading.

1.5. Structure of thesis:

Chapter 2. I examined the reliability of molecular tools and demographic data frequently used to inform conservation and invasion biology, in particular the mitochondrial (mtDNA) control region gene as an indicator of genetic diversity in a population. I conducted a full literature review of avian haplotype diversity measures for control region to test whether such measures are reliable indicators of population size, and sensitive enough to detect population bottlenecks. Establishing the usefulness of such frequently used genetic markers is important prior to their application to infer patterns within and between invasive and endemic parrots.

Chapter 3. Establishing source populations of invasive parrots is an important tool for identifying mechanisms that underpin successful invasions. Here, I construct a time calibrated molecular phylogeny of ring-necked parakeets from museum specimens representative of all four subspecies across a wide geographical representation of their native range, using 868bp of mtDNA (control region and cytochrome *b*). I used microsatellite data in combination with mtDNA data to identify the ancestral origins of ring-necked parakeets in a number of invasive populations in Europe and the Indian Ocean Islands. Finally I examined climatic similarities

between the native and invasive ranges, and import data from the wild bird pet trade to explain observed patterns in ancestral origins.

Chapter 4. Understanding the true severity and impact of a bottleneck effect within an invasive population, against a backdrop of genetic diversity and structure is important for understanding mechanisms that underpin population growth and invasion success. Here I combine a suite of microsatellite loci, originally developed in Mauritius parakeets, with mtDNA sequence data. I used a novel approach to estimate the observed bottleneck effects from contemporary genetic data in a number of European populations of invasive ring-necked parakeets, and examined how those estimates align against expected bottleneck effects from documented demographic data. I examined levels of genetic diversity in comparison to those found in the native range, structure and gene-flow, and tested a suite of genetic, climatic and human-demographic factors for their influence on population growth rates.

Chapter 5. To determine evolutionarily important distinct species requiring direct conservation management, I applied the same molecular techniques and genetic markers used for invasive parrots to construct a molecular phylogeny and examine the evolutionary and morphological distinctiveness of the Seychelles black parrot. Additionally I compared contemporary levels of genetic diversity to historical levels to identify whether the Seychelles black parrot has become genetically impoverished as a result of a range contraction and population bottleneck.

Chapter 6. In addition to data on invasive and endemic parrots, I reconstructed a time calibrated molecular phylogeny of the Indian Ocean parrots to include three previously unstudied extinct endemic parrot species. This phylogeny was used to examine potential biotic homogenisation across the Western Indian Ocean islands by establishing changes in temporal and spatial phylogenetic diversity that is important for evolutionary adaptation, as a result of extinctions and invasions. I also used the reconstructed phylogeny to inform conservation management by way of identifying the most evolutionarily suitable candidates as ecological replacements for the extinct parrots.

Chapter 7. I provide a general discussion of the key findings of this thesis and their contribution to our understanding of the molecular evolution and population genetics of invasive and endemic parrot species, and how these findings can inform conservation management.

Appendix 1. A manuscript incorporating genetic data (from Chapter 3) obtained for ring-necked parakeets into ecological niche modelling. Evolutionary phylogroups across the native range

were identified and included in niche models to predict areas across Europe suitable for future establishment by ring-necked parakeets.

1.6 References

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German manuscript from the 15th Century

Chapter 2.

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How closely do measures of mitochondrial DNA control region diversity reflect recent trajectories of population decline in birds?

Hazel Jackson, Byron J.T. Morgan & Jim J. Groombridge

2.1 Abstract

Monitoring levels of genetic diversity in wildlife species is important for understanding population status and trajectory. Knowledge of the distribution and level of genetic diversity in a population is essential to inform conservation management, and help alleviate detrimental genetic impacts associated with recent population bottlenecking. Mitochondrial DNA (mtDNA) markers such as the control region have become a common means of surveying for within-population genetic diversity and detecting signatures of recent population decline. Nevertheless, little attention has been given to examining the mtDNA control region's sensitivity and performance at detecting instances of population decline. We review genetic studies of bird populations published since 1993 that have used the mtDNA control region and reported haplotype diversity, number of haplotypes and nucleotide diversity as measures of within-population variability. We examined the extent to which these measures reflect differences in known demographic parameters such as current population size, severity of any recent bottleneck and IUCN Red List status. Overall, significant relationships were observed between two measures of genetic diversity (haplotype diversity and the number of haplotypes), and population size across a number of comparisons. Both measures gave a more accurate reflection of recent population history in comparison to nucleotide diversity, for which no significant associations were found. Importantly, levels of diversity only correlated with demographic declines where population sizes were known to have fallen below 500 individuals. This finding suggests that measures of mtDNA control region diversity should be used with a degree of caution when inferring demographic history, particularly bottleneck events at population sizes above N=500.

2.2 Introduction

A fundamental aim of conservation genetic studies is to provide empirical evidence informing conservation management strategy for minimizing the loss of genetic diversity in order to promote individual or within-population fitness or to enhance the evolutionary potential of endangered species (Frankham 1996; Hughes & Hughes 2007). Exposure to a population bottleneck can frequently result in loss of genetic diversity, often followed by associated problems such as inbreeding depression (Heber & Briskie 2010), which in turn can compromise a population's reproductive fitness and survival (Crnokrak & Roff 1999). Monitoring levels of genetic diversity is important as it can reveal the extent to which threatened populations might be genetically impoverished and can identify genetically important individuals or groups of individuals (heterozygotes or carriers of rare alleles) for conservation management (Allendorf *et al.* 2010). Consequently, knowledge of the distribution and level of population genetic diversity and the extent of recent population bottlenecking can help alleviate these detrimental effects and inform the management of endangered species.

While long-term field monitoring of wildlife populations can provide detailed information on their demographic history, DNA markers have increasingly become a common alternative means of surveying within-population genetic diversity and detecting signatures of recent population decline (Allendorf *et al.* 2010). The maternally-inherited mitochondrial DNA (mtDNA) control region is a non-coding genetic marker commonly used in conservation genetic studies to examine genetic structure at the population level because it is considered to be the most rapidly evolving region of the mitochondrial genome (Baker & Marshall 1997; Ruokonen 2002). This locus is broadly considered to be suitable for examining intraspecific relationships among closely related groups of individuals (Sinclair & Pérez-Losada 2005). Among birds, the mtDNA control region is also reasonably straightforward to amplify across different taxonomic groups because the control region is divided into three domains; a central conserved region flanked by two variable A-rich domains which show higher levels of sequence variation (Ruokonen 2002). The genes that lie on either side of it (the tRNA and ND6 genes) are reasonably conserved across Aves (Quinn 1997), allowing for easy PCR primer design and making it a popular choice for use as a molecular marker in population genetic studies of birds. Finally, this stretch of maternally inherited DNA does not recombine and is in linkage disequilibrium (Höglund 2009). The resulting pattern in haplotype distribution within and between populations is believed to be informative of a population's evolutionary history (Höglund 2009).

The number of published studies that apply the mtDNA control region as a within-population marker in birds has increased ten-fold from 1993-2011 (Fig. 2.1). Surprisingly, despite this increase in studies of birds, little attention has been given to examining its sensitivity as a

population level marker. Whether average levels of mtDNA nucleotide diversity across large groups of animals correlate with population size has been subject of intense debate (Bazin *et al.* 2006; Mulligan *et al.* 2006; Nabholz *et al.* 2008, 2009). Here, we aim to inform this debate on a lower taxonomic level for a commonly used mitochondrial marker. We carry out analyses on genetic diversity data from published bird studies to address the question of how well measures of genetic diversity derived from mtDNA control region reflect a recent decline in population size and bottleneck severity. We review genetic studies of bird populations published since 1993 and examine the extent to which the different measures of mtDNA genetic diversity reflect differences in known demographic parameters such as population size, bottleneck size and IUCN Red List status.

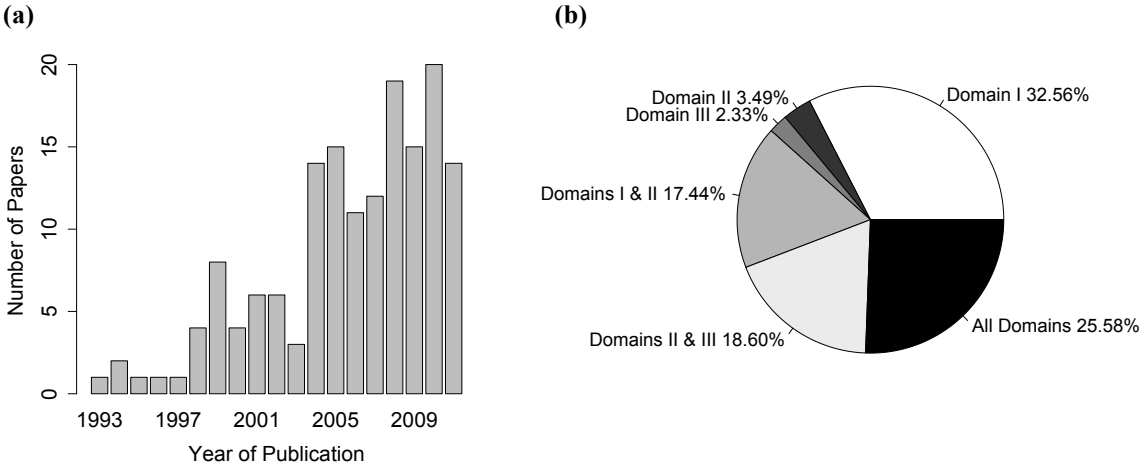


Fig. 2.1. (a) Since 1993, 157 papers have been published on avian population genetics using mitochondrial control region and measures of genetic diversity. A total of 79 populations were included in this study where population data were also available. (b) Proportion of studies using various domains of the control region in the 79 populations included in this paper.

2.3 Methods

The most commonly calculated (and cited) measures of genetic diversity derived from mitochondrial control region sequence data are haplotype diversity (Nei 1987), number of haplotypes (Nei 1987) and nucleotide diversity (Nei & Li 1979). A review of published literature was conducted by searching Google Scholar and the ISI Web of Knowledge databases, to obtain measures of diversity for avian population genetics studies using mitochondrial control region. Published studies were included in our final dataset where all three diversity measures were given. Studies comprising numerous haplotypes observed only once were excluded as potentially high risk of sequencing error and inaccurate calculation of genetic diversity, resulting in a final dataset comprising 79 different populations of bird species (Table. S2.1 in supplementary material). For each population, estimates of population size at the

time of sampling were obtained from the publications used in this study or from current data available from Birdlife International (Birdlife International 2011) that were representative of the geographical area across which the samples were collected (certain studies were used for some comparisons and not others based on available data). As the range of population sizes differed widely, the $\log_{10}$ of these values were used for analysis. For the purpose of this study, rather than quantifying bottleneck shape in terms of duration, rate of decline and recovery, populations were categorised as having experienced a bottleneck if population size was documented as having declined to fewer than 1000 individuals. Subsequently, where information was available, populations were further categorised according to the documented size of the bottleneck (fewer than 100, 101-500 and 501-1000 individuals). The most severe conservation category reached at any time by each species/population under the IUCN Red List was taken for analysis, on the assumption that the most severe category reflected the most extreme extent of a recent bottleneck (categories are 'vulnerable, near threatened, endangered and critically endangered'; the latter being the most severe) (IUCN 2011). Species/populations were also grouped into those originating from mainland or islands and island endemics or island non-endemics, in order to assess any insular effects.

As the DNA sequence length amplified and the number of individuals sampled varied for each population used in this study, linear regressions were performed to ascertain if either should be included in the analysis. The only significant relationship was between the number of individuals sampled and the number of haplotypes ($r = 0.267$, $p < 0.05$). To account for this, an *ad hoc* standardisation method was applied to the number of haplotypes where number of haplotypes was divided by the square root of the number of individuals sampled.

Generalised additive models were used to investigate relationships between haplotype diversity (H), number of haplotypes (nH), nucleotide diversity (π) and population size using the mgcv package in R (Wood 2006), which provides automatic smoothing. These models were found to provide better representations than linear regressions (Wood 2006).

As the log-transformed data were not normally distributed ($W = 0.950$, $p < 0.01$) non-parametric tests were chosen for analysis. Kruskal-Wallis tests were used to identify significant differences present in measures of genetic diversity for populations bottlenecked to different sizes and categorised differently according to their IUCN Redlist status. The Kruskal-Wallis test gives an H statistic that represents the variance in ranks between the groups while making the assumption that the populations being compared share the same shaped distribution, with possibly different medians. Barlett's test for homogeneity of variances was performed between bottleneck sizes and measures of genetic diversity to check for equality of variances (haplotype diversity: $k^2_{(3)} = 1.77$, $p = 0.62$, number of haplotypes: $k^2_{(3)} = 3.53$, $p = 0.32$, nucleotide

diversity: $k^2_{(3)} = 1.55$, $p = 0.67$). *Post hoc* multiple comparison tests were performed using the pgirmess package in R (Giraudoux 2013). Where possible, analyses were performed separately on the different domains of the control region, to explore the extent to which marker sensitivity varied across the three domains.

Unequal variance *t*-tests were used to identify significant differences in mean measures of genetic diversity between island and mainland species, and island endemic and non-endemic species. This test accounts for unequal variances and is robust with respect to departures from normality due to the Central Limit Theorem (Ruxton 2006). Additional non-parametric Wilcoxon rank sum tests were performed in support of the *t*-tests.

2.4 Results

The populations included in this study used six different combinations of the three control region domains. The most commonly used was domain I, followed by a combination of all three domains (Fig. 2.1). Significant relationships were observed between haplotype diversity and the number of haplotypes, with population size ($r^2_{adj} = 0.179$, $\beta = 1.964$, SE = 0.593, $p=0.01$ and $r^2_{adj} = 0.19$, $\beta = 1.675$, SE = 0.389, $p<0.001$ respectively), while no relationship was identified for nucleotide diversity ($r^2_{adj} = 0.0455$, $\beta = 8.352$, SE = 5.709, $p = 0.139$; Fig. 2.2). The generally low values of r^2_{adj} obtained here reflect the degree of scatter about the fitted curves in Fig. 2.2. Although haplotype diversity is significantly correlated with the number of haplotypes ($r = 0.73$, $p <0.01$), we find it useful to consider relationships for both measures with population size. When comparing bottlenecked with non-bottlenecked populations, typical haplotype diversity was significantly lower for populations between 101-500 and fewer than 100 individuals ($H_3 = 15.61$, $p<0.01$, Fig. 2.2 (a)). The standard number of haplotypes was significantly lower for populations below 100 individuals ($H_3 = 19.61$, $p < 0.001$, Fig. 2.2 (b)).

The standard number of haplotypes was significantly lower at a population size below 100 individuals for studies using all control region domains ($H_2 = 9.02$, $p < 0.05$, Fig. 2.3 (c)), while no further associations were present in any other single, or combination of control region domains. No differences were found between any of the three measures of diversity and IUCN listings using the Kruskal-Wallis test. The standard number of haplotypes differed between island and mainland species ($t_{15.8} = -2.179$, $p<0.05$; $W = 237.5$, $p<0.05$; Fig. 2.4). No differences were found between island endemics and island non-endemics. All box-plots and test results are provided in Fig. S2.5.

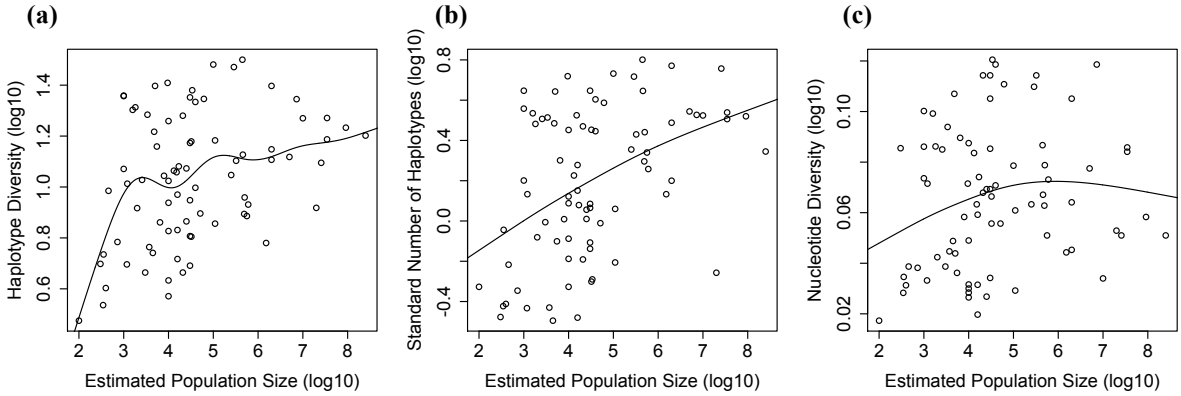
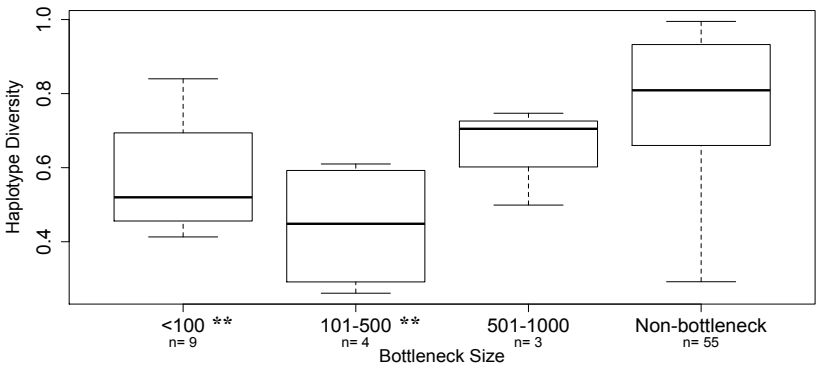
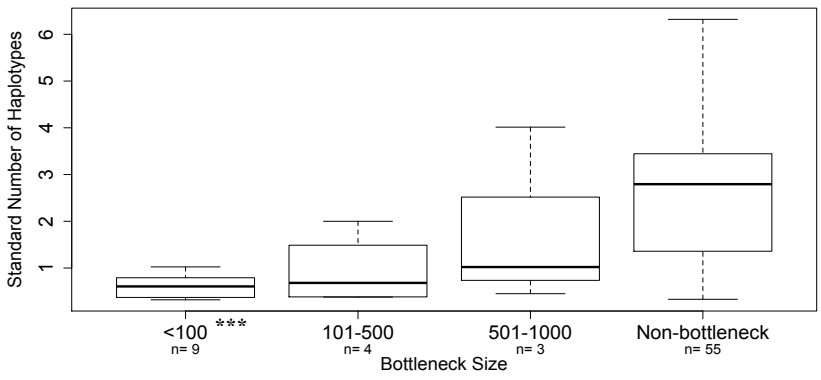


Fig. 2.2. Plots with fitted generalised additive models showing significant associations between $\log_{10}$ population size in the sampled area and (a) $\log_{10}$ haplotype diversity, (b) $\log_{10}$ number of haplotypes (standardised = $nH / (\sqrt{n})$), and no significant association with (c) $\log_{10}$ nucleotide diversity.

(a) Haplotype Diversity: Significant difference present at bottleneck sizes: <100 & 100-500 (H_3=15.61, *p<0.01***)	
(b) Standard Number of Haplotypes: Significant difference present at bottleneck size: <100 (*H_3*=19.61, *p<0.001****)	

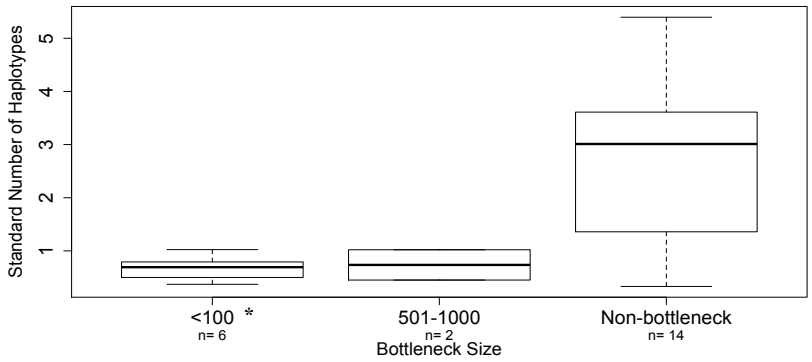
(c) Standard Number of Haplotypes: Control Region all domains *Significant difference present at bottleneck size: <100* *(H_2=9.02, $p<0.05$*)*	

Fig. 2.3. Kruskal-wallis tests showing significant differences between *(a)* haplotype diversity, *(b)* the standard number of haplotypes and *(c)* the standard number of haplotypes and bottlenecked sizes in studies using all three control region domains. Observed significant differences are indicated by * alongside the bottleneck category. No significant differences were found for nucleotide diversity.

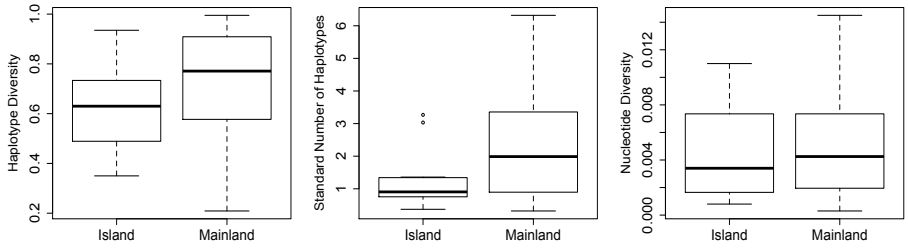
	Haplotype diversity	***Standard Number of haplotypes***	***Nucleotide diversity***
Island or mainland species *(n = 11,68)*			
	$t_{15.8}$=-2.179, $p<0.05$; W=237.5,$p<0.05$		

Fig. 2.4. Unequal t-test and supporting Wilcoxon rank sum test showing significant differences in mean and median values for the standard number of haplotypes between island and mainland species. All box-plots and tests are given in Fig. S2.5.

2.5 Discussion

In principle, demographic effects such as a founder effect, or a population bottleneck should leave a genetic signature in a species' genome (Hartl & Clark 2007). Haplotype diversity and the number of haplotypes appear to perform more consistently than nucleotide diversity in reflecting recent population size. The lack of relationship between nucleotide diversity and

population size, suggests discordance between loss of haplotype diversity and loss of nucleotide diversity during a bottleneck event. Nucleotide diversity is based on an unbiased assumption of neutrality and mutation equilibrium that calculates nucleotide differences between random sequences, as opposed to haplotype diversity that calculates the frequency of unique haplotypes within a population under an infinite-sites model (Kimura 1969). With a rapid reduction in population size, rare haplotypes are lost quickly through drift producing an immediate reduction in haplotype diversity, while the loss of nucleotide diversity, represented across all haplotypes is more gradual (Allendorf 1986).

We examined the sensitivity of these measures of genetic diversity by comparing them against populations that had experienced bottlenecks of differing severity as well as against IUCN Redlist category. No single measure showed a significant decrease in genetic diversity in the bottleneck size category of 501-1000, relative to non-bottlenecked populations. In contrast, haplotype diversity was significantly lower in populations that have experienced a bottleneck size between 101-500 and fewer than 100 individuals, while the number of haplotypes was significantly lower for populations below 100 individuals, suggesting that these two measures can detect a bottleneck so long as it has been sufficiently severe. As a measure of the uniqueness of a given haplotype within a population, haplotype diversity should indicate a reduction in frequency of such unique haplotypes as the population size declines. Similarly, we would expect to find fewer unique haplotypes in a population that has experienced a rapid reduction in size, or a founding event.

Isolated island populations have long been considered to be prone to greater risk of extinction than mainland populations due to lower genetic diversity and elevated levels of inbreeding on islands, with island endemics being at even greater risk (Frankham 1997). However, is this pattern of lower diversity on islands detectable using mtDNA control region sequence data? Neither haplotype or nucleotide diversity measures showed any significant difference between mainland, island or island endemic levels, whereas number of haplotypes was significantly different between island and mainland species, suggesting an inherent signature of evolutionary history irrespective of any recent changes in population size.

The relationship between population size and mtDNA diversity is unlikely to be strictly linear, particularly at generally low population sizes. Our data suggest populations that have not experienced a bottleneck have much larger ranges of genetic diversity in contrast to those that have experienced a bottleneck. As such, haplotype diversity and number of haplotypes appear to be reasonably useful for indicating existence of severe population crashes, but appear less reliable at detecting less severe bottlenecks. The inclusion of available supporting data such as measures of genetic diversity in a population before and after a bottleneck, or between an

original population and reintroduced founders are likely to improve the effectiveness of mtDNA as a tool for detecting and monitoring for changes in population size and genetic diversity.

2.6 Acknowledgements

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2.7 Supplementary material

Table. S2.1

Published studies used in this paper where I = Island, M = Mainland, E = Endemic, NE = not endemic, Pop size = population size in sampled area, NB = Not-bottlenecked, CE = Critically Endangered, EN = Endangered, NT = Near Threatened, LC = Least Concern, n = number sampled in study, Bases = length of genetic sequence used for study, nH = number of haplotypes, H = haplotype diversity, π = nucleotide diversity, - = no data available. A full list of references cited in this table is also included.

Species	Common name	I/M	E	Pop size	Bottleneck size	IUCN	n	Bases	nH	H	π	Author
Nipponia nippon	Crested ibis	M	-	300	<100	CE	36	629	2	0.413	0.0073	(Zhang *et al.* 2004)
Gallirallus okinawae	Okinawa rail	I	E	720	501-1000	EN	177	1056	6	0.499	0.0015	(Ozaki *et al.* 2010)
Hieraaetus fasciatus	Bonelli's eagle	M	-	10000	-	LC	72	253	4	0.542	0.0024	(Cadahía *et al.* 2006)
Anodorhynchus hyacinthinus	Hyacinth macaw	M	-	6500	101-500	EN	16	472	8	0.575	0.0080	(Faria *et al.* 2008)
Loxops coccineus coccineus	Hawaii akepa	I	E	8000	501-1000	EN	47	961	7	0.747	0.0034	(Reding *et al.* 2010)
Psittacula krameri	Mauritius ringed parakeet	I	NE	30000	<100	LC	41	800	5	0.660	0.0073	(Church 2011)
Cygnus buccinator	Trumpetor swan	M	-	5500	<100	LC	129	950	9	0.840	0.0013	(Oyler-McCance *et al.* 2007)
Cygnus buccinator	Trumpetor swan	M	-	24928	<100	LC	61	950	8	0.772	0.0007	(Oyler-McCance *et al.* 2007)
Cygnus buccinator	Trumpetor swan	M	-	459	<100	LC	68	950	5	0.694	0.0015	(Oyler-McCance *et al.* 2007)
Milvus milvus	Red kite	M	-	51000	101-500	NT	105	357	10	0.610	0.0031	(Roques & Negro 2005)
Haliaeetus leucogaster	White-bellied sea eagle	I	E	10000	-	LC	128	499	15	0.350	0.0008	(Shephard *et al.* 2005)
Myiopsitta monachus	Monk parakeet	M	-	32000	<100	LC	64	558	4	0.520	0.0025	(Russello *et al.* 2008)

Harpia harpyja	Harpy eagle	M	-	10000	-	NT	66	417	23	0.906	0.0076	(Lerner *et al.* 2009)
Mergus merganser	European goosander	M	-	21000	NB	LC	203	1047	42	0.918	0.0130	(Hefti-Gautschi *et al.* 2008)
Anser cygnoides	Swan goose	M	-	1000	NB	EN	48	268	11	0.770	0.0074	(Poyarkov *et al.* 2010)
Spizaetus nipalensis orientalis	Hodgson's hawk-eagle	I	NE	1800	NB	-	68	685	25	0.935	0.0074	(Asai *et al.* 2006)
Syrmaticus ellioti	Elliot's pheasant	M	-	100000	NB	VU	33	1154	31	0.992	0.0062	(P. Jiang *et al.* 2007)
Otis tarda	Great bustard	M	-	30000	-	VU	327	657	22	0.850	0.0048	(Alonso *et al.* 2008)
Otis tarda	Great bustard	M	-	25000	-	VU	309	657	20	0.579	0.0048	(Alonso *et al.* 2008)
Otis tarda	Great bustard	M	-	100	-	VU	18	657	2	0.209	0.0003	(Alonso *et al.* 2008)
Progne subis	Purple martin	M	-	2600	NB	LC	214	403	47	0.733	0.0072	(Baker *et al.* 2007)
Charadrius alexandrinus	Snowy plovers	M	-	460000	NB	LC	166	676	57	0.816	0.0045	(Baker *et al.* 2007)
Tetrao urogallus	Western capercaillie	M	-	5000000	NB	LC	112	443	37	0.809	0.0060	(Duriez *et al.* 2006)
Buteo swainsoni	Swainson's hawks	M	-	490000	NB	LC	279	416	33	0.608	0.0039	(Hull *et al.* 2007)
Oxyura leucocephala	White headed duck	M	-	4500	<100	EN	39	574	2	0.456	0.0024	(Muñoz-Fuentes *et al.* 2005)
Hymenolaimus malacorhynchos	New Zealand blue duck	I	E	1200	NB	EN	78	894	12	0.720	0.0051	(Robertson *et al.* 2007)
Branta canadensis occidentalis	Dusky canada goose	M	-	21000	-	LC	154	144	8	0.380	0.0046	(Talbot *et al.* 2003)
Dendroica cerulea	Cerulean warblers	M	-	560000	NB	VU	152	366	27	0.600	0.0026	(Veit *et al.* 2005)
Rissa brevirostris	Red-legged kittiwake	M	NE	321000	NB	VU	27	445	14	0.797	0.0130	(Patirana & Hatch 2002)
Strix occidentalis	Northern spotted owl	M	-	4779	NB	NT	131	522	35	0.880	0.0114	(Haig & Mullins 2004)
Strix occidentalis	California spotted owl	M	-	3050	NB	NT	37	1100	6	0.380	0.0015	(Haig & Mullins 2004)
Strix occidentalis	Mexican spotted owl	M	-	1592	NB	NT	45	522	23	0.930	0.0098	(Haig & Mullins 2004)
Bubo scandiacus	Snowy owl	M	-	290000	NB	LC	40	510	33	0.990	0.0120	(Marthinsen *et al.* 2008)
Parus cinctus	Siberian tit	M	-	2000000	NB	LC	56	911	23	0.832	0.0021	(Uimaniemi *et al.* 2003)
Parus caeruleus	Blue tit	M	-	10000000	NB	LC	181	465	45	0.912	0.0012	(Kvist *et al.* 2004)

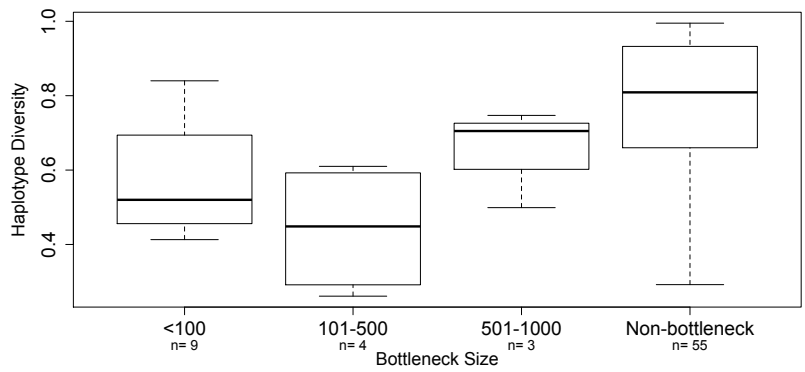
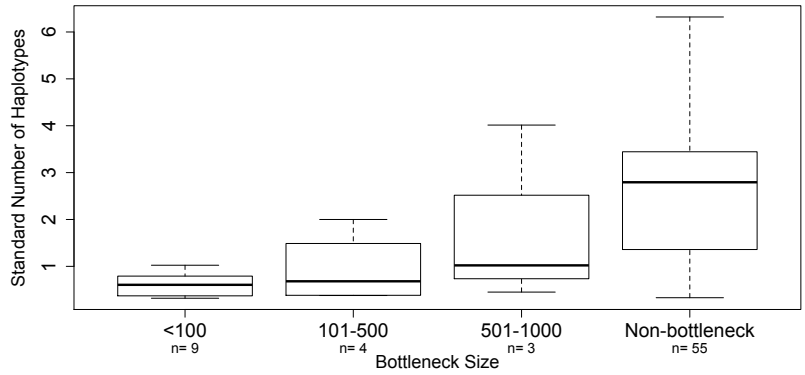
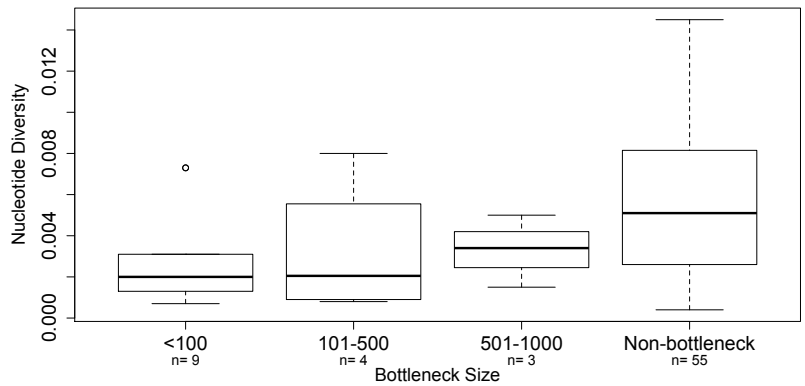
Grus canadensis	Sandhill crane	M	-	450000	NB	LC	73	650	54	0.995	0.0075	(Phymer *et al.* 2001)
Colinus virginianus	Northern bobwhite	M	-	92000000	NB	NT	153	655	41	0.890	0.0034	(Eo *et al.* 2009)
Toxostoma curvirostre	Curve-billed thrasher	M	-	2000000	NB	LC	66	367	48	0.970	0.0110	(Zink & Blackwell-Rago 2000)
Junco phaeonotus	Yellow-eyed junco	M	-	20000000	NB	LC	160	349	7	0.631	0.0028	(Mila *et al.* 2007)
Acrocephalus arundinaceus	Great.reed warbler	M	-	34800000	NB	LC	281	494	58	0.913	0.0074	(Hansson *et al.* 2008)
Lanius minor	Lesser grey shrike	M	-	1500000	NB	LC	78	385	12	0.495	0.0020	(Kvist *et al.* 2011)
Onychostruthus taczanowskii	White-rumped snowfinch	M	-	30000	-	LC	60	530	9	0.406	0.0012	(Yang *et al.* 2006)
Icterus pustulatus	Streak-backed oriole	M	NE	2000000	NB	LC	102	344	16	0.800	0.0041	(Cortes-Rodriguez *et al.* 2008)
Falco rusticolus	Gyrfalcon	M	-	110000	NB	NT	165	458	8	0.570	0.0009	(Johnson *et al.* 2007)
Locustella pryeri sinensis	Marsh grassbird	M	-	15000	NB	VU	75	807	29	0.759	0.0040	(Zhang *et al.* 2010)
Vultur gryphus	Andean condor	M	-	10000	NB	NT	24	501	4	0.650	0.0009	(Hendrickson *et al.* 2003)
Vultur gryphus	Andean condor	M	-	10000	NB	NT	6	501	3	0.730	0.0010	(Hendrickson *et al.* 2003)
Falco cherrug	Saker falcon	M	-	34000	NB	EN	186	412	7	0.964	0.0145	(Nittinger *et al.* 2007)
Falco rusticolus	Gyrfalcon	M	-	110000	NB	NT	19	458	5	0.857	0.0037	(Nittinger *et al.* 2007)
Tympanuchus pallidicinctus	Lesser prairie chicken	M	-	40000	NB	VU	62	394	22	0.945	0.0140	(Johnson & Dunn 2006)
Alectoris rufa	Red-legged partridge	M	-	7200000	NB	LC	121	234	37	0.950	0.0140	(Martínez-Fresno *et al.* 2007)
Chlamydotis undulata	Houbara bustard	M	-	62000	NB	VU	73	854	33	0.950	0.0122	(Idaghdour *et al.* 2004)
Acrocephalus arundinaceus	Great reed warbler	M	-	34800000	NB	LC	106	494	33	0.860	0.0071	(Bensch & Hasselquist 1999)
Ardeotis nigriceps	Great Indian bustard	M	-	350	101-500	CE	63	323	3	0.261	0.0008	(Ishtiaq *et al.* 2011)
Fringilla montifringilla	Brambling	M	-	250000000	NB	LC	10	585	7	0.870	0.0026	(Marshall 1997)

Columba mayeri	Pink pigeon	I	E	355	<100	CE	11	730	3	0.450	0.0012	(Swinnerton *et al.* 2004)
Ammodramus savannarum floridanus	Florida grasshopper sparrow	M	-	992	NB	LC	171	700	58	0.956	0.0100	(Bulgin *et al.* 2003)
Ammodramus savannarum floridanus	Florida grasshopper sparrow	M	-	992	NB	LC	105	700	37	0.955	0.0054	(Bulgin *et al.* 2003)
Jabiru mycteria	Jabiru stork	M	-	32700	NB	NT	60	520	22	0.854	0.0044	(Lopes *et al.* 2010)
Mergus squamatus	Scaly-sided merganser	M	-	10000	NB	EN	38	405	4	0.292	0.0007	(Solovyeva & Pearce 2011)
Mycteria americana	Wood stork	M	-	16000	NB	LC	40	292	12	0.546	0.0035	(Lopes *et al.* 2011)
Haliaeetus albicilla	White-tailed sea eagle	M	-	13200	NB	NT	102	499	17	0.764	0.0070	(Honnen *et al.* 2010)
Egretta rufescens	Reddish egret	M	-	40000	501-1000	NT	149	223	49	0.705	0.0050	(Bates *et al.* 2009)
Platalea ajaja	Roseate spoonbill	M	-	250000	NB	LC	50	483	16	0.750	0.0040	(Santos *et al.* 2008)
Motacilla alba	White wagtail	M	-	26000000	NB	LC	232	436	87	0.790	0.0026	(Pavlova *et al.* 2005)
Columba inornnta	Plain pigeon	I	E	3746	<100	EN	29	730	2	0.479	0.0020	(Young & Allard 1997)
Egretta eulophotes	Chinese egret	I	E	3400	NB	EN	90	433	31	0.920	0.0088	(Zhou *et al.* 2009)
Tragopan caboti	Cabot's tragopan	M	-	5000	NB	VU	53	508	32	0.970	0.0019	(Dong *et al.* 2010)
Accipiter gentilis	Northern goshawk	I	NE	2000	NB	LC	145	625	10	0.630	0.0018	(Asai *et al.* 2008)
Alectoris magna	Rusty-necked partridge	M	-	499999	NB	NT	82	458	25	0.670	0.0062	(Huang *et al.* 2009)
Lagopus mutus	Rock ptarmigan	M	-	16000	NB	LC	82	1150	3	0.432	0.0004	(Bech *et al.* 2009)
Lagopus mutus	Rock ptarmigan	M	-	16000	NB	LC	18	1150	6	0.680	0.0010	(Bech *et al.* 2009)
Ciconia boyciana	Oriental white stork	M	-	30000	NB	EN	66	463	36	0.953	0.0130	(Zan *et al.* 2008)
Ciconia boyciana	Oriental white stork	I	-	30000	NB	EN	17	463	3	0.522	0.0110	(Zan *et al.* 2008)
Larus saundersi	Saunder's gull	M	-	9600	NB	EN	50	550	37	0.974	0.0051	(Jiang *et al.* 2008)
Calidris alpine schinzii	Southern dunlins	M	-	600000	NB	LC	239	474	28	0.644	0.0053	(Wennerberg *et al.* 2008)

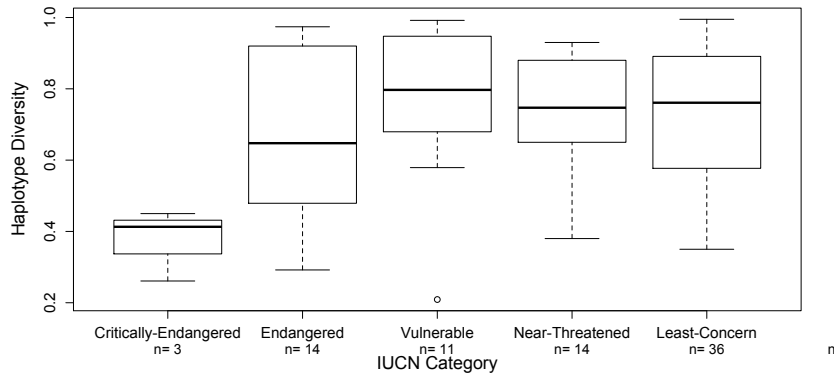
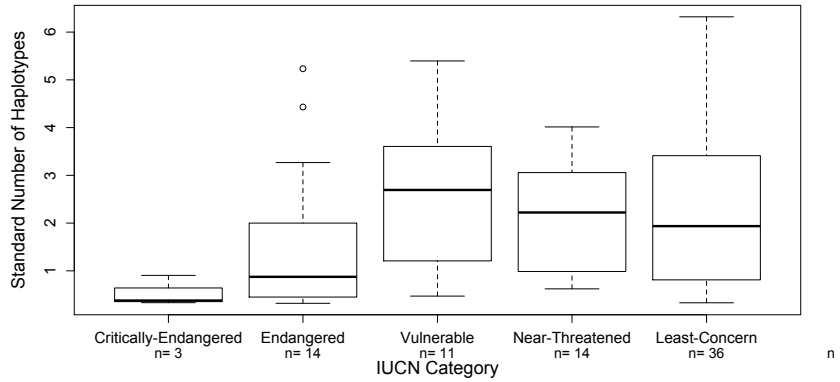
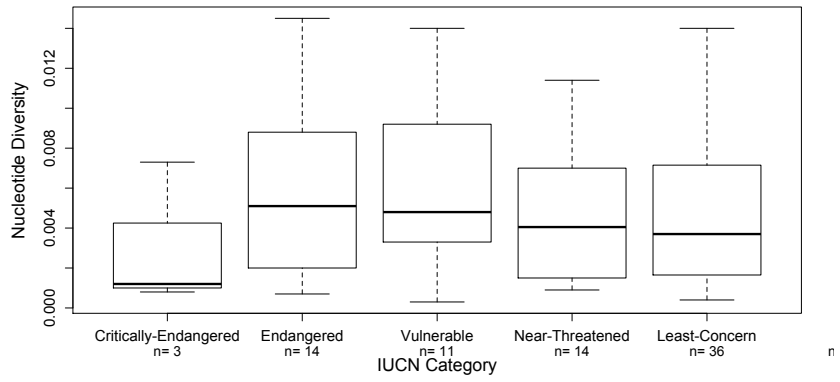
Falco peregrinus	Peregrine falcon	M	-	1168	NB	LC	184	405	5	0.411	0.0011	(Brown *et al.* 2007)
Aquila adalberti	Spanish imperial eagle	M	-	400	101-500	EN	60	345	3	0.322	0.0010	(Martínez-Cruz *et al.* 2004)
Aquila heliaca	Eastern imperial eagle	M	-	16800	NB	VU	34	345	7	0.779	0.0055	(Martínez-Cruz *et al.* 2004)

Fig. S2.5. The full set of Kruskal-Wallis tests to show significant and non-significant differences in values of the three measures of genetic diversity and population size categories (a), IUCN Red List categories (b), and studies using all three control region domains (c). Unequal variance t-tests and supporting Wilcoxon rank sum tests to show significant and non-significant differences in mean and median values of genetic diversity between island, mainland and island endemic species (d).

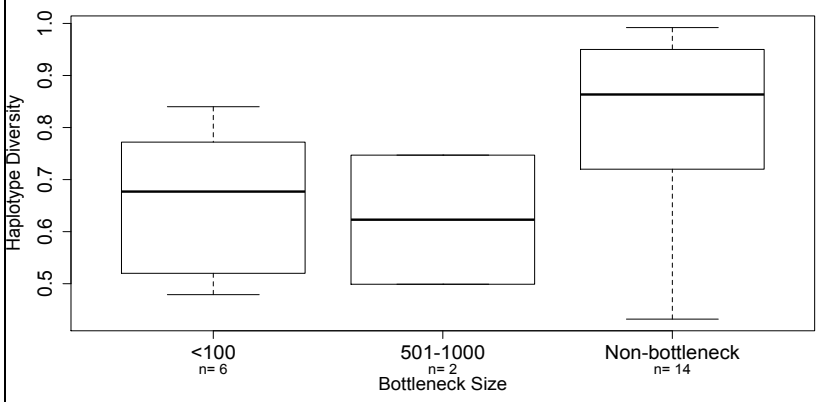
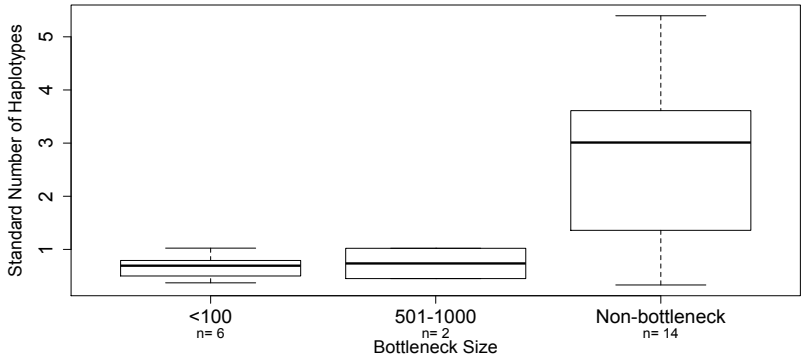
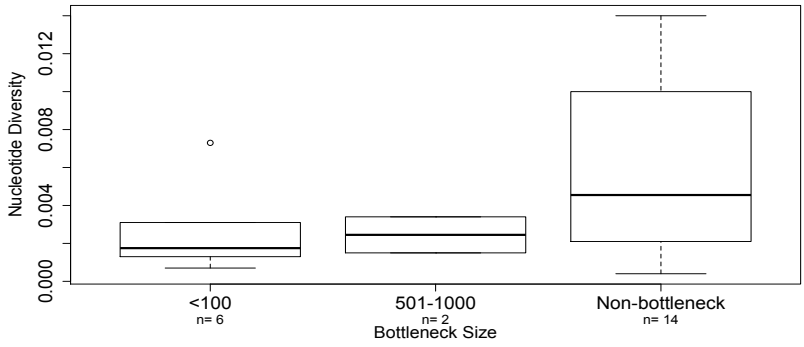
(a)

Haplotype Diversity: Significant difference present at bottleneck sizes: <100 & 100-500 (H_3=15.61, *p<0.01***)	
Standard Number of Haplotypes: Significant difference present at bottleneck size: <100 (H_3=19.61, *p<0.001****)	
Nucleotide Diversity	

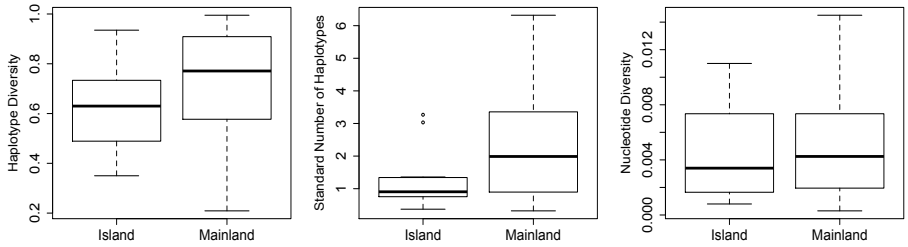
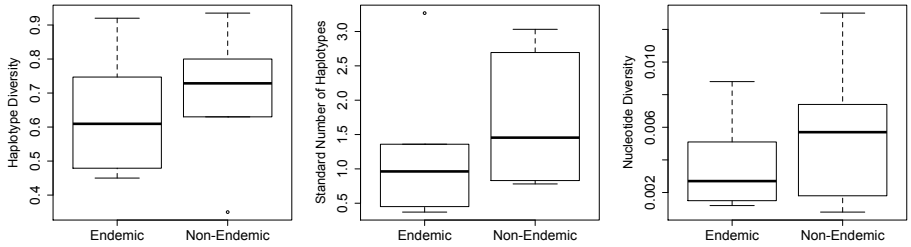
(b)

Haplotype Diversity and IUCN categories	
Standard Number of Haplotypes and IUCN categories	
Nucleotide Diversity and IUCN categories	

(c)

Haplotype Diversity: Control Region all domains.	
Standard Number of Haplotypes: Control Region all domains. Significant difference present at bottleneck size: <100 (H_2=9.02, *p<0.05**)	
Nucleotide Diversity: Control Region all domains	

(d)

	Haplotype diversity	***Standard Number of haplotypes***	***Nucleotide diversity***
Island or mainland species *(n = 11,68)*	 $t_{15.8}$=-2.179, p<0.05; W=237.5,p<0.05		
Island endemics or non-endemics *(n =6,6)*			

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Ring-necked parakeet in the UK.

Photo by Jackie Brunt

Chapter 3.

Version now published in Molecular Ecology (2015).

Molecular phylogeography of a globally invasive bird species reveals invasion pathways that correlate with climate and influences from bird imports for the pet trade.

Hazel Jackson, Diederik Strubbe, Simon Tollington, Robert Prys-Jones, Erik Matthysen & Jim Groombridge

3.1 Abstract

Invasive alien species present a major threat to global biodiversity. Understanding genetic patterns and evolutionary processes that reinforce successful establishment is paramount for elucidating mechanisms underlying biological invasions. Among birds, the ring-necked parakeet (*Psittacula krameri)* is one of the most successful invasive species, established in over 35 countries, however, little is known about the evolutionary genetic origins of this species and what population genetic signatures tell us about patterns of invasion. We resolve evolutionary affinities of native subspecies, reveal the genetic origins and composition of populations across the invasive range, and explore the potential influence of climate and propagule pressure from the pet trade on observed genetic patterns. A total of 928 individuals were sequenced for two mitochondrial DNA markers and ten microsatellite loci to reconstruct and date the phylogenetic history of the species. Samples representing the native range (n=96) were collected from museum specimens and modern samples from the invasive range (n=832) were gathered from across Europe, Mauritius and Seychelles. Ring-necked parakeets diverged from the Asian continent into Africa 1.6-2.6 million years ago, with evidence of phylogeographic structure. Invasive populations originate predominantly from Pakistan and northern areas of India. Haplotypes associated with more northerly distribution limits in the native range were more prevalent in invasive populations in Europe, and the predominance of Asian haplotypes in Europe is consistent with the higher number of Asian birds transported by the pet trade. Successful establishment of invasive species is likely to be underpinned by a combination of environmental and anthropogenic influences.

3.2 Introduction

Invasive alien species represent a global concern to society and the wider environment as a consequence of their rapid spread and competitive nature (Holmes & Simons 1996; Chapin *et al.* 2000; Doody *et al.* 2009), transmission of infectious disease (Wikelski *et al.* 2010) and the risks posed by some species to the global agro-economy (Ziska *et al.* 2011; Keller *et al.* 2011; Caplat *et al.* 2012). Invasive species have also been recognised as having detrimental impacts upon native biodiversity, ecosystems and communities (Sakai *et al.* 2001; Allendorf & Lundquist 2003; Gurevitch & Padilla 2004). It is therefore important to understand the underlying mechanisms that allow invasive species to successfully establish and spread outside of their native range so that strategies can be developed to mitigate their future spread.

The ability of a species to establish and become invasive can be influenced by a variety of factors, including propagule pressure (i.e. propagule sizes, propagule numbers, and temporal and spatial patterns of propagule arrival; Simberloff 2009). In general, the higher the propagule pressure, the greater the likelihood of a successful invasion (Cassey *et al.* 2004; Lockwood *et al.* 2005; Blackburn *et al.* 2009). Increased propagule pressure can buffer against environmental and demographic stochasticity and Allee-effects that may impede successful establishment, while simultaneously mitigating detrimental genetic impacts expected from founding events (Blackburn *et al.* 2009). Alongside this, invasion success is generally higher when species are introduced to climates similar to those occupied in the native range (Williamson 1996), and 'climate matching' therefore is an important component of assessing risk of invasion by invasive alien species (Thuiller *et al.* 2005). However, reported mismatches between native and invasive ranges, termed climate niche shifts (Guisan *et al.* 2014), necessitate research on the ecological and evolutionary processes that allow species to tolerate climates different from their native ranges (Sexton *et al.* 2002; Kelley *et al.* 2013). To identify how such evolutionary, environmental and human-mediated factors combine to underpin successful invasions, reconstructing pathways of invasion and identifying ancestral source populations are essential prerequisites (Sax *et al.* 2005; Estoup & Guillemaud 2010; Ascunce *et al.* 2011; Lombaert *et al.* 2011; Kirk *et al.* 2013; Perdereau *et al.* 2013).

In this study we characterise the evolutionary history for the globally invasive ring-necked parakeet (*Psittacula krameri),* which comprises multiple invasive populations and a well-documented invasion history. The ring-necked parakeet is one of the most widely introduced parrots in the world, with successful breeding populations established in over 35 countries (Butler 2003; Lever 2005). Native to Asia and sub-Saharan Africa, four subspecies are recognised based on geographical and morphological differences (such as wing, tail, beak and tarsus length, Forshaw 2010); two native to Asia (*P.k.borealis*, found in eastern Pakistan,

throughout northern India and from Nepal to Burma, and *P.k.manillensis,* from southern India and Sri Lanka), and two in sub-Saharan Africa (*P.k.krameri*, distributed from Senegal to western Uganda and southern Sudan, and *P.k.parvirostris*, from eastern Sudan to northern Ethiopia and Somalia). In their native range, ring-necked parakeets are found in a variety of woodland habitats, farmlands, urban gardens and parks (Juniper & Parr 1998; Khan 2002), while in their invasive ranges they readily colonise forests and parks surrounded by urban habitats (Strubbe & Matthysen 2007). Tests of niche conservatism based on occurance data ad spatial temperature and precipitation gradients (sensu Broenniman *et al.* 2011) indicate that across the parakeets' native range, phylogeographic lineages exhibit differing niche requirements (Strubbe *et al.* submitted). These authors also found that the invasion of Europe by ring-necked parakeets is accompanied by a climatic shift (sensu Broennimann *et al.* 2011; Petitpierre *et al.* 2012) along a temperature gradient, as in Europe, parakeets have expanded their climatic niche to colonise environments that are significantly colder than their native range.

As one of Europe's top 100 worst alien species **(**DAISIE, 2008**)**, breeding populations of ring-necked parakeets have become established in a number of European countries since the late 1960s, including the UK, Germany, the Netherlands, France, Spain, Italy, Greece and Belgium (Lever 2005), as well as numerous other countries outside of Europe such as Mauritius and the Seychelles. The rapid spread of this species along with evidence of explosive population growth of parakeets in Europe (Butler *et al.* 2013) presents a major cause for agro-economic and environmental concern. These birds are a severe crop pest across their native ranges and are known to decimate maize and fruit crops in India (Ramzan & Toor 1973; Forshaw 2010; Ahmad *et al.* 2012). They have also been shown to compete with native species for nest cavities (Hernández-Brito *et al.* 2014; Strubbe & Matthysen 2007, 2009a) and may have a detrimental impact upon the foraging behaviour of native birds (Peck *et al.* 2014).

We use mitochondrial (mtDNA) markers to construct a molecular time-calibrated phylogenetic framework to characterise the evolutionary origins of the ring-necked parakeet across its native ranges in Asia and Africa. We apply these mtDNA data and a suite of ten microsatellite DNA markers to examine patterns of genetic structure from the native distribution into the invasive range, and identify the genetic composition of invasive populations across Europe, Mauritius and the Seychelles in relation to their native sources. We then examine the observed genetic patterns to address the following questions; (i) is the genetic composition of the invasive populations a random mix of native genes originating across their Asian and African native range, or (ii) given that during invasion, parakeets have expanded their native and climatic niche into colder areas, are genetic patterns related with temperature differences between native and

invasive ranges? and (iii) can propagule pressure (as measured by records of parakeet imports for the bird pet trade) explain patterns of genetic composition of invasive populations ?

3.3 Methods

3.3.1 Sample collection

Feather and blood samples from ring-necked parakeets were collected from invasive populations in Brussels (*n*=69), Heidelberg (*n*=188), Wiesbaden (*n*=80), Bonn (*n*=29), Dusseldorf (*n*=9), Seville (*n*=57), Madrid (*n*=2), Greater London (*n*=164), Tuscany (*n*=1), Marseille (*n*=2), Rotterdam (*n*=75), The Hague (*n*=12), Amsterdam (*n*=17), Utrecht (*n*=2), Mauritius (*n*=116) and Seychelles (*n*=2; Table S3.1 in supplementary material). In response to a media campaign, volunteers across Europe collected naturally shed feathers from known roost sites or local parks and gardens. Experienced researchers acquired blood samples from Seychelles and Mauritius. To study intraspecific phylogenetic relationships among ring-necked parakeets across their native range, toe-pad samples were collected from 96 museum specimens at the Natural History Museum in Tring, UK (Table S3.2). Museum specimens were chosen on the basis of their geographic collection location to maximise geographical coverage of samples from across the species' native range (Fig. 3.1).

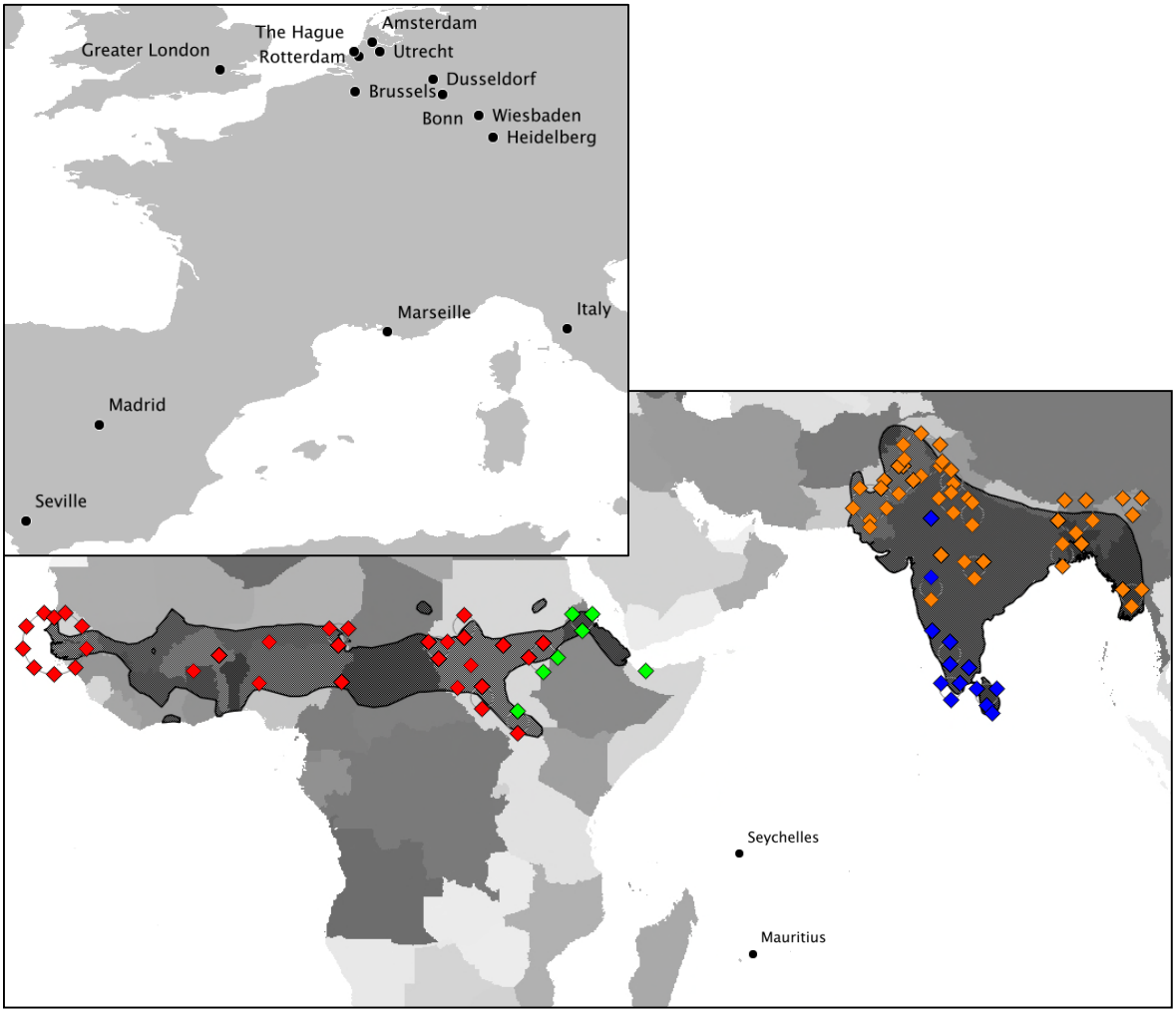

Fig. 3.1. Native range distribution of *Psittacula krameri* (black outlined area across Sub-Saharan Africa and Southern Asia (IUCN 2013)). Sample locations; diamonds = historical specimens from the native range. Colours refer to the subspecies designation given on the label of each museum specimen; *P. k. borealis* = orange, *P. k. manillensis* = blue, *P. k. krameri* = red, *P. k. parvirostris* = green; black dots = invasive populations sampled for this study from the invasive range. Locations of all museum specimens and sampled invasive populations are plotted; where necessary overlapping symbols have been displaced around the true co-ordinate point to display all sample information.

3.3.2 DNA isolation, amplification and sequencing

Processing of the museum specimens, including DNA extraction and PCR amplification, was carried out in a separate laboratory dedicated to ancient DNA work to prevent contamination. All equipment and surfaces were sterilised before and after each use by irradiation with UV light and application of 10% bleach. Negative controls (where template DNA was replaced with

ultrapure ddH_2O) were included during the DNA extraction and PCR process. A selection of negative extractions PCRs were sequenced to ensure there was no traces of contamination in negative controls. DNA was extracted from both contemporary feather samples and historical toe-pad samples using a Bioline ISOLATE Genomic DNA extraction kit (Bioline, UK). Samples were suspended in 400 µl lysis buffer plus 25 µl proteinase K and incubated at 55^{o}C overnight (or until the material had completed digested). DNA was washed through a spin column and historical samples were suspended in 50 µl of elution buffer, while contemporary samples were suspended in 100 µl of elution buffer. Genomic DNA was extracted from blood using an ammonium acetate precipitation method following Nichols *et al.* (2000).

Amplification from contemporary DNA samples was conducted for two mtDNA regions: control region using CR19f and CR19r, and cytochrome *b* using PKCBf and PKCBr (Table S3.3**)**. PCR cycling conditions were 94^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15 secs, 55^{o}C/15 secs and 72^{o}C/10 secs and a final elongation step of 72^{o}C for 10 minutes. For historical samples, amplification of control region and cytochrome *b* was conducted using a specifically designed suite of overlapping short fragment primers to provide replication and ensure no contamination (150-250 bp; Table S3.3). Cycle parameters comprised an initial hot start of 95^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15 secs, 52^{o}C/15 secs and 72^{o}C/10 secs followed by a final 10 minutes 72^{o}C incubation period. All amplicons were examined by agarose gel electrophoresis to check amplification success and no evidence of contamination in the extraction and PCR negative controls. Amplification volumes of 25 µl contained 2 µl of template DNA, 12.5 µl MyTaq HS Redmix (Bioline, UK), 0.5 µl of each primer and 9.5 µl of ddH_20. PCR product was purified and amplified using a 3730xl DNA analyser (Macrogen Inc.). Sequences were edited in 4Peaks (Griekspoor & Groothius 2005) and aligned in Clustal (Larkin *et al.* 2007). Manual edits were made in Jalview (Waterhouse *et al.* 2009). The two genes were concatenated using Sequence Matrix (Vaidya *et al.* 2011).

We used a suite of 21 microsatellite markers available for *Psittacula* parakeets that have been shown to cross-amplify in ring-necked parakeets (Raisin *et al.* 2009). PCR protocols followed Raisin *et al.* 2009. Each PCR contained 1 µl (blood) or 3 µl (feathers) ≈ 10 ng/µl of DNA that was air-dried, 1 µl of primer mix (fluorescently labeled forward) at 0.2 µM and 1 µl QIAGEN Multiplex PCR Master Mix (QIAGEN Inc). PCRs were conducted using differently fluorolabelled forward primers (HEX and 6-FAM; Raisin *et al.* 2009). PCR cycling conditions were 95 ^{o}C for 10 minutes followed by 35 cycles of 93^{o}C/30 secs, 52^{o}C/90 secs and 72^{o}C/90 secs, with a final incubation at 72 ^{o}C for 10 minutes. PCR was performed with a low annealing temperature (52°C) to increase the likelihood of amplification (Primmer *et al.* 2005). Individuals were sex-typed using the Z-002B marker (Dawson 2007) to check for loci that were

sex-linked. PCR products were separated using an Applied Biosystems 3730 DNA Analyser with ROX^{TM} 500 as a size-standard. Alleles were identified and scored using GENEMAPPER 3.7 (Applied Biosystems, UK). Three loci were ambiguous to score and therefore were excluded from the analysis (*Peq06*, *Peq08* and *Peq09)*. Loci identified as sex-linked in echo parakeets *(Psittacula echo)* by Raisin *et al.* (2009) were confirmed to be sex-linked in ring-necked parakeets based on a complete lack of heterozygotes in females and therefore also excluded (*Peq16* and *Peq21*), resulting in a total of 16 loci. Due to the degraded nature and low volume of the DNA extracted from historical museum specimens only 10 of the 16 loci amplified (those with the largest allele size failed to amplify). Ideally when working with museum specimens, samples should be genotyped three times to identify a heterozygote and five times to identify a homozygote (Taberlet *et al.* 1996), however, owing to the restricted quantities of available DNA, here each sample was genotyped twice to ensure consistent scoring of alleles, and to identify potential genotype errors. Genotypes that could not be scored consistently were removed. Such degraded samples are known to be susceptible to genotyping errors due to allelic dropout (Taberlet *et al.* 1996, Hoffman & Amos 2005, Wandeler *et al.* 2007). For comparative analysis between museum and contemporary microsatellite genotype datasets, the contemporary dataset was condensed to the same 10 loci that were genotyped in the museum specimens. Deviations from Hardy-Weinberg equilibrium and null allele frequencies at each locus were estimated using CERVUS. Evidence of genotyping errors (allelic drop-out and stuttering) was assessed using MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004).

Phylogenetic tree inferences were computed on the concatenated dataset from the native range using Bayesian and maximum likelihood methods with *Electus roratus* and *Tanygnathus sumatranus* as outgroups. Additional *Psittacula* species were added to improve resolution, and their topology was constrained. PartitionFinder (Lanfear *et al.* 2012) was used to identify the best-fit models of nucleotide evolution for each partition (control region; 1-521, cytochrome *b*; 522-868), according to Bayesian information criteria (BIC). Bayesian inference was implemented in MrBayes v3.2 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway (Miller *et al.* 2010) with 10 million generations over four parallel Monte Carlo Markov Chains (MCMC**),** under a HKY evolutionary model (Felsentein 1981). Tracer v1.6 (Rambaut & Drummond 2007) was used to assess convergence. After discarding the first 25% (burn-in), tree topologies were summarised in a 50% consensus tree. A maximum likelihood search was conducted in RaxML (Stamatakis 2006). Ten independent runs were performed with 1000 non-parametric bootstrap replicates to obtain the best likelihood score under a GTAGAMMA model and summarised in a majority rule consensus tree. All trees were visualised in FigTree v1.4 (Rambaut 2012). A maximum likelihood phylogeny was then mapped using sample locations in GenGIS (Parks *et al.* 2009).

3.3.3 Molecular dating

Time-calibrated phylogenies were estimated using BEAST v.1.7.5 **(**Drummond & Rambaut 2007**)** using mtDNA data. Given that relative to other bird families the fossil record for parrots is poor (Mayr & Göhlich 2004), we combined our data with cytochrome *b* sequences for other Indian Ocean parrots (Kundu *et al.* 2012) and ran phylogenetic analyses by adopting a similar approach to Wright *et al.* (2008) using two alternative calibration dates. The first calibration used was obtained from the oldest known fossil belonging to a crown group of parrots, *Mopsitta tanta,* dated to approximately 54 Mya in the Tertiary period (Waterhouse *et al.* 2008), while a second calibration of 80 Mya was obtained from a previous dating analysis of extant bird orders, suggesting a Cretaceous date for the divergence of parrots (Hedges *et al.* 1996). This calibration was given a normal distribution with a standard deviation of 10 Mya to ensure the 95% distribution (60.4 Mya and 99.6 Mya) does not exceed the 100 Mya date for the divergence of bird orders (Hedges *et al.* 1996). An uncorrelated strict molecular clock model was used in favour of a lognormal relaxed molecular clock model as identified by Akaike's information criterion (AIC) through MCMC (AICM) comparison of models (Baele *et al.* 2012) with a uniform distribution under the Yule speciation tree prior (Ho *et al.* 2007). MCMC was performed for 10 million generations with sampling every 1000^{th} iteration. Convergence was confirmed by effective sample sizes (ESS) >200 for all parameters using Tracer v1.6 (Drummond & Rambaut 2007). Trees from the first 1000 generations were discarded as burn-in. A maximum clade credibility tree was summarised using TreeAnnotater v1.7.5 (Drummond & Rambaut 2007), and visualised in Figtree v1.4 (Rambaut 2012).

3.3.4 Characterisation of population origin using Bayesian methods

Firstly a median-joining haplotype network was constructed in PopART (Leigh & Bryant 2015; software available at: www.popart.otago.ac.nz), to infer relationships between haplotypes from the ancestral native and invasive ranges. Haplotype frequencies and distributions in the native and invasive range were plotted on a map using QGIS (QGIS Development Team 2014).

We used approximate Bayesian computation methods, executed in DIYABC v 2.02 (Cornuet *et al.* 2014), to infer ancestral source populations. Three scenarios were considered, where each invasive population originated directly from either of the two native sources (Asia or Africa), or from an admixture of both Asia and Africa (Fig. 3.2). A total of 10^6 simulated datasets were used with uniform prior distributions of N_e and bottleneck durations (Table S3.4). We assumed a generation time of 5.6 years based on a genetically confirmed social pedigree from more than 20 years of individual-based life history data of the ring-necked parakeets closet extant relative, the Mauritius parakeet, *Psittacula echo* (Tollingon *et al.* 2013). A stepwise mutation model was assumed for microsatellites with default parameters (Estoup *et al.* 2002) and we used summary

statistics for each population and each population pair comprising the mean number of alleles, mean expected heterozygosity, pairwise F_{ST} (Weir & Cockerham 1984), and genetic distance between two populations (Goldstein *et al.* 1995). Pre-evaluation of each scenario was performed by PCA and to identify the best scenario we used DIYABC's logistic regression to compare posterior probabilities across 500 simulated pseudo-observed data sets. Performance of the scenario parameters was assessed by using DIYABC's functions for estimating type I (false positives) and type II (false negative) errors, and by computing the relative bias across the 500 pseudo-observed simulated datasets. Finally, to verify the goodness of fit for each scenario, model checking was implemented in DIYABC to compare summary statistics between observed and simulated datasets.

As an additional method of determining the ancestral source of each invasive ring-necked parakeet population, a Bayesian clustering approach was utilised to assign individuals in invasive populations to reference source populations, using the program STRUCTURE v2.3.4 (Pritchard *et al.* 2000). To determine the most likely number of clusters (K) across the native range using the microsatellite data from the 10 selected loci, 10 repeated runs were conducted with K ranging from one to 10 for 500,000 iterations with a burn-in of 100,000, under the admixture model and specifying correlated allele frequencies (Falush *et al.* 2003), with prior information included to assist clustering (two separate runs including subspecies and location as prior information; Hubisz *et al.* 2009). The assignment values, log likelihood scores and ΔK were evaluated using Structure Harvester (Earl & vonHolt 2011) to infer the number of genetic clusters. Once the most likely number of clusters in the native range was identified, a further admixture analysis was performed assuming two (K=2) putative ancestral source populations from the native range (Asia, Africa) for the assignment of invasive populations, with the option '*usepopinfo'* activated. Here the native clusters were set as 'known' by activating the '*usepopinfo'* flag, and the invasive populations were included as unknown. This approach requires the analysis to cluster the invasive populations with the native source populations (Pritchard *et al.* 2000), and was conducted separately for each invasive population. CLUMPP (Jakobssen & Rosenberg 2007) was used to obtain proportion averages across the multiple runs and these assignment probabilities were plotted using DISTRUCT (Rosenberg 2004) and mapped using Quantum GIS (Quantum GIS Development Team 2014).

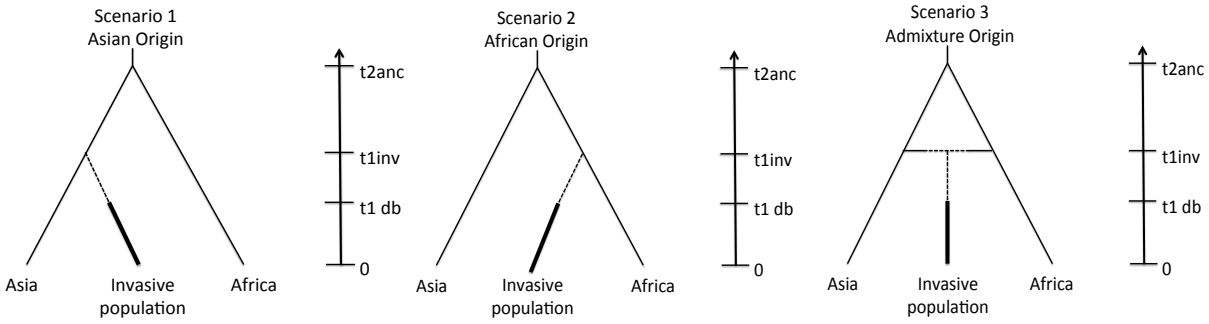

Fig. 3.2. Graphical representation of the three scenarios of ancestral origin examined using approximate Bayesian computation implemented in DIYABC v 2.02 (Cornuet *et al.* 2014). Scenario 1 corresponds to a direct ancestral origin from Asia, scenario 2 corresponds to a direct ancestral origin from Africa, and scenario 3 corresponds to an admixed origin from both Asia and Africa. In each case the black arrow indicates backwards steps in time in generations to when invasive populations were founded (t1inv generations ago), and then back in time to when the native populations diverged (t2anc). Duration of bottlenecks from founding events are included at t1 db. In scenario 3, the admixture rate (ar) represents the genetic contribution of the native populations.

3.3.5 Tests for propagule pressure

To place in context the resulting genetic data and patterns of invasion by ring-necked parakeets alongside the potential contribution to these patterns by unintentional release of escaped pet parakeets, all import records of ring-necked parakeets between 1975 and 2007 from the native ranges (Asia and Africa), were obtained for Spain, Italy, UK, Germany, Netherlands, Belgium and France from the CITES Trade Database (CITES 2014). No equivalent trade information was available for the genetically sampled populations on Mauritius or Seychelles. Chi-square tests were used to verify whether the distribution of Asian versus African haplotypes detected across Europe differed from that expected by propagule pressure, whereby distributions in the invasive range ought to reflect relative quantities of parakeet imports from Asia and Africa (CITES trade data; invasive populations on Mauritius and Seychelles were excluded owing to a lack of available trade data). To compare the observed genetic composition of European parakeet populations derived from microsatellite data with expectations based on propagule pressure, the genetic composition of all populations within a country was averaged, as CITES trade data are only available at the country level. Using the proportion of Asian/African genes observed from the genetic data for each country, a binomial GLM was applied to test for differences between the proportions of imported and observed Asian/African genes.

3.3.6 Genetic patterns and temperature differences between native and invasive ranges.

During the invasion of Europe, ring-necked parakeets have shifted their climatic niche towards colder areas (Strubbe *et al.* submitted). As sample sizes are too limited to carry out haplotype-level tests of niche conservatism (sensu Broennimann *et al.* 2011), we devised two alternative tests to to verify whether the distribution of haplotypes between and within invasive European parakeet populations is associated with temperature differences between native and invasive ranges. Given the species-level niche shift along a temperature gradient, haplotypes characterised by a lower cold niche limit in the native range can be expected to be more successful at invading colder parts of Europe. Using the maximum latitude at which haplotypes occur in the native range as a proxy for tolerance of cold temperatures, and focusing on the predominant Asian haplotypes, the following tests were performed. Firstly, the maximum native-range latitude of haplotypes was correlated with their prevalence across European populations (Table S3.5). Secondly, a weighted average maximum native-range latitude for each European population was calculated (i.e. weighing the maximum native-range latitude of each haplotype present in a European population according to its prevalence in that population). This weighted average latitude was then correlated with the latitude of the European population, to test the hypothesis that haplotypes associated with colder temperatures in the native range will be more prevalent in northern European parakeet populations.

3.4 Results

3.4.1 Molecular phylogeny

A total of 868 bp of DNA sequence was obtained from the mtDNA control region (522 bp) and cytochrome *b* (346 bp) gene. Owing to the fragile nature of the DNA obtained from museum samples, partial sequences (196-868 bp) were obtained for some specimens. However, such partial sequences do not have a detrimental impact on the accuracies of phylogenies, which may in fact benefit from inclusion of additional sequences despite missing data (Wiens 2006, Wiens & Moen 2008, Wiens & Morrill 2011). In total, 44 unique haplotypes were identified from the 96 museum samples and used for phylogenetic reconstruction of the evolutionary history of ring-necked parakeets across their native range.

Topologies recovered from Bayesian and maximum likelihood trees were largely congruent at all major nodes inferring a distinct and well-supported structure between the four subspecies (Fig. 3.3). The Asian ring-necked parakeet subspecies (*P. k. manillensis, P. k. borealis)* were placed as ancestral to the African subspecies (*P. k. krameri, P. k. parvirostris*). A signal of geographical clustering was evident within the haplotypes obtained from the African samples of *P. k. krameri* and *P. k. parvirostris*. Such clusters were apparent for samples from Senegal, Nigeria and Sudan. In contrast, no clear geographical clustering was seen across Asia except for

the *P.k.borealis* haplotypes from Burma that were consistently placed alongside African rather than Asian clades. Finally, a single specimen from Somalia and labelled as *P. k. parvirostris,* appears to have Asian rather than African affinities. Observed phylogeographic clustering patterns were also supported by the median joining haplotype network (Fig. 3.4).

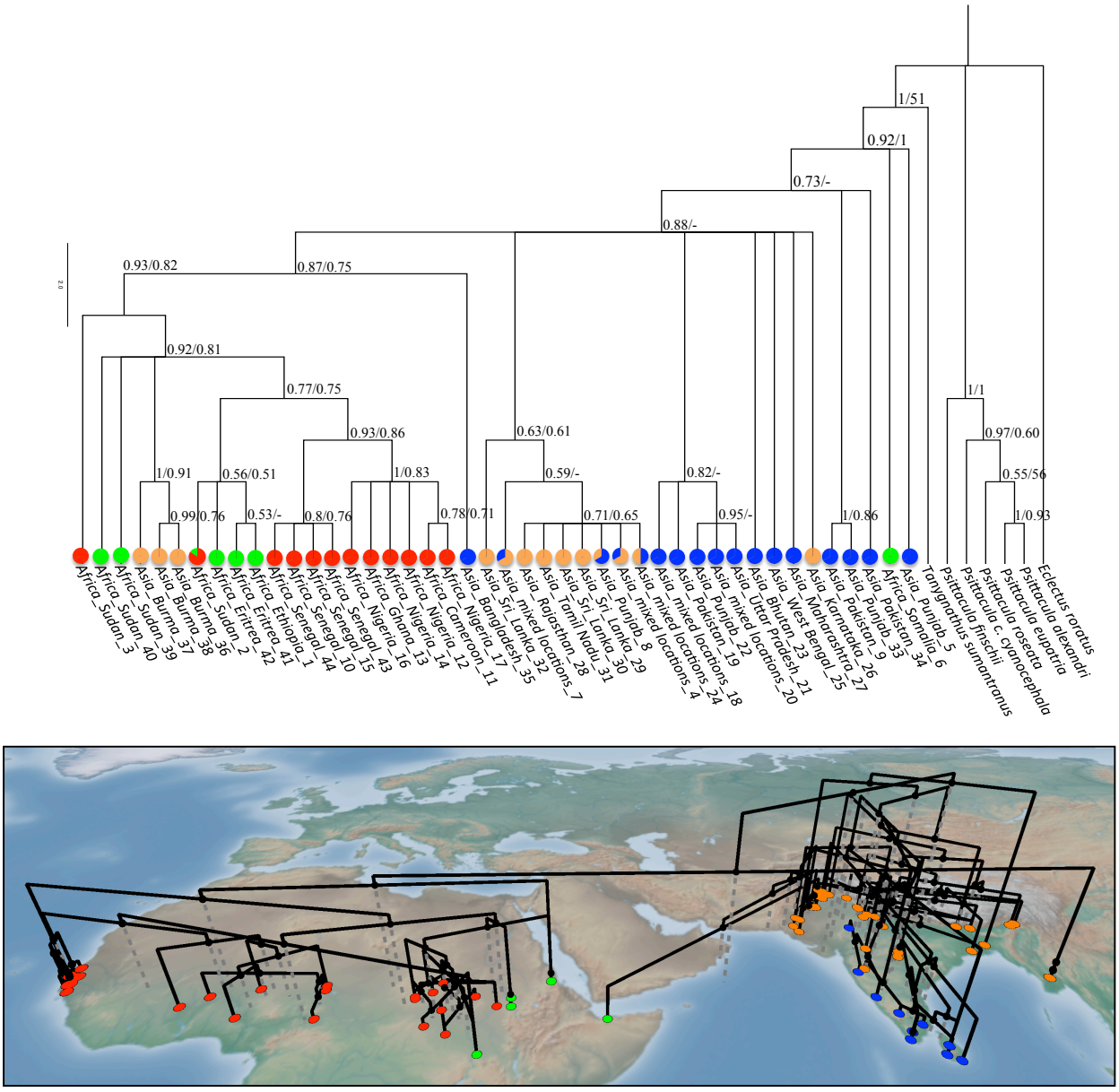

Fig. 3.3. Phylogenetic reconstruction of ring-necked parakets, *Psittacula krameri*, comprising 44 phylogenetically informative mtDNA haplotypes derived from a total of 868bp of mtDNA control region and cytochrome *b* sequence using Bayesian (BI) and maximum likelihood (ML) inference. Both BI posterior probabilities and ML bootstrap values are provided respectively at relevant nodes. Pie charts indicate proportion of different subspecies (information taken from museum specimen labels) that are found to share a single haplotype. Haplotypes were named according to the state or region in which they originate from. Lower panel - ML phylogeny of

mtDNA sequences integrated with each geo-referenced location of sample collection (derived from museum specimen label). Colours identify the subspecies of each sample based on museum label information; *P. k. borealis*, orange, *P. k. manillensis*, blue, *P. k. krameri*, red, *P. k. parvirostris*, green.

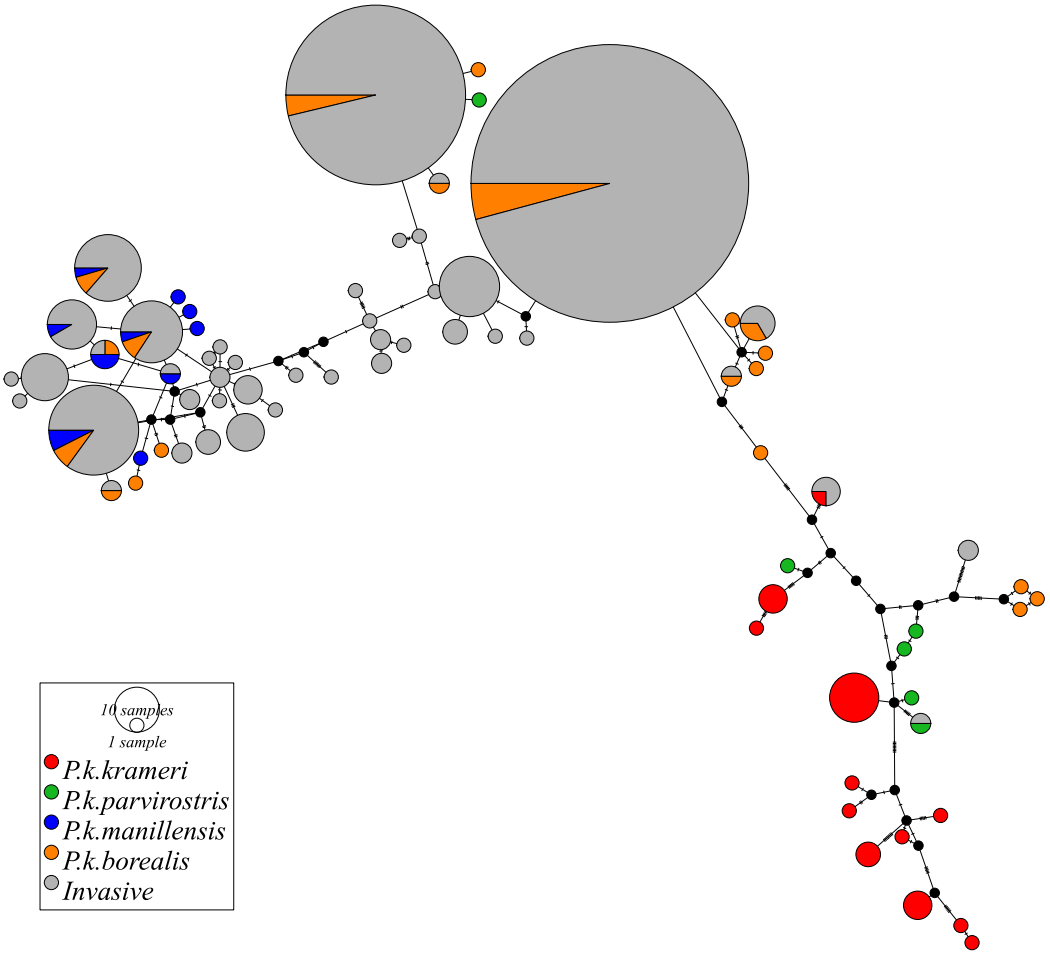

Fig. 3.4. Median joining haplotype network comprising 44 mitochondrial (mtDNA) haplotypes shared between the native and invasive ranges, and an additional 30 haplotypes observed from the invasive range. Haplotypes from the invasive distributions are in grey. Native haplotypes are coloured according to the subspecies designation given on the label of each museum specimen; Asian subspecies= *P. k. borealis,* orange, and *P. k. manillensis*, blue; African subspecies= *P. k. krameri*, red, and *P. k. parvirostris,* green. Circles are proportional to haplotype frequency.

3.4.2 Molecular dating

When using a calibration of 54 Mya, the recovered age of the divergence of the ring-necked parakeet and the Mauritius parakeet (*Psittacula echo),* which cluster together, from the common ancestor, the blue-backed parrot (*Tanygnathus sumatranus),* is 6.23 Mya, after which the ring-necked parakeet splits from the Mauritius form 3.02 Mya. Within *P.krameri,* the molecular dating analysis suggests two divergences towards the end of the Pleistocene. The Asian subspecies (*P.k.borealis* and *P.k.manillensis)* diverged 2.57 Mya, followed by an off-shoot divergence event into Burma 2.21 Mya, before the African subspecies (*P.k.krameri* and *P.k.parvirostris*) diverged 1.61 Mya (Fig. 3.5). When the calibration age of the common ancestor is extended to 80 Mya the divergence dates also move further back in time. The divergence of the ring-necked parakeet and the Mauritius parakeet from the blue-back parrot increases to 10.16 Mya, following which the ring-necked parakeet splits from the Mauritius parakeet 4.9 Mya. The two Asian subspecies of ring-necked parakeet diverged 2.9 Mya, and finally the African subspecies of ring-necked parakeet diverged 2.63 Mya (Fig. S3.1).

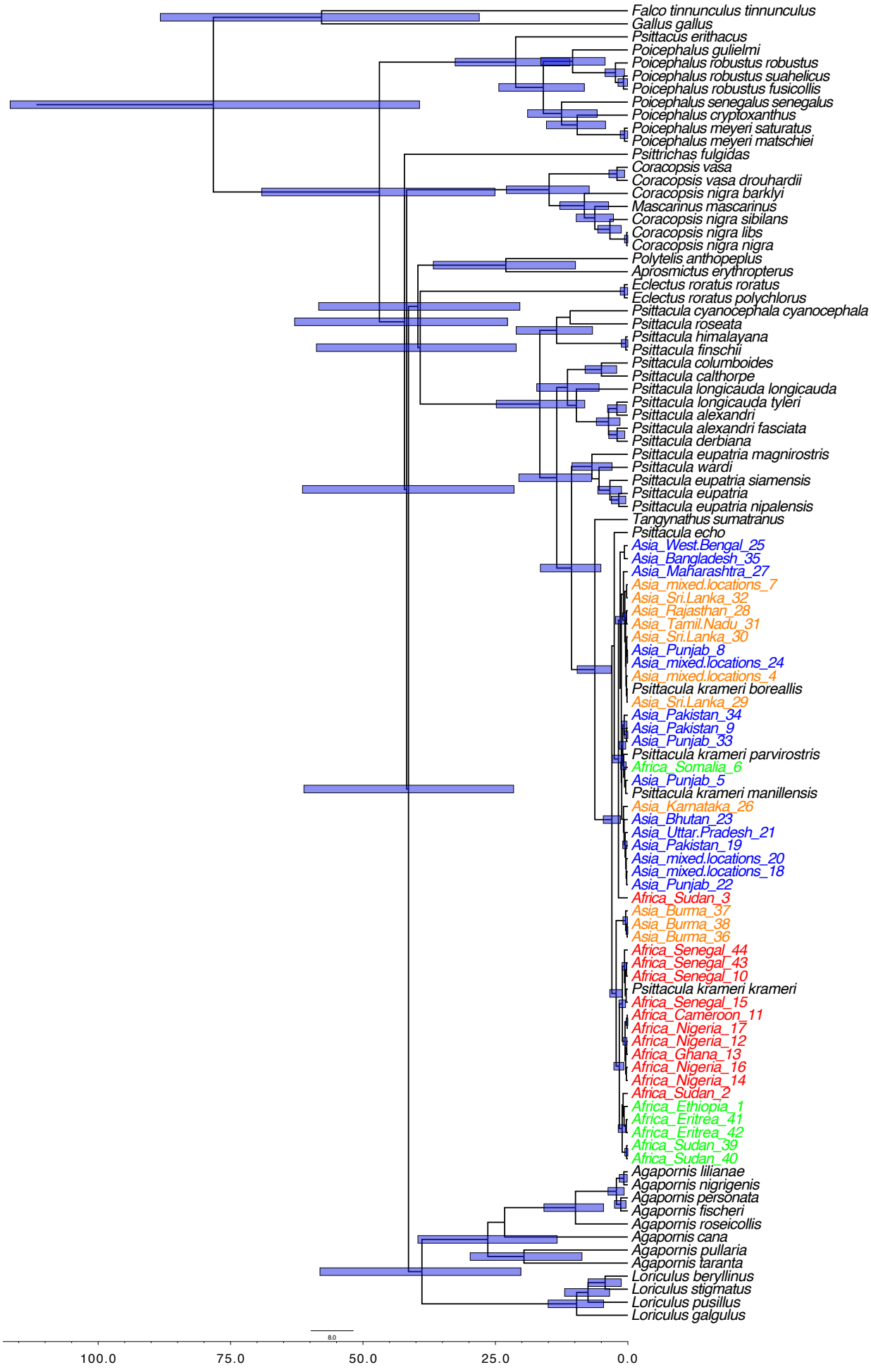
Falco tinnunculus tinnunculus
Gallus gallus
Psittacus erithacus
Poicephalus gulielmi
Poicephalus robustus robustus
Poicephalus robustus suahelicus
Poicephalus robustus fusicollis
Poicephalus senegalus senegalus
Poicephalus cryptoxanthus
Poicephalus meyeri saturatus
Poicephalus meyeri matschiei
Psittrichas fulgidus
Coracopsis vasa
Coracopsis vasa drouhardii
Coracopsis nigra barklyi
Mascarinus mascarinus
Coracopsis nigra sibilans
Coracopsis nigra libs
Coracopsis nigra nigra
Polytelis anthopeplus
Aprosmictus erythropterus
Eclectus roratus roratus
Eclectus roratus polychlorus
Psittacula cyanocephala cyanocephala
Psittacula roseata
Psittacula himalayana
Psittacula finschii
Psittacula columboides
Psittacula calthorpe
Psittacula longicauda longicauda
Psittacula longicauda tyleri
Psittacula alexandri
Psittacula alexandri fasciata
Psittacula derbiana
Psittacula eupatria magnirostris
Psittacula wardi
Psittacula eupatria siamensis
Psittacula eupatria
Psittacula eupatria nipalensis
Tangynathus sumatranus
Psittacula echo
Asia_West.Bengal_25
Asia_Bangladesh_35
Asia_Mañarashtra_27
Asia_mixed.locations_7
Asia_Sri.Lanka_32
Asia_Rajasthan_28
Asia_Tamil.Nadu_31
Asia_Sri.Lanka_30
Asia_Punjab_8
Asia_mixed.locations_24
Asia_mixed.locations_4
Psittacula krameri boreallis
Asia_Sri.Lanka_29
Asia_Pakistan_34
Asia_Pakistan_9
Asia_Punjab_33
Psittacula krameri parvirostris
Africa_Somalia_6
Asia_Punjab_5
Psittacula krameri manillensis
Asia_Karnataka_26
Asia_Bhutan_23
Asia_Uttar.Pradesh_21
Asia_Pakistan_19
Asia_mixed.locations_20
Asia_mixed.locations_18
Asia_Punjab_22
Africa_Sudan_3
Asia_Burma_37
Asia_Burma_38
Asia_Burma_36
Africa_Senegal_44
Africa_Senegal_43
Africa_Senegal_10
Psittacula krameri krameri
Africa_Senegal_15
Africa_Cameroon_11
Africa_Nigeria_17
Africa_Nigeria_12
Africa_Ghana_13
Africa_Nigeria_16
Africa_Nigeria_14
Africa_Sudan_2
Africa_Ethiopia_1
Africa_Eritrea_41
Africa_Eritrea_42
Africa_Sudan_39
Africa_Sudan_40
Agapornis lilianae
Agapornis nigrigenis
Agapornis personata
Agapornis fischeri
Agapornis roseicollis
Agapornis cana
Agapornis pullaria
Agapornis taranta
Loriculus beryllinus
Loriculus stigmatus
Loriculus pusillus
Loriculus galgulus
8.0
100.0
75.0
50.0
25.0
0.0

Fig. 3.5. Estimated divergence times derived from mtDNA data obtained for museum specimens of *P.krameri.* Sequence data were analysed alongside existing sequences for other Old World parrots (*Psittaciformes)* from Kundu *et al.* 2012 (coloured black) and resolved using BEAST with a specified TMRCA of 54 Mya. Error bars at particular nodes display the 95% HPD. Time on the axis is given in millions of years before present. Colours identify the subspecies of each sample based on museum label information; *P. k. borealis*, orange, *P. k. manillensis*, blue, *P. k. krameri*, red, *P. k. parvirostris*, green.

3.4.3 Genetic divergence

Mean nucleotide divergences between subspecies from the complete mtDNA dataset showed that the highest sequence divergences of 2.6% and 2.5% were present between *P. k. krameri* (African) and *P. k. manillensis* and *P. k. borealis* (Asian) respectively. *P. k. parvirostris* (African) differed from both *P. k. manillensis* and *P. k. borealis* (Asian) by 1.9%. The African subspecies (*P. k. krameri* and *P. k. parvirostris*) differed from each other by 1.3%, while the Asian subspecies, *P. k. manillensis* and *P. k. borealis* differed from each other by 1.1% (Table 3.1).

Table 3.1. Mean sequence divergence between *Psittacula krameri* subspecies in the native range (subspecies designations as given on museum specimen labels) based on 868 bp mtDNA.

Concatenated	*P. k. borealis*	*P. k. mallinensis*	*P. k. krameri*	*P. k. parvirostris*
P. k. borealis		0.011	0.025	0.019
P. k. mallinensis			0.026	0.019
P. k. krameri				0.0013
P. k. parvirostris				
Control region				
(522 bp)	*P. k. borealis*	*P. k. mallinensis*	*P. k. krameri*	*P. k. parvirostris*
P. k. borealis		0.009	0.019	0.017
P. k. mallinensis			0.017	0.015
P. k. krameri				0.010
P. k. parvirostris				
Cytochrome b				
(346 bp)	*P. k. borealis*	*P. k. mallinensis*	*P. k. krameri*	*P. k. parvirostris*
P. k. borealis		0.012	0.031	0.022
P. k. mallinensis			0.033	0.022
P. k. krameri				0.017
P. k. parvirostris				

3.4.4 Origins of invasive populations derived from mtDNA

When the mtDNA sequence data derived from the native and invasive populations were combined, a total of 74 haplotypes were identified (Table S3.7). Thirty of these 74 haplotypes were found only in the invasive populations and were not detected in the native range (coloured black; Fig. 3.6). These haplotypes mainly clustered with haplotypes found in the Asian ancestral native range (Fig. 3.4). A total of 14 haplotypes were shared between both the native and invasive ranges, and the majority of these were of Asian origin, predominately from northern regions of India (Fig. 3.6). Of the 14 invasive haplotypes identified from the native range, 12 were of Asian origin (comprising 828 sequenced parakeet individuals) whereas only two were of African origin (comprising four sequenced parakeet individuals); a haplotype from Ethiopia was detected in an individual from the Greater London population and a haplotype from Sudan (Darfur) was detected in two Greater London individuals and in an individual from the Heidelberg population in Germany. In contrast, a single common haplotype detected in numerous locations in Asia (haplotype: Asia_mixed.locations_18) was frequently detected across the invasive range. This haplotype was found in high frequencies in Bonn (37.9%), Brussels (58.4%), Seychelles (100%), Heidelberg (95.8%), Wiesbaden (67.5%), Amsterdam (84.2%), Utrecht (100%), Rotterdam (51.3%), The Hague (46.1%) and Greater London (59.9%). Invasive populations in Seville, Madrid and on Mauritius all predominately shared a second haplotype from Asia (Asia_Punjab_5), which was also detected in the Greater London population (14.9%).

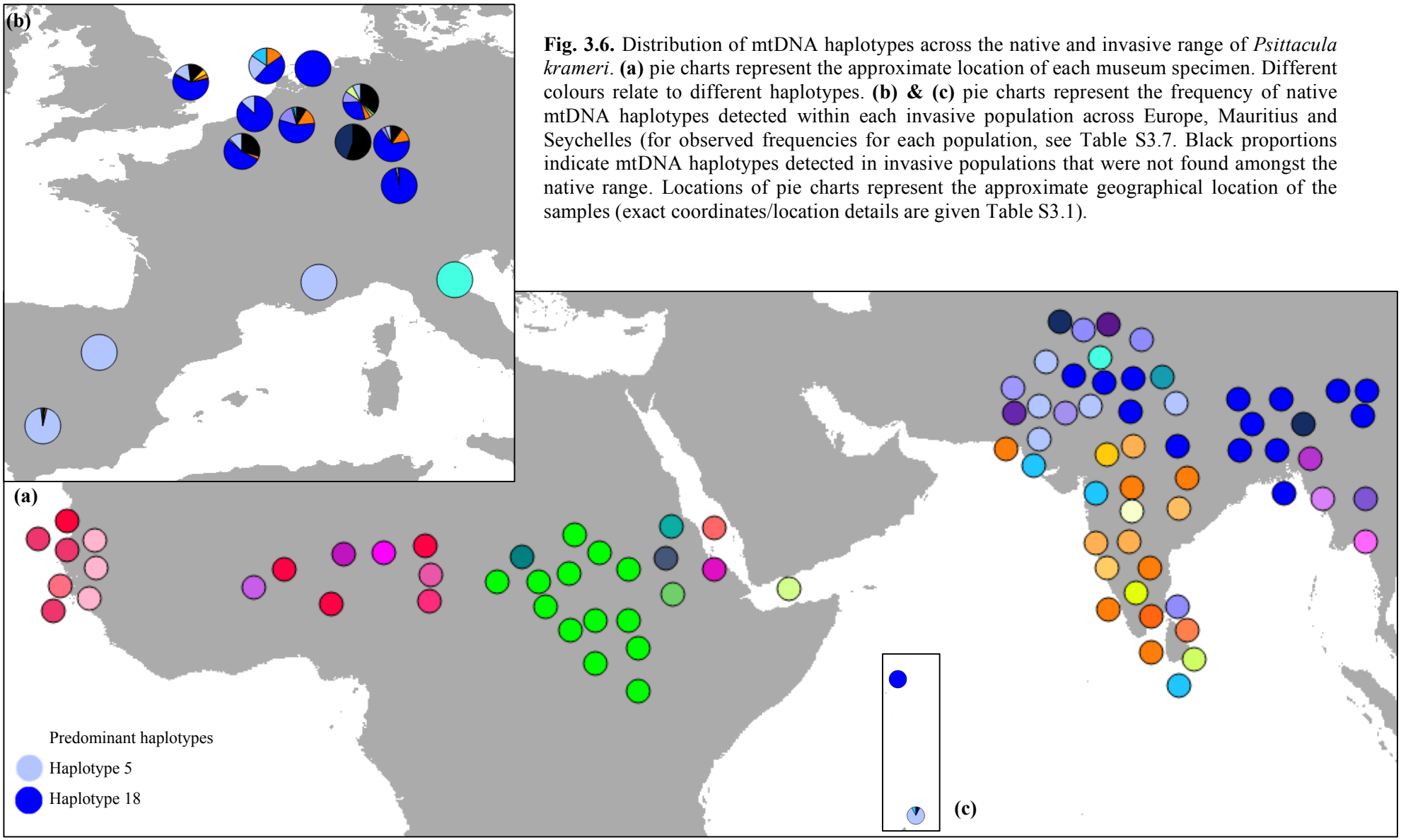

Fig. 3.6. Distribution of mtDNA haplotypes across the native and invasive range of *Psittacula krameri*. **(a)** pie charts represent the approximate location of each museum specimen. Different colours relate to different haplotypes. **(b) & (c)** pie charts represent the frequency of native mtDNA haplotypes detected within each invasive population across Europe, Mauritius and Seychelles (for observed frequencies for each population, see Table S3.7. Black proportions indicate mtDNA haplotypes detected in invasive populations that were not found amongst the native range. Locations of pie charts represent the approximate geographical location of the samples (exact coordinates/location details are given Table S3.1).

3.4.5 Origins of invasive populations derived from microsatellites

A total of 819 specimens from the invasive range were genotyped at 10 microsatellite loci, and 92 specimens from the native range (56 from Asia and 36 from Africa). No linkage disequilibrium was detected between pairs of microsatellite loci following Bonferroni corrections (Rice 1989). Deviations from Hardy Weinberg Equilibrium were detected at two of the 10 loci across all populations combined (*Peq*02 and *Peq*15). Estimated frequencies of null alleles ranged between 0.003-0.084 per locus. The museum specimen genotyping error rates were 3.89% and the estimated frequencies of null alleles per locus ranged between 0.003-0.460. Using approximate Bayesian modelling, implemented with DIYABC, scenario three was the best-supported model under a logistic regression (posterior probability 0.9913), indicating the invasive populations most likely derive from an admixture of both ancestral Asian and African sources. In support of scenario three, all but two of the 162 observed summary statistics were within range of the simulated dataset (Table S3.6), and the priors were not biased (Table S3.4). The type I error from scenario three was 6%, while combining all simulations across the two alternative scenarios resulted in a type II error of 0.6%. Our additional analysis of the microsatellite data using structure also supports the approximate Bayesian computation scenario, and was used to infer the proportions of ancestry from each native source population. Analysis of the native range alone using STRUCTURE revealed a ΔK statistic that suggested two distinct population clusters, one in Asia and one in Africa (K=2; Evanno *et al.* 2005). Assignment probabilities indicated that all individuals from the ancestral range were unambiguously assigned to one of the two clusters, with each cluster corresponding to these two continental ranges (K=2; using subspecies prior; lnK = 12.65 and ΔK = 8.36, using location prior; lnK = 50.54 and ΔK = 9.62). STRUCTURE was then used to infer ancestry of each of the invasive populations from the two native population clusters. All invasive populations demonstrated a higher assignment probability to the Asian cluster, suggesting a stronger Asian (rather than African) contribution to the genetic make-up of the invasive populations (Brussels = 68%, Heidelberg = 71%, Wiesbaden = 66%, Bonn = 66%, Dusseldorf = 64%, Seville = 67%, Madrid = 60%, Greater London = 60%, Tuscany = 94%, Marseille = 86%, Rotterdam = 63%, The Hague = 71%, Amsterdam = 69%, Utrecht = 78%, Mauritius = 68% and Seychelles = 83%; Fig.3.7).

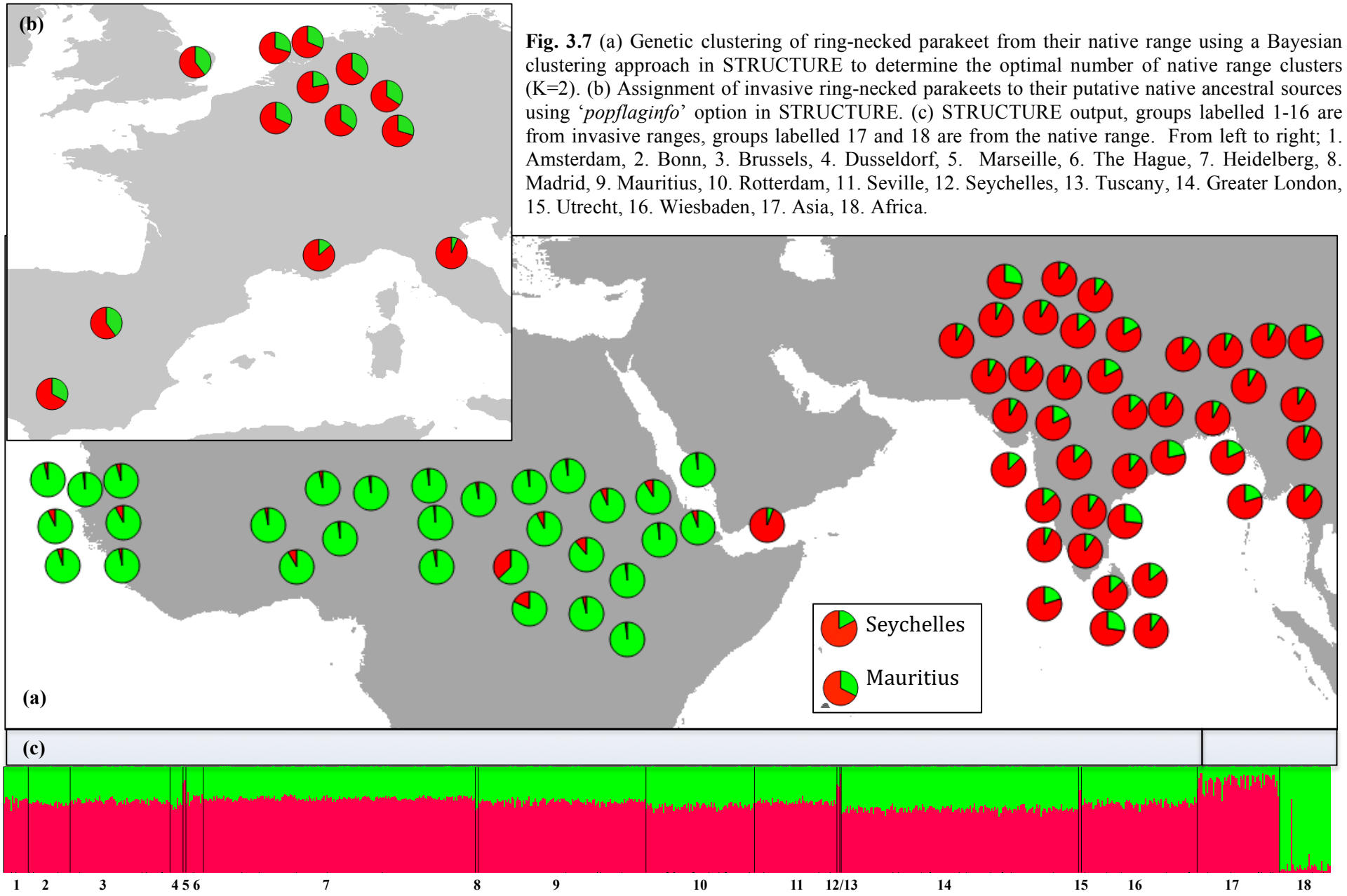

Fig. 3.7 (a) Genetic clustering of ring-necked parakeet from their native range using a Bayesian clustering approach in STRUCTURE to determine the optimal number of native range clusters (K=2). (b) Assignment of invasive ring-necked parakeets to their putative native ancestral sources using '*popflaginfo*' option in STRUCTURE. (c) STRUCTURE output, groups labelled 1-16 are from invasive ranges, groups labelled 17 and 18 are from the native range. From left to right; 1. Amsterdam, 2. Bonn, 3. Brussels, 4. Dusseldorf, 5. Marseille, 6. The Hague, 7. Heidelberg, 8. Madrid, 9. Mauritius, 10. Rotterdam, 11. Seville, 12. Seychelles, 13. Tuscany, 14. Greater London, 15. Utrecht, 16. Wiesbaden, 17. Asia, 18. Africa.

3.4.6 Genetic patterns and information from the bird pet trade

Between 1984 (the earliest trade record available from CITES) and 2007, before the EU ban on the trade of birds was implemented (Commission Regulation (EC) No.318/2007), a total of 109,463 ring-necked parakeets were imported from the Asian native range and 37,072 from the African native range (Table 3.2) into the EU countries included in this study (the proportions equate to 74.7% from Asia and 25.3% from Africa). Imports from Africa into Europe were only received from Senegal, whereas imports from Asia were received from a wider geographic source including India, Pakistan, Sri-Lanka and Bangladesh. The source composition of imported parakeets also varied considerably between country; for example, all imports into France were from Senegal (1053 parakeets), while all Asian imports to Spain and Italy were from Pakistan (48,036 and 45,316 parakeets, respectively). Greater London, Germany and France received larger numbers of ring-necked parakeets from Senegal than from Asia.

Table 3.2. Total number of ring-neck parakeets imported into countries in the invasive range between 1984-2007 (CITES 2014). Imports from the African range are all from Senegal. No import data is available for Mauritius or Seychelles.

Importer	Total global imports	India	Pakistan	Sri Lanka	Bangladesh	Total imports from Asian range	Total imports from African range (Senegal):
Spain	62334	0	48036	0	0	48036	12164
Italy	53167	0	45316	0	4	45320	3556
UK	16520	4607	18	0	2	4627	10396
Germany	11967	2479	753	0	0	3232	7682
Netherlands	7206	4277	1500	4	0	5781	201
Belgium	5639	2469	2	0	0	2471	2020
France	1620	0	0	0	0	0	1053

A comparison of the observed distribution of haplotypes detected across Europe (12 Asian and two African haplotypes) against an equal expectation (seven Asian, seven African) suggests that Asian haplotypes are more common across Europe than expected (χ^2_1=7.14, p<0.01). However, accounting for the known skew in propagule pressure (from Asia: 109,467 birds imported [75%] versus from Africa: 37,072 birds [25%], Table 3.2) indicates (after rounding) that the corrected expected proportion is 10 Asian versus 4 African haplotypes, and against this null

expectation, the observed difference fails to reach significance (χ^2_1=1.40, p=0.24). No relationships were observed between microsatellite-based estimates of the proportion of Asian/African genes observed in European countries and the proportion of Asian/African birds imported into each country (GLM coefficient = 0.121, SE = 0.764, t = 0.159, p=0.880)

Compared to expectations based on propagule pressure, Asian genes made up a larger than expected percentage of the population in Italy (propagule pressure: 93% Asian imports versus 94% Asian genes observed in the population), the UK (31% imports versus 60% genes), Germany (30% imports versus 67% genes), Belgium (55% imports versus 68% genes and France (0% imports versus 86% genes). The proportion of Asian genes detected was lower than expected in Spain (80% imports versus 64% genes) and the Netherlands (97% imports versus 70% genes).

3.4.7 Genetic patterns and temperature differences between native and invasive ranges

Haplotypes with higher maximum native-range latitude (i.e. associated with colder temperatures in the native range) are more prevalent in invaded Europe (r_{23}=0.58, p<0.01; haplotype prevalence across Europe log transformed). This result holds when comparing the maximum native-range latitude of haplotypes detected in Europe with haplotypes not detected ($t_{17.11}$=3.74, p<0.01). The weighted average maximum native-range latitude of haplotypes present in a European parakeet population was positively and significantly correlated with the latitude of that population (r_{12}=0.54, p<0.05; Fig. 3.8). Yet, despite our efforts to collect as much data as possible from southern European populations, our dataset is biased towards more northern populations (i.e. eight northern European populations versus four southern). Moreover, of the four southern populations, all but Seville have limited sample sizes. We therefore carried out a jackknife resampling in which each southern European parakeet population was omitted from the dataset. Our findings that haplotypes with higher maximum native-range latitude are more prevalent in invaded Europe and that those haplotypes detected in Europe have higher maximum native-range latitudes than haplotypes not detected are robust to jackknife resampling. However, the correlation between the weighted average maximum native-range latitude of haplotypes present in a European parakeet population and the latitude of that population is only robust to the removal of the Tuscany population. The correlation fails to achieve statistical significance when omitting any of the other southern European parakeet populations (Table S3.6.10 and Table S3.6.11). Lastly, it should be noted that in our dataset, latitude is strongly correlated with temperature (correlation between minimum temperature of the coldest month (derived from Hijmans *et al.* 2005) and latitude: r=0.94, p<0.0001). Replacing latitude with temperature consequently does not affect our results (Table S3.6.12).

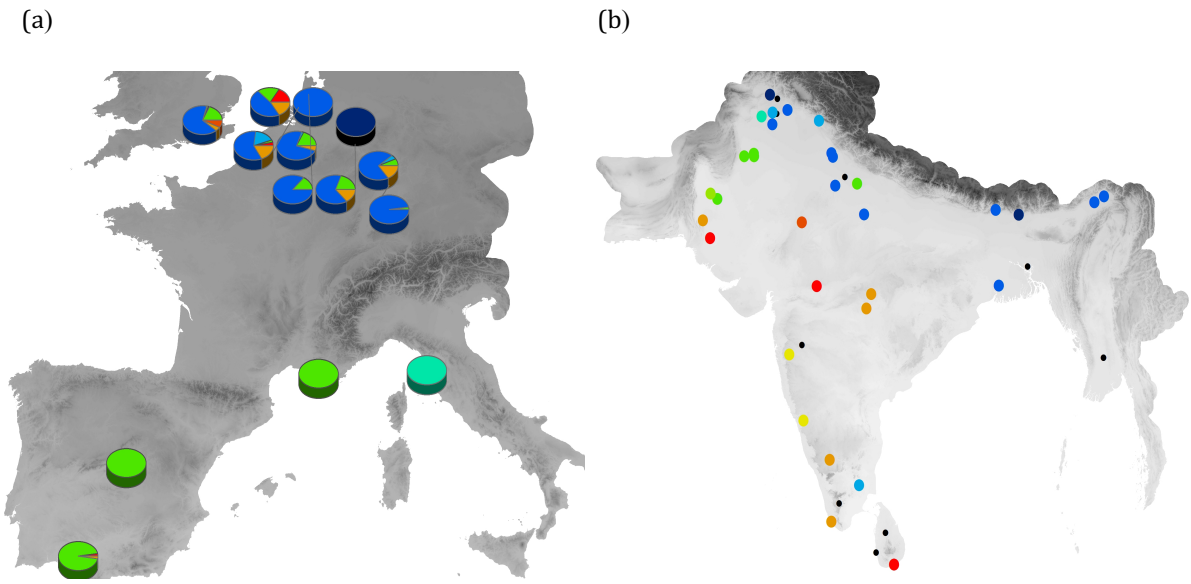

Fig. 3.8. (a) Occurrence and frequency of native Asian mtDNA haplotypes in Europe according to their (b) native Asian range maximum cold niche limits. Specimens belonging to mtDNA haplotypes with high maximum latitudes (i.e. cold-tolerance) are depicted in blue. Specimens belonging to haplotypes present in Asia but not detected in Europe are shown by smaller black dots. The shaded background of each map indicates mean annual temperature (darker shading represents colder temperatures).

3.5 Discussion

By combining extensive sampling across native and invasive geographical ranges, this study is the first to establish the evolutionary history and genetic origin of a top global avian invader. Both mtDNA and nuclear markers reveal the Asian native range to be the predominant ancestral source for the invasive populations established across Europe and the Indian Ocean islands. This observed genetic pattern in the invasive range is consistent with propagule pressure, in the form of parakeets imported from the native Asian versus African ranges for the pet trade. Our results also indicate that, in Europe, haplotypes originating from northern Asia occur in higher frequencies, which may be explained by smaller differences in temperature between the native and invasive ranges of these haplotypes. Below we discuss the supporting evidence for each alternative explanation and their likely levels of influence on the observed patterns.

3.5.1 Genetic patterns and temperature differences between native and invasive ranges

Strubbe *et al.* (2015) found that intra-specific climatic niche variation is present among native-range ring-necked parakeet phylogeographic lineages, and that including this niche variation

into ecological niche models (which are statistical techniques that link the occurrence of species to aspects of the environment, Elith *et al.* 2006) improves the accuracy of predictions of parakeet occurrence across Europe. Here, we show that the most prevalent mtDNA haplotypes in the invasive populations either have a wide native distribution, spanning almost the entire Indian subcontinent or originate from across the northern areas of India and Pakistan, the native range of *P.k.borealis*. As these haplotypes tolerate colder parts of the native range, our results suggest that their higher prevalence across Europe (and the higher accuracy of ecological niche model forecasts accounting for intra-specific niche variation found by Strubbe *et al.* (2015)) can potentially be explained by a higher establishment success and subsequent survival of these haplotypes in the cooler parts of the parakeets' invasive range. Theory predicts that because of physiological limits, for endotherms, tolerance to cold is more likely than tolerance to high temperatures (Sunday 2012; Araujo 2013). Indeed, given the temperature differences between native and invasive ranges, parakeets have not been introduced to areas warmer than their native range. Even haplotypes from the coldest parts of the native range experience, in their native range, temperatures similar to those of the warmer parts of the invasive range (such as Seville, Mauritius and Seychelles). The fact that haplotypes associated with warmer parts of the native range (i.e. southern Asia) are more frequently found in more southern and thus warmer invasive populations suggest that for these haplotypes, temperature differences with the more northerly invasive populations in Greater London, Netherlands, Belgium and Germany may be too large to tolerate.

Several eco-evolutionary scenarios have been proposed to explain the invasiveness of populations. Evolutionary changes can occur independently at each introduction location, adaptation may take place at a first site of invasion with other areas subsequently invaded from this site or alternatively, key evolutionary changes for invasion may arise in the native range, before introduction in the invaded range (Huffbauer *et al.* 2012). For example, by combining ecological niche modelling techniques with common-garden experiments and genetic data, Rey *et al.* (2012) showed that the invasion of Mediterranean Israel by the tropical ant *Wasmannia auropunctata* could be explained by adaptation to cold at the southern limit of the native range before introduction to Israel. Such an invasion scenario, termed 'prior-adaption' by Huffbauer *et al.* (2012) (not to be confused with pre-adaption, which implies a change of function), may also explain the invasion of ring-necked parakeets, as our results point to a prior-adaptation of certain haplotypes to cold conditions in the northern parts of the parakeets native Asian range.

Yet, certainly given our limited sampling of southern European parakeet populations, more research is needed to conclusively rule out alternative explanations. More data on the distribution of parakeet haplotypes across Europe are needed to run meaningful ecological niche

models (sensu Hernandez *et al.* 2006) aimed at delineating candidate geographical source areas for haplotypes across the native range. Such models could provide another, independent line of evidence for climatic similarities between native and invaded regions as driver of parakeet invasion success in Europe. European parakeet populations experience climates colder than their native range, but experimental common-garden experiments for verifying whether cold adaptation occurred during invasion are impractical with such a long-lived vertebrate species. However, recent advances in genomic methods permit using genomic signatures of selection to identify genes associated with adaptation during invasion and in response to differencing climates, without requiring common-garden experiments (Chown *et al.* 2015). Future population sampling for genetic analyses should focus specifically on parakeet populations established within geographical regions identified by ecological niche models. Genome-wide analyses of both contemporary and historic (i.e. from museum specimens) ring-necked parakeet DNA allow assessing the temporal dynamics of evolutionary change during invasion (Smith *et al.* 2011), as well as elucidating demographic footprints of past climate change in native populations (Miller *et al.* 2012). Such analyses can strongly improve the inference of invasion history and provide a long-term perspective for understanding how selection pressures during invasion impact species invasion success (Chown *et al.* 2015, Hofman & Sgro 2011).

3.5.2 Evidence for an influence of wild bird trade

Analyses of nuclear and mtDNA evidence a predominantly Asian origin for the invasive populations of ring-necked parakeets, which is consistent with expectations from the bird pet trade, as about 75% of the 158,453 ring-necked parakeets imported into Europe originated from Asia. Cassey *et al.* (2004) already reported that successful colonisations of invasive parrots are most likely to be attributed to those species that are traded and kept as pets. Recent research suggests that wild-caught parrots demonstrate acute stress responses, behaviours which can make them more adept at escaping from captivity and surviving in novel wild environments, thus contributing to their success as an invasive species (Cabezas *et al.* 2012, Carrette & Tella 2008). Strubbe & Matthysen (2009b) found that most ring-necked parakeet populations in Europe stem from unintentional escapes of cage birds, supporting the use of the proportion of Asian versus African birds imported as (an admittedly rough) proxy of propagule pressure. Similarly, Russello et al (2008) found that geographic origins of monk parakeets (*Myiopsitta monachus*) introduced across the US were concordant with trapping records from the native range, suggesting that propagule pressure exerted by the international pet bird trade contributed to the distribution of genetic diversity across the monk parakeets' invasive range.

At the European level, genetic patterns are thus in line with expectations based on propagule pressure, but interestingly, some countries, such as the UK, received much larger numbers of

imports from Africa compared to other countries, yet this difference is not reflected in their mtDNA and nuclear composition. Conversely, Seville, the warmest European parakeet population, exhibits a larger amount of African genes than expected based on trade patterns. Such mismatches may be due to the fact that populations such as Greater London were established prior to the earliest available trade records (ring-necked parakeets were recorded as breeding in 1971; Balmer *et al.* 2013), subjected to stochastic genetic processes such as founder effects or genetic drift, or may alternatively be caused by Asian and African ring-necked parakeets differing in their 'prior-adaptation' to one or more aspects of the environment (e.g. climate, association with human-disturbance, breeding or phenology).

Lastly, it should be noted that to fully unravel how eco-evolutionary scenarios lead to the emergence of invasive populations, higher resolution data on the origin of source populations and propagule pressure may be needed. For example, although we have no evidence to assume this, theoretically speaking, it could be possible that the higher prevalence of north Asian haplotypes in Europe is not due to a prior-adaptation to colder environments, but to larger amounts of northern versus southern Indian parakeets introduced and escaped in Europe. CITES trade data are only available at the country level, and this crude proxy for propagule pressure thus does not allow testing for such a scenario.

3.5.3 Evolutionary history of native populations

The molecular phylogenetic analysis suggests that ring-necked parakeets diverged into Asia approximately 2.57 Mya and subsequently into Africa around 1.61 Mya. Previous studies found the African subspecies *P. k. krameri* as basal to the Asian and African ring-necked parakeets (Groombridge *et al.* 2004; Kundu *et al.* 2012), however the inclusion of additional genetic data for a markedly larger sample set suggests an Asian origin and should be considered more robust. The clustering of ring-necked parakeets from Burma with African clades is intriguing, and may be a result of misidentification of subspecies designation during collection, incorrect labelling at the museum or specimen mix up. Alternatively this clustering pattern may suggest the true nature of relationships between ring-necked parakeet subspecies is complex. While the sample from Somalia is labelled *P. k. parvirostris* the geographic location appears outside the native African range, suggesting ring-necked parakeets in Somalia may in fact be a result of historical trade or transport of parakeets from India rather than the result of establishment of a natural population (Jennings in prep). The overall levels of nucleotide divergence between Asian and African subspecies are congruent with earlier literature reporting mean nucleotide divergences for ring-necked parakeets based on cytochrome *b* data (Groombridge *et al.* 2004). These patterns of divergence are consistent with ancient patterns of environmental and climatic changes in Asia following the uplift of the Tibetan Plateau, and the formation of Indian and

African monsoon systems (Ruddiman & Kubacch 1990; Zhisheng *et al.* 2001). Major areas of inhospitable habitat such as the Tibetan Plateau and Sahara Desert resulted in divergence events of ancestral parrots (Schweizer *et al.* 2011), while cold climates governed the pattern of radiation from Asia to Africa, allowing ring-necked parakeets to spread across northern Africa (Groombridge *et al.* 2004). While there is a basic geographic structure of haplotypes within the Asian range (*P. k. borealis* in the north, *P. k. manillensis* in the south), a clear geographic pattern of clusters can be seen in northern Africa across Senegal, Nigeria and Sudan. This pattern could be explained by historical changes in climate in the Sahara where lush forests have slowly become transformed to deserts (Kropelin *et al.* 2008), creating barriers to ring-necked parakeet dispersal and confining these birds to geographical regions across their African range. Interestingly, 30 haplotypes were identified in the invasive ranges but not in the native ranges. We suspect that these haplotypes may be present in the Asian native ranges but that they were not detected by our sampling.

3.6 Conclusions

Our study has provided the first substantial insights into the evolutionary history and genetic origins of the ring-necked parakeet across its widespread invasive range. Our findings provide strong evidence that the invasive populations predominately originate from Asia, in particular from the northern areas of the ring-necked parakeets' range in Asia. Our data also suggest that a combination of non-random effects from prior-adaptation to cold in the northern parts of the native Asian range, along with high levels of bird trade, have influenced the signatures of ancestral origin within the invasive populations of ring-necked parakeets. This eco-evolutionary scenario, shaped by a large number of importations from Asia and subsequently influenced by prior-adaptaion to cold climates in the native range, represents an important first step in reconstructing pathways of invasion. Our identification of the ancestral origins of invasive ring-necked parakeets provides crucial information that can be applied to further studies such as ecological niche models aimed at predicting areas at risk of invasion by this species. Our findings provide an important contribution for understanding the evolutionary adaptation of a globally invasive species in a novel environment.

3.7 Acknowledgements

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3.8 Supplementary material

Table S3.1. Sample size and location information for invasive populations of *Psittacula krameri* included in this study.

Population	N	Lat	Long
Amsterdam	17	52.37	4.89
Bonn	29	50.73	7.1
Brussels	69	50.8411	4.3564
Dusseldorf	9	51.2256	6.7828
Greater London	164	51.5171	0.1062
Heidelberg	188	49.4034	8.6792
Madrid	2	40.4	-3.6833
Marseille	2	43.2975	5.3772
Mauritius	116	-20.1625	57.5
Rotterdam	75	51.9217	4.4811
Seville	57	37.3833	-5.9833
Seychelles	2	-4.6308	55.4619
The Hague	12	52.0799	4.3111
Tuscany	1	43.41	11
Utrecht	2	52.091	5.122
Wiesbaden	80	50.0856	8.2387

Table S3.2. Sample and location information for historical specimens of *Psittacula krameri* sampled from the Natural History Museum at Tring, UK

Subspp. designation	Museum ID	Sample location	Lat	Long
P.k.borealis	1889.1.26.271	Assam	27.83	95.67
P.k.borealis	1889.1.26.258	Bhutan	26.7481	89.7498
P.k.borealis	1889.1.26.288	Bhutan	26.7481	89.7498
P.k.borealis	1941.5.30.2913	Burma	18.3374	95.6239
P.k.borealis	1908.5.30.84	Burma	18.3374	95.6239
P.k.borealis	1948.80.3671	Burma Myanmar	18.3374	95.6239
P.k.borealis	1889.1.26.289	Calcutta Bhutan	22.5697	88.3697
P.k.borealis	1889.1.26.290	Calcutta India	22.5697	88.3697
P.k.borealis	1889.1.26.286	Dacca Bangladesh	23.7	90.375
P.k.borealis	1889.1.26.254	Darjeeling India	27.03	88.16
P.k.borealis	1889.1.26.245	Dehli India	28.4667	77.0333
P.k.borealis	1889.1.26.273	Dibrugarh India	27.4805	94.9999
P.k.borealis	1889.1.26.291	Dibrughur India	27.4805	94.9999
P.k.borealis	1889.1.26.252	Etawah India	26.77	79.03
P.k.borealis	1889.1.26.253	Etawah India	26.77	79.03
P.k.borealis	1889.1.26.237	Jhelum Pakistan	32.9286	73.7314
P.k.borealis	1875.7.13.72	Kamrtee India	21.2333	79.2
P.k.borealis	1875.7.13.73	Kamthi India	21.2333	79.2
P.k.borealis	1889.1.20.360	Lawrencepur Pakistan	33.8347	72.5078
P.k.borealis	1889.1.20.355	Maunbhoom India	19.0808	74.7299
P.k.borealis	1897.12.10.1842	Meerut India	28.99	77.7
P.k.borealis	1884.10.8.50	Mhow India	22.55	75.76
P.k.borealis	1889.1.26.256	Nepal	13.4774	5.8749
P.k.borealis	1949.Whi.1.16880	Punjab	30.16	76.87
P.k.borealis	1949.25.853	Punjab	32.7187	72.9843
P.k.borealis	1949.25.854	Punjab India	30.2039	70.7227
P.k.borealis	1949.25.856	Punjab India	30.35	71.39
P.k.borealis	1949.Whi.1.16883	Punjab Pakistan	30.2	71.4167
P.k.borealis	1881.5.1.4725	Punjab Pakistan	32.5417	71.9333
P.k.borealis	1949.25.850	Punjab Pakistan	32.0836	72.6711
P.k.borealis	1949.25.851	Punjab Pakistan	32.7833	72.7
P.k.borealis	1949.25.855	Punjab Pakistan	26.3	74.73
P.k.borealis	1949.Whi.1.16956	Rawalpindi Pakistan	11.83	32.8
P.k.borealis	1889.1.26.240	Sambhal India	14.8333	-17.1
P.k.borealis	1889.1.26.278	Seoni India	28	68.4
P.k.borealis	1860.4.16.550	Shikarpur Pakistan	26.4254	67.8607
P.k.borealis	1898.12.12.320	Sind India	27.6831	68.8678
P.k.borealis	1889.1.20.235	Sindh Punjab	28.4667	77.0333
P.k.borealis	1889.1.26.246	Sindh Punjab	32.3	75.9
P.k.borealis	1889.1.26.248	Sindh Punjab	30.38	76.78
P.k.borealis	1889.1.20.358	Sindh Punjab	25.3792	68.3683
P.k.borealis	1860.4.16.557	Sindh Punjab	14.6195	74.8354
P.k.borealis	1889.1.26.262	Suddya Bhutan	27.6833	68.8667
P.k.borealis	1889.1.26.263	Suddya India	27.6833	68.8667
P.k.borealis	1889.1.26.236	Sukkur Pakistan	14.8333	-17.1
P.k.krameri	1915.12.24.510	Bahr el Ghazal Sudan	7.7	28
P.k.krameri	1907.12.23.77	Bahr el Ghazal Sudan	7.7	28
P.k.krameri	1929.2.18.122	Bakalari Gambia	13.4217	-16.4267
P.k.krameri	1923.10.26.67	Cameroon	9.3	13.4
P.k.krameri	1920.12.22.105	Dafur Sudan	11.07	26.85

P.k.krameri	1922.12.8.406	Dafur Sudan	12.9	23.4833
P.k.krameri	1922.12.8.408	Dafur Sudan	12.5947	23.6192
P.k.krameri	1922.12.8.410	Dafur Sudan	12.9	23.4833
P.k.krameri	1922.12.8.412	Dafur Sudan	15.1304	26.1709
P.k.krameri	1922.12.8.414	Dafur Sudan	13.6306	25.35
P.k.krameri	1929.2.18.121	Gambia	13.4217	-16.4267
P.k.krameri	1899.9.20.5	Ghana	10.5021	-1.9651
P.k.krameri	1910.5.6.154	Guinea	12.3103	-15.7874
P.k.krameri	1910.5.6.153	Gunnal Guinea Bissau	12.3103	-15.7874
P.k.krameri	1930.3.4.371	Haute Volta	12.1389	0.6552
P.k.krameri	1939.12.9.3243	Kael Senegal	14.7077	-15.8991
P.k.krameri	1929.2.18.120	Kerewan Gambia	13.5	-16.0833
P.k.krameri	1922.12.8.405	Kurdufan Sudan	13.1833	30.2167
P.k.krameri	1926.8.8.136	LakeChad Nigeria	14.2528	13.1108
P.k.krameri	1915.12.24.512	Mongalla Sudan	5.1989	31.7695
P.k.krameri	1928.7.20.26	Nigeria	9.1539	4.812
P.k.krameri	1900.8.4.29	Nigeria	13.5561	13.233
P.k.krameri	1911.12.23.535	Nigeria	18.5203	73.8567
P.k.krameri	1923.8.7.7000	Renk Sudan	11.83	32.8
P.k.krameri	1923.8.7.7001	Renk Sudan	28.58	78.55
P.k.krameri	1939.12.9.3241	Senegal Gambia	14.6667	-16.2501
P.k.krameri	1918.8.26.33	Senegal Gambia	16.0333	-16.5
P.k.krameri	1889.1.20.331	Senegal Gambia	22.08	79.53
P.k.krameri	1907.12.23.76	Sudan	26.7481	89.7498
P.k.krameri	1939.12.9.3240	Thies Senegal	8.6626	76.7646
P.k.krameri	1911.12.23.536	Yo Nigeria	27.83	95.67
P.k.mallinensis	1889.1.26.283	Aujango India	8.6626	76.7646
P.k.mallinensis	1940.12.3.211	Kalawewa Sri Lanka	8.0166	80.5164
P.k.mallinensis	1949.Whi.1.16889	Madras India	9.74	77.3
P.k.mallinensis	1884.7.28.38	Mysore India	12.3	76.65
P.k.mallinensis	1860.4.16.558	Mysore India	12.3	76.65
P.k.mallinensis	1919.1.12.61	Pune India	30.35	71.39
P.k.mallinensis	1949.Whi.1.16884	Rajasthan India	33.6	73.0333
P.k.mallinensis	1925.12.23.1106	Sirsi Kanara	8.0166	80.5164
P.k.mallinensis	1940.12.3.209	Sri Lanka	6.1244	81.1225
P.k.mallinensis	1946.28.234	Sri Lanka	6.8397	79.8758
P.k.mallinensis	1953.16.31	Sri Lanka	8.7047	25.4579
P.k.mallinensis	1937.12.21.94	Travancore	13.5561	13.233
P.k.mallinensis	1889.1.26.281	Travancore India	15.7833	38.45
P.k.parvirostris	1890.10.10.4	Anseba River Ethiopia	15.7833	38.45
P.k.parvirostris	1927.5.3.1	Berbera Somalia	10.4333	45.0167
P.k.parvirostris	1915.12.24.515	BlueNile Sudan	11.85	34.3833
P.k.parvirostris	1889.1.20.334	Eritrea Africa	15.9027	38.4522
P.k.parvirostris	1878.12.31.663	Eritrea Africa	15.9027	38.4522
P.k.parvirostris	1919.12.17.751	Kamisa Sudan	13.1206	34.2279
P.k.parvirostris	1915.12.24.513	Mongalla Sudan	5.1989	31.7695

Table 3.5. Suite of mtDNA PCR primers used to amplify cytochrome *b* and control region fragments in historical and contemporary *Psittacula krameri* specimens.

Primer name	Sequence (5' – 3')
Historical specimen primers	
Cb1f	CTA CCA TTC ATA ATC ACC AGC C
Cb1r	GTG AGG GAG AGG AGT ATG ATA G
Cb2f	CTA TCA TAC TCC TCT CCC TCA C
Cb2r	TAG GAT CAG TAC GGA GGC AG
Cb3f	AAC AAC TCC CCC ACA CAT C
Cb3r	CGG CGA GTG TTC AGA ATA G
CR1f	CGT TCG TGT TTG CTT ACA TTT C
CR1r	GGT CCG TGT TGT TTG TTT TG
CR2f	CAC TGA TGC ACT TTT TCT GAC
CR2r	GGT GAA ATG TAA GCA AAC ACG
MCR2f	GAT GCA CTT TTT CTG ACA TCT G
MCR2r	GTT TCT TGA AAT GAA TCA CAG
CR3f	GAA CAA ACA AAC GTC TCC TTC
CR3r	GGA TAT TTG AGT GCG AGT GAC
Contemporary specimen primers	
PKCBf	CGGCCTACTCCTAGCCGCCC
PKCBr	GGGAAGCAGGCCGGAAGGC
CR19f	CACAGGCTCATTTGGTTCGC
CR19r	TAAGCTACAGGGACATTCGGGG

Table S3.4. Prior distributions for the three competing senarios scenarios modelled using DIYABC v 2.02 (Cornuet *et al.* 2014) to determine ancestral origins of invasive ring-necked parakeets.

Prior distributions	Min	Max	Bias	RRMISE
N1 – Brussels	1.0	10000.0	0.117	1.955
N2 – Heidelberg	1.0	10000.0	0.127	2.938
N3 – Wiesbaden	1.0	10000.0	0.040	2.183
N4 - Bonn	1.0	10000.0	0.076	2.497
N5- Dusseldorf	1.0	10000.0	0.052	3.162
N6 – Seville	1.0	10000.0	0.075	3.753
N7 - Madrid	1.0	10000.0	0.022	3.328
N8- UK	1.0	10000.0	0.065	2.284
N9 – Tuscany	1.0	10000.0	0.034	5.918
N10 – Marseille	1.0	10000.0	0.033	2.755
N11- Rotterdam	1.0	10000.0	0.048	3.290
N12 – The Hague	1.0	10000.0	0.112	2.718
N13 - Amsterdam	1.0	10000.0	0.018	2.552
N14 - Utrecht	1.0	10000.0	0.051	4.080
N15 – Mauritius	1.0	10000.0	0.106	2.961
N16 – Seychelles	1.0	10000.0	0.036	3.820
N17 – native Asia	10.0	10000.0	0.029	1.983
N18 – native Africa	10.0	10000.0	-0.005	1.500
t1db – bottleneck	1.0	100.0	-0.014	1.493
db –duration of bottleneck	1.0	10.0	-0.081	1.177
t1inv - invasion	1.0	15.0	-0.012	0.786
t2anc - historical	200000.0	300000.0	-0.001	0.166
Ar - admixture	0.001	0.999	-0.002	0.585

Table S3.5. Latitudes for native range Asian mtDNA haplotypes, and their prevalence in invasive European populations of ring-necked parakeet.

Native Asian haplotype number	No of individuals in each haplotype	Latitude (min)	Latitude (max)	Latitude (mean)	Prevalence in invasive European populations
4	3	6.124443934	25.37916699	18.0179	11
5	6	27.68313799	30.35000521	29.5612	153
7	3	14.61949631	26.77000251	19.9699	1
8	3	10.80501746	32.78333308	25.2961	17
9	1	27.99999999	27.99999999	28	1
18	16	22.56972206	32.92861481	27.7964	366
19	1	33.60000001	33.60000001	33.6	0
20	2	26.7480604	33.834722	30.2914	4
21	1	28.99000359	28.99000359	28.99	0
22	1	32.71868815	32.71868815	32.7187	0
23	1	27.68333272	27.68333272	27.6833	1
24	6	8.662577648	26.425432	16.5606	34
25	1	19.08080632	19.08080632	19.0808	0
26	1	12.30000564	12.30000564	12.3	0
27	1	21.23330443	21.23330443	21.2333	0
28	1	26.30000023	26.30000023	26.3	10
29	1	8.016645888	8.016645888	8.0166	0
30	1	6.839716414	6.839716414	6.8397	0
31	1	9.739977287	9.739977287	9.74	0
33	1	32.54166705	32.54166705	32.5417	1
34	1	27.68333272	27.68333272	27.6833	0
35	1	23.70000015	23.70000015	23.7	0
36	2	0	18.337448	9.1687	0
37	1	18.337448	18.337448	18.3374	0
38	1	18.337448	18.337448	18.3374	0

Table S3.6. Model Checking for DIYABC based upon 1,000,000 simulated datasets. For each summary statistic we calculated the proportion of datasets in which the statistic was less than the observed value. Values <5% (bold) are indicative of a poor match.

Summary statistics	Observed value	Proportion (simulated<observed)
NAL_1_1	9.9	0.723
NAL_1_2	8.6	0.779
NAL_1_3	8.8	0.697
NAL_1_4	8.5	0.7225
NAL_1_5	6.7	0.7725
NAL_1_6	9.9	0.802
NAL_1_7	2.2	0.322
NAL_1_8	11.5	0.7345
NAL_1_9	1.5	0.2855
NAL_1_10	2.6	0.5465
NAL_1_11	10.1	0.7355
NAL_1_12	5.7	0.6425
NAL_1_13	7.1	0.696
NAL_1_14	2.5	0.4835
NAL_1_15	6.7	0.649
NAL_1_16	2	0.236
NAL_1_17	8	0.396
NAL_1_18	6.7	0.886
HET_1_1	0.79	0.7295
HET_1_2	0.765	0.775
HET_1_3	0.8029	0.7895
HET_1_4	0.8079	0.7845
HET_1_5	0.8261	0.834
HET_1_6	0.7964	0.8
HET_1_7	0.5667	0.3235
HET_1_8	0.8243	0.8335
HET_1_9	0.5	0.2855
HET_1_10	0.7167	0.59
HET_1_11	0.7897	0.7455
HET_1_12	0.6841	0.519
HET_1_13	0.7385	0.6165
HET_1_14	0.6667	0.493
HET_1_15	0.6228	0.405
HET_1_16	0.45	0.183
HET_1_17	0.6286	0.1235
HET_1_18	0.5781	0.6545
N2P_1_1&17	10.9	0.534
N2P_1_1&18	11	0.718
N2P_1_2&17	10.5	0.5305
N2P_1_2&18	10.3	0.7445

N2P_1_3&17	10.5	0.475
N2P_1_3&18	10.2	0.667
N2P_1_4&17	10.4	0.498
N2P_1_4&18	10.1	0.71
N2P_1_5&17	9.6	0.4455
N2P_1_5&18	9.4	0.771
N2P_1_6&17	11.2	0.604
N2P_1_6&18	11.2	0.7855
N2P_1_7&17	8.6	0.393
N2P_1_7&18	7.4	0.7545
N2P_1_8&17	12.1	0.6015
N2P_1_8&18	12.3	0.747
N2P_1_9&17	8.2	0.3695
N2P_1_9&18	7.2	0.816
N2P_1_10&17	8.4	0.353
N2P_1_10&18	7.3	0.7445
N2P_1_11&17	11.1	0.5625
N2P_1_11&18	11	0.7185
N2P_1_12&17	9.4	0.3955
N2P_1_12&18	8.5	0.636
N2P_1_13&17	10	0.4715
N2P_1_13&18	9.5	0.699
N2P_1_14&17	8.6	0.387
N2P_1_14&18	7.3	0.745
N2P_1_15&17	10.5	0.493
N2P_1_15&18	10.1	0.701
N2P_1_16&17	8.7	0.407
N2P_1_16&18	7.5	0.7685
H2P_1_1&17	0.7823	0.6595
H2P_1_1&18	0.7911	0.7285
H2P_1_2&17	0.7785	0.7695
H2P_1_2&18	0.7766	0.749
H2P_1_3&17	0.7824	0.6845
H2P_1_3&18	0.7936	0.73
H2P_1_4&17	0.736	0.384
H2P_1_4&18	0.7565	0.6835
H2P_1_5&17	0.6742	0.1755
H2P_1_5&18	0.6578	0.5825
H2P_1_6&17	0.775	0.6455
H2P_1_6&18	0.7866	0.756
H2P_1_7&17	0.6388	0.1305
H2P_1_8&17	0.8189	0.8295
H2P_1_8&18	0.8192	0.798
H2P_1_9&17	0.6365	0.129
H2P_1_9&18	0.5948	0.6575
H2P_1_10&17	0.6438	0.1405
H2P_1_10&18	0.6079	0.65

H2P_1_11&17	0.7857	0.696
H2P_1_11&18	0.7916	0.729
H2P_1_12&17	0.6951	0.2435
H2P_1_12&18	0.6948	0.6475
H2P_1_13&17	0.7134	0.2885
H2P_1_13&18	0.7153	0.6345
H2P_1_14&17	0.6386	0.1265
H2P_1_14&18	0.6013	0.631
H2P_1_15&17	0.7376	0.5285
H2P_1_15&18	0.7132	0.4625
H2P_1_16&17	0.6383	0.1315
H2P_1_16&18	0.6013	0.6345
V2P_1_7&18	2.1954	**0.006**
FST_1_1&17	0.1528	0.606
FST_1_1&18	0.1806	0.1815
FST_1_2&17	0.1453	0.4745
FST_1_2&18	0.1705	0.128
FST_1_3&17	0.1251	0.52
FST_1_3&18	0.1557	0.104
FST_1_4&17	0.1283	0.5545
FST_1_4&18	0.1748	0.1475
FST_1_5&17	0.1006	**0.4605**
FST_1_5&18	0.1166	0.027
FST_1_6&17	0.1465	0.5525
FST_1_6&18	0.1752	0.1415
FST_1_7&17	0.1798	0.759
FST_1_7&18	0.1569	0.065
FST_1_8&17	0.1299	0.54
FST_1_8&18	0.1409	0.0945
FST_1_9&17	0.2252	0.894
FST_1_9&18	0.2999	0.326
FST_1_10&17	0.1974	0.7925
FST_1_10&18	0.2484	0.2205
FST_1_11&17	0.1538	0.611
FST_1_11&18	0.1772	0.1725
FST_1_12&17	0.2283	0.8345
FST_1_12&18	0.2715	0.362
FST_1_13&17	0.1988	0.7705
FST_1_13&18	0.2274	0.274
FST_1_14&17	0.132	0.58
FST_1_14&18	0.2027	0.139
FST_1_15&17	0.2888	0.8445
FST_1_15&18	0.3144	0.572
FST_1_16&17	0.2192	0.842
FST_1_16&18	0.2901	0.327


Falco tinnunculus tinnunculus
Gallus gallus
Psittacus erithacus
Poicephalus senegalus senegalus
Poicephalus cryptoxanthus
Poicephalus meyeri matschiei
Poicephalus meyeri saturatus
Poicephalus gulielmi
Poicephalus robustus robustus
Poicephalus robustus fusicollis
Poicephalus robustus suahelicus
Psittrichas fulgidas
Coracopsis vasa drouhardii
Coracopsis vasa
Coracopsis nigra barklyi
Mascarinus mascarinus
Coracopsis nigra sibilans
Coracopsis nigra nigra
Coracopsis nigra libs
Aprosmictus erythropterus
Polytelis anthopeplus
Eclectus roratus polychlorus
Eclectus roratus roratus
Psittacula roseata
Psittacula cyanocephala cyanocephala
Psittacula finschii
Psittacula himalayana
Psittacula longicauda longicauda
Psittacula calthorpe
Psittacula columboides
Psittacula derbiana
Psittacula alexandri fasciata
Psittacula longicauda tyleri
Psittacula alexandri
Psittacula wardi
Psittacula eupatria magnirostris
Psittacula eupatria siamensis
Psittacula eupatria
Psittacula eupatria nipalensis
Tangynathus sumatranus
Psittacula echo
Asia_Pakistan_34
Asia_Punjab_33
Asia_Pakistan_9
Psittacula krameri parvirostris
Psittacula krameri manillensis
Africa_Somalia_6
Asia_Punjab_5
Africa_Sudan_3
Asia_Bangladesh_35
Asia_West.Bengal_25
Asia_Bhutan_23
Asia_Karnataka_26
Asia_Uttar.Pradesh_21
Asia_mixed.locations_20
Asia_Pakistan_19
Asia_Punjab_22
Asia_mixed.locations_18
Asia_Maharashtra_27
Asia_Tamil.Nadu_31
Psittacula krameri boreallis
Asia_Sri.Lanka_32
Asia_mixed.locations_7
Asia_Sri.Lanka_30
Asia_Sri.Lanka_29
Asia_Rajasthan_28
Asia_mixed.locations_4
Asia_Punjab_8
Asia_mixed.locations_24
Asia_Burma_37
Asia_Burma_36
Asia_Burma_38
Africa_Sudan_40
Africa_Sudan_39
Africa_Ethiopia_1
Africa_Sudan_2
Africa_Eritrea_41
Africa_Eritrea_42
Africa_Senegal_15
Psittacula krameri krameri
Africa_Senegal_44
Africa_Senegal_43
Africa_Senegal_10
Africa_Ghana_13
Africa_Cameroon_11
Africa_Nigeria_17
Africa_Nigeria_14
Africa_Nigeria_12
Africa_Nigeria_16
Agapornis taranta
Agapornis pullaria
Agapornis cana
Agapornis roseicollis
Agapornis nigrigenis
Agapornis lilianae
Agapornis fischeri
Agapornis personata
Loriculus galgulus
Loriculus pusillus
Loriculus stigmatus
Loriculus beryllinus
20.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0

Fig. S3.1. Estimated divergence times derived from mtDNA data obtained for museum specimens of *Psittacula krameri.* Sequence data were analysed alongside existing sequences for other Old World parrots (*Psittaciformes)* from Kundu *et al.* 2012 and resolved using BEAST and specifying a 80 Mya TMRCA. Node bars display the 95% HPD and time on the axis is given in millions of years before the present.

Table S3.7. Haplotype frequencies for each of the 74 haplotypes discovered across the invasive populations of ring-necked parakeet.

Pop/Hap No	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Bonn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	1	0
Brussels	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dusseldorf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marseille	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seychelles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tuscany	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mauritius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heidelberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Madrid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seville	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Greater London	0	0	10	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
Wiesbaden	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Rotterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
The Hague	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amsterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utrecht	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3.7. Continued.

Pop/Hap No	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Bonn	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	4	1	0	4	0	0
Brussels	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Dusseldorf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marseille	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seychelles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tuscany	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mauritius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heidelberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Madrid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seville	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G.London	10	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	2
Wiesbaden	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
Rotterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0
The Hague	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amsterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utrecht	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3.7. Continued.

Pop/Hap No	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
Bonn	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
Brussels	0	0	0	0	8	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Dusseldorf	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Marseille	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seychelles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tuscany	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mauritius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Heidelberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Madrid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seville	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
G.London	0	1	0	1	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wiesbaden	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0
Rotterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
The Hague	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amsterdam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utrecht	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3.8. Despite our efforts to collate as much information as possible from southern Europe, our dataset is biased towards more northerly parakeet populations. We have made this explicit in the thesis so that the reader can judge the quality of our dataset and the implications thereof. Concerning the analysis of proportion of haplotypes, the low sample size of southern populations indeed makes our results vulnerable to the Seville population. We have repeated the analysis omitting each of the southern European populations, showing that only Tuscany can be removed from the dataset. Removing any other population results in P-values > 0.05.

Population removed	R	P-value
Seville	0.41	**0.17**
Marseille	0.48	**0.09**
Tuscany	0.63	0.02
Madrid	0.45	**0.12**

Table S3.9. We carried out similar 'sensitivity' test on our analyses of haplotypes prevalence or presence-absence in invaded Europe, showing that these analyses are robust concerning the inclusion of southern European populations.

Haplotypes prevalence in invaded range			Haplotype presence/absence in the invaded range		
Seville	0.538832	0.00545	Seville	3.7413	0.00161
Marseille	0.5382809	0.005508	Marseille	3.7413	0.00161
Tuscany	0.481604	0.01479	Tuscany	3.2663	0.004142
Madrid	0.5382809	0.005508	Madrid	3.7413	0.00161

Thus, our first finding that haplotypes with higher maximum native-range latitudes (i.e. a higher cold niche limit) are more prevalent in Europe/that haplotypes detected in Europe have a higher maximum native-range latitude than haplotypes not detected in Europe is not dependent on a specific southern European population. Our second finding that within European parakeet populations, the weighted average maximum native-range latitude of haplotypes present is positively and significantly correlated with the latitude of that population does however depend on specific southern European populations (i.e. only Tuscany can be excluded from the analysis).

Table S3.10. About the use of latitude as a proxy for temperature: we opted for latitude as a proxy to facilitate interpretation of Fig. 3.4.7, which illustrates that invasive populations

originate predominantly from northern areas of the Asian native range. We derived the 'minimum temperature of the coldest month' from the WorldClim database (variable 'bio6') and find that in our dataset, minimum temperature and latitude are very strongly correlated (r = -0.97, P-value < 0.0001). Consequently, replacing latitude with minimum temperature does not affect our main conclusions. The table below compares the results obtained using latitude (as mentioned under the header *Influence of 'native range climatic niche limits and the bird trade'*) with the results obtained using minimum temperature of the coldest month.

Latitude		Minimum temperature coldest month	
r: 0.5382809	P-value: 0.005508	r: -0.5718359	P-value: 0.002822
t: 3.7413	P-value: 0.00161	t: -3.7761	P-value: 0.001341
r: 0.5367374	P-value: 0.04782	r: 0.5381287	P-value: 0.04714

3.9 References

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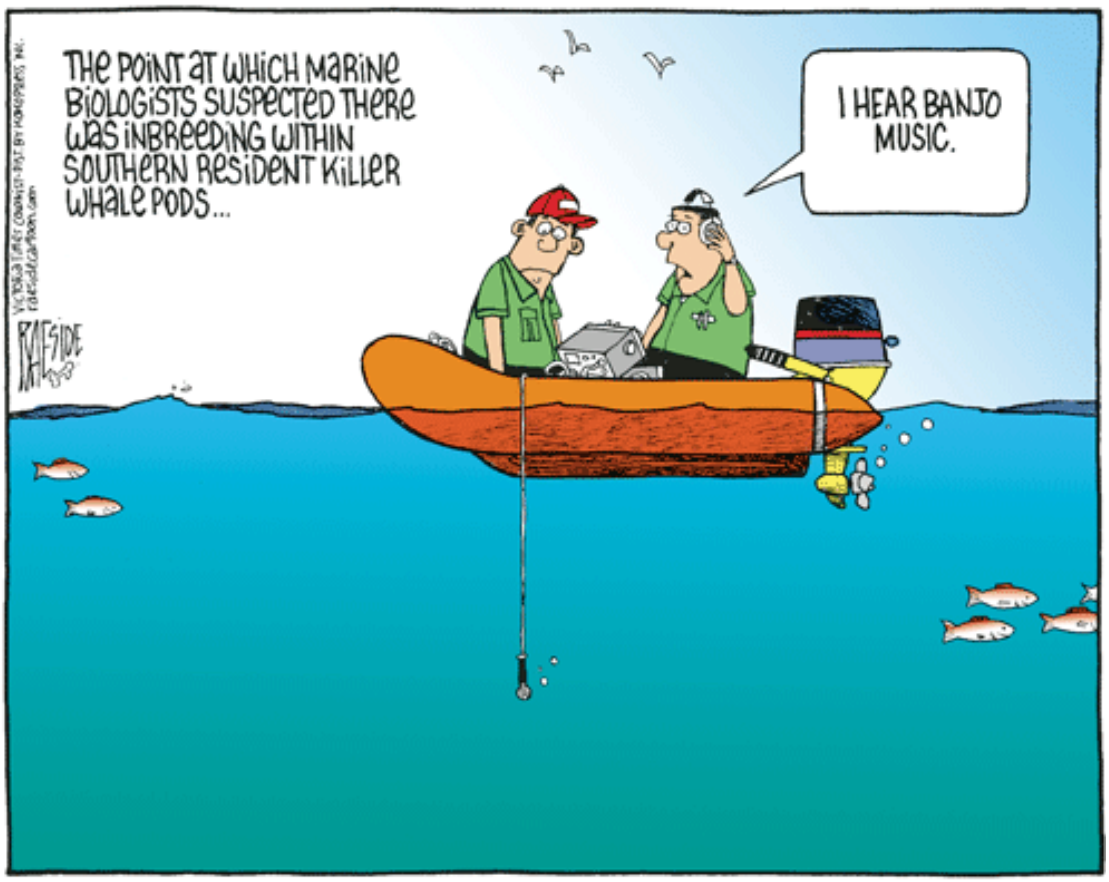
THE POINT AT WHICH MARINE BIOLOGISTS SUSPECTED THERE WAS INBREEDING WITHIN SOUTHERN RESIDENT KILLER WHALE PODS...
I HEAR BANJO MUSIC.

Chapter 4.

In prep

Characterising factors important for invasion success for invasive ring-necked parakeets (*Psittacula krameri)* in Europe; bottleneck effects, genetic structure and drivers of population growth.

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4.1 Abstract

The rapid growth and spread of invasive alien species presents an insidious threat to global biodiversity. Invasive populations are predicted to suffer severe bottleneck effects during founding prior to their successful establishment, however historical information on the number of individuals released into the wild and subsequent patterns of population growth are often anecdotal We apply a novel approach to estimate the severity of population bottlenecks using genetic data from 10 populations of invasive ring-necked parakeets (*Psittacula krameri)* in Europe, against a backdrop of population genetic diversity and structure. We furthermore examine a suite of environmental, human-demographic and genetic variables to identify drivers of population growth. European individuals (n=700) were genotyped at 15 polymorphic microsatellite loci, museum specimens (n=92) from the native range were genotyped at 10 loci, and all samples were sequenced for two mtDNA genes. Genetic bottlenecks were substantially milder than would be expected from the demographic records of population growth. High levels of genetic diversity were observed in the invasive and native range, but there was no obvious geographic structure across the invasive range. Population growth rates were found to correlate with available suitable habitat based on climate and niche structure. The discrepancies between the severe population bottlenecks from records of population size, mild genetic bottlenecks in combination with high contemporary levels of genetic diversity, suggest that the invasive populations have been 'topped-up' by continuing escapes from captive populations, while higher growth rates in warmer European populations can be explained by climatic and niche similarities between the native and invasive areas.

4.2 Introduction

Invasive alien species present an insidious threat to global biodiversity (Holmes & Simons 1996; Chapin *et al.* 2000; Doody *et al.* 2009), impacting native species and ecosystems through a multitude of mechanisms including competition for resources, transmission of infectious diseases and hybridisation (Sakai *et al.* 2001; Allendorf & Lundquist 2003; Gurevitch & Padilla 2004; Wikelski *et al.* 2010). Invasive alien species are also of economic concern and present a significant risk to global agriculture and public health (Ziska *et al.* 2011; Keller *et al.* 2011; Caplat *et al.* 2012). In response to this, national and international legislations have been adopted, such as the recent European Commission Regulation on the prevention and management of the introduction and spread of invasive alien species (Regulation (EU) No.1143/2014). To mitigate such devastating impacts on native biota, it is imperative to understand the evolutionary and ecological processes that enable species to become successful invaders. Indeed, determining the genetic structure of invasive populations, such as patterns of genetic diversity, structure, gene flow (Sax *et al.* 2005; Estoup & Guillemaud 2010), in combination with information on the severity of associated bottleneck effects (loss of heterozygosity, accumulation of level of inbreeding) can provide information about the history and underlying drivers of successful invasions. Such information also has a practical application, for developing management strategies and for predicting future invasions (Sakai *et al.* 2001; Miura 2007; Ross & Shoemaker 2008). For example, knowledge of which factors underpin invasion success may also help identify populations and regions that are likely to become invasive and invaded, respectively, in the future (Ramstad *et al.* 2004).

Populations of invasive alien species frequently appear to avoid problems commonly associated with small founding population size, such as loss of genetic diversity and inbreeding depression. It has been argued that severe bottlenecks typically reduce a species' potential for evolutionary adaptation to new and changing environments, making establishment in novel environments unlikely (Dlugosch & Parker 2008), but invasive alien species seem to buck this trend. Recent evidence indicates that invasions can often be fuelled by continued propagule pressure, whereby subsequent waves of 'top-up' introductions can mitigate the detrimental genetic impacts predicted from an initial severe bottleneck (Lockwood 2005; Drake *et al.* 2007; Ross & Shoemaker 2008). However, such mitigation against genetic barriers to invasion success can be offset by environmental and demographic stochastic effects, which may hinder successful establishment (Sakai *et al.* 2001; Lee 2002; Cassey *et al.* 2004; Frankham 2005; Dlugosch and Parker 2008; Blackburn *et al.* 2009; Gaudeul *et al.* 2011). Unfortunately demographic data (such as information on the number of founders) during the early years of establishment is commonly unavailable or difficult to accurately derive from incomplete field records. This means that repeated 'top-up' introductions of individuals into already-founded populations may

occur undetected, which may alleviate inbreeding depression and low levels of diversity often experienced within small populations. Therefore determining the true severity and impact of a bottleneck effect on invasion success is important and challenging. The bottleneck effect within an invasive population is not only defined by the size of the founding population (the smaller the number of founders, the more severe the effect), but also by the rate of subsequent population growth. Indeed, the length of a bottleneck event also determines how much genetic diversity is lost (Frankham *et al.* 1993; Reed & Frankham 2003). On the other hand, low genetic divergence and inbreeding depression associated with bottleneck events can depress population growth rate. Consequently, it is difficult to tease apart whether variation in population growth rate is a cause or a consequence of bottleneck effects in invasive alien species.

Here, we examine the spatial genetic structure of a number of invasive populations of a globally invasive bird species, the ring-necked parakeet (*Psittacula krameri)*, which has a particularly well-documented invasion history across Europe. To determine the severity of the genetic bottleneck that each population has endured, we develop a novel approach that estimates the observed bottleneck effect from contemporary genetic data sampled from each population following its establishment. We then examine the degree to which those estimates align with the equivalent effects predicted from documented bottlenecks. Finally, we test a suite of climatic, demographic and genetic variables for their influence on population growth rates.

Listed as one of Europe's top 100 worst alien species **(**DAISIE European Invasive Alien Species Gateway, 2008), the ring-necked parakeet is one of the most widely introduced parrots in the world. Native to southern Asia and sub-Saharan Africa, they are established in more than 35 countries spread over five continents, while across Europe this species can be found in 65 independently established populations in cities (Lever 2005; Strubbe & Matthysen 2009a). Current breeding populations vary in size from tens to tens of thousands of individuals (Strubbe & Matthysen 2007), and many of them show a trajectory of explosive population growth and rapid spread (Butler *et al.* 2013). Ring-necked parakeets in Europe are a major cause of agro-economic and environmental concern, and across their native Indian range, are considered a severe crop pest, destroying maize and fruit crops (Ramzan & Toor 1973; Forshaw 2010, Ahmad *et al.* 2012). In Europe, evidence is accumulating that they compete with native bird species for nest cavities and have a detrimental impact upon the foraging success of native birds (Hernández-Brito *et al.* 2014; Strubbe & Matthysen 2007, 2009a; Peck *et al.* 2014). With the future prospect of a warmer European climate providing a more favourable environment for ring-necked parakeets, there is a need to understand what factors underpin their invasion success (Loss *et al.* 2011; Bellard *et al.* 2013**).** Interestingly, ring-necked parakeets in warmer regions of Europe experience higher reproductive success in comparison to those from colder

northern areas of Europe, supporting a climate-matching hypothesis which argues that climatic similarity between the native and invasive regions is important for invasion success (Shwartz *et al.* 2009).

The growth and spread of populations of these birds has been recorded across Europe since the first recorded breeding populations in the late 1960s, largely by citizen-scientists (Butler 2013). This documented invasion history, including detailed population growth profiles for some individual invasive populations across Europe, provides an ideal opportunity to quantify genetic bottleneck effects, and describe current genetic structure including patterns of diversity, population structure and gene flow. We use this study system to demonstrate how difficulties frequently encountered with poorly documented founder size can be overcome by utilising genetic data derived from samples from contemporary populations. Furthermore, we investigate what factors underpin rates of population growth in invasive ring-necked parakeets. Here we use mitochondrial (mtDNA) and microsatellite markers for 10 invasive populations established across Europe, and 98 museum samples spanning the entire geographic native distribution of southern Asia and sub-Saharan Africa, to determine factors important for invasion success by addressing the following questions: (i) do invasive populations in Europe have low levels of genetic diversity in comparison to the native range?, (ii) how accurate is demographic data for inferring severity of bottleneck effects in invasive populations and (iii) what factors influence rates of population growth?

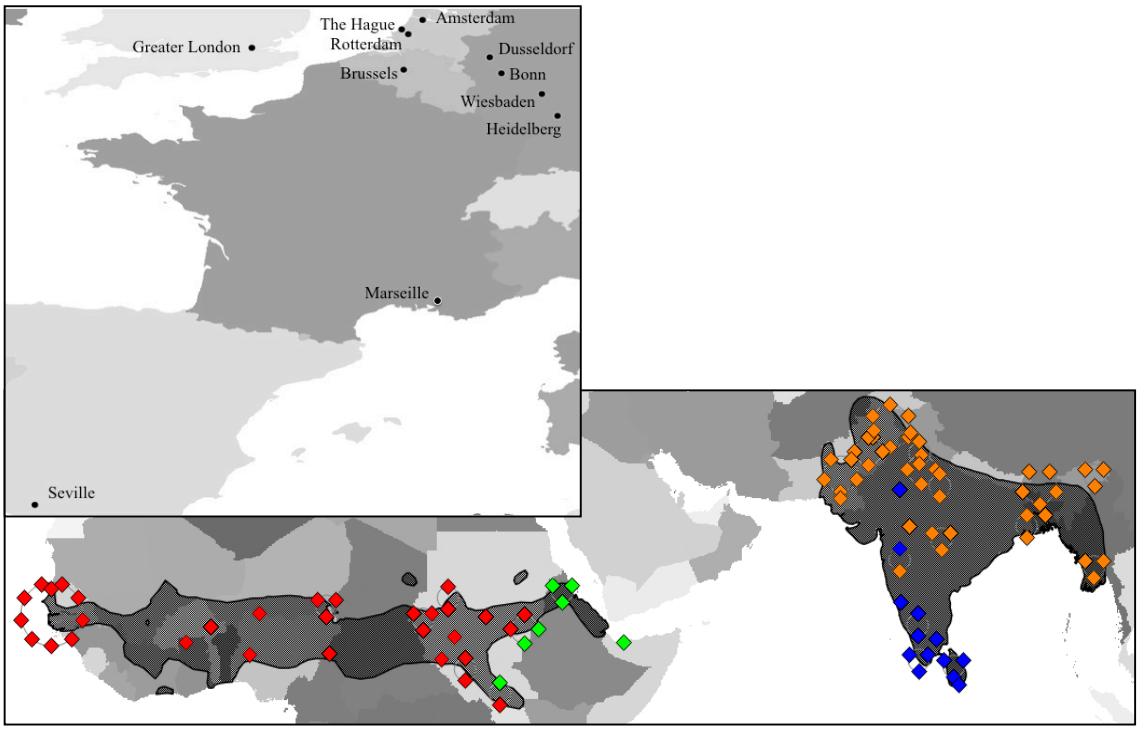


Fig. 4.1. Location of the ten invasive populations sampled for this study (with the additional Marseille population that has population growth data but no associated genetic data; upper panel). Native range distribution of ring-necked parakeets, *Psittacula krameri* (lower panel); boundaries outlined in black and encompassing areas across Sub-Saharan Africa and southern Asia (IUCN 2013); diamonds = location of individual samples from native range acquired from historical specimens collected from the native range; colours refer to the subspecies designation given on the label of each museum specimen; *P. k. borealis*, orange, *P. k. manillensis*, blue, *P. k. krameri*, red, *P. k. parvirostris*, green. Locations of all specimens are plotted; where necessary overlapping symbols have been displaced around the co-ordinate point to more clearly display the full sample size.

4.3 Methods

4.3.1 Sample collection

Volunteers and researchers collected naturally shed feathers from known roost sites or local parks and gardens from ten invasive European locations/populations: Brussels (*n*=69), Heidelberg (*n*=188), Wiesbaden (*n*=80), Bonn (*n*=29), Dusseldorf (*n*=9), Seville (*n*=59), Greater London (*n*=164), Rotterdam (*n*=75), The Hague (*n*=12), Amsterdam (*n*=19) (Table S4.4.1 in supplementary material). In addition, 98 toe-pad samples were collected from museum specimens at the Natural History Museum in Tring, UK (Table S4.4.2). Museum specimens were chosen on the basis of their geographic collection location to obtain a wide geographical representation of the species' native range, and for statistical purposes were assigned to four groups representative of each subspecies geographical range (Fig. 4.1).

4.3.2 DNA isolation, amplification and genotyping

Processing of the museum specimens, including DNA extraction and PCR amplification, was carried out in a separate laboratory dedicated to ancient DNA work to prevent contamination. All equipment and surfaces were sterilised before and after each use by irradiation from UV light and with 10% bleach. Negative controls (where DNA was replaced with ultrapure ddH_2O) were included during the DNA extraction and PCR process and finally, a selection of negative extractions PCRs were sequenced to ensure there was no contamination. DNA was extracted from both contemporary feather samples and historical toe-pad samples using a Bioline ISOLATE Genomic DNA extraction kit (Bioline, UK). Samples were suspended in 400 µl lysis buffer plus 25 µl proteinase K and incubated at 55^{o}C overnight (or until the material had completed digested). DNA was washed through a spin column and historical samples were suspended in 50 µl of elution buffer, while contemporary samples were suspended in 100 µl of elution buffer.

We used a suite of 21 microsatellite markers available for *Psittacula* parakeets that have demonstrated cross-species amplification in ring-necked parakeets (Raisin *et al.* 2009). PCR protocols were as per Raisin *et al.* (2009). Each PCR contained 3 µl ≈ 10 ng/µl of DNA that was air-dried, 1 µl of primer mix (fluorescently labelled forward) at 0.2 µM and 1 µl QIAGEN Multiplex PCR Master Mix (QIAGEN Inc). PCRs were conducted using differently fluorolabelled forward primers (HEX and 6-FAM; Raisin *et al.* 2009). PCR cycling conditions were 95 ^{o}C for 10 minutes followed by 35 cycles of 93^{o}C/30 secs, 52^{o}C/90 secs and 72^{o}C/90 secs, with a final incubation at 72 ^{o}C for 10 minutes. PCR was performed with a low annealing temperature (52°C) to increase the likelihood of amplification (Primmer *et al.* 2005). Individuals were sex-typed using the Z-002B marker (Dawson 2007) to check for loci that were sex-linked. PCR products were separated using an Applied Biosystems 3730 DNA Analyser with ROX^{TM} 500 as a size-standard. Alleles were identified and scored using GENEMAPPER 3.7 (Applied Biosystems, UK). Three loci were ambiguous to score and therefore were excluded from the analysis (*Peq06*, *Peq08* and *Peq09)*. Loci identified as sex-linked in echo parakeets *(Psittacula echo)* by Raisin *et al.* (2009) were confirmed to be sex-linked in ring-necked parakeets based on a complete lack of heterozygotes in females and therefore also excluded (*Peq16* and *Peq21*), while finally, one marker failed to amplify in all populations and was also excluded resulting in a final dataset comprising 15 loci. Due to the degraded nature and low volume of the DNA extracted from historical museum specimens only 10 of the 15 loci amplified (those with the largest allele size failed to amplify). Each sample was genotyped twice to ensure consistent scoring of alleles, and to identify potential genotype errors. Such degraded samples are known to be susceptible to genotyping errors due to allelic dropout (Taberlet *et al.* 1996, Hoffman & Amos 2005, Wandeler *et al.* 2007). Deviations from Hardy-Weinberg

equilibrium and null allele frequencies at each locus were estimated using CERVUS (Marshall *et al.* 1998). Sequential Bonferroni corrections for multiple tests were applied (Rice 1989). Evidence of genotyping errors (allelic drop-out and stuttering) was assessed using MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004).

Amplification from contemporary DNA samples was conducted for two mtDNA regions: control region using CR19f and CR19r, and cytochrome *b* using PKCBf and PKCBr (Table S4.4.3**)**. PCR cycling conditions were 94^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15 secs, 55^{o}C/15 secs and 72^{o}C/10 secs and a final elongation step of 72^{o}C for 10 minutes. For historical samples, amplification of control region and cytochrome *b* was conducted using a specifically designed suite of overlapping short fragment primers to provide sequence replication and ensure no contamination (150-250 bp; Table S4.4.3). Cycle parameters comprised an initial hot start of 95^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15 secs, 52^{o}C/15 secs and 72^{o}C/10 secs followed by a final 10 minutes 72^{o}C incubation period. All amplicons were examined by agarose gel electrophoresis to check amplification and no evidence of contamination in the extraction and PCR negative controls. Amplification volumes of 25 µl contained 2 µl of template DNA, 12.5 µl MyTaq HS Redmix (Bioline, UK), 0.5µl of each primer and 9.5 µl of dd$H_2$0. PCR product was purified and amplified using a 3730xl DNA analyser (Macrogen Inc.). Sequences were edited in 4Peaks (Griekspoor & Groothius 2005) and aligned in Clustal (Larkin *et al.* 2007). Manual edits were made in Jalview (Waterhouse *et al.* 2009). The two genes were concatenated using Sequence Matrix (Vaidya *et al.* 2011).

4.3.3 Genetic diversity, structure and gene flow

Mean number of effective alleles (Na), observed (H_O) and expected (H_E) heterozygosity for each sample location was estimated with GENALEX 6.41 (Peakall & Smouse 2006). Allelic richness (Ar) and private allelic richness (Par) were calculated in HP-RARE, which uses rarefaction to correct for unequal sample sizes (Kalinowski 2005). Haplotype diversity (H) was calculated in DNAsp v5 (Librado & Rozas 2009). Kruskal-wallis tests were applied to identify significant differences in genetic diversity between invasive locations. Inbreeding coefficients (F_{IS}) and pairwise differentiation (F_{ST}) with statistical significance were calculated in FSTAT. A second genetic differentiation statistic, D_{jost} (Jost 2008), based on the number of effective alleles, was calculated using the package DEMEtics in R v3.0.2 (Gerlach *et al.* 2010), which provides complementary information (Whitlock 2011, Meirmans & Hedrick 2011, Edelaar & Björklund 2011, Verity & Nichols 2014). Isolation by distance was assessed using a Mantel test by testing the correlation between genetic (F_{ST}) and linear geographical distances (logged) in GENALEX 6.5 (Peakall & Smouse 2012). A Bayesian clustering approach was used to identify genetic structure of invasive ring-necked parakeets in STRUCTURE v2.3.4 (Pritchard *et al.*

2000). To determine the most likely number of clusters (K), ten repeated runs were conducted with K ranging from one to 10 for 500,000 MCMC iterations with a burn-in of 100,000, under the admixture model and specifying correlated allele frequencies (Falush *et al.* 2003). The assignment values, log likelihood scores and ΔK were evaluated using STRUCTURE HARVESTER (Earl & vonHolt 2011) to infer the number of genetic clusters. Migration rates between invasive European populations were estimated using Bayesian inference in BAYESASS v3.0.3 (Wilson & Rannala 2003). A total of $5x10^6$ MCMC iterations were performed, with sampling every 1000^{th} iteration and delta values were adjusted following BAYESASS recommendations. We calculated 95% credible intervals associated with each migration rate and classified rates as negligible if the credible intervals included zero.

4.3.4 Statistical modelling of observed bottleneck effects

The model behind the analysis of the genetic data to determine the bottleneck severity for each population is presented in Fig. 4.2. The calculation starts with vector ***S***, which records the observed count of each allele in a sample. These observations were modelled as descending from N_a ancestral lineages in the founder population. The probability of the genotypes in the sample is given by

$$\mathrm{P}(S \mid \alpha) = \frac{\prod_{\mathrm{i}} \binom{S_i - 1}{\alpha_i - 1}}{\binom{\sum_i (S_i - 1)}{\sum_i (\alpha_i - 1)}} \quad (1);$$

in which the genotypes of these N_a ancestral lineages are represented by a vector α. Each element, α_i, is the number of the i^{th} allele, which has left descendants in the present-day sample. In equation (1) the notation $\binom{n}{k}$ represents the binomial coefficient: the number of ways of choosing k objects from a sample of size n. Note that Saccheri *et al.* (1999) and Leblois & Slatkin (2007) have outlined the logic for deriving (1) but both give a different denominator to (1); a difference which does not have a substantial effect on the results for large sample sizes, but results in probabilities that do not sum to one over the allowed combinations in α (specified below). We do not know the values of α, so for a given number of ancestral lineages, N_a, the probability of the genotypes must be calculated by summing over A, the set of possible ancestral combinations of alleles in α. The possible combinations are constrained to sum to N_a, must contain at least one copy of each allele, and cannot exceed the values in S – because each ancestor must have at least one descendant (by definition of being an ancestor). Hence $\Sigma_i\, \alpha_i = N_a$, $0 < \alpha_i \leq S_i$. It is this last inequality that leads to the difference of the denominator in (1) from

the previously published values. Having defined *A,* the likelihood of the genotypes in the sample can be calculated as

$$\mathrm{P}(S \mid N_a, p, F_{ST}) = \sum_{\alpha \in A} \mathrm{P}(S \mid \alpha)\mathrm{P}(\alpha \mid p, F_{ST}) \quad (2).$$

Equation (2) makes explicit that the likelihood of the sample depends on the vector of average allele frequencies p in the European pet trade, and F_{ST} – the population genetics parameter that quantifies the variation of the allele frequencies in the local captive populations from p. The term $\mathrm{P}(\alpha|p,F_{ST})$ is the probability of the α allele counts in the ancestors. Balding & Nichols (1995) showed that this probability could be obtained from the multinomial Dirichlet distribution for a wide range of assumptions. It can be calculated as

$$\mathrm{P}(\alpha \mid p, F_{ST}) = \frac{\Gamma(\lambda)\Gamma(N_a + 1)\prod_i \Gamma(\lambda p_i + \alpha_i)}{\Gamma(N_a + \lambda)\prod_i \Gamma(\alpha_i + 1)\prod_i \Gamma(\lambda p_i)} \quad (3),$$

where $\lambda = (1/F_{ST})-1$, and $\Gamma(\ )$ is the gamma function. For each locus, the value of equation (2) was calculated, N_a in the range $1 \ldots N_s$, where N_s is the sample size ($\Sigma_i S_i$), and for a range of F_{ST} values, the results being stored in a matrix, L_l, as an intermediate step in the analysis. The chosen F_{ST} values were ten equally spaced values across the prior distribution on F_{ST}, which ranged from 0.001 to 0.1. The value of p was obtained from the pooled frequencies from all populations apart from the focal population – the posterior mode assuming a uniform Dirichlet prior, calculated as $(x_a+1)/(N_t+l_t)$; where x_a is the allele count, N_t the total count and l_t the total number of alleles at the locus. For large values of N_s the set A is too large (at intermediate values of N_a) to allow the calculation of (2) for all possible values. In those cases, 200 realisations of α were drawn with probability proportional to $\mathrm{P}(\alpha|p,F_{ST})$ and the value of (2) estimated as the average value of (1) for these α. This procedure gave smooth likelihood curves corresponding to longer runs of the exact calculation when tested on a subset of the data (not shown).

The next step in the calculation requires the function $Dn(\ )$, which returns the expected distribution of N_a values, for a given bottleneck. This distribution has previously been calculated by Leblois & Slatkin (2007) in a continuous time model, as a function of a bottleneck intensity parameter τ (their equation 2). However for the larger sample sizes, the straightforward coding of their equation led to arithmetic overflow errors in our implementation. Instead we obtained a numerical estimate of this distribution, $Dn(n_b)$, the probability density for $N_a = 1 \ldots N_s$, given a bottleneck intensity parameter, n_b.

The numerical estimate of $Dn(n_b)$ the probability density of N_a, as function of the bottleneck intensity parameter n_b, was obtained by a discrete-time calculation stepping over an arbitrary number of time slices (100) from the sampled generation back to the founder generation. At each step the probability of coalescence is determined by a notional populations size n_b. As this is a discrete-time model we must allow for multiple coalescent events in any one time-slice. The probability of transitioning from n descendant lineages to j ancestral lineages is calculated by observing that a Stirling numbers of the second kind $S(n,k)$ is, by definition, the number of ways of allocating n objects to k unlabeled subsets; in our case, n lineages into k ancestral lineages. In a population of size n_b there are $\binom{N_b}{j}$ ways of choosing j ancestors from n_b possibilities, and $j!$ ways of allocating labels. The total number of ways of allocating the n descendants to labelled ancestors is $(n_b)^n$. Hence the transition probability from $n \rightarrow j$ is

$$\mathrm{P(n \rightarrow j)} = \frac{S(n,j)\, j! \binom{n_b}{j}}{(n_b)^n} \quad (4).$$

These values were entered into a matrix, T, in which $t[i,j]$ gives the probabilty of transitioning from n-i to n-j lineages in one time slice. The vector Dn^t contains the probability density of 1… N_s ancestors at time-slice t. As the at sample at the time of sampling is known to have contained N_s lineages, Dn^1 has value one in element Dn^1_{Ns} and zero elsewhere. The probability densities are then given by $Dn^{t+1}=T.Dn^t$. The function $Dn(n_b)$ returns Dn^{100}.

The value of n_b is inversely related to the expected loss in heterozygosity during the bottleneck $(H_s/H_f)=(1\text{-}1/2n_b)^{100}$, where H_s is the heterozygosity in the sampled generation and H_f in the founding generation. Recall that the values of $P(S|N_a,p,F_{ST})$ have been stored in a matrix L_l. In the following we suppress the conditioning on p, since it is estimated from the dataset excluding the focal population (above). The matrix L_l is organised so the product $L_l Dn(n_b)$ returns a vector in which the ith element gives $P(S|F_{ST}= F_{ST(i)},n_b)$:

$$\begin{bmatrix} \mathrm{P}(S \mid F_{ST} = F_{ST(1)}, N_a = 1) & \cdots & \cdots & \mathrm{P}(S \mid F_{ST} = F_{ST(1)}, N_a = N_s) \\ \vdots & \ddots & & \vdots \\ \vdots & & \ddots & \vdots \\ \mathrm{P}(S \mid F_{ST} = F_{ST(10)}, N_a = 1) & \cdots & \cdots & \mathrm{P}(S \mid F_{ST} = F_{ST(10)}, N_a = N_s) \end{bmatrix} \begin{pmatrix} \mathrm{P}(N_a = 1 \mid n_b) \\ \vdots \\ \vdots \\ \mathrm{P}(N_a = N_s \mid n_b) \end{pmatrix},$$

since $P(\boldsymbol{S}|F_{ST}= F_{ST(i)},n_b)=\Sigma_j P(\boldsymbol{S}|F_{ST}= F_{ST(i)},N_a=j)\, P(N_a=j \mid n_b)$.

The total likelihood conditional on the bottleneck intensity was then obtained as

$$\mathrm{P}(S \mid n_b) = 1' \prod_l L_l Dn(n_b).$$

The product across loci combines the likelihoods in each row, corresponding to the same F_{ST} value for each locus. The notation 1' indicates summation of the elements in the vector. This amounts to summation across the values obtained for different F_{ST} values, i.e. integration across the prior distribution on F_{ST}. The maximum likelihood value and support limits for n_b (the bottleneck intensity parameter) were obtained using the mle function in R**.**

To examine the accuracy of inferring genetic bottleneck effects (ie, loss of heterozygosity) from demographic data (population count records), the expected bottleneck effect (from demographic data) and observed bottleneck effect (from genetic modelling detailed above) was calculated for each invasive population using Wright´s inbreeding coefficient (Wright 1922).

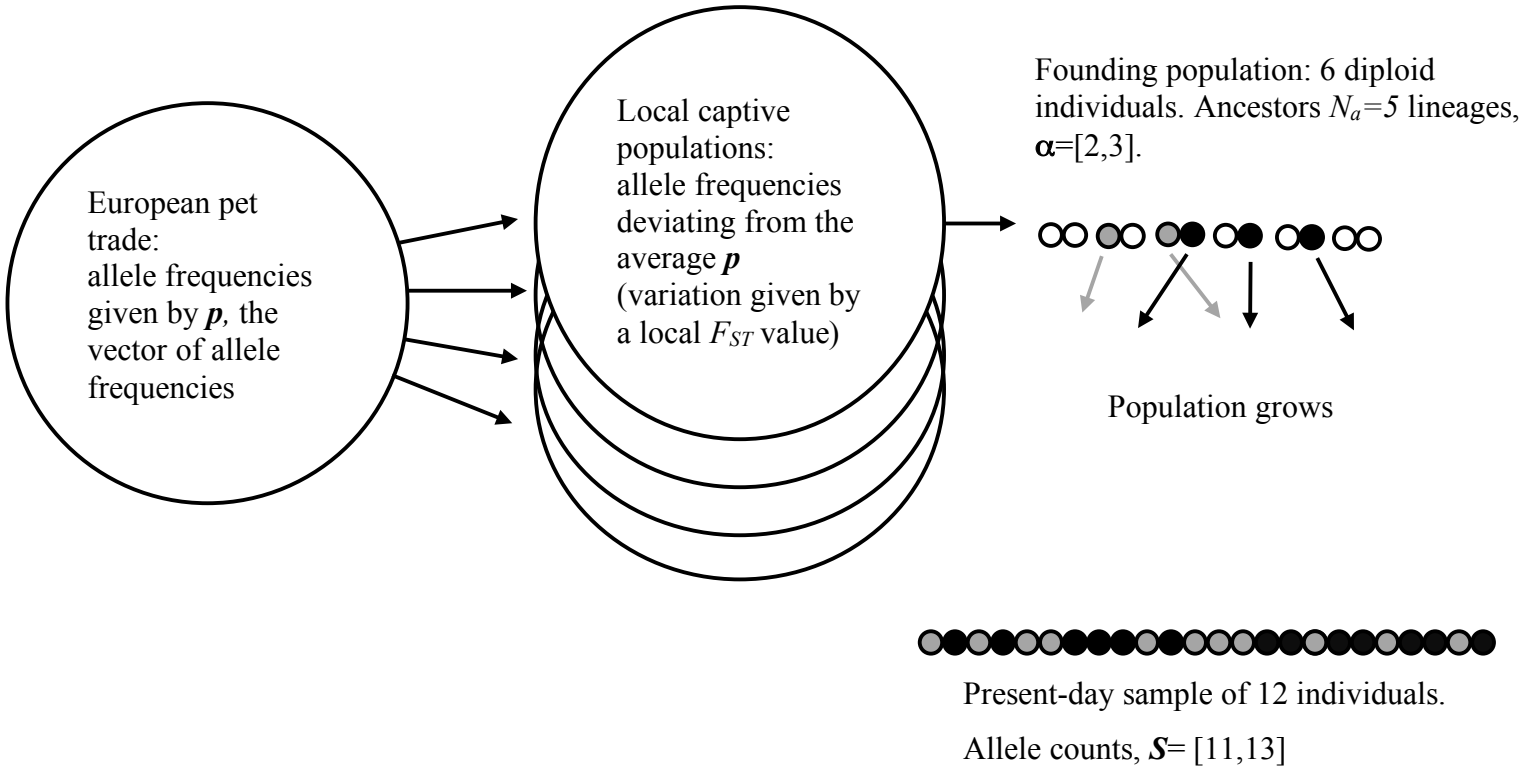


Fig. 4.2. The model used in the genetic analysis of the population bottleneck at each location. The present-day population is assumed to have grown from a founder population derived from the local captive population. The allele frequencies of that captive population are assumed to deviate from the average throughout Europe, and the magnitude of those deviations is quantified by a local F_{ST} value (common to all loci). A number of individuals (here: 6) have founded a population, but not all their alleles have left descendants in the current population: only a subset comprising N_a of the genetic lineages (here: 5 alleles coloured grey and black) in the founders with allelic counts $\boldsymbol{\alpha}$, have left descendants in the present-day sample, which has allelic counts $\boldsymbol{S}$. The white-filled circles represent lineages that coincidentally do not have descendants. The value of N_a is expected to differ among loci, and the distribution of these N_a values depends on the bottleneck intensity due to the change in population size from foundation to the present day.

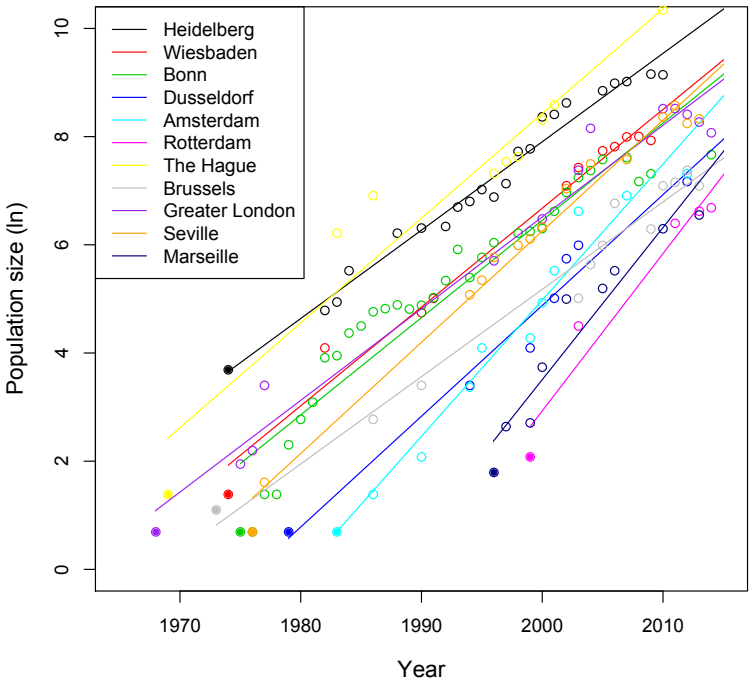
4.3.5 Drivers of population growth

Population count data was obtained via personal communications or published sources (data available on request) for each of the 10 invasive populations. Additional growth data was obtained for a population in Marseille, France, although no genetic data was available for this population; the growth data was included in our analysis to provide an extra data point representing a different region in Europe (Fig. 4.3). Population growth rates were estimated from the slope of a regression line fitted to the observed count data (logged) over time since introduction. We used generalised linear models (GLMs) implemented using Rcommander in R v3.02 (Fox 2005) to examine variables that may explain population growth.

Strubbe & Matthysen (2009) showed that, in Europe, ring-necked parakeet establishment success (i.e. whether a parakeet introduction resulted in a self-sustaining population or not) was influenced by climatic and anthropogenic factors. Warmer temperatures and a higher human population density correlated positively with establishment success. Recently, Strubbe *et al.* (2015) found that Species Distribution Models (SDM, which are statistical techniques that link the occurrence of species to aspects of the environment; Araujo & Peterson 2012) based on climatic and anthropogenic factors recorded in the native range yield accurate predictions of invasion risk across Europe (i.e. habitat suitability, varying between 0 and 100), especially when accounting for the differing niche requirements of (mtDNA based) phylogeographic lineages (hereafter 'niche structure'). Based on these findings, we constructed GLMs to assess whether such climatic and anthropogenic factors also affect the population growth rates of successfully established populations. Population growth rates were tested against a number of 'raw' climatic and anthropogenic data derived for European cities (i.e. mean temperature of the coldest and warmest month, human population density and total human population), and a number of SDM-predictions of habitat suitability derived from Strubbe *et al.* (2015). SDM predictions include predictions based on climate only, climate + anthropogenic factors, and with and without phylogeographic structure. Climatic variables considered by Strubbe *et al.* (2015) are annual mean temperature (bio_1), mean temperature of the warmest month (t_max), mean temperature of the coldest month (t_min), temperature seasonality (bio_4), annual precipitation (bio_12), precipitation of the wettest month (bio_13), precipitation of the driest month (bio_14) and precipitation seasonality (bio_15). Climatic variables were derived from the WorldClim database (Hijmans *et al.* 2005) and represent mean values over the 1961-1990 period. As anthropogenic variable, the 'human footprint', which is a quantitative measure of human alteration of terrestrial environments based on human population size, land use and infrastructure was used (Sanderson *et al.* 2002). For further explanations of each variable see Table S4.4)

Climatic and demographic explanatory variables were log-transformed before analysis to meet assumptions of normality. The initial set of explanatory variables was checked for collinearity using correlation matrices. Where correlation coefficients >±0.7 were observed between two variables, one was removed from the final analysis, as such collinearity is know to confound GLM analyses (Zuur *et al.* 2010). The final GLM also excluded the genetic diversity variables, as relationships are likely to be an effect of the bottleneck rather than a cause of population growth. The final GLM was constructed with all remaining explanatory variables fitted, and utilising a stepwise variable selection procedure by progressively eliminating non-significant variables, until only the significant variables were retained under the best fitting model (full details of which variables were included and excluded in the final GLM see Table S4.4). Such a stepwise procedure is applied to estimate the minimal adequate model that explains the highest proportion of variation with the smallest number of variables (Barni *et al.* 2012)

a)



b)

Pop	*GrowthR*	*SE*
He	0.18	0.01
Wi	0.18	0.01
Bo	0.2	0.02
Du	0.25	0.02
Am	0.205	0.007
Ro	0.16	0.008
Ha	0.17	0.009
Br	0.16	0.005
GL	0.2	0.02
Se	0.29	0.04
Ma	0.28	0.005

Fig. 4.3. Population growth rates for 11 invasive populations of ring-necked parakeet populations in Europe. (a) Logged population growth data; year of founding is indicated where regression lines end dots are filled, (b) population growth rates (GrowthR) calculated from the slope of the fitted regression.

4.4 Results

A total of 700 individuals were genotyped from 10 locations across Europe. After removing sex-linked loci and one poorly amplifying locus the final dataset comprised 15 polymorphic loci and 1500 bp of mtDNA sequence data comprising control region (735 bp) and cytochrome *b* (765 bp). No linkage disequilibrium was detected between pairs of loci following Bonferroni corrections (Rice 1989). Deviations from Hardy Weinberg Equilibrium were detected at three of the fifteen loci across all populations combined (*Peq*02, *Peq*12 and *Peq*15). Estimated frequencies of null alleles ranged between 0.003 - 0.084% per locus. A total of 218 alleles were detected with a mean of 14.53 alleles per locus. Ninety-two of the 98 museum specimens were successfully genotyped representing the native range in Southern Asia and sub-Saharan Africa. For these specimens, 10 of the 15 microsatellite loci were genotyped (larger sized alleles failed to amplify), along with a total of 868 bp mtDNA sequence data for each specimen comprising 522 bp control region and 346 bp cytochrome *b*.

4.4.1 Genetic diversity, structure and gene flow

Average microsatellite heterozygosity ranged between 0.69 (The Hague) and 0.82 (Greater London). Allelic richness ranged between 4.44 at The Hague and 5.36 in Greater London, while haplotype diversity ranged from 0.51 for Wiesbaden to 0.79 for Dusseldorf (Table 4.1). No significant differences were found between invasive populations for mean heterozygosity ($k^2_{(7)}$=6.27, p=0.51), allelic richness ($k^2_{(7)}$=8.89, p=0.26) or haplotype diversity ($k^2_{(8)}$=8.51, p=0.38). Levels of heterozygosity in the native range (0.60 in East Africa and 0.66-0.68 across the Asian native range) were similar to those in Europe, except for western Africa where heterozygosity was lower (0.45) (Fig. 4.4a). Similar levels of allelic richness were observed between native and invasive populations; however, Heidelberg, Wiesbaden, Bonn and Brussels showed slightly higher levels of allelic richness in comparison to the native ranges (Fig. 4.4b). Haplotype diversity in Europe ranged from 0.51 to 0.79, with higher levels observed in the native range; Asia = 0.80 and 0.91 and Africa = 0.91 and 0.95 (Fig. 4.4c). Estimated levels of inbreeding ranged from -0.01 to 0.09 within European populations (Table 4.1). Genetic differentiation between pairs of populations was always significant when using D_{jost}, while for F_{ST} most comparisons were significant except for a number involving The Hague and Dusseldorf (the two populations with the smallest sample sizes). Heidelberg showed the highest levels of differentiation, for both F_{ST} (0.038 - 0.096) and D_{jost} (0.017 – 0.327), while other populations exhibited lower levels of differentiation ranging between 0.020 – 0.070 (F_{ST}) and 0.111 – 0.276 (D_{jost}; Table 4.2).

Table 4.1. Sample locations and mean measures of genetic diversity for invasive ring-necked parakeet populations in Europe; sample size (*n*), number of effective alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), allelic richness (Ar), private allelic richness (Par), inbreeding coefficient (Fis), and haplotype diversity (H).

Location	ID	*n*	Na	Ho	He	Ar	Par	Fis	H
Heidelberg	He	188	9.33	0.74	0.76	4.65	0.25	0.03	0.64
Wiesbaden	Wi	80	9.46	0.80	0.80	5.11	0.25	0.00	0.51
Bonn	Bo	29	8.20	0.78	0.77	4.87	0.18	0.02	0.75
Dusseldorf	Du	9	6.00	0.73	0.74	5.02	0.24	0.09	0.79
Amsterdam	Am	19	6.93	0.76	0.78	4.87	0.18	0.05	0.78
Rotterdam	Ro	75	9.67	0.74	0.78	5.05	0.14	0.07	0.71
The Hague	Ha	12	5.80	0.70	0.69	4.44	0.14	0.04	0.69
Brussels	Br	69	10.27	0.75	0.79	5.02	0.17	0.05	0.66
Greater London	GL	164	11.80	0.75	0.82	5.36	0.40	0.09	0.71
Seville	Se	59	10.07	0.81	0.80	5.22	0.20	-0.01	0.55

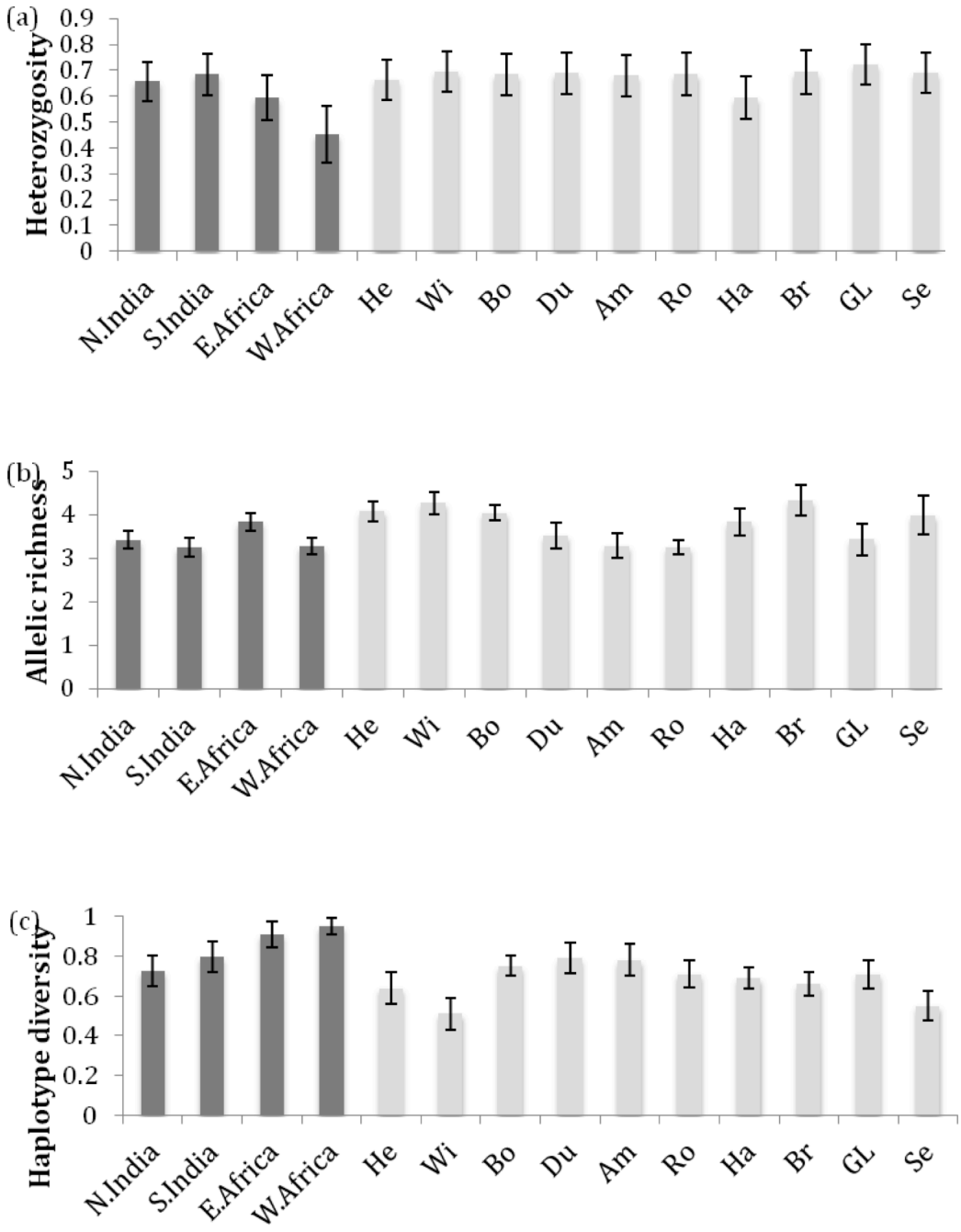


Fig. 4.4. Comparison of measures of genetic diversity between the native source populations (delineated here into the main geographical distributions of each subspecies of ring-necked parakeet, shaded dark grey, as seen in Fig. 4.1) and the invasive populations of ring-necked parakeets; (a) heterozygosity, (b) allelic richness and (c) haplotype diversity. Population abbreviations are detailed in Table 4.1

Table 4.2. Genetic differentiation between invasive populations of ring-necked parakeet. Pairwise F_{ST} (below the diagonal) and pairwise D_{jost} values (above the diagonal), values in bold are significantly different from zero at $p < 0.01$ (values significant at $p < 0.05$ are also highlighted *).

	He	Wi	Bo	Du	Am	Ro	Ha	Br	GL	Se
He	-	**0.231**	**0.199**	**0.234**	**0.219**	**0.226**	**0.327**	**0.179**	**0.168**	**0.213**
Wi	**0.058**	-	**0.145**	**0.143**	**0.220**	**0.177**	**0.270**	**0.111**	**0.161**	**0.162**
Bo	**0.055**	**0.055**	-	**0.156**	**0.197**	**0.149**	**0.234**	**0.119**	**0.165**	**0.156**
Du	0.062	0.031	0.040	-	**0.198**	**0.149**	**0.210**	**0.113**	**0.131**	**0.160**
Am	**0.055**	**0.047**	**0.044**	0.048	-	**0.221**	**0.296**	**0.177**	**0.196**	**0.193**
Ro	**0.057**	**0.036**	**0.033**	0.033	**0.052**	-	**0.123**	**0.195**	**0.276**	**0.232**
Ha	**0.096**	0.070	0.070	0.054	0.084	0.039	-	**0.195**	**0.276**	**0.232**
Br	**0.044**	**0.025**	**0.025**	0.024	**0.038**	**0.020**	**0.057**	-	**0.144**	**0.118**
GL	**0.038**	**0.033**	**0.034**	**0.029**	**0.038**	**0.033**	**0.068**	**0.027**	-	**0.143**
Se	**0.054**	**0.034**	**0.034**	0.038	**0.044**	**0.025**	**0.059***	**0.024**	**0.027**	-

The log likelihood of the STRUCTURE simulations increases sharply to K=2 then shows a more gradual increase (Fig. S4.1a). Delta K clustered the European invasive populations predominantly into two groups (K=2), but also indicates a peak at K=3, 5 and 8 (Fig. S4.1b). For K=2, almost all populations demonstrated a higher assignment probability to group one while Heidelberg clustered predominately with group two. For K=3, Greater London is also identified as a separate cluster, with additional clusters identified in Wiesbaden, Seville, Rotterdam and the Hague for K=5 and K=8. After K=5 no additional clusters are identified (Fig. 4.5).

We found no significant signal of isolation by distance based on a correlation between genetic differentiation (F_{ST}) and geographic distance (r=-0.198 p=0.28), nor did we detect significant evidence for dispersal between populations, as all estimations of migration rates contained zero (Table 4.3).

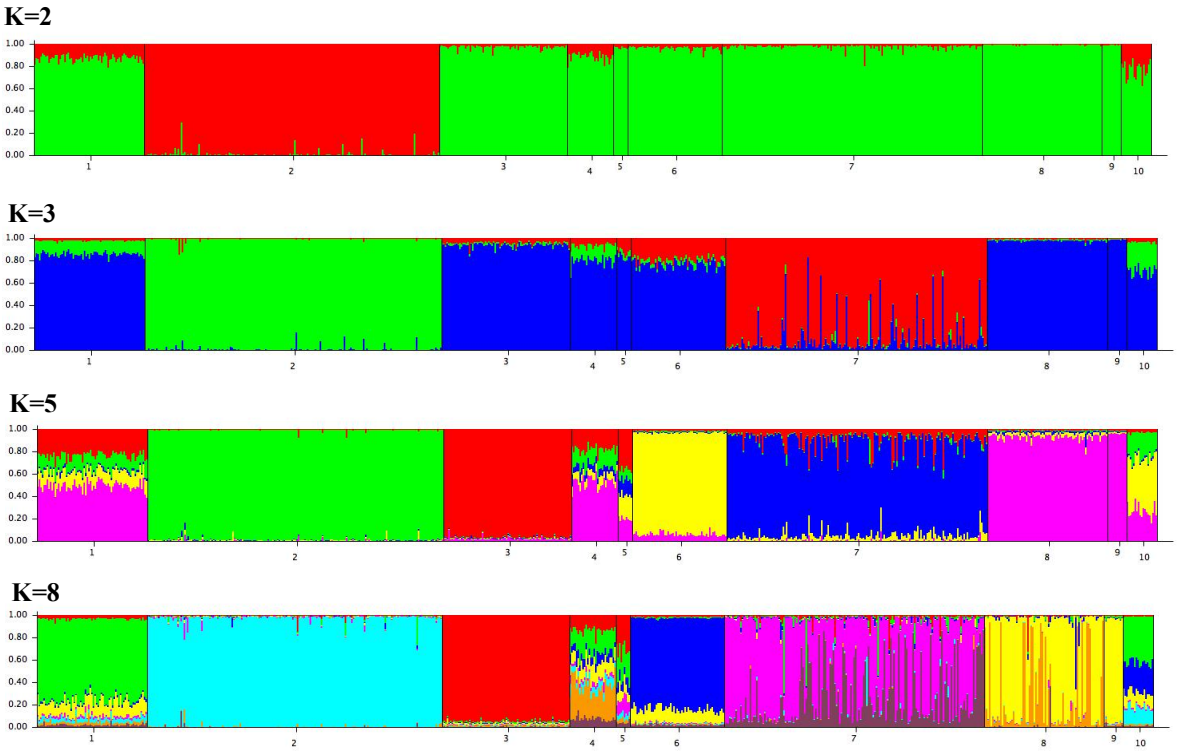


Fig. 4.5. Analysis of genetic structure for ten invasive European ring-necked parakeet populations reveals clusters K=2,3,5 & 8 based on genotypes of 700 individuals at 15 microsatellite loci. Each individual parakeet is represented by a vertical line coloured to indicate the probability of its cluster assignment, and individuals are grouped into each of the ten European populations; 1=Brussels, 2=Heidelberg, 3=Wiesbaden, 4=Bonn, 5=Dusseldorf, 6=Seville, 7=Greater London, 8=Rotterdam, 9=The Hague, 10=Amsterdam.

Table 4.3. BAYESASS estimated migration rates (±95% credible values) among the ten invasive populations of ring-necked parakeet. Diagonal values in italics represent the proportions of non-migrant ring-necked parakeets. All values include zero indicating no significant levels of gene flow between any populations.

	To:									
From:	He	Wi	Bo	Du	Am	Ro	Ha	Br	GL	Se
He	*-0.06–1.93*	-0.01–0.00	-0.01–0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.00-0.00	-0.04-1.81	-0.01-0.00	-0.01-0.00
Wi	-0.01-0.00	*-0.04-1.84*	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.03-0.02	-0.01-0.00	-0.01-0.00
Bo	-0.02–0.00	-0.02–0.01	*-0.02-1.33*	-0.01-0.00	-0.02-0.00	-0.03-0.00	-0.01-0.00	-0.05-0.43	-0.02-0.00	-0.02-0.00
Du	-0.03–0.00	-0.06–0.01	-0.03-0.00	-0.04-1.34	-0.03-0.00	-0.05-0.01	-0.03-0.00	-0.10-0.19	-0.06-0.00	-0.03-0.00
Am	-0.04–0.00	-0.02–0.00	-0.02-0.00	-0.02-0.00	*-0.03-1.34*	-0.03-0.00	-0.02-0.00	-0.07-0.36	-0.02-0.00	-0.02-0.00
Ro	-0.01–0.00	-0.01–0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	*-0.10-1.62*	-0.01-0.00	-0.01-0.09	-0.02-0.00	-0.01-0.00
Ha	-0.02–0.00	-0.02–0.00	-0.02-0.00	-0.02-0.00	-0.02-0.00	-0.07-0.36	*-0.03-1.34*	-0.03-0.00	-0.02-0.00	-0.02-0.00
Br	-0.01–0.00	-0.02–0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.03-0.01	-0.01-0.00	*-0.04-1.81*	-0.02-0.00	-0.01-0.00
GL	-0.01–0.01	-0.02–0.01	-0.01-0.00	-0.00-0.00	-0.00-0.00	-0.01-0.00	-0.00-0.00	-0.03-0.08	*-0.03-1.79*	-0.01-0.00
Se	-0.02–0.00	-0.01–0.00	-0.01-0.00	-0.01-0.00	-0.01-0.00	-0.07-0.00	-0.01-0.00	-0.09-0.42	-0.01-0.00	*-0.03-1.33*

4.4.2 Bottleneck effects in invasive European populations

The demographic records suggested that all populations of ring-necked parakeets in Europe had founder sizes of between n=2–4 individuals (Wiesbaden, Bonn, Dusseldorf, Seville, The Hague and Amsterdam, n=2; Rotterdam, n=3; Heidelberg and Greater London, n=4), with the exception of Brussels which has a recorded founder size of n=40 individuals (a deliberate release of 40 parakeets by the Meli Zoo in Brussels is considered the founding population; Strubbe & Matthysen 2009b). The expected bottleneck effects were compared with the estimates from the genetic data (Fig 4.6). While expected and observed bottleneck effects were correlated (r = 0.436, $p < 0.05$), observed bottleneck effects were milder than expected (they fall below the 1:1 line) in all populations except Brussels, suggesting that founder effects based on demographic data were underestimates in 90% of the cases.

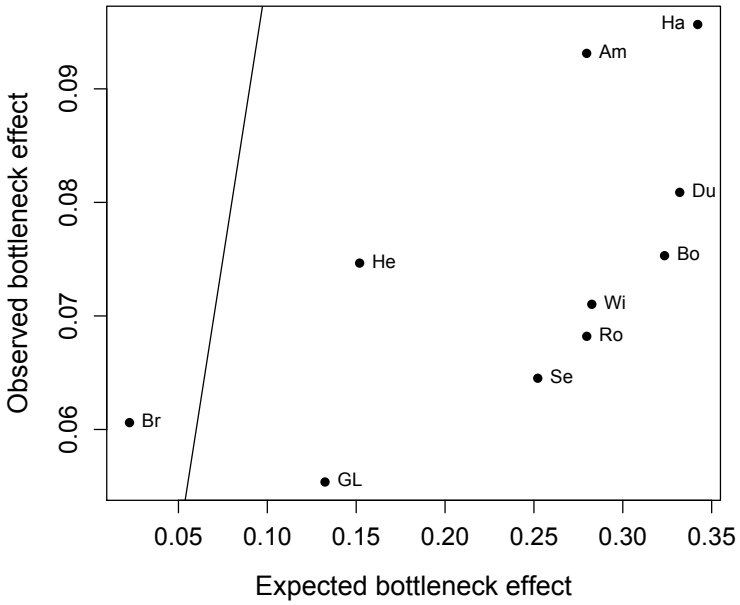


Fig. 4.6. Relationship between observed bottleneck effect (derived from genotype data) and expected bottleneck effect (estimated from demographic records) in invasive populations of ring-necked parakeet. The solid line indicates y=x. The demographically-derived expected bottleneck effect is an overestimate in all populations (founder number, n=2/3/4) apart from Brussels (founder number, n=40) when compared to the genetically-derived observed bottleneck effect.

4.4.3 Influences on population growth rates

In general population growth rates were highest in more southerly European populations, Seville (29% per year) and Marseille (28% per year), compared to the lowest rates observed in Brussels and Rotterdam (16% per year; Fig. 4.3). Population growth rates were significantly correlated with predicted habitat suitability in the invasive range based on climate and niche structure in the native range (Table 4.4. & Fig. 4.7.). No relationships were found between population growth rate and demographic data on human population size. Although no relationships were observed in our initial analysis between population growth rates, bottleneck effect or genetic variables, these explanatory variables were excluded from the final GLM, as it is impossible to determine whether they are a cause or an effect of population growth.

Table 4.4. Final GLM for factors influencing population growth rates (GrowthR) of invasive populations of ring-necked parakeets. Population growth rates are significantly correlated with predicted habitat suitability in the invasive range based on climate and niche structure in the native range (Clim_n).

Response variable: *GrowthR*	Estimate ± SE	Test	*p*
(intercept)	0.627 ± 0.212	2.948	0.021
Clim_n	**0.085 ± 0.033**	**2.562**	**0.037**
Clim_ghf_n	0.019 ± 0.053	0.374	0.719
Clim	0.016 ± 0.90	0.176	0.865

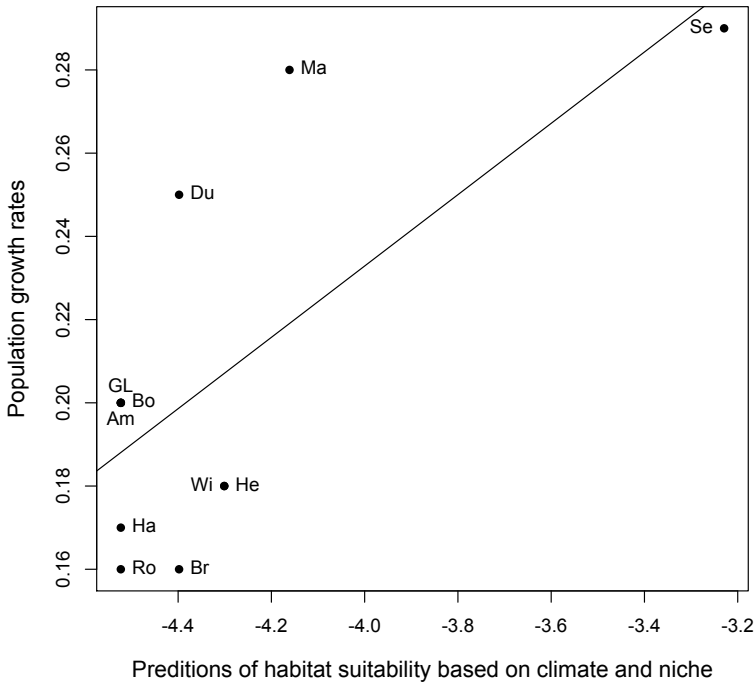


Fig. 4.7. Positive relationship between population growth rates (GrowthR) in invasive populations of ring-necked parakeets, and predicted habitat suitability in the invasive ranges based on climate and niche structure in the native range (clim_n).

4.5 Discussion

Our extensive sampling of ring-necked parakeets from their native and invasive geographical ranges has enabled an in-depth examination of the importance of observed bottleneck effects, genetic structure and growth rates as underlying driving forces of invasion success. Our molecular findings for this highly successful global avian invader provide independent support for the suspected influence of multiple 'top-up' introductions in areas that share climatic similarities with the species' native range, in underpinning growth and invasion success of invasive alien species.

4.5.1 Milder than expected bottleneck effects

The number of founders that contributes to the contemporary genetic composition of an invasive population is commonly unknown or at best is limited to inference from historical demographic data (such as anecdotal observations or routine population surveys). Furthermore, invasive alien species are frequently under-reported or not recorded as part of native wildlife surveys, and data is often sporadic with regards to a recently established population, meaning

initial founder sizes and associated bottleneck effects are difficult to confirm (Strubbe & Matthysen 2009b). Our genetic estimates of the bottleneck effects clearly illustrate that demographic data would overestimate the severity of bottleneck effects endured by invasive alien species. This discrepancy could be due to under-reporting of demographic data, but also by 'top-up' introductions, which are perhaps more likely and would explain why European parakeet populations, and perhaps invasive species in general, avoid the detrimental genetic impacts associated with the expected severe bottlenecks.

Continuing introductions into the wild would be consistent with the unregulated nature of the captive population. Ring-necked parakeets are popular as pets owing to their variety of colour mutations (Feare 1996; Pithon & Dytham 2001; Forshaw 2006). Prior to the European Union introduction of a ban on the importation of wild birds in 2007 (Regulation (EU) No.318/2007), large numbers were transported into Europe from Asia and Africa (approximately 158,453 parakeets; CITES 2014; Jackson *et al.* submitted). In addition to trade from the native range, large numbers of ring-necked parakeets have been bred and cross-bred under captive conditions within the invasive range (Morgan 1993). Indeed, data from the UK Parrot Society indicates that at least 20,105 individuals were bred in captivity in the UK between 1990 and 2004 (Fletcher & Askew 2010). This is a substantial population, kept by a diverse range of owners and some of these parakeets may have escaped or been purposefully-released into existing invasive populations (Pithon & Dytham 2001; Fletcher & Askew 2010).

Such continual releases into wild populations as suggested by our observation of milder than expected bottleneck effects, is a process which we suspect plays a highly influential role in the invasion success of ring-necked parakeets (Cassey *et al.* 2004; Lockwood *et al.* 2005; Dlugosch & Parker 2008; Signorile *et al.* 2014). The repetitive genetic contributions from captive birds into the wild populations suggests that mitigation against further growth and expansion of ring-necked parakeet populations may be assisted by changes in legislation that prohibit private breeding or ownership of pet parakeets, as implemented in Spain, where it is now illegal to keep ring-necked parakeets as pets (Regulation: Real Decreto 630/2013).

4.5.2 Genetic structure of invasive populations of ring-necked parakeets

Classic invasion theory suggests that introduced populations should be genetically impoverished as a result of a founding bottleneck event (Allendorf & Lundquist 2003; Frankham 2005). Such small, genetically impoverished populations with reduced evolutionary potential and fitness should therefore struggle to establish and become a viable or successful invasive population (Sakai *et al.* 2001). Under such a scenario, invasive populations should experience lower levels of genetic diversity when compared to their native counterparts (Dlugosch *et al.* 2008).

However, all invasive populations of ring-necked parakeets in Europe exhibited high levels of within-population genetic diversity, comparable to or even higher than levels observed across their native range. Ring-necked parakeets in Europe predominantly originate from across the northern Indian regions of their native range (Jackson *et al.* 2015), and as such exhibit levels of genetic diversity similar to their native Indian conspecifics. Such high levels of diversity may have been important for the avoidance of detrimental impacts associated with an expected severe bottleneck effect, leading to the establishment success of ring-necked parakeets across Europe. The levels of gene flow between European populations are negligible, suggesting among-population dispersal has not contributed to high levels of genetic diversity observed within each population. Signals of geographical differentiation and structure were observed among the invasive populations of ring-necked parakeets, especially in Heidelberg, for which there is a strong signal of differentiation.

Various mechanisms are thought to resolve the genetic paradox of how invasive alien species avoid detrimental impacts from bottleneck effects, such as high reproductive rates, purging of deleterious alleles or repeated 'top-up' introduction events (Frankham 2005). Current research suggests that invasive populations are rarely a result of a single founding event, but instead are associated with propagule pressure from multiple introductions which reduces genetic risks associated with severe bottleneck effects (Sakai *et al.* 2001; Kolbe 2004; Frankham 2005; Signorile 2014). The high levels of diversity, low rates of gene flow and genetic similarity between European populations of ring-necked parakeet are consistent with a scenario of multiple introductions from the same, ancestral source (Prentis 2009; Kelager *et al.* 2013). Identification of the ancestral origins of invasive European ring-necked parakeets indeed suggests that they each predominately originate from the same northern regions of the species' Asian native range, and share high frequencies of ancestral mitochondrial haplotypes (Jackson *et al.*, submitted). These shared haplotypes occur across widespread parts of the native range, which supports a scenario whereby the invasive populations have received genetic contributions from across large parts of their native range. High levels of genetic diversity within invasive populations stemming from introductions derived from multiple native sources have been observed before, i.e. in *Anolis* Lizards introduced to Florida (Kolbe *et al.* 2004).

4.5.3 Factors influencing variation in population growth rates

Broad spectrums of abiotic and biotic factors such as species traits and environmental characteristics are known to influence invasion success (Simberloff *et al.* 2013). Our examination of the influence of climatic variables and human demographic data on rates of population growth for invasive ring-necked parakeets in Europe support the climate matching hypothesis (Shwartz *et al.* 2009) by showing higher growth rates in populations with climate

conditions that are more similar to the native range, and are therefore expected to have a higher degree of habitat suitability. This result suggests that population growth in European populations relies upon the availability of climatic niches (ie, the range of temperature and precipitation gradients) being similar to those prevailing in the native range ring-necked parakeets.

Strubbe & Matthysen (2009b) found that ring-necked parakeet establishment success was higher in warmer and more densely human population areas, however propagule pressure did not appear to be important in their study. The higher rates of population growth we observe in warmer European populations supports this finding, but our results demonstrate that the estimates of propagule pressure used by Strubbe & Matthysen (2009b) were likely to be underestimates, suggesting the role of propagule pressure (the number of founders) is likely to be important for invasion success (Blackburn *et al.* 2013). While it is important to note that our observed correlations are driven by two populations from warmer regions in Europe (Seville and Marseille), our results are consistent with previous research on reproductive output. A previous study found that in a northern European population, approximately half of the eggs laid by parakeets failed to hatch due to a colder climate, while in Mediterranean climate all eggs hatched, suggesting that more northern European populations should experience slower population growth rates (Schwartz *et al.* 2009). Our observation of higher rates of population growth in both Seville and Marseille, compared to the colder more northerly European populations support the influence of climate matching on the population growth phase of the invasion process, however, more data from additional populations of ring-necked parakeets from warmer regions will be an important future focus to support these findings.

4.6 Conclusion

Determining actual bottleneck effects for populations of invasive alien species is challenging, particularly because detailed demographic information is rarely available. Our study has used a novel approach to estimate the bottleneck effect for ten invasive populations of ring-necked parakeets in Europe, and has demonstrated how, for this species at least, the bottleneck effects are not as severe as demographic profiles would suggest. Consequently, expected detrimental genetic effects are likely to have been offset by repeat 'top-up' introductions of additional individuals since foundation. These findings complement a growing number of studies indicating that multiple 'top-up' introductions play a strong role in invasion success. Furthermore, provide evidence of the influence that climate matching has on population growth and invasion success, highlighting the importance of identifying areas in Europe suitable for future invasions by ring-necked parakeets (Strubbe *et al.* 2015). For policy-makers tasked with managing the spread of invasive alien species such as the ring-necked parakeet, the important

role of genetic contributions from additional individuals in successful invasions would suggest that policies to prevent such accidental releases, especially in areas that are ideal for parakeets under the climate matching hypothesis, can ameliorate the invasive alien species problem.

4.7 Acknowledgments

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4.8 Supplementary material

Table S4.1. Sample size and location information for invasive populations of ring-necked parakeets included in this study.

Population	*n*	Latitude	Longitude
Brussels	69	50.8411	4.3564
Heidelberg	188	49.4034	8.6792
Wiesbaden	80	50.0856	8.2387
Bonn	29	50.73	7.1
Dusseldorf	9	51.2256	6.7828
Seville	59	37.3833	-5.9833
Greater London	164	51.5171	0.1062
Rotterdam	75	51.9217	4.4811
The Hague	12	52.0799	4.3111
Amsterdam	19	52.37	4.89

Table S4.2. Sample and location information for historical specimens of ring-necked parakeet sampled from the Natural History Museum (Tring, UK).

Subspp. designation	Museum ID	Sample location	Latitude	Longitude
P.k.borealis	1889.1.26.271	Assam	27.83	95.67
P.k.borealis	1889.1.26.258	Bhutan	26.7481	89.7498
P.k.borealis	1889.1.26.288	Bhutan	26.7481	89.7498
P.k.borealis	1941.5.30.2913	Burma	18.3374	95.6239
P.k.borealis	1908.5.30.84	Burma	18.3374	95.6239
P.k.borealis	1948.80.3671	Burma Myanmar	18.3374	95.6239
P.k.borealis	1889.1.26.289	Calcutta Bhutan	22.5697	88.3697
P.k.borealis	1889.1.26.290	Calcutta India	22.5697	88.3697
P.k.borealis	1889.1.26.286	Dacca Bangladesh	23.7	90.375
P.k.borealis	1889.1.26.254	Darjeeling India	27.03	88.16
P.k.borealis	1889.1.26.245	Dehli India	28.4667	77.0333
P.k.borealis	1889.1.26.273	Dibrugarh India	27.4805	94.9999
P.k.borealis	1889.1.26.291	Dibrughur India	27.4805	94.9999
P.k.borealis	1889.1.26.252	Etawah India	26.77	79.03
P.k.borealis	1889.1.26.253	Etawah India	26.77	79.03
P.k.borealis	1889.1.26.237	Jhelum Pakistan	32.9286	73.7314
P.k.borealis	1875.7.13.72	Kamrtee India	21.2333	79.2
P.k.borealis	1875.7.13.73	Kamthi India	21.2333	79.2
P.k.borealis	1889.1.20.360	Lawrencepur Pakistan	33.8347	72.5078
P.k.borealis	1889.1.20.355	Maunbhoom India	19.0808	74.7299
P.k.borealis	1897.12.10.1842	Meerut India	28.99	77.7
P.k.borealis	1884.10.8.50	Mhow India	22.55	75.76
P.k.borealis	1889.1.26.256	Nepal	13.4774	5.8749
P.k.borealis	1949.Whi.1.16880	Punjab	30.16	76.87
P.k.borealis	1949.25.853	Punjab	32.7187	72.9843
P.k.borealis	1949.25.854	Punjab India	30.2039	70.7227
P.k.borealis	1949.25.856	Punjab India	30.35	71.39
P.k.borealis	1949.Whi.1.16883	Punjab Pakistan	30.2	71.4167
P.k.borealis	1881.5.1.4725	Punjab Pakistan	32.5417	71.9333
P.k.borealis	1949.25.850	Punjab Pakistan	32.0836	72.6711
P.k.borealis	1949.25.851	Punjab Pakistan	32.7833	72.7
P.k.borealis	1949.25.855	Punjab Pakistan	26.3	74.73
P.k.borealis	1949.Whi.1.16956	Rawalpindi Pakistan	11.83	32.8
P.k.borealis	1889.1.26.240	Sambhal India	14.8333	-17.1
P.k.borealis	1889.1.26.278	Seoni India	28	68.4
P.k.borealis	1860.4.16.550	Shikarpur Pakistan	26.4254	67.8607
P.k.borealis	1898.12.12.320	Sind India	27.6831	68.8678
P.k.borealis	1889.1.20.235	Sindh Punjab	28.4667	77.0333
P.k.borealis	1889.1.26.246	Sindh Punjab	32.3	75.9
P.k.borealis	1889.1.26.248	Sindh Punjab	30.38	76.78
P.k.borealis	1889.1.20.358	Sindh Punjab	25.3792	68.3683
P.k.borealis	1860.4.16.557	Sindh Punjab	14.6195	74.8354
P.k.borealis	1889.1.26.262	Suddya Bhutan	27.6833	68.8667
P.k.borealis	1889.1.26.263	Suddya India	27.6833	68.8667
P.k.borealis	1889.1.26.236	Sukkur Pakistan	14.8333	-17.1
P.k.krameri	1915.12.24.510	Bahr el Ghazal Sudan	7.7	28
P.k.krameri	1907.12.23.77	Bahr el Ghazal Sudan	7.7	28
P.k.krameri	1929.2.18.122	Bakalari Gambia	13.4217	-16.4267
P.k.krameri	1923.10.26.67	Cameroon	9.3	13.4
P.k.krameri	1920.12.22.105	Dafur Sudan	11.07	26.85

P.k.krameri	1922.12.8.406	Dafur Sudan	12.9	23.4833
P.k.krameri	1922.12.8.408	Dafur Sudan	12.5947	23.6192
P.k.krameri	1922.12.8.410	Dafur Sudan	12.9	23.4833
P.k.krameri	1922.12.8.412	Dafur Sudan	15.1304	26.1709
P.k.krameri	1922.12.8.414	Dafur Sudan	13.6306	25.35
P.k.krameri	1929.2.18.121	Gambia	13.4217	-16.4267
P.k.krameri	1899.9.20.5	Ghana	10.5021	-1.9651
P.k.krameri	1910.5.6.154	Guinea	12.3103	-15.7874
P.k.krameri	1910.5.6.153	Gunnal Guinea Bissau	12.3103	-15.7874
P.k.krameri	1930.3.4.371	Haute Volta	12.1389	0.6552
P.k.krameri	1939.12.9.3243	Kael Senegal	14.7077	-15.8991
P.k.krameri	1929.2.18.120	Kerewan Gambia	13.5	-16.0833
P.k.krameri	1922.12.8.405	Kurdufan Sudan	13.1833	30.2167
P.k.krameri	1926.8.8.136	LakeChad Nigeria	14.2528	13.1108
P.k.krameri	1915.12.24.512	Mongalla Sudan	5.1989	31.7695
P.k.krameri	1928.7.20.26	Nigeria	9.1539	4.812
P.k.krameri	1900.8.4.29	Nigeria	13.5561	13.233
P.k.krameri	1911.12.23.535	Nigeria	18.5203	73.8567
P.k.krameri	1923.8.7.7000	Renk Sudan	11.83	32.8
P.k.krameri	1923.8.7.7001	Renk Sudan	28.58	78.55
P.k.krameri	1939.12.9.3241	Senegal Gambia	14.6667	-16.2501
P.k.krameri	1918.8.26.33	Senegal Gambia	16.0333	-16.5
P.k.krameri	1889.1.20.331	Senegal Gambia	22.08	79.53
P.k.krameri	1907.12.23.76	Sudan	26.7481	89.7498
P.k.krameri	1939.12.9.3240	Thies Senegal	8.6626	76.7646
P.k.krameri	1911.12.23.536	Yo Nigeria	27.83	95.67
P.k.mallinensis	1889.1.26.283	Aujango India	8.6626	76.7646
P.k.mallinensis	1940.12.3.211	Kalawewa Sri Lanka	8.0166	80.5164
P.k.mallinensis	1949.Whi.1.16889	Madras India	9.74	77.3
P.k.mallinensis	1884.7.28.38	Mysore India	12.3	76.65
P.k.mallinensis	1860.4.16.558	Mysore India	12.3	76.65
P.k.mallinensis	1919.1.12.61	Pune India	30.35	71.39
P.k.mallinensis	1949.Whi.1.16884	Rajasthan India	33.6	73.0333
P.k.mallinensis	1925.12.23.1106	Sirsi Kanara	8.0166	80.5164
P.k.mallinensis	1940.12.3.209	Sri Lanka	6.1244	81.1225
P.k.mallinensis	1946.28.234	Sri Lanka	6.8397	79.8758
P.k.mallinensis	1953.16.31	Sri Lanka	8.7047	25.4579
P.k.mallinensis	1937.12.21.94	Travancore	13.5561	13.233
P.k.mallinensis	1889.1.26.281	Travancore India	15.7833	38.45
P.k.parvirostris	1890.10.10.4	Anseba River Ethiopia	15.7833	38.45
P.k.parvirostris	1927.5.3.1	Berbera Somalia	10.4333	45.0167
P.k.parvirostris	1915.12.24.515	BlueNile Sudan	11.85	34.3833
P.k.parvirostris	1889.1.20.334	Eritrea Africa	15.9027	38.4522
P.k.parvirostris	1878.12.31.663	Eritrea Africa	15.9027	38.4522
P.k.parvirostris	1919.12.17.751	Kamisa Sudan	13.1206	34.2279
P.k.parvirostris	1915.12.24.513	Mongalla Sudan	5.1989	31.7695

Table S4.3. Suite of mtDNA PCR primers used to amplify cytochrome b and control region sequence from historical museum specimens and from contemporary *Psittacula krameri* samples.

Primer name	Sequence (5' – 3')
Primers used for historical specimens	
Cb1f	CTA CCA TTC ATA ATC ACC AGC C
Cb1r	GTG AGG GAG AGG AGT ATG ATA G
Cb2f	CTA TCA TAC TCC TCT CCC TCA C
Cb2r	TAG GAT CAG TAC GGA GGC AG
Cb3f	AAC AAC TCC CCC ACA CAT C
Cb3r	CGG CGA GTG TTC AGA ATA G
CR1f	CGT TCG TGT TTG CTT ACA TTT C
CR1r	GGT CCG TGT TGT TTG TTT TG
CR2f	CAC TGA TGC ACT TTT TCT GAC
CR2r	GGT GAA ATG TAA GCA AAC ACG
MCR2f	GAT GCA CTT TTT CTG ACA TCT G
MCR2r	GTT TCT TGA AAT GAA TCA CAG
CR3f	GAA CAA ACA AAC GTC TCC TTC
CR3r	GGA TAT TTG AGT GCG AGT GAC
Primers used for contemporary specimens	
PKCBf	CGGCCTACTCCTAGCCGCCC
PKCBr	GGGAAGCAGGCCGGAAGGC
CR19f	CACAGGCTCATTTGGTTCGC
CR19r	TAAGCTACAGGGACATTCGGGG

Table. S4.4. Full explanation of variables obtained for GLMs examining influences on growth rates in invasive populations of ring-necked parakeets.

Response variable:

GrowthR: Population growth rates

Explanatory variables included in final GLM:

Climatic variables

clim_ghf_n: Native-range based prediction of parakeet habitat suitability based on climatic and anthropogenic factors taking niche structure into account.

clim_n: Native-range based prediction of parakeet habitat suitability based on climatic factors ONLY taking niche structure into account

clim: Native-range based prediction of parakeet habitat suitability based on climatic factors ONLY taking NOT niche structure into account

Explanatory variables excluded from the final GLM:

Climatic variables

clim_dist Euclidian distances (in climatic space) between native and invasive haplotypes. Strubbe *et al.* (2015) determined for each parakeet haplotype the climatic niche it occupies in the native range (i.e. the range of temperature and precipitation gradients it currently tolerates, sensu Broennimann *et al.* 2012). For each haplotype in each European city, Euclidean distance between the position of that city in the climatic space and the centroid of the native-range haplotype niche was calculated. This distance was weighted according to the prevalence of each haplotype in each city to obtain a single 'climatic distance' for each European city studied here.

clim_ghf: Native-range based prediction of parakeet habitat suitability based on climatic and anthropogenic factors NOT taking niche structure into account. Accurate/reliable estimate

Lat Highest latitude for each population (see Table S4.1)

meantcold: European data (NOT a native range based model prediction - raw climate data): average mean temperature of the coldest month

meantwarm: European data (NOT a native range based model prediction - raw climate data): average mean temperature of the warmest month

Human demographic variables

humanpopde: European data (NOT a native range based model prediction - just raw socio-economic data) - human population density (Balk & Yetman 2004)

totalpop: Total human population density (source: Wikipedia)

Genetic variables

Bottleneck effect: Bottleneck effect calculated from genetic data.

HapD: Haplotype diversity

He: Heterozygosity

Ar Allelic richness

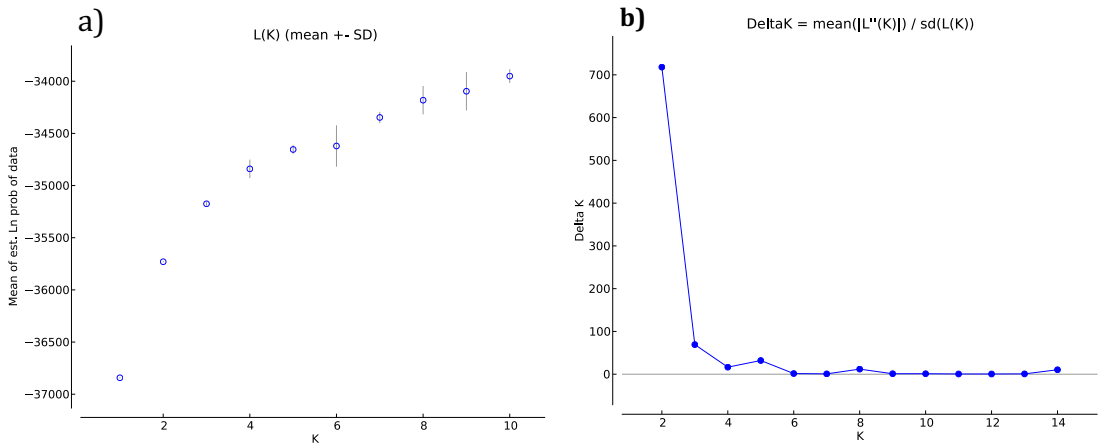

Fig. S4.1. a) mean log-likelihood and b) Delta K (Evanno *et al.* 2005) for genetic clustering simulations run in STRUCTURE (Pritchard *et al.* 2000), for each value of K calculated in STRUCTURE HARVESTER (Earl *et al.* 2012) in invasive populations of ring-necked parakeets.

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Seychelles black parrot on Praslin, the National bird of the Seychelles

Photo by Wokoti

Chapter 5.

In prep for publication in Ibis

Evolutionary distinctiveness and historical decline in genetic diversity within the Seychelles black parrot *Coracopsis nigra barklyi.*

Hazel Jackson, Nancy Bunbury, Natalia Przelomska & Jim Groombridge

5.1 Abstract

The Seychelles black parrot (*Coracopsis nigra barklyi)* is an island endemic threatened with extinction. The total population of 520–900 individuals is restricted to the 38 km^2 island of Praslin, and is considered to be one of the last few remaining endemic island parrots that survive in the Indian Ocean. We used mitochondrial and microsatellite DNA markers to examine the evolutionary distinctiveness of *C. n. barklyi* within *Coracopsis*, an evolutionarily ancient group of parrots, and to compare levels of genetic diversity between historical and contemporary specimens. Finally we examined morphological differences between the four *Coracopsis nigra* subspecies. Phylogenetic and molecular clock analyses revealed *C. n. barklyi* to be basal to the remaining three *C. nigra* subspecies, having diverged approximately 8 Mya. Discriminant function analysis of morphological measurements suggests the Seychelles black parrot is the smallest of the four *Coracopsis* subspecies. Higher levels of genetic diversity were observed in historical specimens (100–150 years old) while only one *mt*DNA haplotype was observed in the contemporary specimens, suggesting that *C. n. barklyi* has lost genetic diversity as a consequence of substantial recent population decline. This study provides a first insight into the evolutionary, genetic and morphological processes that have shaped *C. n. barklyi* and provides an important perspective on this parrot's current genetic status in order to guide its future conservation management. While further ecological studies are essential, we suggest that *C. n. barklyi* should be managed as an evolutionary significant unit to conserve its novel evolutionary pathway.

5.2 Introduction

With current extinction rates estimated to be up to 1000 times greater than their pre-human levels (Pimm *et al.* 1995) and unevenly distributed on a global scale, there is an urgent need to prioritise the allocation of conservation resources (Hazevoet 1996; Myers 2000; Butchart *et al.* 2004; Isaac 2007). While some approaches to prioritising species for conservation focus on endemism and restricted range, evolutionary distinctiveness remains an essential consideration (Witting & Loeschcke 1995; Crozier 1997; Isaac 2007; Villegér *et al.* 2008). Indeed, genetic diversity is the raw material essential for adaptation to change and is considered a priority for conservation by the IUCN (McNeely *et al.* 1990; Faith 1992; IUCN 2012).

Oceanic island ecosystems are a particularly rich source of evolutionary diversity in the form of endemic populations and this diversity makes a substantial contribution to global biodiversity (Whittaker & Fernández-Palacios 2007). Islands support endemic populations that exhibit patterns of adaptive radiation, and are often the last refuges for such taxonomically distinct relict populations that are a high priority for conservation (Cronk 1997). Such island populations can also harbor high levels of phylogenetic diversity, a valuable measure in conservation priority setting (Vane-Wright *et al.* 1991). Unfortunately, endemic populations on small, isolated islands are affected by deterministic factors that include habitat loss and invasive species, as well as stochastic factors, making them acutely vulnerable to extinction (Shaffer 1981; Nott *et al.* 1995; Pimm 1995; Frankham 1997, 2005).

Low levels of genetic diversity within populations on islands can be due to recent small population size and/or ancestrally low effective population size relative to continental populations. The former inevitably leads to loss of genetic diversity due to the effects of genetic drift, inbreeding and ultimately loss of fitness, key factors which increase extinction risk (Lande 1988; Frankham 1995) and can contribute to population decline (Berger 1990; Bijlsma *et al.* 2000; Hedrick & Kalinowski, 2000; Frankham *et al.* 2010). Importantly, signatures of low genetic variation can persist long after populations have recovered or until conservation genetic intervention (Mucci *et al.* 1999; Groombridge *et al.* 2000, 2009). Consequently, conservation managers tasked with recovering endemic island populations, and particularly island populations, need to identify the type, extent and distribution of genetic diversity to promote its retention and enhance the evolutionary potential of the population.

One such example is the Seychelles black parrot (*Coracopisis nigra barklyi,* Newton 1867), an endangered subspecies endemic to the Seychelles archipelago and restricted to Praslin (Evans 1979; Watson 1984; Reuleaux *et al.* 2013), a small island of just 38 km^2. *Coracopsis* parrots display dull plumage but are remarkable in their reproductive biology; not only do they exhibit

a socially polygamous mating system characterised by female promiscuity and cooperative breeding, but the males are also unique among parrots in having an intromittent organ for copulation (Wilkinson & Birkhead 1995; Ekstrom 2007; Reuleaux *et al.* 2013). Seychelles black parrots occur in their highest densities in the mature palm forest on Praslin including the UNESCO World Heritage site of the Vallée de Mai (Fig. 5.1). This area comprises a rare habitat containing all six of the palm species endemic to Seychelles (Usher 1993), which also form a substantial part of the parrot's diet and nesting habitat (Gaymer *et al.* 1969; Evans 1979; Reuleaux 2011; Reuleaux *et al* 2014).

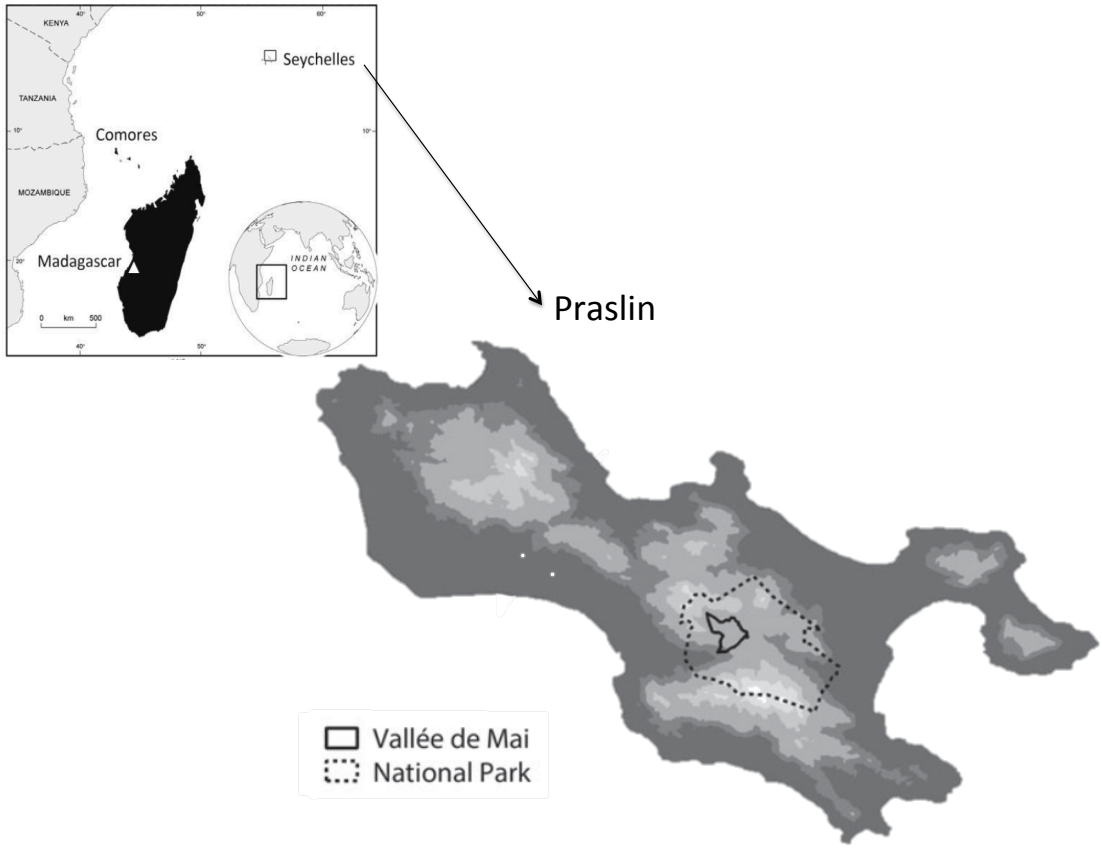


Fig. 5.1. Island of Praslin, Seychelles, off the coast of Africa and Madagascar. Here the National park and Vallee de Mai are shown where Seychelles black parrots (*Coracopsis nigra barklyi)* occur in their highest densities.

The population of *C. n. barklyi*, estimated at only 520–900 individuals (Reuleaux *et al.* 2013), is one of only two endemic island parrot populations remaining in the Western Indian Ocean. This region was formerly a rich source of parrot diversity, with at least five other endemic island parrot species described prior to their extinction (Temple 1981; Hume 2008; Cheke & Hume 2009), including Newton's parakeet on the Seychelles (*Psittacula wardi). Coracopsis n. barklyi*

may have experienced recent range contraction; there are previous records of it occurring on Curieuse, Aride and Marianne islands. It is not known whether any of these were breeding populations but *C. n. barklyi* is now only resident on Praslin (Reuleaux *et al.* 2013). Field surveys over the last 40 years suggest that the population may have declined between the 1960s and 1980s, prior to an estimate of 200–300 birds in 2001 and 520–900 by 2011 (Gaymer *et al.* 1969; Merritt *et al.* 1986; Rocamora & Skerrett 2001; Reuleaux *et al.* 2013).

The history of possible range contraction and field observations for *C. n. barklyi* are suggestive of a recent population bottleneck and have raised concerns about whether this island subspecies is now genetically impoverished, thereby compromising its evolutionary potential (Soulé 1972; Nunney 1993; Amos & Harwood 1998). Surprisingly, however, despite their unusual mating system and biogeographical distribution, the population genetics of *Coracopsis* parrots have not been examined, due in part perhaps to a lack of available DNA markers. Therefore we applied mtDNA and nuclear DNA markers available for a closely-related parrot, the endangered Mauritius parakeet (*Psittacula echo*; Raisin *et al.* 2009) to survey levels of genetic diversity within and between the *Coracopsis nigra* subspecies.

We examine evolutionary divergence, morphological differentiation and population-level genetic diversity using two mitochondrial and five microsatellite DNA markers in the four *Coracopsis nigra* subspecies. We also examine measures of population genetic diversity in the historical population of *C. n. barklyi* on the Seychelles in comparison to the contemporary population. Our aims were to: (1) resolve patterns of molecular genetic and morphological divergence between the four subspecies and quantify levels of genetic diversity within them, (2) evaluate whether genetic diversity is low in the contemporary *C. n. barklyi* population in comparison to historical levels and quantify any population bottleneck, and (3) use this information to suggest guidance for future conservation management of the *C. n. barklyi* population.

5.3 Methods

5.3.1 Sample Collection

The Seychelles Islands Foundation (SIF) field team collected blood samples from 42 *C. n. barklyi* specimens over two breeding seasons from November 2009 to June 2010 and October 2010 to April 2011. Individuals were sampled at the nest or caught by mist- netting in several locations across Praslin (Fig. 5.2). The majority (n=28) of the birds were sampled within the Vallée de Mai on Praslin. Of the remaining 14 individuals sampled, three were sampled within the Praslin National Park and the others originated from outside the park (Fig. 5.2). All blood samples were stored at –20°C in 90–95% ethanol prior to analysis. In addition, a total of 81 toe-

pad samples were obtained from *Coracopsis nigra* specimens collected from Madagascar, Comoros and Seychelles (Fig. 5.2.), from the Natural History Museum at Tring in the UK (n=25) and the Muséum National d'Histoire Naturelle in Paris (n=56) (Table S5.1 in supplementary material). For all analyses samples from museum specimens were assigned a subspecies based on their taxonomic name and location information given on the museum specimen label. Morphological data for total body length, wing length, tail length, beak length and beak width were available for the majority of the museum specimens from the *Coracopsis* Vasa Breeding Group, Germany (Asmus 2004), with additional specimens from the Tring collection measured by JG.

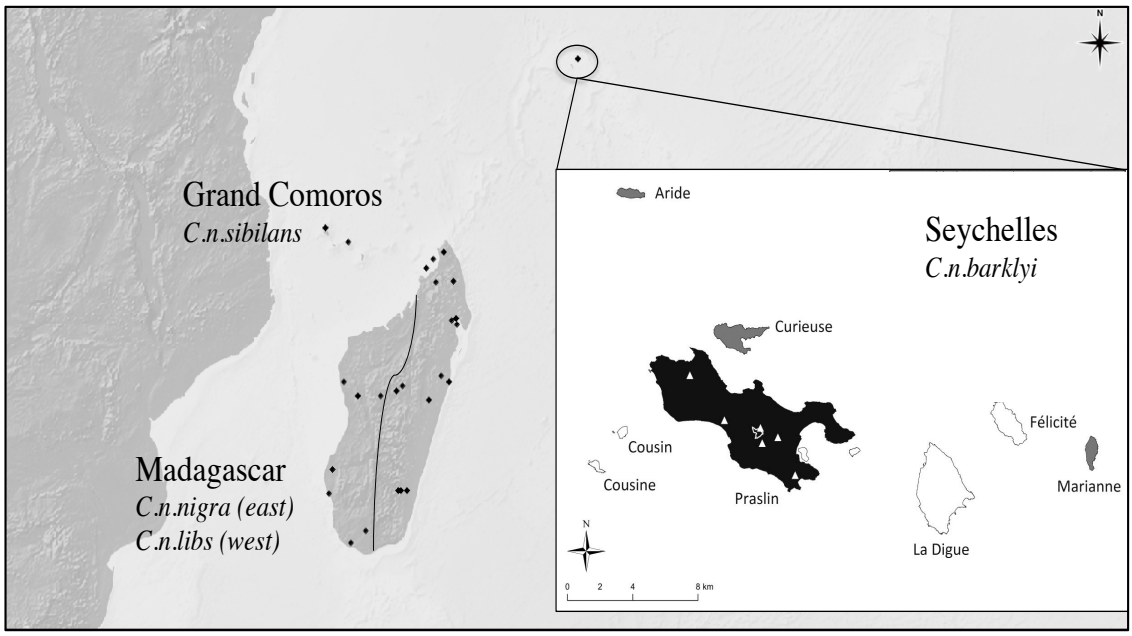


Fig. 5.2. a) Sample location for museum specimens used as part of this study (black dots), covering the native ranges of all four *Coracopsis nigra* subspecies. On Madagascar, the two subspecies, *C. n. nigra* and *C. n. libs,* native ranges are delineated by the black line. Extent of occurrence of the Seychelles black parrot (current range indicated in inset by black shading; historic sightings by grey shading). Sampling locations for contemporary specimens are indicated by white triangular symbols.

5.3.2 DNA isolation, amplification and sequencing

DNA was extracted from contemporary blood samples using either an ammonium acetate salt precipitation method (Nicholls *et al.* 2000) or by DNeasy® Tissue Extraction Kits (QIAGEN) following manufacturer's protocols (Qiagen, UK) and suspended in 200 µl elution buffer. Negative controls were included to ensure no contamination during the extraction and PCR

process. Amplification from contemporary *C. n. barklyi* samples was conducted for control region (spanning across the conserved central Domain II and Domain III) using CoraF3 and H1248, and cytochrome *b* using CoraCB2F and CoraCB2R (Table S5.2.2**)**. PCR protocols for each gene were optimised using different taq reagents. PCR cycling conditions for *mt*DNA control region consisted of 94°C for 3 min followed by 30 cycles of 94°C/30 secs, 58°C/30 secs and 72°C/45 secs and 10 minutes of final elongation at 72°C with amplification in 10 µl reactions containing 1 µl NH_4, 0.3 µl $MgCl_2$, 1.6 µl dNTPs (2.5 mM), 0.2 µl of each primer, 6.22 µl of ddH_2O and 0.8 µl of Taq Polymerase (Bioline, UK). PCR cycling conditions for *mt*DNA cytochrome *b* consisted of an initial hot start of 95°C for 1 min followed by 35 cycles of 95°C/15 secs, 58°C/15 secs and 72°C/10 secs followed by a final 10 min 72°C incubation period with amplification volumes of 25 µl contained 1 µl of template DNA, 12.5 µl MyTaq HS redmix (Bioline, UK) containing dNTPs and $MgCL_2$, 0.5 µl of each primer and 8.5 µl of dH20.

Processing of the historical specimens, including DNA extraction and PCR amplification, was carried out in a separate laboratory dedicated to ancient DNA work, under a UV-irradiated fume hood to ensure no contamination. All equipment and surfaces were sterilised before and after each use by irradiation from UV light and with 10% bleach. Negative controls were included during the DNA extraction and PCR process and a selection of negative extractions and PCRS were sequenced to ensure there was no contamination. DNA was extracted from historical toe-pad samples using a Bioline Isolate Genomic DNA extraction kit (Bioline, UK). Samples were suspended in 400 µl lysis buffer plus 25 µl proteinase K and incubated at 55^oC overnight (or until the material had completed digested). DNA was washed through a spin column and suspended in 50 µl of elution buffer. Amplification of cytochrome *b* (635 bp) was conducted using a suite of short fragment primers specifically designed for *Coracopsis* (Kundu *et al.* 2012). A second suite of primers was designed to amplify control region (280 bp, spanning the conserved central domain II, and domain III; Table S5.2) The short fragment primers were designed to overlap each other providing sequence replication reducing the risk of contamination. Amplification volumes of 25 µl contained 2 µl of template DNA, 12.5 µl MyTaq HS redmix (Bioline, UK), 0.5 µl of each primer and 9.5 µl of dH20. Cycle parameters comprised an initial hot start of 95°C for 1 min followed by 35 cycles of 95°C/15 secs, 52°C/15 secs and 72°C/10 secs followed by a final 10 min 72°C incubation period.

All amplicons were examined by agarose gel electrophoresis to ensure clean single bands and to check for any signs of contamination. All PCR products were purified and amplified using a 3730xl analyser (Macrogen Inc.). Sequences were edited in 4Peaks (Griekspoor & Groothius 2005) and aligned in Clustal (Larkin *et al.* 2007). Manual edits were made in Jalview (Waterhouse *et al.* 2009). The two genes were concatenated into a complete dataset of 915 bp

using Sequence Matrix (Vaidya *et al.* 2011).

Given that no microsatellite markers have been developed for *Coracopsis* parrots, and indeed few exist for parrot taxa from the Old World, we used a suite of microsatellite markers available for *Psittacula* parakeets, which have been shown to cross-amplify in *Coracopsis* parrots (Raisin *et al.* 2009). All loci were tested for cross-species amplification on four samples from the contemporary *C. n. barklyi* population. PCRs were conducted on five loci that amplified in both contemporary and museum specimens (*Peq01*, *Peq05*, *Peq07*, *Peq08* and *Peq11*; Table S5.2), using differently fluorolabelled forward primers (HEX and 6-FAM). For contemporary samples, cycling conditions were 94°C for 3 min followed by 30 cycles of 94°C/30 secs, 56°C/30 secs and 72°C/45 secs and 10 minutes of final elongation at 72°C with amplification in 10 µl reactions containing 1 µl NH_4, 0.3 µl $MgCl_2$, 1.6 µl dNTPs (2.5 mM), 0.2 µl of each primer, 6.22 µl of ddH_2O and 0.8 µl of Taq Polymerase (Bioline, UK). PCRs on museum specimens were carried out in a separate dedicated museum lab to minimise contamination. PCR cycling conditions were 95°C for 10 min followed by 35 cycles of 94°C/30 secs, 52°C/90 secs and 72°C/90 secs, with a final incubation at 72°C for 10 min. PCR products were genotyped using an Applied Biosystems 3730 DNA Analyser and ROX 500 ROX^{TM} as a size-standard. Alleles were identified and scored using GENEMAPPER 3.7 (Applied Biosystems, UK). Due to the degraded nature and low volume of DNA derived from historical museum specimens, each sample was genotyped twice to confirm identification of a heterozygote or homozygote, and to identify potential genotype errors. Degraded samples are known to be susceptible to genotyping errors due to allelic dropout of the larger-sized alleles (Taberlet *et al.* 1996; Hoffman & Amos 2005, Wandeler *et al.* 2007). Consequently historical samples can sometimes underestimate ancestral levels of genetic diversity in comparison to contemporary samples (Wandeler *et al.* 2007).

5.3.3 Phylogenetic analysis

Our concatenated dataset was combined with sequences for other Indian Ocean parrots (Kundu *et al.* 2012) and phylogenetic tree inferences were computed using Bayesian and maximum likelihood methods. PartitionFinder (Lanfear *et al.* 2012) was used to identify the best-fit models of nucleotide evolution for each partition (control region 1-280, cytochrome *b* 281-915), according to Bayesian information criteria (BIC). Bayesian inference was implemented in MrBayes v3.2 (Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway (Miller *et al.* 2010) with 10 million generations over four parallel Monte Carlo Markov Chains (MCMC**),** under a HKY+I evolutionary model (Felsenstein 1981). Tracer v1.6 (Rambaut & Drummond 2007) and AWTY (Nylander *et al.* 2008) were used to assess convergence. After discarding the first 25%, tree topologies were summarised in a 50% consensus tree. A maximum likelihood

search was conducted in RaxML (Stamatakis 2006). Ten independent runs were performed with 1000 non-parametric bootstrap replicates to obtain the best likelihood score under a GTAGAMMA model and summarised in a majority rule consensus tree. All trees were visualised in FigTree v1.4 (Rambaut 2012). Additionally, a UPGMA phylogeny was generated based on D_{ST} distances using microsatellite data, with 500 bootstrap replications, computed using POPTREE (Takezaki *et al.* 2014). A median joining haplotype network was constructed in NETWORK v4.612 (www.fluxus-engineering.com) using all 915 bp of *mt*DNA, to estimate relationships between different *C. nigra* subspecies.

Time-calibrated phylogenies were estimated using BEAST v.1.7.5 **(**Drummond & Rambaut 2007**)**. Given that relative to other bird families the fossil record for parrots is poor (Mayr & Göhlich 2004), we combined our data with cytochrome *b* sequences for other Indian Ocean parrots obtained from Genbank (Kundu *et al.* 2012) and ran phylogenetic analyses by adopting a similar approach to Wright *et al.* (2008) and using two alternative calibration dates. The first calibration used was obtained from the oldest known fossil belonging to a crown group of parrots, *Mopsitta tanta,* dated to around 54 Mya in the Tertiary period (Waterhouse *et al.* 2008), while a second calibration of 80 Mya was taken from previous dating analysis of extant bird orders, suggesting the Cretaceous period may be more suitable (Hedges *et al.* 1996). This calibration was given a normal distribution with a standard deviation of 10 Mya to ensure the 95% distribution (60.4 and 99.6 Mya) did not exceed the 100 Mya date for the divergence of bird orders (Hedges *et al.* 1996). An uncorrelated lognormal relaxed molecular clock model was used in favor of a strict molecular clock model as identified by Akaike's information criterion (AIC) through MCMC (AICM) comparison of models (Baele *et al.* 2012) with a uniform distribution under the Yule speciation tree prior (Ho *et al.* 2007). MCMC was performed for 10 million generations with sampling every 1000th iteration. Convergence was confirmed by effective sample sizes (ESS) >200 for all parameters using Tracer v1.6 (Drummond & Rambaut 2007). Trees from the first 1000 generations were discarded as burn-in. A maximum clade credibility tree was summarised using TreeAnnotater v1.7.5 (Drummond & Rambaut 2007), and visualised in Figtree v1.4 (Rambaut 2012).

5.3.4 Genetic analysis

Known related individuals (parents/siblings) were removed from the contemporary *C. n. barklyi* dataset prior to analysis. Diversity metrics were calculated on subspecies groups, based on taxonomic designations assigned by the museum and collection location information. Mitochondrial haplotype diversity (H) and nucleotide diversity (π) were calculated using DNAsp (Librado & Rozas 2009). For the microsatellite data, linkage disequilibrium and departure from Hardy-Weinberg equilibrium (HWE) was tested for using GENEPOP (Raymond

& Rousset 1995) and CERVUS (Kalinowski *et al.* 2007) was used to assess the frequency of null alleles. For the historical dataset, FreeNA (Chapuis & Estoup 2007) was used to generate a dataset corrected for null alleles to examine their potential influence on our analysis. The number of private alleles (Na), observed, expected and unbiased heterozygosity (H_O, H_E and UH_E) were calculated using GENALEX (Peakall & Smouse 2012). Allelic richness (Ar) was obtained using the R program STANDARICH 1.0 (Alberto *et al.* 2006), which rarefied the number of alleles in each population, standardised to the smallest complete sample number (here eight) across loci. Genetic distance between populations was calculated using two methods, pairwise F_{ST} in GenAlEx (Peakall & Smouse 2012) and Jost's measure of differentiation (D_{jost}) in the R package diveRsity (Keenan *et al.* 2013). Population differentiation between subspecies was analysed using Fisher's exact test in GENEPOP (Raymond & Rousset 1995) and Kruskal-wallis tests were performed to identify significant differences in nuclear genetic diversity measures among subspecies. Net between-group mean genetic distances were calculated using MEGA 5.05 (Tamura *et al.* 2011) under the Kimura 2-parameter model (Kimura 1980) with gamma distribution of rates among sites. Standard error estimates were calculated using 500 bootstrap replicates. All measures of genetic diversity and distance were calculated using the uncorrected dataset. To examine temporal changes in effective population size (N_e) for *C. n. barklyi*, a Bayesian method was implemented using the programme *tmvp* (Beaumont 2003), an approach that uses both historical and contemporary genotypes. Historical and contemporary N_e was estimated using likelihoods based on allele frequencies, accounting for unequal sample sizes across loci and the sampling periods. A mean generation time of two years was specified with rectangular priors of 1-1000 for historical and contemporary N_e. A range of smoothing parameters (alpha) were used, α=0.3-0.7, with α=0.3 chosen for the final analysis.

5.3.5 Morphological analysis

To identify differentiation between the four subspecies, a discriminant function analysis was performed (SPSS 2012), using five morphological measurements; total body length, wing length, tail length, beak length and beak width. Wing length, tail length, beak length and beak width) were standardised again body size by dividing each measurement by total body length.

5.4 Results:

5.4.1 Molecular phylogeny

A total of 915 bp was obtained including mtDNA control region (280bp) and cytochrome *b* (635 bp). Of 81 historical samples, 66 were successfully extracted and used in this study. Owing to the fragile nature of the museum samples, some partial sequences were obtained (209–915 bp); however, missing data does not have a detrimental impact on the accuracy of phylogenies,

which may in fact benefit from the inclusion of sequences from additional individuals despite the overall effect of missing data across the sequence dataset (Wiens 2006, Wiens & Moen 2008, Wiens & Morrill 2011). Twenty-six unique haplotypes were identified from the concatenated dataset and used for phylogenetic reconstruction. Two haplotypes were shared by the two subspecies on Madagascar, *C. n. nigra* and *C. n. libs,* while one sample of *C. n. libs* shared a haplotype with a sample taken from a specimen from Comoros. Only a small number (n=9) of historical *C. n. barklyi* specimens from Seychelles exist in museum collections. As a consequence of restricted quantities of available sample material and limited eluted volumes of extracted genomic DNA we amplified *mt*DNA from four of these specimens (comprising two haplotypes), while microsatellite genotypes were obtained from seven of the nine specimens. A single haplotype was identified for the contemporary *C. n. barklyi* population.

Topologies recovered from Bayesian and maximum likelihood trees were largely congruent at all major nodes, inferring a distinct and well supported structure between the four subspecies (Fig. 5.3.). *Coracopsis. n. barklyi* is placed as being ancestral to the three remaining subspecies, with *C. n. sibilans* from Comoros forming a distinct sister clade alongside the mixed group of individuals from the two more closely-related subspecies on Madagascar. The UPGMA phenogram (Fig. 5.4.) provides additional support from the nuclear data for the placement of *C. n. barklyi* as basal to the remaining subspecies. This clustering pattern is also supported in the median joining haplotype network (Fig. 5.5.), where *C. n. barklyi* and *C. n. sibilans* cluster separately from the two subspecies found on Madagascar, *C. n. nigra* and *C. n. libs,* which collectively contain a considerable number of different haplotypes.

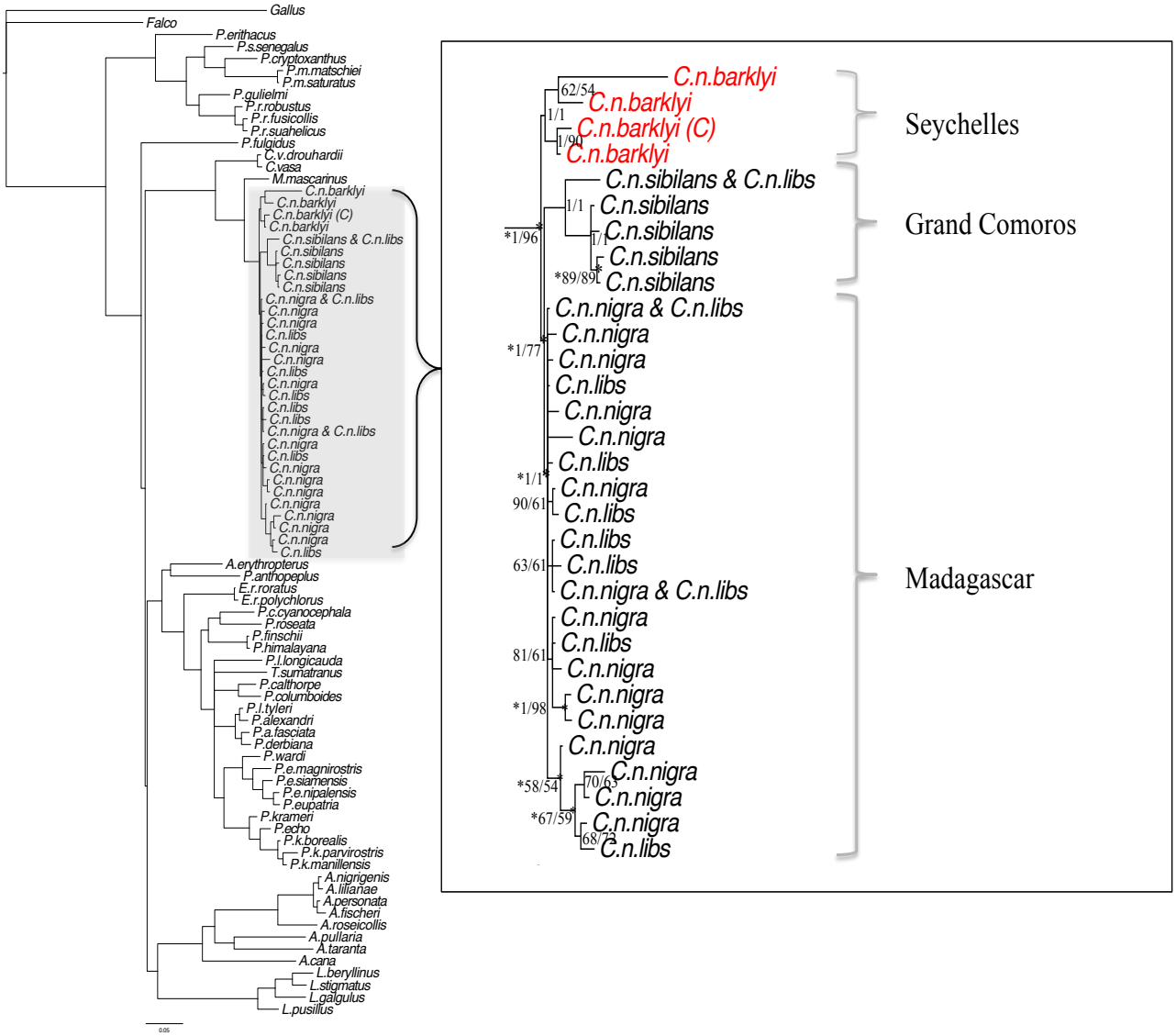


Fig. 5.3. Phylogenetic reconstructions of the four *Coracopsis nigra* subspecies, comprising 26 phylogenetically informative haplotypes derived from a total of 915 bp of control region and cytochrome *b* mtDNA using two approaches: Bayesian and maximum likelihood inference. The single mtDNA haplotype found in the modern population of *C. n. barklyi* (C) along with sequences from Kundu *et al.* (2012), have also been included. Both Bayesian posterior probabilities and maximum likelihood bootstrap values are provided respectively at relevant nodes.

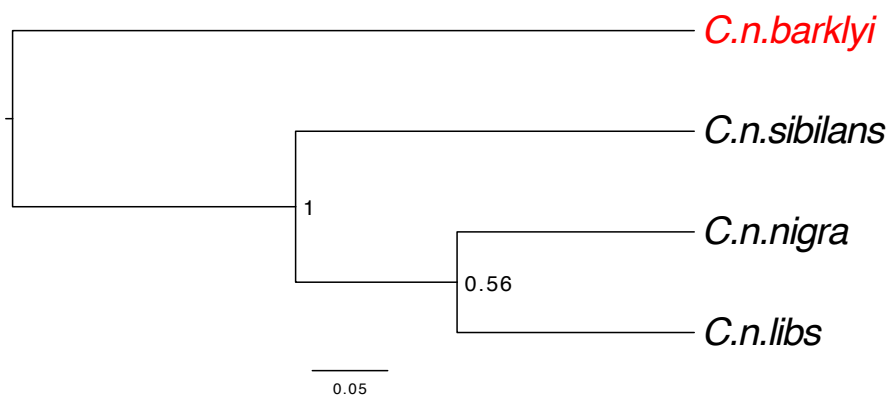


Fig. 5.4. UPGMA phylogeny of the four *C. nigra* subspecies, generated from uncorrected microsatellite Dst distances with 500 bootstrap replications.

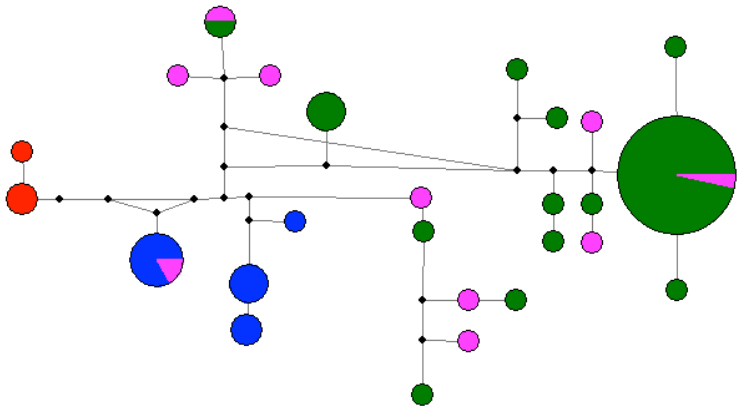
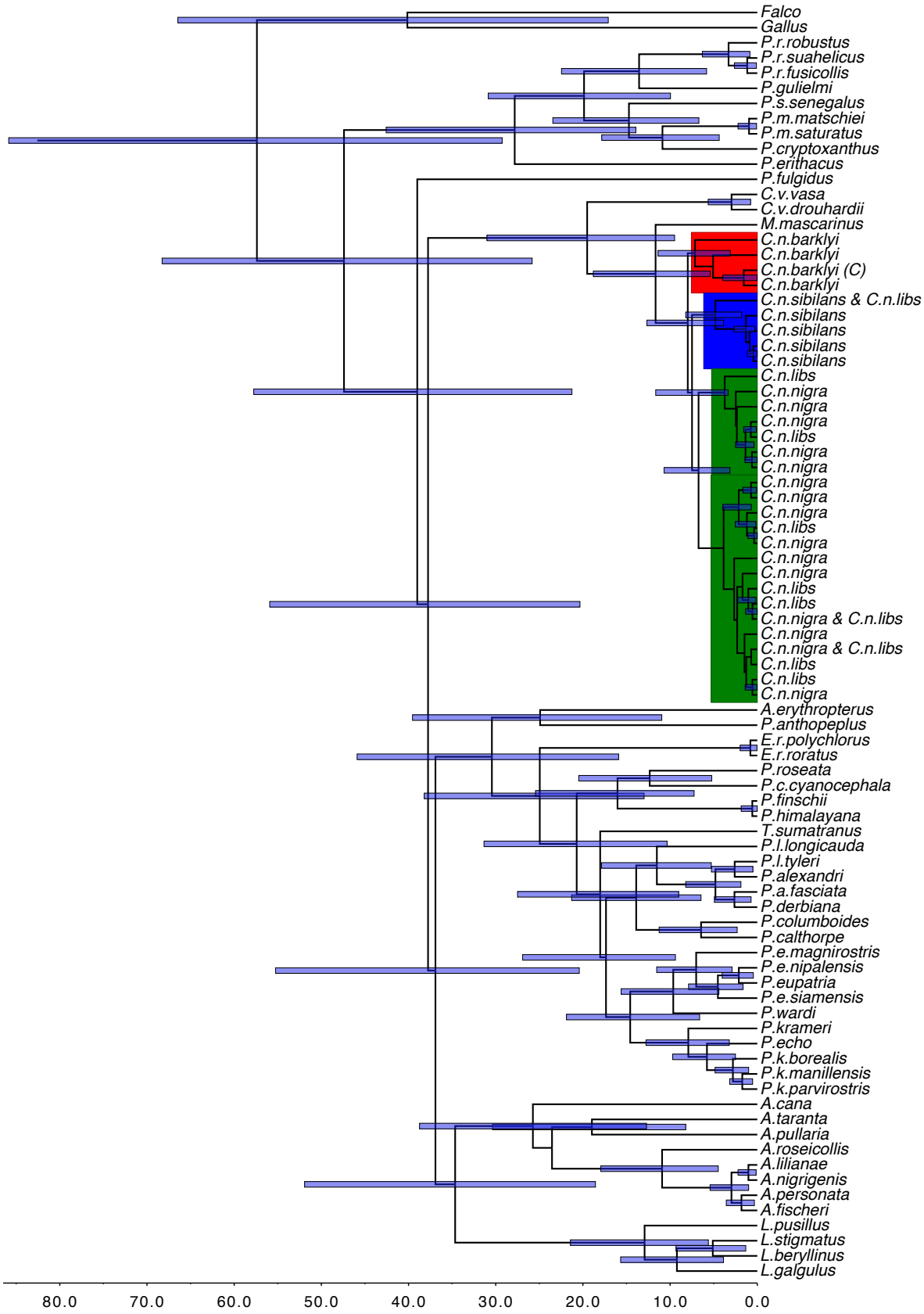


Fig. 5.5. MtDNA median joining haplotype network of historical specimens of *C. nigra*. Coloured nodes correspond to haplotypes comprising each subspecies (green = *C. n.nigra*, purple = *C. n. libs*, blue = *C. n. sibilans,* red = *C. n. barklyi*), black squares represent hypothetical mutational steps between haplotypes that were not observed within the sequence dataset. The size of the coloured node represents the frequency of each haplotype.

5.4.2 Molecular-based estimates of divergence

When using a calibration of 54 Mya, the recovered date of divergence of the genus *Coracopsis* from the common ancestor *P. fulgidus,* is estimated to be 39 Mya (95% confidence interval 21.2–57.7 Mya), while *C. nigra* split from *C. vasa* 19.5 Mya (95% confidence interval 9.4–31 Mya). Within *C. nigra,* the analysis indicates *C. n. barklyi* diverged from the remaining *C. nigra* subspecies 8 Mya (95% confidence interval 3.8–12.6 Mya). Subsequently, *C. n. sibilans* split from *C. n. barklyi* 7.5 Mya (95% confidence interval 3.3–11.6 Mya) and finally the two

Madagascan subspecies, *C. n. libs and C. n. nigra,* diverged 6.7 Mya (95% confidence interval 3.1–10.7 Mya), (Fig. 5.6.). When the calibration age of the common ancestor is changed to 80 Mya the divergence dates also move further back in time. The divergence date of *Coracopsis* from *P. fulgidus* increases to 60.6 Mya (95% confidence interval 41.7–78.7 Mya), *C. nigra* splits from *C. vasa* 31 Mya (95% confidence interval 19.2–44.7 Mya), while *C. n. barklyi* diverges from the other *C. nigra* subspecies 13 Mya (95% confidence interval 7.8–18.3 Mya), (Fig. S5.1).


Falco
Gallus
P.r.robustus
P.r.suahelicus
P.r.fusicollis
P.gulielmi
P.s.senegalus
P.m.matschiei
P.m.saturatus
P.cryptoxanthus
P.erithacus
P.fulgidus
C.v.vasa
C.v.drouhardii
M.mascarinus
C.n.barklyi
C.n.barklyi
C.n.barklyi (C)
C.n.barklyi
C.n.sibilans & C.n.libs
C.n.sibilans
C.n.sibilans
C.n.sibilans
C.n.sibilans
C.n.libs
C.n.nigra
C.n.nigra
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C.n.libs
C.n.libs
C.n.nigra
A.erythropterus
P.anthopeplus
E.r.polychlorus
E.r.roratus
P.roseata
P.c.cyanocephala
P.finschii
P.himalayana
T.sumatranus
P.l.longicauda
P.l.tyleri
P.alexandri
P.a.fasciata
P.derbiana
P.columboides
P.calthorpe
P.e.magnirostris
P.e.nipalensis
P.eupatria
P.e.siamensis
P.wardi
P.krameri
P.echo
P.k.borealis
P.k.manillensis
P.k.parvirostris
A.cana
A.taranta
A.pullaria
A.roseicollis
A.lilianae
A.nigrigenis
A.personata
A.fischeri
L.pusillus
L.stigmatus
L.beryllinus
L.galgulus
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

Fig. 5.6. Estimated divergence times derived from cytochrome *b* mtDNA data obtained for museum specimens of *C. n. barklyi* from the Seychelles (red), *C. n. sibilans* from Grand Comoros (blue), and *C. n. libs* and *C. n. nigra* from Madagascar (Green). Sequence data were analysed alongside existing sequences for other Old World parrots (*Psittaciformes*) from Kundu *et al.* 2012, and resolved using BEAST, specifying a 54 Mya TMRCA. Node bars display the 95% HPD and time on the axis is given in millions of years before the present.

5.4.3 Genetic differentiation

Mean nucleotide divergences between subspecies derived from the complete *mt*DNA dataset show a highest sequence divergence of 3.3% to be present between the subspecies *C. n. barklyi* (Seychelles) and *C. n. sibilans* (Comoros). Mean sequence divergences indicate that *C. n. barklyi* differs by 1.8% and 1.9% from the two subspecies on Madagascar (*C. n. nigra* and *C. n. libs* respectively), while *C. n. siblians* differs by 2.1% and 2.2% from the two Madagascar subspecies. The smallest divergence, of 0.6%, is observed between the two Madagascan subspecies (Table 5.1.). Both measures of genetic differentiation, F_{ST} and D_{jost} derived from microsatellite genotypes of the museum specimens indicate the highest level of differentiation being between the two island subspecies, *C. n. barklyi* and *C. n. sibilans* (F_{ST} = 0.429, D_{jost} = 0.337), while the lowest levels are between *C. n. nigra and C. n. libs* on the Madagascan mainland (Table 5.2.).

Table 5.1. Estimates of evolutionary divergence over sequence pairs between *C. nigra* subspecies based on mtDNA. Samples were designated to a specific subspecies following the information given on museum specimen labels.

Concatenated	*C.n.barklyi*	*C.n.sibilans*	*C.n.nigra*	*C.n.libs*
C.n.barklyi		0.030	0.018	0.019
C.n.sibilans			0.021	0.022
C.n.nigra				0.006
C.n.libs				
Cytochrome b	*C.n.barklyi*	*C.n.sibilans*	*C.n.nigra*	*C.n.libs*
C.n.barklyi		0.033	0.019	0.018
C.n.sibilans			0.028	0.028
C.n.nigra				0.008
C.n.libs				
Control region	*C.n.barklyi*	*C.n.sibilans*	*C.n.nigra*	*C.n.libs*
C.n.barklyi		0.014	0.009	0.009
C.n.sibilans			0.004	0.006
C.n.nigra				0.000
C.n.libs				

Table 5.2. Genetic differentiation between *C. nigra* subspecies; F_{ST} (lower half) and D_{jost} (upper half).

	C.n.barklyi	*C.n.libs*	*C.n.nigra*	*C.n.sibilans*
C.n.barklyi	-	0.154	0.153	0.337
C.n.libs	0.190	-	2e-04	0.259
C.n.nigra	0.179	0.028	-	0.334
C.n.sibilans	0.429	0.232	0.257	-

5.4.4 Levels of genetic diversity

Among the historical samples the mean microsatellite amplification success was 55% across all loci. Of the five loci, *Peq*08 and *Peq*11 yielded the lowest amplification success rate as both loci produce alleles over 200 bp; failure to amplify loci greater than 200 bp is not uncommon from degraded historical specimens (Neilsen *et al.* 1999, Paabo *et al.* 2004, Wandeler 2007). Following repeat amplifications, only three samples suffered from genotype error (mismatched

alleles); two samples for loci *Peq*08 and one sample for *Peq*11; these were excluded from the analysis. Amplification success was also hindered by the use of non-species specific primers, a common issue that is problematic with degraded DNA (Wandeler 2007). Despite the presence of null alleles (*Peq*01 = +0.26, *Peq*05 = +0.12, *Peq*07 = +0.22, *Peq*08 = +0.29 and *Peq*11 = +0.13), the corrected FreeNA dataset produced similar levels of pairwise genetic differentiation, suggesting that the genotype data derived from the museum specimen samples are robust. Among the contemporary *C. n. barklyi* samples amplification success of the microsatellite loci was 96.7% and the mean null allele frequency was; *Peq*01 = -0.029, *Peq*05 = –0.054, *Peq*07 = 0, *Peq*08 = +0.041 and *Peq*11 = –0.005.

All four groups of subspecies were significantly differentiated from each other (Fisher's exact test: $p < 0.001$) and nuclear levels of historical expected heterozygosity (H_E) were significantly different between subspecies ($K^2_{(3)} = 7.87$, $p < 0.05$; Table 5.3.). All measures of genetic diversity in the historical *C. n. barklyi* population were higher than in the contemporary population. No polymorphic sites were observed in the *mt*DNA data for the contemporary *C. n. barklyi* population, resulting in no measures of haplotype or nucleotide diversity (Table 5.4.).

Table 5.3. Genetic diversity for each of the four *C. nigra* subspecies based on genotyping of the museum specimens using five microsatellite loci. N=number of samples, Na= number of private alleles, Ar=standardised allelic richness, H_O=observed heterzygosity, H_E=expected heterozygosity, UH_E=unbiased expected heterozygosity, H=haplotype diversity, π=nucleotide diversity

	Microsatellite						**mtDNA**	
Pop	**N**	**Na**	**Ar**	**H_O**	**H_E**	**UH_E**	**H**	**π**
C.n.barklyi	8	0.800	3.400	0.200	0.410	0.459	0.400	0.00620
C.n.sibilans	9	0.547	2.900	0.108	0.200	0.214	0.436	0.01897
C.n.nigra	30	2.000	3.772	0.254	0.589	0.613	0.846	0.01166
C.n.libs	17	1.200	3.978	0.205	0.606	0.659	0.769	0.00707

Table 5.4. Temporal comparison of genetic diversity between historical museum specimens and contemporary samples of *C. n. barklyi* from Praslin, Seychelles.

	Historical population	Contemporary population
Number of samples	8	33
Number of private alleles	0.800	0.400
Standardised allelic richness	3.400	1.828
Observed heterozygosity	0.200	0.170
Expected heterozygosity	0.410	0.165
Unbiased expected heterozygosity	0.459	0.167
Haplotype diversity	0.400	0
Nucleotide diversity	0.00620	0

5.4.5 Temporal changes in historical and contemporary N_e

The posterior distribution of the temporal change between historical and contemporary effective population size (N_e) for *C. n. barklyi* on the Seychelles is given in Fig. 5.7. The density of points is proportional to the probability density of population size at two different time periods (the time of the oldest sample and of the most recent sample). An 'off-diagonal' distribution indicates a change in N_e; the resulting output for *C. n. barklyi* therefore shows a strong signal of a severe decline in N_e over the last 146 years, from an N_e of 864 (90% higher posterior density [HPD] limits, 258–1000) in 1878 to a contemporary N_e of only six individuals (90% HPD limits, 3–17) in 2011.

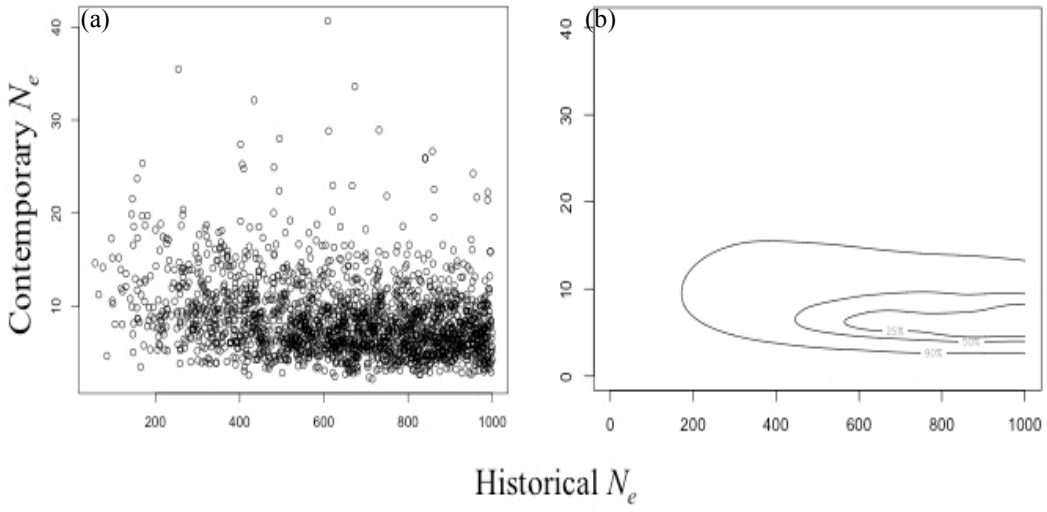

Fig. 5.7. Posterior distribution of the historical and contemporary effective population size (N_e) of *C.n.barklyi* calculated using *tmvp* analysis (Beaumont 2003). (a) The density of points is proportional to the probability density of population size at the two different times; an 'off-diagonal' distribution indicates a change in N_e. (b) 25%-95% higher posterior density limits of the posterior distribution.

5.4.6 Morphological differentiation

Discriminant function analysis revealed there to be morphological differences present between subspecies from different islands (Wilks' lambda=0.162, Chi-square = 332.65, df=12, p<0.001; Fig. 5.8.). The analysis resulted in three discriminant functions accounting for 100% of variation, with one of those functions accounting for 91.7% of the variation between groups. Total length is the strongest variable for discriminating between groups (canonical discriminant function coefficient = 1.636: Table 5.5). Overall the proportion of individuals correctly classified into their original groups were, C. n. nigra = 63.3%, C. n. libs = 71.8%, C. n. sibilans = 82.6% and C. n. barklyi = 89.3%. Both subspecies from Madagascar are larger in size across all five measurements (total body length, wing length, tail length, beak length and beak width) in comparison to C. n. siblians and C. n. barklyi (Table 5.5).

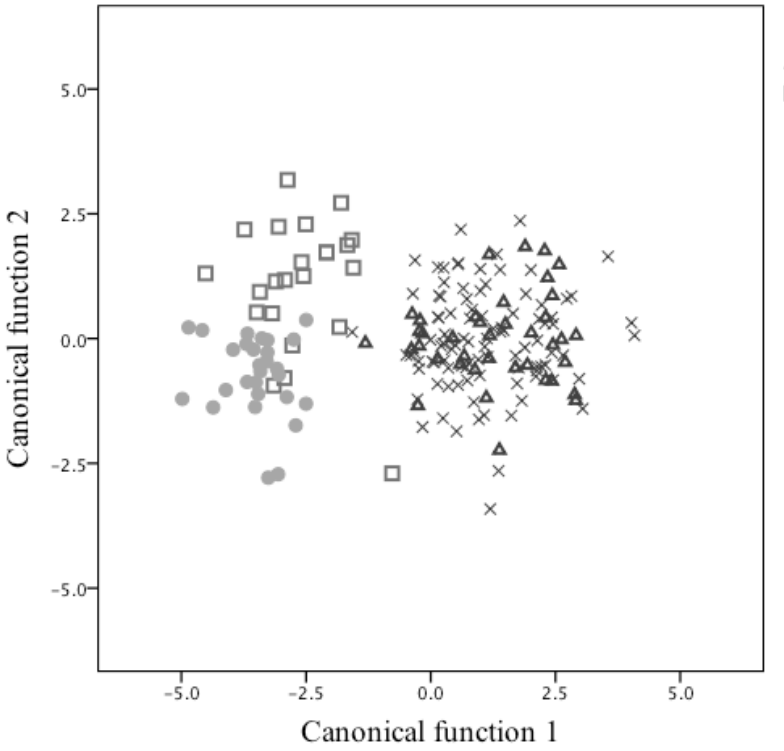

Fig. 5.8. Plot of first two canonical functions resulting from the discriminant function analysis accounting for morphological differences between *C. nigra* subspecies. *C. n. barklyi* = •, *C. n. sibilans* = □, *C. n. nigra* = ×, *C. n. libs* = Δ

Table 5.5. Mean morphological measurements (prior to standardisation) ± standard error for each of the four *C. nigra* subspecies. Standardised canonical discriminant function coefficient for function 1, which accounts for 91.7% of variation, indicates total length is the predominant variable for discriminating between groups (coefficient = 1.636).

Variable	*C. n. nigra* (*n*=98)	*C. n. libs* (*n*=39)	*C. n. sibilans* (*n*=23)	*C. n. barklyi* (*n*=28)	Standardised canonical discriminant function coefficient (Function 1)
Total body length	34.45 ± 2.186	35.00 ± 2.677	29.78 ± 1.853	29.52 ± 2.818	1.636
Wing length	23.48 ± 1.144	23.82 ± 1.187	19.40 ± 0.956	18.88 ± 0.722	1.160
Tail length	15.69 ± 1.282	15.84 ± 1.135	13.70 ± 0.663	12.84 ± 0.895	-
Beak length	2.22 ± 0.160	2.15 ± 0.141	1.89 ± 0.169	1.90 ± 0.102	0.51
Beak width	1.38 ± 0.062	1.37 ± 0.061	1.29 ± 0.064	1.19 ± 0.044	0.307

5.5 Discussion

This study is the first to apply DNA markers to examine the evolutionary and morphological differentiation and patterns of population genetic diversity in the *Coracopsis nigra* parrots, a comparatively ancient and ecologically and morphologically unusual genus of Psittaciformes. The conservation status of the black parrot population on Seychelles, shown by this study and elsewhere to be a comparatively basal, evolutionary relict population among the *Coracopsis* genus (Kundu *et al.* 2012), can now be re-evaluated in light of population genetic and morphological comparisons and observed low levels of contemporary genetic diversity revealed by this study.

5.5.1 Evolutionary history

The molecular phylogenetic analysis suggests that *C. n. barklyi* is ancestral to the remaining three subspecies. The placement of *Coracopsis nigra* within the broader genus suggests following an initial radiation to Madagascar from Africa by the precursors of the *Coracopsis* genus, an outward pattern of radiation to the Seychelles (*C. n. barklyi*) and Comoros (*C. n. sibilans*), followed by a recolonisation of Madagascar (*C. n. nigra* and *C. n. libs*). During this radiation, Madagascar may have acted as a 'stepping stone' to the Seychelles and Comoros, alternatively, *C. vasa* (which is still found on Madagascar) may have been the ancestral precursor of *C. nigra*. Estimates of divergence times reveal that *C. nigra* split from *C. vasa* 19.5–31 Mya, and that *C. n. barklyi* diverged from the other *C. nigra* subspecies 8–13 Mya, indicative of a substantial period of island isolation for the Seychelles black parrot. Following colonisation of the Seychelles, the Comoros islands were colonised shortly afterwards, with a subsequent divergence event 6.75 Mya on to Madagascar where the population finally split to form the two most closely related subspecies.

The results from the phylogenetic analysis support the idea that the subspecies on Seychelles, and indeed the subspecies on Comoros, are each on different evolutionary trajectories compared to the two subspecies on Madagascar. This phylogenetic pattern is supported by morphological differences between the subspecies, with each of the two island subspecies, *C. n. barklyi* and *C. n. sibilans,* being of very different size to those on Madagascar. *C. n. barklyi* is the smallest of the four subspecies, and it appears that body size has increased as *Coracopsis nigra* has radiated from Seychelles and Comoros back into Madagascar. This observed size difference may be directly linked to the size of the island inhabited by each subspecies. A larger wing size may be required to traverse the larger continental sized island of Madagascar, opposed to smaller wings required for the small island of Praslin. Indeed, the two subspecies on Madagascar cluster together morphologically and genetically, indicative of relatively recent gene flow that could have been facilitated by their adjacent (and in some areas, sympatric) distributions.

The mean *mt*DNA divergence between *C. n. barklyi* and *C. n. sibilans* of 3.3% for cytochrome b is higher than that observed between the Mauritius parakeet (*Psittacula echo)* and the Indian ring-necked parakeet (*Psittacula krameri manillensis)* which are classified as two separate species (Groombridge *et al.* 2004; Kundu *et al.* 2012). Elsewhere, lower levels of nucleotide divergence of 0.7–1.5%, have been observed between subspecies of *Poicephalus* parrots (Perrin 2005). In combination, the molecular phylogenetic and morphological findings from this study, together with observed differences in plumage and behavior among the subspecies (Rocamora & Skerrett 2001; Asmus 2004), provide evidence to support the assignment of the Seychelles black parrot as an evolutionarily significant unit (ESU), with a recommendation that this island population be managed as if it were a separate species to maintain its unique evolutionary trajectory (*C. n. barklyi* is now referred to as *C. barklyi* by Birdlife International and the IUCN*;* del Hoyo *et al.* 2014).

5.5.2 Genetic signature of a recent population crash on Seychelles

Our observed levels of within-population genetic diversity on Seychelles add to the growing body of literature that shows island populations to have lower levels of genetic diversity than continental-sized populations and consequently to be at greater risk of extinction due to genetic factors associated with genetic impoverishment (Frankham 1997; Eldridge *et al.* 2004; Boessenkool *et al.* 2007). Furthermore, the markedly reduced levels of genetic diversity observed in the contemporary *C. n. barklyi* population compared to the historical population that existed 140 years ago on the Seychelles support the claim that genetic factors can potentially adversely affect threatened species vulnerable to extinction (Spielman *et al.* 2004).

Our finding of substantially reduced N_e within the contemporary population in comparison to historical levels indicates that this population has experienced a recent population decline. Indeed, the earliest historical sample in our study was collected in 1878, whereas human colonisation and forest clearance began in the Seychelles in the 1770s (Cheke & Hume 2008), suggesting that our estimate of an historical N_e of 864 may be representative of a population already in decline. Substantial historical range contraction may have occurred on Seychelles. While the contemporary population on Seychelles is restricted to Praslin, recent field records document the presence (albeit in low numbers and of non-resident birds) of parrots on the neighbouring island of Curieuse. Furthermore, historical records suggest that the population's range previously included other islands of the Seychelles archipelago including Marianne (1875) and Aride (1907) (Skerrett *et al.* 2001). This larger range would have provided more land area for an ancestrally larger population size. Seychelles black parrots have not, however, been recorded to breed on any islands other than Praslin, nor have there been any recent

sightings on these islands (Rocamora and Laboudallon 2009). Alternatively, specialised nesting requirements of these parrots may have restricting the ancestral population size to very few islands, curtailing long-term ancestral N_e; black parrots only nest in cavities in dead trees, primarily in the rare Coco de Mer (*Lodoicea maldivica*) (Merritt *et al.* 1986, Reuleaux *et al.* in press), an endemic palm occurring exclusively on Praslin and Curieuse (Rist *et al.* 2010). Endemic palm forest on Praslin is largely restricted to the Praslin National Park, which also contains the highest proportion and density of parrots (Reuleaux 2011).

Our detection of a recent population decline is also supported by observations from field records and surveys (Rocamora & Laboudallon 2009; Reuleaux 2013). Similar genetic analyses of temporal changes in N_e for other Seychelles endemic birds such as the Seychelles kestrel (*Falco araea*; Groombridge *et al.* 2009) and the Seychelles paradise flycatcher (*Terpsiphone corvine;* Bristol *et al.* 2013) suggest substantial population declines within the last century are not uncommon on these islands. Indeed, the severe decline to a contemporary N_e of only six individuals for the Seychelles black parrot is comparable to that genetically derived for the kestrel which experienced a decline to an N_e of eight individuals prior to subsequent recovery (Groombridge *et al.* 2009).

5.5.3 Conservation management

While loss of genetic diversity may act too slowly to contribute directly to extinction risk of island populations (Jamieson 2007), the extent of genetic impoverishment that remains detectable within the Seychelles black parrot population implies that genetic problems associated with small population size, such as accumulation of deleterious alleles, inbreeding depression and compromised evolutionary potential, may still arise in the recovering population and could increase extinction risk (Frankham 1995; Crnokrak & Roff 1999; Brook *et al.* 2002; Frankham *et al.* 2010). In view of the evolutionary relict status of these parrots confirmed by our phylogeny and elsewhere (Kundu *et al.* 2012), and its restricted range, the surviving population warrants close conservation attention (Reuleaux *et al.* 2013). Consequently, we recommend maintaining and potentially expanding the habitat and range of black parrots via continued protection and intensified restoration of endemic palm forest on Praslin and on islands identified as being appropriate for potential reintroduction. These practices should promote increased population size and subsequently minimise further loss of genetic diversity (Soulé 1976, Lacy 1987; Hedrick 2005). Encouragingly, a research programme focused on monitoring the population was launched in 2009 and has provided an increasing body of information on the ecology of these black parrots. Our findings highlight the need for continued ecological and genetic monitoring of this island parrot to restore its population, which together with the endangered Mauritius parakeet (*Psittacula echo*) comprises one of the last few

remaining endemic island parrots that survive in the Indian Ocean.

5.6 Acknowledgements

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5.7 Supplementary material

Table S5.1. Sample information for historical specimens from the NHM Tring, UK and NHM Paris.

Subspp.	Museum Label	Sample location information	Museum
C.n.barklyi	1878-484	Seychelles	Paris
C.n.barklyi	1878-486	Seychelles	Paris
C.n.barklyi	1878-491	Praslin, Seychelles	Paris
C.n.barklyi	1878-492	Seychelles	Paris
C.n.barklyi	1906.12.21.189	Praslin, Seychelles	Tring
C.n.barklyi	1927.12.18.129	Praslin, Seychelles	Tring
C.n.barklyi	1946.75.19	Praslin, Seychelles	Tring
C.n.barklyi	1994-228	Seychelles	Paris
C.n.barklyi	1889.1.20.640	Seychelles	Tring
C.n.libs	1909-297	West coast, Madagascar	Paris
C.n.libs	1931.12.9.592	Marovato, SW Madagascar	Tring
C.n.libs	1931.8.18.415	Tsiandro, WC Madagascar	Tring
C.n.libs	1931.8.18.416	Tsiandro, WC Madagascar	Tring
C.n.libs	1931.8.18.423	Lake Ihotry, SW Madagascar	Tring
C.n.libs	1931.8.18.424	Lake Ihotry, SW Madagascar	Tring

C.n.libs	1932-888	Tsiandro, Madagascar	Paris
C.n.libs	1932-889	Tsiandro, Madagascar	Paris
C.n.libs	1932-890	Ampotaka, Madagascar	Paris
C.n.libs	1932-891	Ampotaka, Madagascar	Paris
C.n.libs	1932-892	Tsiandro, Madagascar	Paris
C.n.libs	1932-893	Tsiandro, Madagascar	Paris
C.n.libs	1932-895	near Tsiroanomandidy, Madagascar	Paris
C.n.libs	1932-896	Tsiandro, Madagascar	Paris
C.n.libs	1932-897	Tsiandro, Madagascar	Paris
C.n.libs	1994-316	Ankaboa, Madagascar	Paris
C.n.libs	1994-317	Forest Park South Bekitro, Madagascar	Paris
C.n.nigra	1882-1634	Madagascar	Paris
C.n.nigra	1912-175	Ankazobe, Madagascar	Paris
C.n.nigra	1913-260	near Toamasina, Madagascar	Paris
C.n.nigra	1913-261	near Toamasina, Madagascar	Paris
C.n.nigra	1913-262	near Toamasina, Madagascar	Paris
C.n.nigra	1931.8.18.412	Tsarakibany, Northern Madagascar	Tring
C.n.nigra	1931.8.18.400	Montagne d'Ambre, Madagascar	Tring
C.n.nigra	1931.8.18.403	Vondrozo, SE Madagascar	Tring
C.n.nigra	1931.8.18.404	Vondrozo, SE Madagascar	Tring
C.n.nigra	1931.8.18.408	Vondrozo, SE Madagascar	Tring
C.n.nigra	1932-871	30 km w of Vondrozo, Madagascar	Paris
C.n.nigra	1932-872	Marotony, Nosy Bé, Madagascar	Paris
C.n.nigra	1932-873	Maroantsetra, Madagascar	Paris
C.n.nigra	1932-874	20 km w of Vendrozo, Madagascar	Paris
C.n.nigra	1932-875	Montagne d'Ambre, Madagascar	Paris
C.n.nigra	1932-876	Farafangana, Madagascar	Paris
C.n.nigra	1932-877	Marotony, Nosy Bé, Madagascar	Paris
C.n.nigra	1932-878	40 km sw of Maroantsetra, Madagascar	Paris
C.n.nigra	1932-879	30 km w of Vendrozo, Madagascar	Paris
C.n.nigra	1932-880	Maroantsetra, Madagascar	Paris
C.n.nigra	1932-881	20 km w of Vondrozo, Madagascar	Paris
C.n.nigra	1932-882	Maroantsetra, Madagascar	Paris
C.n.nigra	1932-883	Maroantsetra, Madagascar	Paris
C.n.nigra	1932-884	30 km w of Vendrozo, Madagascar	Paris
C.n.nigra	1932-885	20 km w of Vendrozo, Madagascar	Paris
C.n.nigra	1932-886	Montagne d'Ambre, Madagascar	Paris
C.n.nigra	1932-887	Tsiroanomandidy, Madagascar	Paris
C.n.nigra	1964-1101	Perinet, Madagascar	Paris
C.n.nigra	1974-157	Taolagnaro Madagascar	Paris
C.n.nigra	1994-232	Toamasina, Madagascar	Paris
C.n.nigra	1994-233	Toamasina, Madagascar	Paris
C.n.nigra	1994-234	Antongila Bay, Toamasina, Madagascar	Paris
C.n.nigra	1994-318	Perinet, Madagascar	Paris
C.n.nigra	1994-319	Perinet, Madagascar	Paris
C.n.nigra	2005-2279	Farafangana, Madagascar	Paris
C.n.nigra	2006-198	Montagne des francais, Madagascar	Paris

C.n.nigra	2006-199	Antsiranana Rural, Madagascar	Paris
C.n.sibilans	1959-405	Near Dindi, Anjouan	Paris
C.n.sibilans	1959-406	La convalesence, Grand Comoroe	Paris
C.n.sibilans	1959.5.130	Nioumbadjou, Grand Comoroe	Tring
C.n.sibilans	1959.5.131	Nioumbadjou, Grand Comoroe	Tring
C.n.sibilans	1959.5.132	Nioumbadjou, Grand Comoroe	Tring
C.n.sibilans	1964-953	Nioumbadjou, Grand Comoroe	Paris
C.n.sibilans	1994-229	Grand Comoroe	Paris
C.n.sibilans	1994-230	Grand Comoroe	Paris
C.n.sibilans	1994-231	Grand Comoroe	Paris
C.n.sibilans	90.10.10.37	Anjouan	Tring
C.n.sibilans	90.10.10.38	Grand Comoroe	Tring

Table S5.2. Suite of mtDNA and microsatellite primers used to amplify cytochrome *b*, control region and five loci in historical and contemporary *Coracopsis nigra* specimens.

Primer name	Sequence (5' – 3')	Tm (ºC)
mtDNA primers		
H1248	CATCTTCAGTGTCATGCT	51
CoraF3	TCATTCGGCACTTCCAGTGCG	64
CoraCB2F	TTACCAGAGGATTTGCTGGAGT	59.4
CoraCBR	ACCAGAGGATTTGCTGGAGT	57.3
mF1	GGGTTGCTTTAATGAGACGG	57
mR1	TGTCGAAAATCATGTCCCAC	55
mF2	CTACTTAATCGTGGTGTTA	50
mR2	AAATGACTATTCGAGTAATGTG	52
mF3	ATCTTCTCAAGTTTTTAAC	49
mR3	GATGACGAAAAATGGCAGGG	57
mF4	CCCTGCCATTTTTCGTCATC	57
mR4	TAAGCTACAGGGACAGGGTC	59
MC44-81F	TTTGTGGTCCTCAGGGGTTG	59.4
MC44-81R	AGAGGGAACGGAAATGTCAA	55.3
MC185-309F	CGACATTTCCGTTCCCTCTA	57.3
MC185-309R	TTCGAGTAATGTGTTTGTTGTTTG	55.9
MC273-438F	ATTTTTCTTTAACAAACAACAAACAC	54.3
MC273-438R	CGAAAAATGGCAGGGAAATA	53.2
Cora F20_39	ACCCACTACACCGCAGATAC	59
Cora R241_261	AGGACGTATCCAACGAAGGCT	59

Cora F146_164	TACCTCCACATCGCCCGAG	61
Cora R282_304	TGTGATGACTGTAGCCCCTCAGA	62
Cora F255_273	ACGTCCTACCATGAGGCCA	59
Cora R481_499	TGAGATGCCTAGGGGGTTG	59
Cora F389_409	ACTCGATTCTTCGCCCTACAC	60
Cora F490_510	AGGCATCTCATCAAACTGCGA	58
Cora R633_655	AGTTACCAGAGGATTTGCTGGAG	61
microsatellite primers:		
Peq01	F: AGGCTTAACAGATGTAGGACA	63
	R: TGTGCTTTTCCATCACAAG	
Peq05	F: GGAATTGTAGGTTTTAAATGCAC	56
	R: AGCTCATAAACAGCCATATCTC	
Peq07	F: AAACAAACATACCCACAGAAAC	66
	R: GGAGGATAAGCAGAACTTGAG	
Peq08	F: AGTCGGGAACAGTTTCATTAG	58
	R: GACATGATGCTGACACAGATAG	
Peq11	F: CTCAAGGAGAATCTGAAGTCTG	56
	R: TGGGAGGTTAGAGTGAAAAAC	

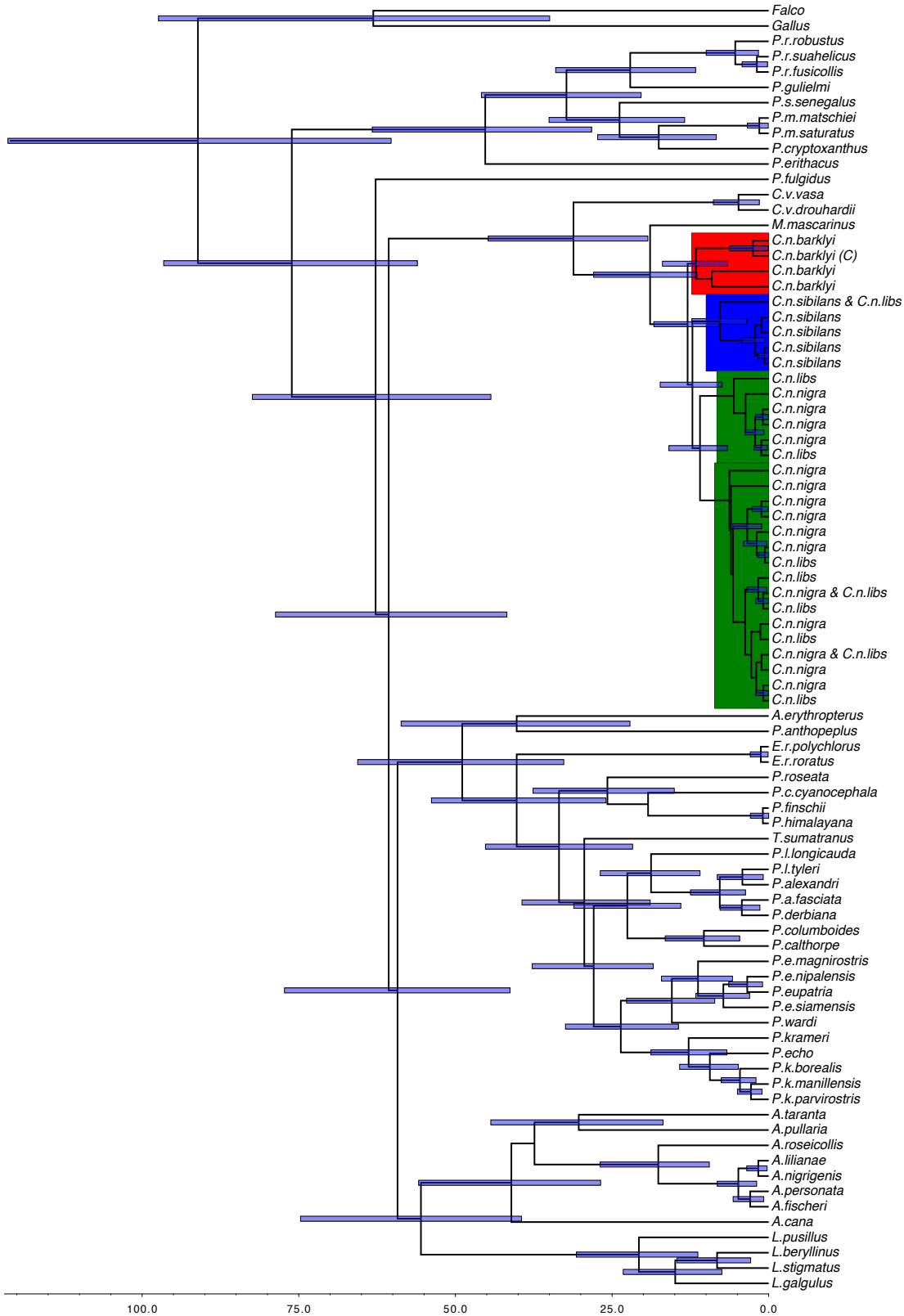

Fig. S5.1. Molecular divergence times of *C.n.barklyi* from the Seychelles (red), *C.n.sibilans* from Grand Comoroe (blue), *C.n.libs* and *C.n.nigra* both from Madagascar (Green), in context of Indian Ocean parrots (*Psittaciformes)*, based on Cytochrome b sequence data and resolved in BEAST using 80 MYA TMRCA. Node bars display the 95% HPD and time on the axis is given in millions of years before the present.

5.8 References

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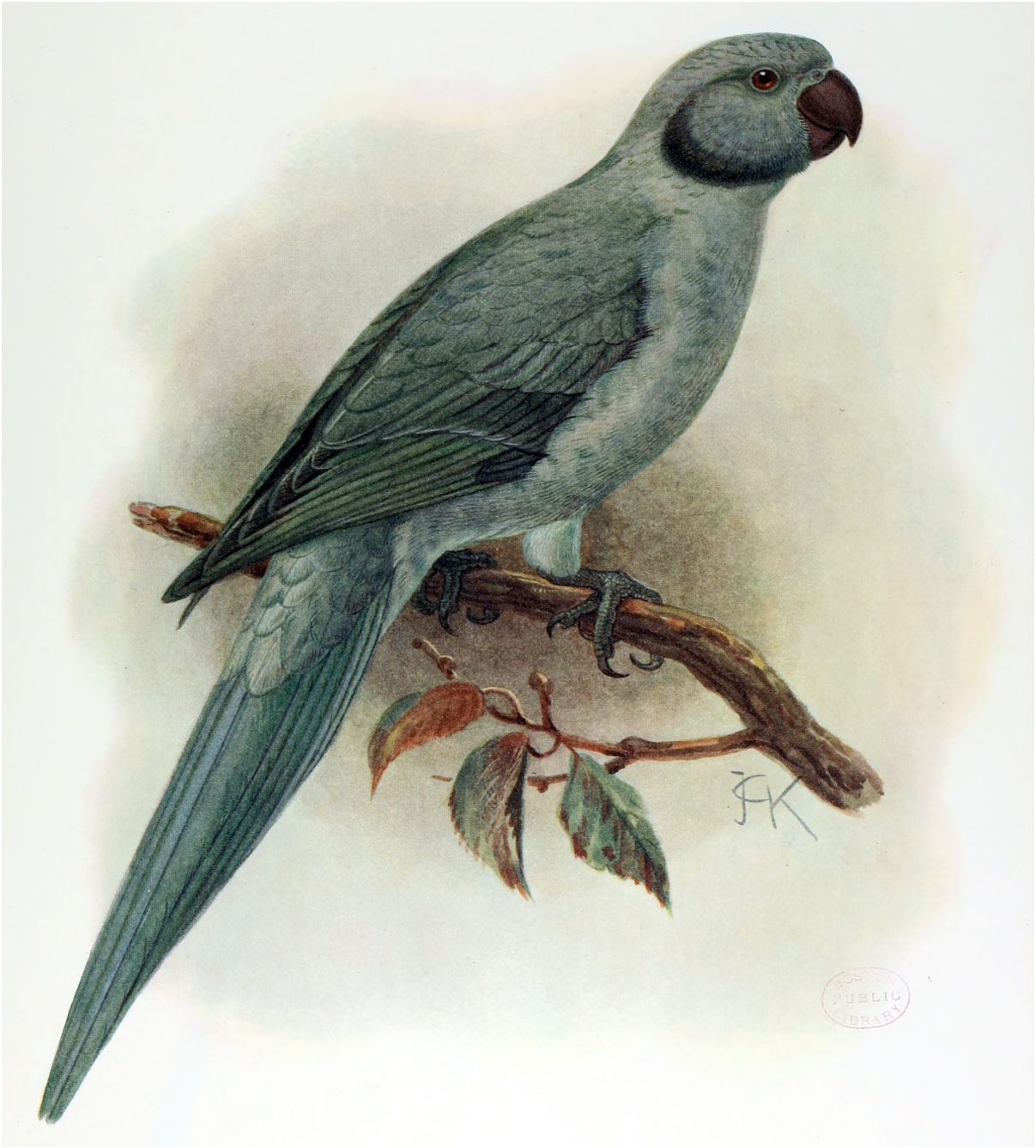

The extinct Rodrigues parakeet, *Psittacula exsul,*

Image by John Gerrad Keuleman 1875

Chapter 6.

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Micro-evolutionary diversification among Indian Ocean parrots; temporal and spatial changes in phylogenetic diversity as a consequence of extinction and invasion

Hazel Jackson, Carl G. Jones, Paul-Michael Agapow, Vikash Tatayah, & Jim. J. Groombridge

6.1 Abstract

More than 89% of global bird extinctions have occurred on islands. The loss of endemic species from island systems can dramatically alter evolutionary trajectories of insular species assemblages, resulting in a loss of evolutionary diversity important for species adaptation to changing environments. The Western Indian Ocean islands have been the scene of evolution for a large number of endemic parrots. Since their discovery in the 16th century many of these parrots have become extinct or have declined in numbers. Alongside the extinction of species, a number of the Indian Ocean islands have experienced colonisation by highly invasive parrots, such as the ring-necked parakeet. Such extinctions and invasions can, on an evolutionary timescale, drive changes in species composition, genetic diversity, and turnover in phylogenetic diversity, all of which can have important impacts on community-level adaptation to changing environmental and climatic conditions. Using mtDNA cytochrome *b* data we resolve the taxonomic placement of three extinct Indian Ocean parrots: the Rodrigues, Seychelles and Reunion parakeets. This case study quantifies how the extinction of these species has resulted in lost historical endemic phylogenetic diversity and reduced levels of species richness, and illustrates how it is being replaced by non-endemic invasive forms such as the ring-necked parakeet. Finally we use our phylogenetic framework to identify and recommend a number of phylogenetically appropriate ecological replacements for the extinct parrots, for introduction once invasive forms have been cleared, in order to rejuvenate ecosystem function and restore lost phylogenetic diversity.

6.2 Introduction

Ecological communities are experiencing simultaneously elevated rates of extinction and invasions by non-native species as human activities continue to affect biodiversity on a global scale (Cassey *et al.* 2007; McKinney 2006). As a consequence of these dual processes, biotic homogenisation (the increase in the taxonomic similarity of biotas over time; Olden 2006) can disrupt the net biological distinctiveness and diversity of a region by replacing unique endemic species with already widespread non-indigenous species (McKinney & Lockwood 1999). In particular, extinctions and invasions can have detrimental consequences on endemic taxa in oceanic island ecosystems, which are a rich source of evolutionary diversity (Whittaker & Fernandez-Palacis 2007). The isolated nature of these environments means endemic species are acutely vulnerable to extinction as a consequence of habitat loss, predation by introduced mammals, introduced disease and other human impacts on islands (Steadman 1995; Blackburn *et al.* 2004; Frankham 2005). Indeed, human activities have resulted in the extinction of as many as 2000 bird species across the Pacific islands (Steadman & Martin 2003; Boyer 2008), and these extinctions are resulting in significant losses of ecological function and functional diversity (Boyer & Jetz 2014). The loss of such endemic island species can dramatically alter evolutionary trajectories of species assemblages as a result of reduced species interactions (Mooney & Cleland 2001; Rosenzweig 2001). In this way, extinctions and invasions can disrupt species communities, affecting their composition, genetic and phylogenetic diversity (Olden & Poff 2003; Cassey *et al.* 2006). For example, high levels of endemic population genetic and phylogenetic diversity are important to allow adaptation to changing environmental and climatic conditions on an ecological and evolutionary timescale (Maherali & Klironomos 2007; Jump *et al.* 2009). In contrast, a disruption in the level of species diversity may result in a decreased capacity to adapt to environmental change (Olden & Poff 2003; Olden 2006). Fundamentally, extinctions and invasions may compromise the potential for future evolutionary diversification and persistence of endemic species assemblages (Day & Young 2004).

Phylogenetic diversity (Faith 1992; Crozier 1997) is a good predictor of ecological assemblage function (Flynn *et al.* 2011; Cadotte 2013; Jetz *et al.* 2014) and can be used to quantify these changes in community-level evolutionary diversification. Based on phylogenetic species assemblages, they are a measure of the evolutionary history of a group of taxa (Vane-Wright *et al.* 1991) and prioritise species or environments of high conservation value (Rodrigues & Gaston 2002; Jetz *et al.* 2014). Phylogenetic diversity can be used as a biodiversity measure and applied to a variety of conservation situations (Winter *et al.* 2013; Fenker *et al.* 2014; Pio *et al.* 2014). For example, phylogenetic diversity has been used to assess conservation value based on how much of the encompassing phylogeny of a species is preserved (Crozier 1997; Crozier *et al.* 2005) by describing evolutionary distinctiveness of a group of taxa (Faith 1992; Helmus *et*

al. 2007; Cadotte *et al.* 2010; Jetz *et al.* 2014). Broader multi-species approaches, which can embrace changes in evolutionary processes within a context of priority-setting for conservation, are also valuable because they can help to identify and preserve stable species communities which may have higher productivity and ecosystem function (Crozier *et al.* 2005; Thomasson *et al.* 2011; Rolland *et al.* 2012). Phylogenetic diversity has recently been applied to conservation strategies such as the Evolutionary Distinct and Globally Endangered (EDGE) programme (Isaac *et al.* 2007; Jetz *et al.* 2014), the evolutionary framework for biodiversity science, bioGENEIS (www.bioGENEIS.diversitias.org), and the Intergovernmental Platform on Biodiversity and Ecosystem Services, IPBES (www.IPBES.net). Phylogenetic diversity is also important in conservation management for assessing biodiversity change. The loss of phylogenetic diversity following human-mediated extinctions is often much greater than from natural random extinctions, as the entire network of unique evolutionary branches from which extinct species descend are lost (Purvis *et al.* 2000). Island systems such as the Pacific and Indian Ocean islands, are often subjected to human-induced extinctions and invasions, and as these events are often well documented (Steadman & Martin 2003; Cheke & Hume 2008), they provide an ideal framework for quantifying non-random changes in phylogenetic diversity over the past few hundred years.

The parrots (Psittaciformes) are one of the most endangered groups of birds in the world with 95 (26.8%) of the 354 known parrot species currently threatened with extinction, accounting for 2.4 billion years of global avian phylogenetic diversity (of 82.1 billion years total avian phylogenetic diversity; Jetz *et al.* 2014). Over the past 500 years approximately 163 avian extinctions have occurred across the globe, comprising some 20 parrot species (12%), half of which were island endemics (Collar 2000; Butchart *et al.* 2006). In this study we examine the Western Indian Ocean islands of Mauritius, Seychelles, Madagascar, Reunion, Rodrigues and Grand Comoros, which have been the evolutionary source for a large number of endemic parrot species (Hume 2007). These islands remained largely pristine until the 16th century (Hume 2007; Fig. 6.1.), resulting in numerous extinctions and invasions, driven predominately by human impacts such as habitat destruction (Synder *et al.* 2000; Cheke & Hume 2008). Subsequently, intense hunting and the introduction of predatory exotic mammals led to the extinction of many of the endemic parrots including the Reunion parakeet (*Psittacula eques*) which was last recorded in 1732, the Rodrigues parakeet (*Psittacula exsul*) by 1875, the Seychelles parakeet (*Psittacula wardi*) between 1881 and 1906, and the Mascarene parrot (*Mascarinus mascarinus)* from Reunion by the end of the 19th century (Hume & Waters 2012).

Alongside these extinctions, the islands have been colonised by invasive parrots. Invasive species are of global concern as they have detrimental impacts upon native species, ecosystems

and communities (Sakai *et al.* 2001; Allendorf & Lundquist 2003; Gurevitch & Padilla 2004). In particular, the invasive ring-necked parakeet (*Psittacula krameri)* is recognised as one of the top 100 worst invasive alien species in Europe (DAISIE, European Invasive Alien Species Gateway 2008). Native to Asia and Sub-Saharan Africa, these birds are a major agro-economic and environmental concern; they are a severe crop pest in their native range, decimating maize and fruit crops (Ramzan & Toor 1973; Forshaw 2010; Ahmad *et al.* 2012) and they also act as secondary cavity-nesters and compete with native species for nesting holes (Strubbe & Matthysen 2007; 2009a). Breeding populations of *P. krameri* have established in over 35 countries across five continents, where the species has become widespread with evidence of rapid population growth (Butler 2003; Butler *et al.* 2013). *Psittacula krameri* has invaded some of the Western Indian Ocean islands, including Mauritius where they compete with the endangered Mauritius parakeet (*Psittacula echo)* for nest sites and food resources (Tatayah *et al.* 2007; Jones *et al.* 2013). On Mauritius, *P. krameri* are a suspected source of Psittacine beak and feather disease (PBFD), caused by the highly infectious Beak and Feather Disease Virus which threatens the population of the endangered endemic *P. echo* (Kundu *et al.* 2012a). *Psittacula krameri* also occur on the Seychelles where their recent establishment (Jones *et al.* 2013) presents a potential disease threat to the endemic Seychelles black parrot, *Coracopsis barklyi* (Seychelles Islands Foundation, 2012).

As a consequence of the small number of museum specimens of the extinct endemic parrots from the Western Indian Ocean, there is taxonomic uncertainty surrounding their evolutionary affinities. For example, the taxonomic placement of *P. exsul* and *P. eques* within the Indian Ocean parrots has remained unresolved. *Psittacula exsul* was hunted to extinction by the mid-19th century and only two museum specimens remain, a female collected in 1871 and a male collected in August 1874 (Cheke & Hume 2008). Osteological characteristics suggest it shares a close relationship with other Mascarene species of *Psittacula* (Hume 2007). *Psittacula eques* had become extinct by 1770 and only one specimen is held, at the National Museums, Scotland, collected in 1750 (Hume & Waters 2012). A number of documents explicitly refer to *Psittacula eques*, however, this specimen is considered to be the only material proof of the existence of the Reunion island form and taxonomists remain unsure whether *P. eques* was a distinct species or conspecific with the endangered *P. echo*.

To date, there have been few attempts to quantify the historical loss of endemic phylogenetic diversity across a region and its insidious replacement by non-endemic invasive forms (Winter *et al.* 2009; Graham & Fine 2008). Here, we describe new molecular phylogenetic data for extinct and invasive *Psittacula* parrots and integrate them with existing data (Kundu *et al.* 2012b) into a detailed phylogenetic framework to quantify changes in phylogenetic diversity

over the past 260 years. Specifically, we (i) use mitochondrial (mtDNA) cytochrome *b* sequence to resolve phylogenetic affinities of the extinct *Psittacula exsul, Psittacula eques* and *Psittacula wardi*, (ii) determine whether *P. eques* warrants distinct species status or can be considered as conspecific to the extant *P. echo*, and (iii) examine the effect of these extinctions and invasions of parrots on phylogenetic diversity. We apply our findings to identify potential ecological replacement species for introduction onto appropriate Western Indian Ocean islands where historical extinctions have occurred in order to rebuild lost ecosystem function.

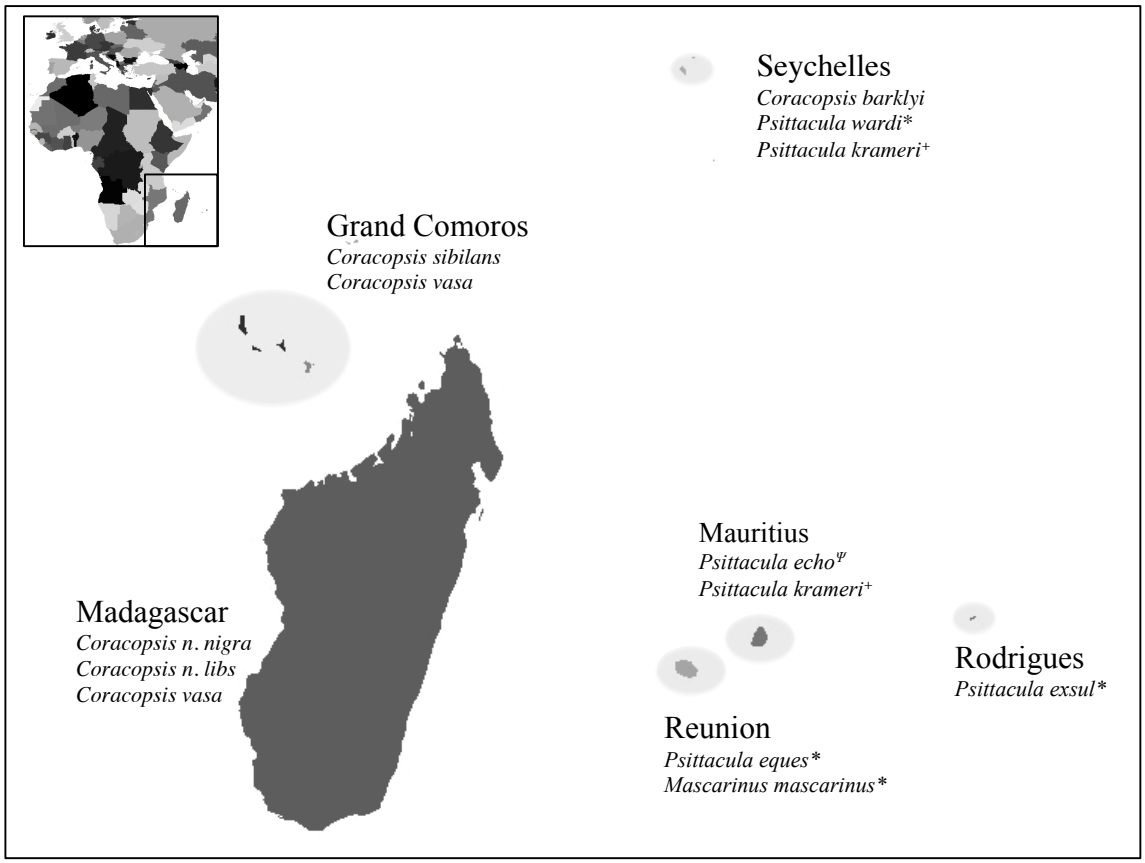

Fig. 6.1. Distribution of extinct*, invasive+, endangered endemic Ψ, and other endemic parrots across the Indian Ocean Islands. Small islands are highlighted with grey shading.

6.3 Materials and methods

6.3.1 Sample collection

Toepad samples for *P. exsul* and *P. wardi* were obtained from Cambridge Museum of Zoology. A toepad sample was obtained from *P. eques* from the specimen held at the National Museums Scotland. To establish whether *P. eques* was a distinct species, samples were obtained from

three historical *P. echo* museum specimens from the Natural History Museum in Tring and the Muséum National d'Histoire Naturelle in Paris for comparison. For invasive populations of *P. krameri*, contemporary blood specimens were obtained from Seychelles (*n*=2) and Mauritius (*n*=25) (Table 6.1.). We combined our data with cytochrome *b* sequences for other Indian Ocean parrots, including the extinct *M. mascarinus,* obtained from Genbank (Kundu *et al.* 2012b).

Table 6.1. Museum samples from which DNA was successfully extracted, along with two contemporary samples representing invasive ring-necked parakeet haplotypes. All three sampled individuals of the Mauritius parakeet produced a single identical haplotype, which was submitted to Genbank using sample CG1911 No 2114 as this individual produced the longest sequence.

Taxon	Common name	Source	Sample	Ref	ENA Accession No
Psittacula exsul	Rodrigues parakeet	Cambridge	Toepad	18/PSI./67/h/1	LN614516
Psittacula eques	Reunion parakeet	Edinburgh	Toepad	N/A	LN614517
Psittacula wardi	Seychelles parakeet	Cambridge	Toepad	18/PSI/67/g/1869	LN614515
Psittacula echo	Mauritius parakeet	Paris	Toepad	CG1911 No 2114	LN614518
Psittacula echo	Mauritius parakeet	Paris	Toepad	CG1936 No 1695	n/a
Psittacula echo	Mauritius parakeet	Tring	Toepad	90.10.10.7	n/a
Psittacula krameri manillensis	Ring-necked parakeet	Mauritius	Blood	N/A	LN614520
Psittacula krameri borealis	Ring-necked parakeet	Seychelles	Blood	N/A	LN614519

6.3.2 DNA isolation, amplification and sequencing

Processing of the museum specimens, including DNA extraction and polymerase chain reaction (PCR) amplifications were carried out in a laboratory dedicated to ancient DNA work, under a UV-irradiated fume hood to ensure no contamination. All equipment and surfaces were sterilised before and after each use by irradiation from UV light and with 10% bleach. Negative controls were included during the DNA extraction and PCR process and a selection of negative

extractions and PCRs were sequenced to ensure there was no contamination. DNA was extracted from both contemporary blood and historical toepad samples using a Bioline Isolate Genomic DNA extraction kit (Bioline, UK). Samples were suspended in 400 µl lysis buffer plus 25 µl proteinase K and incubated at 55^{o}C overnight (or until the material had completed digested). DNA was washed through a spin column and blood specimens were suspended in 200 µl of elution buffer while historical specimens were suspended in 40 µl of elution buffer.
Amplification from contemporary blood samples was conducted for cytochrome *b* using PKCBf and PKCBr (Table S6.1.**)**. PCR cycling conditions were 94^{o}C for one minute followed by 35 cycles of 94^{o}C/15 secs, 55^{o}C/15 secs and 72^{o}C/10 secs and a final elongation step of 72^{o}C for 10 minutes. For historical samples, amplification of cytochrome *b* was conducted using a suite of short overlapping primers (100-200 bp; Table S6.1**)**. PCR cycling conditions were 94^{o}C for one minute followed by 35 cycles of 94^{o}C/15 secs, 52^{o}C/15 secs and 72^{o}C/10 secs and an elongation step of 72^{o}C for 10 minutes. All amplicons were examined by agarose gel electrophoresis. Amplification volumes of 25 µl contained 1 µl template DNA from contemporary samples or 2 µl of template DNA from historical samples, 12.5 µl MyTaq HS Red Mix, containing dNTPs and $MgCL_2$ (Bioline, UK), 0.5 µl of each primer and 10.5 µl (contemporary PCR) or 9.5 µl (historical PCR) of dH_20. PCR product was purified and amplified using a 3730xl analyser (Macrogen Inc.). Sequences were edited in 4Peaks (Griekspoor & Groothius 2005) and aligned in Clustal (Larkin *et al.* 2007). Manual edits were made in Jalview (Waterhouse *et al.* 2009).

6.3.3 Phylogenetic analysis

Phylogenetic trees were reconstructed using Bayesian and maximum likelihood methods with *Falco* and *Gallus* as outgroups. PartitionFinder (Lanfear *et al.* 2012) was used to identify the best-fit models of nucleotide evolution according to Bayesian information criteria (BIC). Bayesian inference was implemented in MrBayes v3.2 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway (Miller *et al.* 2010) with 10 million generations over four parallel Monte Carlow Markov Chains (MCMC**),** under a HKY evolutionary model (Felsentein 1981). Tracer v1.6 (Rambaut & Drummond 2007) was used to assess convergence. After discarding the first 25%, tree topologies were summarised in a 50% consensus tree. A maximum likelihood search was conducted in RaxML (Stamatakis 2006). Ten independent runs were performed with 1000 non-parametric bootstrap replicates to obtain the best likelihood score under a GTAGAMMA model and summarised in a majority rule consensus tree. All trees were visualised in FigTree v1.4 (Rambaut 2012).

6.3.4 Molecular dating

Time-calibrated phylogenies were estimated using BEAST v.1.7.5 **(**Drummond & Rambaut 2007**)** using cytochrome *b* data. Given that relative to other bird families the fossil record for

parrots is poor (Mayr & Göhlich 2004), we combined our data with cytochrome *b* sequences for other Indian Ocean parrots obtained from Genbank and ran phylogenetic analyses by adopting a similar approach to Wright *et al.* (2008) using two alternative calibration dates for the origin of the parrots. The first calibration used was obtained from the oldest known fossil belonging to a crown group of parrots, *Mopsitta tanta,* dated to approximately 54 Mya in the Tertiary period (Waterhouse *et al.* 2008), while a second calibration of 80 Mya was obtained from a previous dating analysis of extant bird orders, suggesting a Cretaceous date for the divergence of parrots (Hedges *et al.* 1996). This calibration was given a normal distribution with a standard deviation of 10 Mya to ensure the 95% distribution (60.4 and 99.6 Mya) does not exceed the 100 Mya date for the divergence of bird orders (Hedges *et al.* 1996). An uncorrelated strict molecular clock model was used in preference to a lognormal relaxed molecular clock model as identified by Akaike information criterion (AIC) through MCMC (AICM) comparison of models (Baele *et al.* 2012) with a uniform distribution under the Yule speciation tree prior (Ho *et al.* 2007). MCMC was performed for 20 million generations with sampling every 1000 iterations. Convergence was confirmed by effective sample sizes (ESS) >200 for all parameters using Tracer v1.6 (Drummond & Rambaut 2007). Trees from the first 1000 generations were discarded as burn-in. A maximum clade credibility tree was summarised using TreeAnnotater v1.7.5 (Drummond & Rambaut 2007), visualised in Figtree v1.4 (Rambaut 2012), and edited in Inkscape (www.inkscape.org).

6.3.5 Genetic divergences

Net between-group mean genetic distances were calculated using MEGA 5.05 (Tamura *et al.* 2011) under the Kimura 2-parameter model (Kimura 1980) with gamma distribution of rates among sites. Standard error estimates were calculated using 500 bootstrap replicates.

6.3.6 Phylogenetic diversity

Phylogenetic diversity is a distance-based method that measures the phylogenetic information of a species assemblage by summing up the branch lengths of the subtree that includes the communities' species (Faith 1992). Branch lengths are indicative of molecular characteristics accumulated over evolutionary time (Schweiger *et al.* 2008), hence phylogenetic diversity was calculated using our time-calibrated phylogeny and is reported in millions of years (Myr). Phylogenetic diversity was calculated using the 'Picante' package in R (Kembel *et al.* 2010), for 1000 replications to obtain standard errors. Diversity metrics were calculated for the following three scenarios involving the inclusion of; (i) all (endemic) Indian Ocean island parrots, extinct and extant (referred to as 'historical PD'), (ii) the historic taxa less the four extinct species; ie, *P. exsul, P. eques, P. wardi* and *M. mascarinus* (referred to as 'lost PD'), and (iii) the extant *P. echo* and invasive *P. krameri* (referred to as 'current PD').

6.4 Results

DNA was amplified from one specimen of *P. exsul*, *P. wardi*, *P. eques* and three *P. echo* specimens. The DNA sequences obtained from the three Mauritius *P. echo* samples were identical and condensed into a single haplotype. The sequence data from invasive *P. krameri* on the Seychelles were identical and condensed into a single haplotype, while the sequence data derived from *P. krameri* sampled from Mauritius were collapsed into five different haplotypes. For the purpose of this study, the most common haplotype was chosen to capture prevalent levels of phylogenetic diversity within Mauritius *P. krameri*.

6.4.1 Phylogenetic reconstruction and molecular dating

A total of 1000 bp of cytochrome *b* was used for phylogenetic reconstruction, taxonomic placement and molecular dating of the extinct Indian Ocean parakeets. While this study is based on a single gene, the cytochrome *b* gene has been shown to produce phylogenies that are congruent at major nodes when compared to phylogenies built with other mitochondrial and nuclear genes (Faulkes *et al.* 2004), suggesting it is a robust choice of marker. Furthermore, we chose cytochrome *b* based on the availability of a large number of cytochrome *b* sequences for other Indian Ocean Parrots (Kundu *et al.* 2012b). Since the museum specimens were up to 260 years old, for some specimens only partial sequences (503-760bp) of the cytochrome *b* gene could be amplified (European Nucleotide Archive accession numbers LN614515-LN614520). Topologies reconstructed from Bayesian and maximum likelihood trees were largely congruent at all major nodes inferring a distinct and well supported phylogenetic structure of the Indian Ocean parrots (Fig. 6.2).

Assuming a calibration of 54 Mya, *P. wardi* clustered deep within the Alexandrine parakeet (*P. eupatria)* clade and diverged 3.9 Mya. *Psittacula exsul* clusters as ancestral to *P. eques* and *P. echo*, which all fall within the *P. krameri* clade. *Psittacula exsul* diverged 5.15 Mya, while *P. eques* and *P. echo* split from it 3.89 Mya. The invasive Seychelles and Mauritius *P. krameri* both cluster with their native counterparts from southern Asia (*P.k.manillensis* and *P.k.borealis*; Fig. 6.2.). When the calibration age is extended to 80 Mya, the divergence dates also move further back in time. The *P. wardi* divergence increases to 6.28 Mya and *P. exsul* is estimated to have diverged 8.29 Mya, while *P. eques* and *P. echo* diverged from *P. exsul* 6.26 Mya (Fig. S6.1.).

6.4.2 Genetic divergences

Table 6.2. gives the uncorrected nucleotide distances between Indian Ocean endemic parrots (extinct and extant) and invasive *P. krameri*. The highest observed divergences were between the *Psittacula* parakeets and Vasa (*Coracopsis)* parrots for which divergences ranged between

9.6% and 14.8%. The extinct *P. exsul* and *P. wardi* differ by 5.4%, while *P. exsul* and *P. echo* are closely related with only 2.9% difference. The extinct *P. eques* differs by 0.2% from historical *P. echo*. The invasive *P. krameri* found on Mauritius and the Seychelles differ by only 2.2-2.6% with *P. eques* and *P. echo*, and 3.5- 3.6% with *P. exsul*.

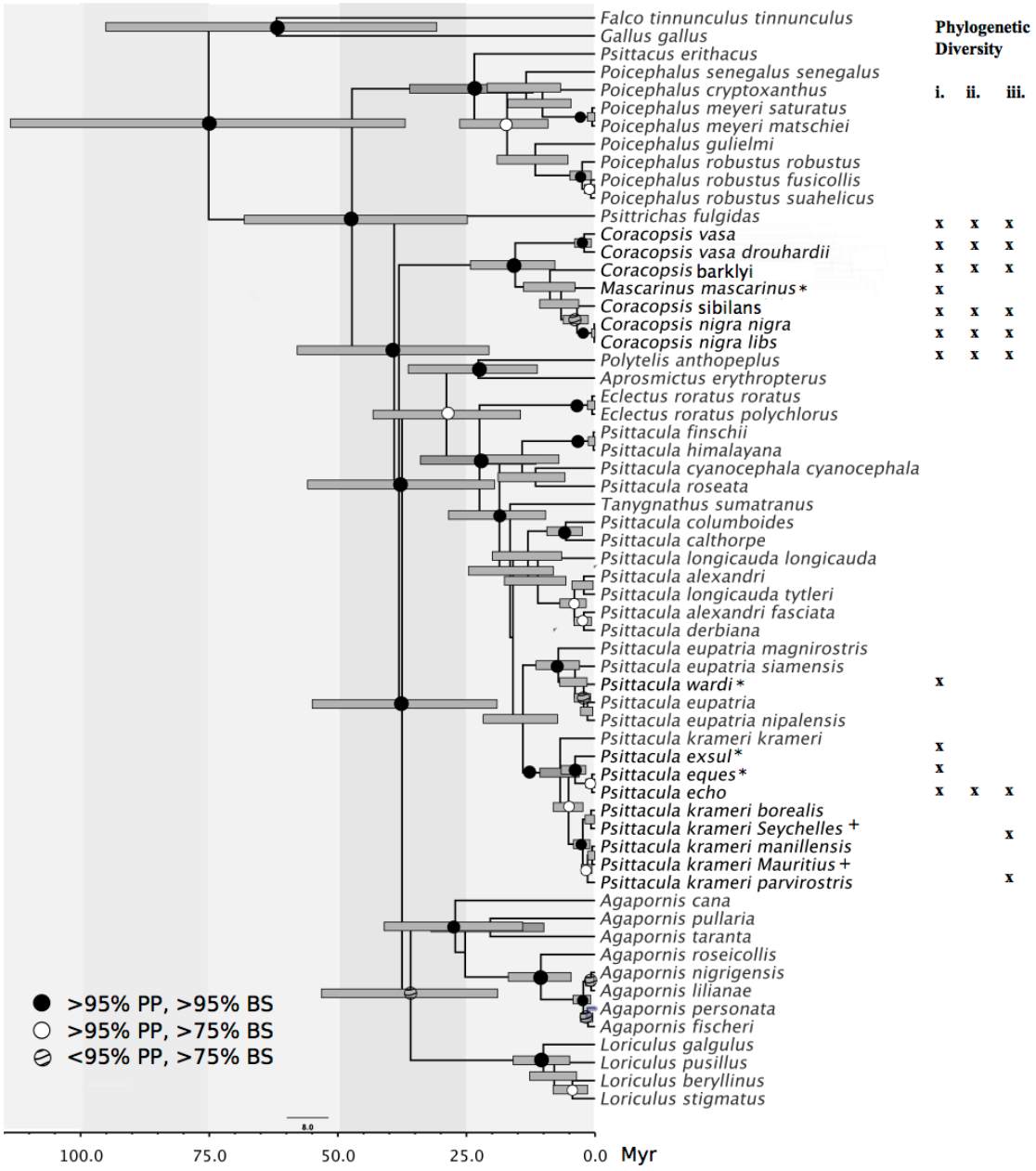

Fig. 6.2. Phylogenetic placement of extinct Indian Ocean parrots. Estimated divergence times resolved using BEAST with a specified TMRCA of 54 Mya. Error bars display the 95% HPD, and the axis is given in millions of years (Myr) before present. Black dots indicate nodes with Bayesian posterior probability (PP) >95% and maximum Likelihood boostrap support (BS)

>95%, white dots indicate >95% PP and >75% BS, striped dots indicate <95% PP and >75% BS. Node values both lower than 95% PP and 75% BS repsectively are not given. Crosses indicate which species are included within the different phylogenetic diversity scenarios including extinct*, invasive^{+}, and endangered endemic $^{\Psi}$ parrots.

Table 6.2. Uncorrected nucleotide distances between Indian Ocean parrot taxa. Extinct *, invasive[+], and endangered endemic [Ψ] parrots are included.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*C.vasa*		0.014	0.090	0.085	0.086	0.087	0.090	0.148	0.131	0.139	0.133	0.143	0.133
2	*C.v.drouhardii*			0.081	0.079	0.079	0.084	0.084	0.136	0.118	0.136	0.127	0.137	0.134
3	*C.barklyi*				0.035	0.038	0.039	0.041	0.124	0.100	0.103	0.096	0.128	0.117
4	*M.mascarinus**					0.036	0.038	0.045	0.111	0.096	0.099	0.097	0.117	0.111
5	*C.n.libs*						0.000	0.022	0.141	0.119	0.128	0.129	0.139	0.135
6	*C.n.nigra*							0.023	0.145	0.123	0.134	0.129	0.142	0.139
7	*C.sibilans*								0.148	0.116	0.126	0.127	0.133	0.131
8	*P.wardi**									0.054	0.052	0.051	0.064	0.065
9	*P.exsul**										0.020	0.029	0.036	0.035
10	*P.echo* [Ψ]											0.002	0.022	0.022
11	*P.eques**												0.022	0.026
12	*P.krameri Seychelles*[+]													0.007
13	*P.krameri Mauritius*[+]													

6.4.3 Phylogenetic diversity

Prior to the extinction of the four parrot species from these Indian Ocean islands, phylogenetic diversity was 168.00 million years (Myr) with a species richness value of 11. Following the extinction events, species richness was reduced to seven, with a concomitant reduced level of phylogenetic diversity by 14% to 143.28 Myr. The establishment of invasive *P. krameri* on Mauritius and Seychelles and their introduction of non-endemic phylogenetic diversity resulted in a net increase of 5% to 150.67 Myr. Species richness increased to nine (Fig 6.3.). When comparing changes over time on a spatial scale, the extinction of endemic parrots from Rodrigues and Reunion has resulted in a complete loss of phylogenetic diversity and species richness. On Rodrigues phylogenetic diversity has been reduced from 75.15 Myr to zero Myr, and species richness from one to zero. Similarly on Reunion, phylogenetic diversity has decreased from 113.65 Myr to zero Myr, and species richness from two to zero. The Seychelles has experienced a 33% reduction of phylogenetic diversity from 113.65 Myr to 75.25 Myr and species richness from two to one following extinctions. The introduction of ring-necked parakeets to Seychelles has increased phylogenetic diversity by just 6% to 80.29 Myr and species richness to two. Following the invasion of *P. krameri* on Mauritius, phylogenetic diversity increased by 6% from 75.25 Myr to 80.29 Myr and species richness from one to two (Fig.6.4.).

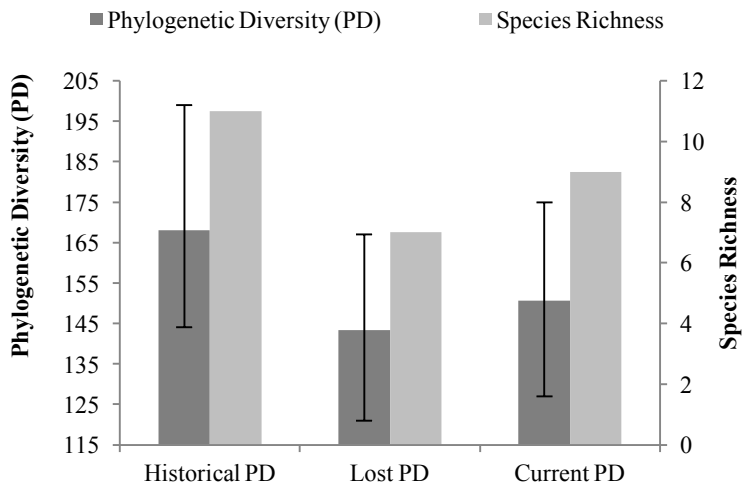

Fig. 6.3. Phylogenetic diversity (PD; dark grey columns, with standard error bars calculated over 1000 replicates) and species richness (light grey columns) for Indian Ocean parrots, under each of the three temporal grouping scenarios.

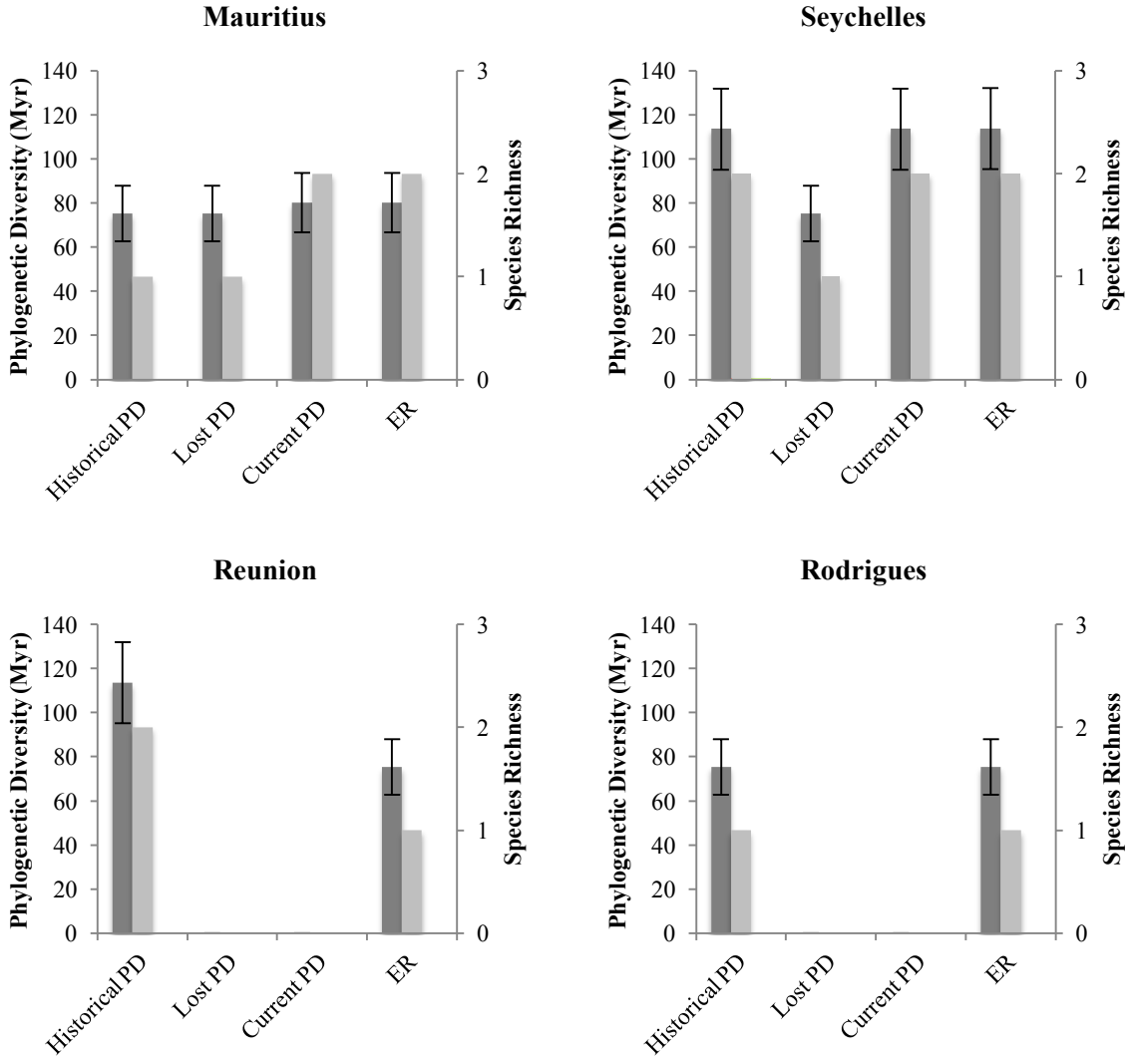

Fig. 6.4. Phylogenetic Diversity (PD; dark grey columns with standard error bars calculated over 1000 replicates) in Myr, and species richness (light grey columns), under each of the three grouping scenarios for four Indian Ocean islands that have experienced extinctions and invasions (Seychelles, Mauritius, Rodrigues and Reunion). An additional fourth scenario for the inclusion of ecological replacements 'ER' gives predicted levels of phylogenetic diversity and species richness as a result of the use of the Mauritius parakeet (*P.echo*) as an analogue on Rodrigues and Reunion, the use of the Alexandrine parakeet (*P.eupatria)* as an analogue on the Seychelles and the removal of invasive parakeets (*P.krameri)* from the Seychelles.

6.5 Discussion

This study has provided a resolution for the phylogenetic placement of the extinct *P. exsul*, *P. eques* and *P. wardi* within the Indian Ocean *Psittacula* parrot radiation. It has also quantified

the temporal and spatial effects on phylogenetic diversity due to historical extinctions and the insidious introduction of invasive parrots across the Western Indian Ocean islands.

6.5.1 Evolution of the Indian Ocean parrots

The molecular phylogenetic analysis suggests the extinct island parrots experienced recent divergences within their clades, implying the Indian Ocean islands have played a key role in the evolutionary radiation of *Psittacula* parakeets. *Psittacula exsul* diverged 5.15 Mya and *P. eques* diverged 3.89 Mya from their respective most recent common ancestor. *Psittacula wardi* groups within the Alexandrine parakeets (which originate from Asia) and diverged 3.9 Mya, while *P. exsul* and *P. eques* group with *P. krameri* (native to Asia and Africa). While islands are usually colonised from their nearest mainland source, a high proportion of biota found across the western Indian Ocean islands show affinities with Asia rather than the African continent (Warren *et al.* 2010). The low sea levels over the previous 10 million years may have facilitated radiations by 'island-hopping' from Asia towards Madagascar, allowing colonisation of the Indian Ocean islands (Cheke & Hume 2008; Warren *et al.* 2010).

The close phylogenetic relationship and low but detectable nucleotide divergence between the single specimen of the extinct *P. eques* and the extant *P. echo* (0.2%) suggest that these island populations had evolutionarily diverged, but the low level of divergence suggests it is likely the populations on Reunion and Mauritius were only divergent at a sub-specific level. Comparable levels of nucleotide divergence are seen between some of the species of *Coracopsis* black parrots of the Indian Ocean; within this genus values range from 0.28% between the sympatric *C.n.libs* and *C.n.nigra* found on Madagascar, and 1.79-4.29% between them and *C. sibilans* on Grand Comoros and *C. barklyi* on Seychelles, although recent accounts describe the Madagascan subspecies as a single species and the Grand Comoros and Seychelles forms as separate species (del Hoyo *et al.* 2014; Jackson *et al.* submitted).

6.5.2 Historical and spatial changes in phylogenetic diversity

Losing evolutionarily divergent taxa can result in phylogenetic homogenisation of species assemblages. Such losses of unique phylogenetic and taxonomic information may have detrimental impacts upon the capability of species' assemblies to respond to changing environments, leaving an impoverished and more homogeneous global biota (Webb *et al.* 2001, Winter *et al.* 2009). Our study has demonstrated how the extinction of four endemic parrot species and establishment of the invasive *P. krameri* has resulted in biotic homogenisation across the Indian Ocean islands, reflected by the overall decrease of parrot species assemblage. In the last 500 years, a majority (>89.3%) of the 163 documented avian extinctions have occurred on islands, with large numbers of recent avian extinctions occurring on Mauritius

(n=18) and Reunion (n=11). Such islands have recently been highlighted as effective priority areas for the conservation of evolutionary distinctiveness and phylogenetic diversity (Jetz *et al.* 2014). Parrots have suffered a high number of extinctions (Butchart *et al.* 2006), of which half were endemic parrots from islands (Collar 2000). Our study demonstrates that the extinction of just four island forms of parrot across the Western Indian Ocean islands has resulted in a 14% loss (25 Myr) in phylogenetic diversity, with a complete loss of phylogenetic diversity in this group on the islands of Reunion and Rodrigues, suggesting that global parrot assemblages may have experienced substantial reduction in phylogenetic diversity from the documented extinction of 20 species of parrot, many of them from island systems.

Our phylogenetic analysis suggests invasive *P. krameri* found on Mauritius and Seychelles originate from southern Asia and comprise two subspecies; *P.k.borealis* (introduced on Seychelles) and *P.k.manillensis* (introduced on Mauritius*)*. This establishment of invasive *P. krameri* on Mauritius and Seychelles has replaced lost endemic phylogenetic diversity with non-endemic forms, which are representatives of a globally widespread continental form (Frankham 1997).

6.5.3 Using ecological replacements to restore lost parrot diversity

More recently, ecologists have begun to embrace evolutionary perspectives based upon the idea that closely related species are ecologically similar (Losos 2008). Our phylogenetic framework provides an opportunity to use evolutionary information to inform long-term conservation efforts. Using ecological replacements to replace extinct species is a conservation tool used to restore lost ecological function in disrupted ecosystems (Griffins *et al.* 2013; Hunter *et al.* 2013). This approach involves deliberately introducing a species into an environment to fill an ecological niche formerly occupied by a now extinct species (Donlan *et al.* 2006; Griffiths *et al.* 2010). Ecological replacements are generally considered to be acceptable where the benefits of their expected ecological function outweigh the potential risks of them becoming detrimental to the ecosystem (Parker *et al.* 2010; IUCN/SSC 2013), for example by introducing unintended pathogens or becoming an invasive species. Despite these risks, the use of ecological replacements as a conservation management strategy has proven successful, for example the Aldabra giant tortoise (*Aldabrachelys gigantea)* has been introduced to a number of offshore islands in Mauritius to successfully refill herbivory and seed-dispersal niches left vacant by the extinction of endemic Mauritian tortoises (Griffiths *et al.* 2010; 2011).

The extinct parrots of the Western Indian Ocean, in particular *P. exsul*. *P. wardi* and *P. eques*, represent phylogenetic diversity within *Psittacula* which is irreplaceable. However, our molecular phylogeny can inform the initial identification of the most closely related extant taxa

that might form appropriate candidates. Such phylogenetically close species may exhibit patterns of phylogenetic niche conservatism (the tendency of taxa to retain ancestral niche-related traits over macro-evolutionary time: Wiens *et al.* 2010; Crisp & Cook 2012), having experienced allopatric speciation. Such divergence constraints on ecological traits between closely related species may enable successful introductions of ecological replacements into ecologically similar environments, however introductions into contrasting environments are likely to be unsuccessful as species are unable to adapt to their new environments (Losos 2008; Crisp & Cook 2012). Evolutionarily proximate species, which may have diverged as a result of strong ecological influences, are therefore less likely to be appropriate ecological replacements. The identification of such appropriate candidates for introduction on to these islands as ecological replacements may help restore ecosystem function (Griffin *et al.* 2013; Hunter *et al.* 2013) and, on an evolutionary timescale, enable endemic phylogenetic diversity to re-evolve *in situ* (potential increases in phylogenetic diversity and species richness for each island are given under our ecological replacements scenario; see Fig.6.4.).

In this way, our phylogeny identifies the most evolutionarily appropriate ecological replacement candidate for *P. exsul* and *P. eques* as being the extant *P. echo*, given that this species is the last-remaining island representative of the *P. exsul/P. echo/P. eques* phylogenetic lineage. *Psittacula echo* was the world's rarest parrot in the 1980s when the total population consisted of fewer than 20 individuals prior to an intensive conservation management programme which restored the species' wild population to over 500 individuals by 2010 (Raisin *et al.* 2012; Tollington *et al.* 2013). Establishment of populations of *P. echo* on Rodrigues and Reunion, by way of a conservation introduction, could therefore help to secure the short-to-medium term future of this recently restored parrot population while at the same time providing phylogenetically appropriate material for longer-term evolutionary forces to act upon to return an endemic parrot form to those islands.

The introduction of endemic *P. echo* from Mauritius to Reunion and Rodrigues would likely reactivate the ecological roles that the extinct parakeets had within their ecosystems. There is accumulating evidence that there has been co-evolution on Mauritius between some of the endemic trees and the endemic parrots that fed on their fruit. Many canopy trees produce fruits that are dispersed by fruit bats (Cheke & Hume 2008) and parrots (Jones *et al.* 2013). *Psittacula echo* feeds on the fruit of canopy trees with a fleshy epicarp and very hard seeds. This parrot eats the epicarp and then discards the seeds thereby acting as probable dispersal agent. Introducing *P. echo* to Reunion and Rodrigues as an ecological replacement would likely rejuvenate this function (Jones 1987; Jones *et al.* 2013).

Our phylogeny also suggests *P. eupatria,* from Southern Asia, as a phylogenetically appropriate potential ecological replacement for the extinct *P. wardi* on Seychelles. However, in contrast to *P. echo*, which has phylogenetic affinities to the extinct Indian Ocean parrots of Reunion and Rodrigues, and has evolved within an island ecosystem, *P. eupatria* originates from the Asian mainland and may therefore bring risks associated with invasiveness because of the fact that the worst invasive species tend to be continental forms (Blackburn *et al.* 2009). Ideally, ecological replacements ought to be selected for their ecological and evolutionary similarity to the extinct species they are replacing, in order to reduce the possible unwanted risks that could accompany such introductions (Seddon & Soorae 1999, Parker *et al.* 2010). For example, evolutionarily close existing island forms should replace extinct island species. Here, our phylogeny indicates that the most suitable candidate may be *P. e. magnirostris* from the Andaman Islands, which is basal within the *P. eupatria* clade. Our phylogenetic framework has addressed the evolutionary component of this issue, but clearly detailed ecological studies would be required to further refine the choice of any ecological replacement.

The invasion of *P. krameri* from Southern Asia across the Indian Ocean presents a concern for conservationists. *Psittacula krameri* poses a serious threat to the surviving endemic parrot species in the Indian Ocean; they are currently being controlled on the Seychelles (Seychelles Islands Foundation 2012) while the populations on Mauritius are more widely established and, as with many invasive bird populations, present a longer-term challenge. Elsewhere, *P. krameri* are known to be a crop pest across large parts of their native and invasive range (Ramzan & Toor 1973; Forshaw 2010, Ahmad *et al.* 2012). Therefore local communities on Rodrigues who grow subsistence maize crops may be justifiably apprehensive about the purposeful introduction of the endangered *P. echo* as an ecological replacement given that it looks very similar to *P. krameri* and might therefore be anticipated to behave like *P. krameri* when introduced to a new environment. Our phylogenetic framework however, lends support from an evolutionary perspective to the idea of using an endangered species from a neighbouring island as an ecological replacement, a concept which is relatively novel but is gaining wider acceptance in modern ecological restoration (Hansen 2010, Griffiths 2010).

6.6 Conclusions

The Indian Ocean islands are an important source of endemic species that contribute substantially to global biodiversity (Whittaker & Fernandez-Palacios 2007). The extinction of endemic species from islands results in a loss of historical phylogenetic diversity and reduced levels of species richness. The arrival of invasive alien species replaces lost phylogenetic diversity with non-endemic diversity represented by globally widespread continental forms. Phylogenetic frameworks can inform conservation strategies such as the use of ecological

replacements to restore island ecosystems. On an evolutionary timescale these conservation initiatives may result, through natural selection, in the evolution of novel island forms and the restoration of lost phylogenetic diversity (Cadotte *et al.* 2009, Gravel *et al.* 2012).

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6.8 Supplementary material

Table S6.1. Suite of short fragment (150-200 bp), overlapping PCR pri.mers designed for historical specimens, and contemporary specimen PCR primers to amplify approximately 1000 bp of cytochrome *b*.

Primer	Sequence (5' -> 3')
Historical specimen primers	
Mcb1a – F	CTACCATTCATAATCACCAGCC
Mcb1a – R	GTGAGGGAGAGGAGTATGATAG
Mcb2 – F	CTATCATACTCCTCTCCCTCAC
Mcb2 - R	TAGGATCAGTACGGAGGCAG
Mcb3 – F	AACAACTCCCCCACACATC
Mcb3 – R	CGGCGAGTGTTCAGAATAG
Cb99-238F	CCACTACACCGCAGACACC
Cb99-238R	GGGCGATGTGGAGGTAGATG
Cb114-258F	AACCTACATGCAAACGGAGC
Cb114-258R	CGAAAGCGGTTGCTATGAGG
Cb247-397F	CAACCGCTTTCGTTGGCTAT
Cb247-397R	AAGGTGGGGTTGTCTACGGA
Cb250-426F	CCGCTTTCGTTGGCTATGTC
Cb250-426R	GGAGGAAGTGTAGGGCGAAG
Cb359-521F	ATGAGCCTGAGGCGGATTCT
Cb359-521F	GTCGCAGTTTGATGGGATGC

Cb662-743F	CCCTTGCCCTATTCTCACCC
Cb662-743R	ATGTGCGGGGGAGTTGTTAG

Contemporary specimen primers

PKCBf	CGGCCTACTCCTAGCCGCCC
PKCBr	GGGAAGCAGGCCGGAAGGC

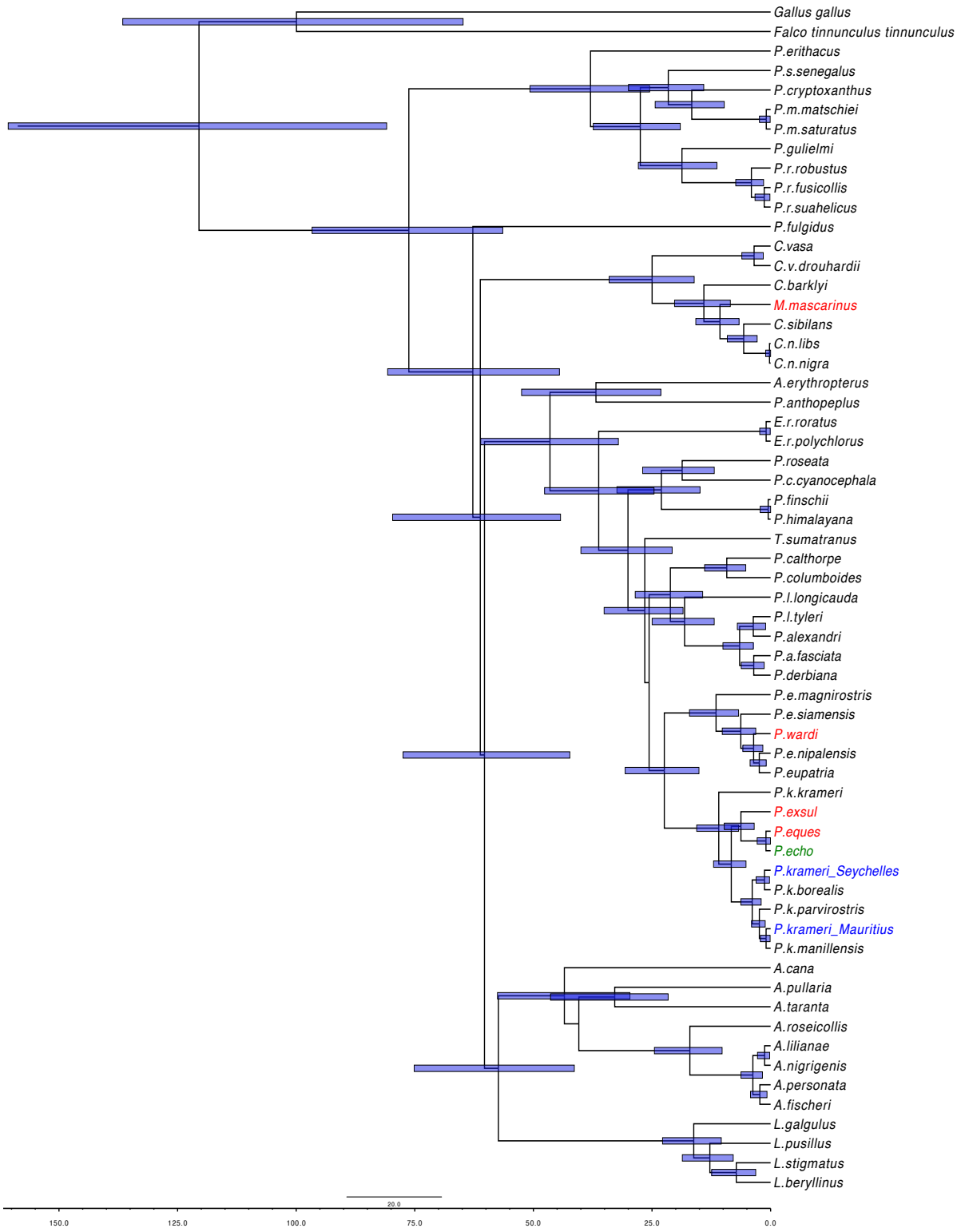

Fig. S6.1. Estimated divergence times derived from cytochrome *b* mtDNA data obtained for museum specimens for extinct *P.exsul, P.wardi* and *P.eques.* Sequence data were analysed alongside existing sequences for other Old World parrots (*Psittaciformes*) from Kundu *et al.* (2012), and resolved using BEAST with a specified TMRCA of 80 Mya. Node error bars display the 95% HPD and the axis is in millions of years before.

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Ring-necked parakeet on a bird feeder in Chiswick, London

Photo by Andrew Hodson

Chapter 7.

General discussion

7.1 Patterns of evolution and genetic diversity

Understanding patterns of evolution and genetic diversity in both invasive and endemic species is important for conservation management at a population level and on a broader scale. The phylogenetic reconstructions within this thesis highlight how information on evolutionary patterns can be applied to conservation management strategies. Observed patterns of evolution provide useful insights into the diversification of invasive species, and an understanding of invasion pathways. The identification of evolutionary phylogroups has now been incorporated into ecological niches models, improving predictions of areas suitable for future invasions. This fundamental understanding of invasive species evolutionary history is also important for future examination of signals of evolutionary change and adaptation to their new environments. The phylogenetic reconstructions on endemic and extinct species within this thesis have, in combination with evidence of genetic and morphological differences, not only informed conservation management action plans, but have importantly led to the reclassification of the Seychelles black parrot to full species status. This is a successful outcome that provides justification for directing conservation management efforts towards this evolutionarily distinct species.

As the raw material needed for evolutionary change, measuring genetic diversity is of high importance for establishing the genetic health and status of wild populations and communities. Conservation management must include strategies to monitor levels of genetic diversity and maintain genetically viable populations whilst determining levels of genetic diversity within invasive populations is important to understanding how such populations thrive in novel areas. The increasingly common use of mitochondrial markers in conservation management, demonstrates the important role of such markers for answering questions about the genetic structure of wild populations. Surprisingly however, our results show that not all commonly used measures of genetic diversity are useful for making inferences about the demographic history of wild populations. With this in mind, conservation genetics studies should consider which genetic diversity measure is most suitable for testing their chosen hypothesis. In using such genetic markers (both mtDNA and microsatellites) to obtain measures of genetic diversity, endemic species and invasive species demonstrate different patterns and responses to small population sizes. The endemic Seychelles black parrot has suffered a loss of genetic diversity over time as a result of a substantial population decline, and may be at risk of extinction as a result of genetic problems associated with being a small island population with low levels of

genetic diversity (Frankham 1997, 2005). In contrast, despite predictions of low levels of diversity and problems associated with founding events, populations of invasive ring-necked parakeets in Europe exhibit high levels of genetic diversity, comparable with levels observed from their native ranges, increasing the likelihood of successful establishment in novel environments (Roman & Darling 2007; Dlugosch & Parker 2008).

While single species approaches are commonplace, the novel examination of phylogenetic diversity within this study demonstrates how understanding patterns of genetic diversity on a broader scale are becoming important for conservation genetics on a community or regional scale (Flynn *et al.* 2011; Meynard *et al.* 2011; Peralta *et al.* 2014). Phylogenetic diversity is an indicator of ecological assemblage function and has recently been recognised as an important measure to identify and prioritise areas of high biodiversity value (Jetz *et al.* 2014). By examining phylogenetic diversity on a temporal and spatial scale it can be seen that the extinction of endemic parakeets from the Western Indian Ocean islands has resulted in lost phylogenetic diversity and reduced levels of species diversity. The loss of such endemic evolutionary diversity can reduce remaining extant species adaptive potential in response to changing environments. This lost phylogenetic diversity is now being replaced by a single invasive species, ultimately leading to taxonomic homogenisation in a once very diverse region.

7.2 Understanding successful invasions

Understanding genetic factors that underpin successful invasions is vitally important for the management of invasive species, and prevention of future invasions. Obtaining evolutionary insights into the genetic structure of invasive species may reveal how they respond to novel environments, by way of adaptation or plasticity (Lee 2002). Such information is important to identifying factors that play an important role in facilitating invasion success. Species that become invasive go through a multi-stage invasion process (Blackburn *et al.* 2013). Although the large majority of species fail to establish in novel environments during this invasion process, a few succeed. To fully understand the process of becoming invasive, it is important to examine each stage including transportation, releases or introductions, establishment, spread and becoming a pest or an 'invasive' species. Initially, establishing source populations for invasive species is important for understanding routes of invasions and evolutionary adaptations of invasive species that enable them to establish in novel environments (Blackburn *et al.* 2009). Prior to the ban on the trade of wild birds in 2007 (European Commission Regulation (EC) No.318/2007), trade records show ring-necked parakeets were imported all across Europe from both the Asian and African ranges (CITES 2014). Interestingly, a high degree of mixed ancestral origins (comprising all four subspecies) was therefore anticipated, however, invasive ring-necked parakeets across Europe and the Indian Ocean predominately originate from their

Asian range. While observed levels of imports from the native range are likely to have influenced these signatures of ancestral origins within invasive populations (proportionally higher numbers of birds were imported from Asia), the observed patterns can also be explained by the prevalence of mtDNA haplotypes within the invasive populations that are characterised by a lower cold niche limit from the native range. This association with colder parts of the native range may have preferentially assisted the successful establishment of ring-necked parakeets in colder areas of their invasive range, with parakeets with warmer niche limits failing to survive in novel environments. These findings highlight the role of human-mediated transport in facilitating invasions (Meyerson & Mooney 2007; Hulme 2009), but also demonstrates how identifying ancestral origins of invasive species enables an examination of the importance of patterns of climate matching between native and invasive ranges for invasion success (Shwartz et al 2009; Strubbe et al, submitted). Additionally, the importance of climate matching was further highlighted as population growth rates of European ring-necked parakeets were driven by the availability of suitable habitat, based upon patterns of climate and niche structure predictions from the native range (such as temperature and precipitation). Interestingly, no relationships were observed between population growth rates and genetic diversity. It is likely, however, that this is due to the lack of variation in genetic diversity among invasive populations. Furthermore, it would be very difficult to tease apart whether any variation in genetic diversity and growth rates was a cause or consequence of associated bottleneck effects.

Classic invasion biology predicts that new populations, founded with a low number of individuals should be genetically impoverished and struggle to establish. However, invasive species appear to avoid these issues. Recent research suggests multiple or repeat introductions help resolve this genetic paradox (Frankham 2004; Roman & Darling 2007), however the true number of individuals released that genetically contribute to an invasive population is often unknown or difficult to accurately infer due to a lack of observations, records or field data. Therefore our novel method to to accurately estimate true bottleneck effects from genetic data is important for understanding how invasive populations avoid detrimental impacts associated with a small founding population. Genetic bottleneck effects in a number of invasive populations of parakeet in Europe were generally milder than the severe bottleneck effects expected from demographic records. In addition to mild bottleneck effects, no gene flow was detected amoungst European populations. These findings, in combination with high levels of genetic diversity within each population compliment a growing body of literature indicating that multiple 'top-up' introductions play a strong role in invasion success (Cassey *et al.* 2004; Lockwood *et al.* 2005; Dlugosch & Parker 2008; Signorile *et al.* 2014).

7.3 Conservation genetics informing conservation management and policy

Conservation management approaches often focus on endemism and species with restricted ranges, however, the conservation of evolutionary distinctiveness and genetic diversity at a species level but also on a broader regional or global scale, should also be prioritised to prevent homogenisation (McNeely *et al.* 1990; Faith 1992; Witting & Loeschcke 1995; Crozier 1997; Isaac 2007; Villegér *et al.* 2008; IUCN 2012). Conservation genetics can identify important taxonomic units or genetically impoverished populations for prioritisation under conservation management plans. The genetic techniques applied in this research on endemic parrots can continue to be applied to monitor the future genetic health and viability of such populations. Continued studies are important to ensure endemic species do not suffer any additional detrimental impacts from the population bottleneck, and low levels of diversity, while avoiding increased levels of inbreeding. The new species status of the Seychelles black parrot is important for conservation management to focus efforts on preserving this evolutionarily distinct parrot, one of the few remaining in the Indian Ocean Islands. Additionally reconstructing phylogenies by incorporating sequence data from extinct species can be used to inform conservation strategies, to reinvigorate lost phylogenetic diversity and ecosystem function, while providing new habitat to encourage population growth and recovery endemic endangered species. The use of such evolutionary information must be in combination with ecological studies, but may be an important tool for conservation management strategies planning the restoration of novel island species and lost phylogenetic diversity.

The identification of drivers of successful invasions is important to inform policy and conservation management. Globalisation of trade has led to the estalishment of large numbers of invasive species around the world. As a major driver of biodiversity loss and often a cause of damage to economics and human health (Keller *et al.* 2011), there is an increasingly urgent need to create and implement policies to reduce the transport and release of non-native species while managing those that have already become invasive. In 2007 the European Union introduced a ban on the importation of wild birds (Commission Regulation (EC) No.318/2007), however prior to this over 158,000 ring-necked parakeets (just one of the 16 non-native species of parrot now established across Europe), were imported into Europe. Such bans fail to address the continued breeding of such species that are popular as pets, within European countries. In response to the lack of measures to deal with invasive species in Europe, the European Commission have recently adopted a Regulation on the prevention and management of the introduction and spread of invasive alien species (Regulation (EU) No.1143/2014). Importantly, our evidence of continued releases of individuals into the wild populations of invasive parakeets, demonstrates the need to incorporate measures within this or new policy to control or prohibit the commercial breeding and owning of pets that are invasive species, as seen in Spain

where it is now illegal to keep ring-necked parakeets as pets (Regulation: Real Decreto 630/2013). Furthermore, the inclusion of genetic data into ecological niche models now provides a robust model for predictions of areas suitable for invasion. Such models should be utilised to ensure measures are included in policy and management to prevent the introduction and spread of non-native species in areas predicted to be highly suitable for invasion.

7.4 Closing remarks and future research

This thesis has examined the evolution and population genetics of invasive and endemic parrot species, to highlight differences in genetic responses that are important for evolutionary adaptation to a changing world. This research has provided a deeper understanding of such responses, that enable some species to thrive in novel environments despite experiencing population bottlenecks, or founding events, while other species suffer from reduced levels of genetic diversity and require intensive conservation efforts. Biological invasions are indeed a highly significant problem for conservation management, acting as a cause of major biodiversity loss on a global scale. The importance of identifying source populations, pathways of invasion, multiple introductions, drivers of population growth, patterns of climate matching, alongside an understanding of the genetic structure of invasive populations, is essential for constructing sound management strategies, informing policy makers towards dealing with invasive alien species and to prevent further invasions. Further research is now needed to understand what mechanisms facilitate invasion success by examining how species respond to their new environments. The use of common garden experiments, genome wide studies and epigenetics to test whether colonising species experience rapid adaption following introduction to a novel environment, will be useful towards understanding whether invasive species experience genetic adaptation or phenotypic plasticity.

An interesting direction for future research is to identify signals of adaptation in invasive parakeets. The ability to respond to selection by evolutionary adaptation may be an important mechanism allowing species to establish in non-native environments. By comparing measures of genetic differentiation (F_{ST} and Q_{ST}) in invasive populations, signals of adaptation can be detected (if $Q_{ST} > F_{ST}$: Leinonen *et al.* 2013). Similar signals of adaptation can be obtained from morphometric data, to combine and corroborate genetic data. Additionally evolutionary and genetic data on invasive parakeets can be applied to different types of modelling, such as genetic dissimilarity modelling (GDM). The use of combined genetic and morphological data in GDMs can indicate how native range climate influences population structure, and can then be extrapolated onto the invasive range to identify similarities and explanations for observed patterns in invasive populations. Physiological niche models (PNM) are much more complex and can elucidate factors underlying invasion success and traits under selection. Such models

incorporate physiological data, morphological data and genetic data in the form of genetic sequence data on genes under selection, MHC genes to identify immune function and disease resistance and data from stable isotopes to identify what invasive parakeets eat, especially during winter to survive. New methodologies and tools in conservation genomics are now becoming more available and the use of next generation sequencing techniques will enable significantly larger datasets to be obtained, and improve the ability to detect signals of adaptation at specific loci.

Our novel recommendation of the use of phylogenetics to identify closely related species (on an evolutionary timescale) for use as ecological replacements, requires further research into the ecological suitability of our suggested species, in particular the use of the Mauritius parakeet for translocation onto Rodriguez and Reunion. In addition to ensuring there is suitable habitat provisions, and that local communities have been consulted to address any concerns, further genetic data will be useful to identify suitable individuals for translocation. To increase their chances of survival, choosing unrelated, genetically diverse individuals is essential to avoid unwanted genetic problems such as inbreeding, low levels of genetic diversity, and increased mortality rates.

Despite recent criticisms that the field of invasion science is too restricted in its nature (to just the study of non-native species: Valery *et al.* 2013), this multidisciplinary research is an important rapidly evolving field that draws insights from other scientific fields including palaeontology, immunology, human geography and history (Richardson & Riccardi 2013). The application of sophisticated techniques, such as the molecular genetics tools applied throughout this research, enable invasion scientists to evaluate new concepts for not only understanding invasions but also managing biodiversity, novel ecosystems and endemic species (Richardson & Riccardi 2013). This thesis has provided an in-depth understanding of population genetics that underpin successful invasions and inform conservation management of endemic species.

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Appendix 1.

Copy of a manuscript resulting from a collaboration during this PhD research. Genetic data from this thesis on evolutionary phylogroups of *Psittacula krameri* was incorporated into ecological niche models for predicting areas suitable for invasion.

Invasion success of a global avian invader is explained by within-taxon niche structure and association with humans in the native range.

Diversity and Distributions (2015) **21***,* 675-685

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Abstract

Aim To mitigate the threat invasive species pose to ecosystem functioning, reliable risk assessment is paramount. Spatially explicit predictions of invasion risk obtained through bioclimatic envelope models calibrated with native species distribution data can play a critical role in invasive species management. Forecasts of invasion risk to novel environments however remain controversial Here, we assess how species' association with human-modified habitats in the native range and within-taxon niche structure shape the distribution of invasive populations at biogeographical scales and influence the reliability of predictions of invasion risk.

Location Africa, Asia and Europe

Methods: We use ~1,200 native and invasive ring-necked parakeet (*Psittacula krameri*) occurrences and their associated data on establishment success in combination with mtDNA-based phylogeographic structure to assess niche dynamics during biological invasion and to generate predictions of invasion risk. Niche dynamics were quantified in a gridded environmental space while bioclimatic models were created using the biomod2 ensemble modelling framework.

Results: Ring-necked parakeets show considerable niche expansion into climates colder than their native range. Only when incorporating a measure of human modification of habitats within the native range do bioclimatic envelope models yield credible predictions of invasion risk for parakeets across Europe. Invasion risk derived from models that account for differing niche requirements of phylogeographic lineages and those that do not achieve similar statistical accuracy, but there are pronounced differences in areas predicted to be susceptible for invasion.

Main conclusions Information on within-taxon niche structure and association with humans in the native range can substantially improve predictive models of invasion risk. In order to

provide policy-makers with robust predictions of invasion risk, including these factors into bioclimatic envelope models should become standard practice.

Introduction

Biological invasions are a major global environmental and economic problem (Sala *et al.*, 2000). As eradication is frequently costly and sometimes impossible, attempting to limit the further introduction and spread of invasive species is the most effective and cost-efficient management strategy (Leung *et al.*, 2002). To identify potentially invasive species, risk assessment protocols based on species traits associated with invasiveness have been developed (Keller *et al.*, 2011). Spatially explicit predictions of invasion risk derived from bioclimatic envelope models calibrated with native species distributions are increasingly incorporated into such invasive species risk assessments (Beaumont *et al.*, 2014). To assess potential invasion risk, bioclimatic envelope models estimate the geographical distribution of climates suitable for invasive species (Araujo & Peterson, 2012). Applications of these models to invasive species however fail to consider how association with human-modified habitats in the native range, a species trait strongly associated with invasion success (Keller *et al.*, 2011), might modify the distributional limits sets by climate. Also, models typically do not appreciate how the existence of phylogeographic lineages with differing niche requirements can influence forecasts of invasion risk (Pearman *et al.*, 2010). Ignoring these factors may result in mismatches between predicted potential and realised invasive distributions, fuelling doubts about the suitability of bioclimatic envelope models for anticipating biological invasions (Guisan *et al.*, 2014).

Therefore, in this study, we assess three key assumptions underlying bioclimatic envelope models: (i) that species' distributions are largely governed by climate (Araujo & Peterson, 2012), (ii) that a species' current native distribution corresponds with the total set of climate conditions under which it can persist (Peterson, 2003), and (iii) that the climatic niche remains conserved across time and space (Broennimann *et al.*, 2007). Climate is generally recognised as a chief driver of species' distributions at large spatial scales (Araujo & Peterson, 2012), although the broad distributional limits governed by climate may be modified by factors such as habitat availability, biotic interactions and dispersal limitations (Soberon, 2007). Erroneous predictions of the potential distribution of invasive species are often attributed to species adaptations in response to selection pressures imposed by the novel environment (Whitney & Gabler, 2008). However, within the native range, species may also evolve pre-adaptations to invasiveness; strong selection imposed by human modification of habitats within the native range is likely to lead to adaptation prior to introduction elsewhere (Hufbauer *et al.*, 2012). As human activities tend to promote similar ecological conditions across biogeographical areas (Savard *et al.*, 2000), species or populations associated with human-modified habitats in the

native range can be expected to successfully invade similar areas elsewhere. It is therefore surprising that predictions of invasion risk obtained from bioclimatic envelope models have not yet explicitly considered how human modification of habitats might modify the distributional limits set by climate.

Bioclimatic envelope models assume that a species' invasive distribution can be predicted from its native niche characteristics (Peterson, 2003). Niche theory indeed predicts that for relatively recent events such as biological invasions, conservatism of the fundamental native niche is expected (Peterson, 2011), although species may, in the invaded range, occupy different portions of their fundamental niche compared to the native range (Guisan *et al.*, 2014). Empirical studies on the prevalence of (realised) niche conservatism have yielded mixed results. Two large scale studies on European plants introduced to North America found niche conservatism was the dominant pattern for weedy, widespread plant species (Petitpierre *et al.*, 2012) while niche expansion into climates not occupied in the native range was common for plants with smaller native ranges (Early & Sax, 2014). Niche conservatism was the norm for non-native vertrebrates introduced to Europe and North America (Strubbe *et al.*, 2013; Strubbe *et al.*, 2014), whereas a global study on amphibians and reptiles found widespread evidence for niche expansion (Li *et al.*, 2014). To better understand the mechanisms underlying patterns of niche conservatism, here, we question the inherent assumption that pooling occurrence data from across the entire native range of a species adequately describes the full range of climatic conditions in which invasive populations can establish and survive. This assumption may be violated when phylogeographic lineages with differing niche requirements are present (D'Amen *et al.*, 2013). Species may not represent a single evolutionary entity (Pearman *et al.*, 2010), and as species-level models smooth across environmental response curves of specific lineages, ignoring within-taxon niche structure risks erroneous predictions of a species' potential distribution (D'Amen *et al.*, 2013). Despite their potential to improve predictions of invasion risk, within-taxon niche structures have only received scant attention in invasive species management (Beaumont *et al.* 2014).

Here, using a unique dataset on the distribution of a global avian invader, the ring-necked parakeet (*Psittacula krameri*), we test whether accounting for within-taxon niche structure and association with humans in the native range leads to more accurate predictions of invasion risk. Ring-necked parakeets are native to large parts of Africa and Asia. They have benefited from the conversion of natural habitats to agro-ecosystems (Bruggers & Beck, 1979; Khan, 2002), and reach their highest breeding densities near human settlements and cultivated crops (Khan *et al.*, 2004). These parakeets are a globally widespread invasive species, especially in Europe, where they rank among the top 100 worst invasive species as they compete with native birds

and bats and cause damage to crops (DAISIE, 2009; Strubbe & Matthysen, 2009a; Hernández-Brito *et al.*, 2014; Peck *et al.*, 2014). In this study, we present the most complete information on the distribution of ring-necked parakeets to date, comprising a set of about 1,200 (686 native and 513 invasive) occurrences collected at a finer resolution than has previously been reported, 123 failed and successful introduction events across Europe, plus a high-resolution mtDNA molecular phylogeny derived from 98 museum specimens geospatially selected to cover the parakeet's native range and from feather samples collected at 13 invaded sites across Europe. We expect that incorporating within-taxon niche structure into bioclimatic envelope models will result in important differences in the geographical distribution of climate predicted as suitable for parakeets across Europe, and that accounting for association with human-modified habitats in the native range will allow for more accurate predictions of the potential European distribution of this ubiquitous avian invader.

Methods

DNA isolation, amplification and sequencing

DNA was extracted from toe-pad samples (n=98) collected from specimens at the Natural History Museum (Tring, UK) and from contemporary feather samples collected in Europe (n=13), using a Bioline Isolate Genomic DNA extraction kit (Bioline, UK). Finely-chopped samples were suspended in 400ul lysis buffer and 25ul proteinase K and incubated at 55^{o}C overnight (or until the material had completed digested). Processing of samples from museum specimens was carried out in a dedicated museum DNA laboratory, under a UV-irradiated fume hood to destroy any contaminants. Negative controls were included to ensure no contamination during the DNA extraction and PCR procedures. Amplification of mtDNA control region and cytochrome b was conducted using a specifically designed suite of short fragment primers (see Appendix S1 in Supporting Information). Cycle parameters comprised an initial hot start of 95^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15secs, 52^{o}C/15secs and 72^{o}C/10secs, followed by a final 10 minutes 72^{o}C incubation period. All amplicons were examined by agarose gel electrophoresis and PCR product was purified and amplified using a 3730xl analyser (Applied Biosystems; Macrogen Inc.). The concatenated DNA sequence dataset was condensed into haplotypes using TCS (Clement *et al.*, 2000).

Phylogenetic analysis

To identify native phylogroups, Bayesian phylogenetic inference was implemented in MrBayes v3.2 (Ronquist & Huelsenbeck, 2003) using the CIPRES Science Gateway (Miller *et al.*, 2010) with 10 million generations over four parallel Monte Carlo Markov Chains (MCMC**),** under an HKY evolutionary model (Felsenstein, 1981). Tracer v1.6 (Rambaut & Drummond, 2007) was used to assess convergence. After discarding the first 25% as burn-in, tree topologies were

summarised in a 50% consensus tree. To identify native haplotypes in the invasive range, the combined native and invasive dataset was condensed into haplotypes using TCS (Clement *et al.*, 2000, APP 1). All node values with a posterior probability of >50 were used to identify phylogroups.

Occurrence data and environmental variables

Ring-necked parakeet occurrence data (i.e. longitude-latitude coordinates) in Europe and the native range were extracted from a range of databases (GBIF, ORNIS and natural history museums), scientific papers and grey literature (such as government or NGO reports, bird trip reports and parakeet observations posted on the image hosting website Flickr.com). Occurrence data were retained only when their spatial resolution was ≤ 5' (i.e. 0.083° or ~10x10km, assessment of spatial accuracy based on information present in the source data, or through pers. comm. with observers). In total, we gathered 8,667 ring-necked parakeet occurrences (Europe: 6,634, Africa: 515, Asia: 1,518), but as we used only one occurrence per 5' grid cell, the final database comprised 1,199 observations (Europe: 513, Africa: 211 and Asia: 475; Appendix S2). Data on parakeet introduction success were taken from Strubbe & Matthysen (2009b) (n=123 introduction events). Datasets are available upon request from D.S. and will be made public through the European Monitoring Centre for invasive parrots (COST Action ES1304). Minimum convex and Thiessen polygons circumscribing the geographic distribution of each mtDNA clade were then applied to assign parakeet occurrences to phylogroups (Appendix S2).

Environmental variables considered are a set of eight climatic variables assumed to impose direct and indirect constraints on avian distributions (Araújo *et al.*, 2009): annual mean temperature (bio_1), mean temperature of the warmest month (t_max), mean temperature of the coldest month (t_min), temperature seasonality (bio_4), annual precipitation (bio_12), precipitation of the wettest month (bio_13), precipitation of the driest month (bio_14) and precipitation seasonality (bio_15). These variables were derived from the WorldClim database (Hijmans *et al.*, 2005) and represent mean values over the 1961-1990 period at a 0.083° resolution. The 'human footprint', a quantitative measure of human alteration of terrestrial environments based on human population size, land use and infrastructure was derived from Sanderson *et al.* (2002) at a resolution of 30'' and resampled to the 0.083° resolution of the climate and parakeet occurrence data.

Niche analyses

To assess niche differences between phylogroups and between native and invasive parakeet populations, we used Broennimann *et al.* (2012) framework. This framework applies kernel smoothers to densities of species occurrence in a gridded environmental space to calculate

metrics of niche overlap (quantified by Schoener's D, 0: no overlap, 1: complete overlap). Using a randomization test whereby the measured niche overlap is compared against a null distribution of 100 simulated overlap values, we test whether parakeet niches are more similar to each other than expected by chance (i.e. niche similarity, Broennimann *et al.*, 2012). We first assessed whether ring-necked parakeet climatic niches differed significantly between phylogroups (i.e. Africa vs. Asian, and phylogroups within each continent), using all biomes occupied by parakeets across their native range as background area (Guisan *et al.*, 2014). Second, native and invasive ring-necked parakeet occurrences were used to assess whether native niche characteristics are conserved during the invasion process (using a niche similarity test), and to determine whether parakeets have colonised in the invaded range climates not occupied in the native range (i.e. niche expansion, Petitpierre *et al.*, 2012). Niche metrics are calculated on the climate space shared by native and invasive ranges (sensu Petitpierre *et al.* 2012). Background areas should reflect the set of areas a species could potentially have encountered since its presence in the region (Barve *et al.*, 2011). Therefore, in Europe, we buffered each locality where parakeets have been introduced with a distance equal to the minimum invasion speed recorded for birds (i.e. 4,59 km/year, derived from Blackburn *et al.*, 2009) multiplied by the number of years since introduction (see Strubbe *et al.*, 2013 for details). In doing so, we obtained an ecologically realistic European background (models were also run using the whole of Europe as background, but this did not affect our main results, Appendix S3).

Bioclimatic envelope models

Bioclimatic envelope models were run in R using the ensemble modelling framework biomod2 (Thuiller *et al.*, 2013). We applied five different modelling algorithms: generalised linear models (GLM), generalised boosted models (GBM), multivariate adaptive regression splines (MARS), random forest (RF) and maximum entropy (MaxEnt) to identify areas at risk of invasion. Models were fitted with default settings unless stated otherwise. Following Barbet-Massin *et al.* (2012) models were run with a single set of 10,000 pseudo-absences drawn from the same native-range background area as used for the niche analyses described above. Pseudo-absences were generated randomly from all grid cells in background area that were not presences, with no minimum or maximum distance to presence locations (Wisz & Guisan 2009). For each modelling algorithm, presences and pseudo-absences used to calibrate the model were weighted such as to ensure neutral (0.5) prevalence (Petitpierre *et al.* 2012). Each model was subjected to 10-fold cross validation with a 80-20% random split of the presence data for training-testing each replicate, respectively. Models were evaluated using the True Skill Statistic (TSS), and to exclude inaccurate models, only those with TSS > 0.7 were kept for generating ensemble projections (Thuiller *et al.*, 2013) of parakeet invasion risk in Europe,

using unweighted averaging across models. Relative variable importance (0 to 1) was obtained through the randomization procedure described by Thuiller *et al.* (2013).

Following the procedures described above, we first fitted a 'clade' model, using as presences all native-range grid cells occupied by parakeets (i.e. occurrences pooled across all phylogroups). Then, we built separate models for each phylogroup, using as presences all occupied grid cells located within phylogroup range boundaries. A composite 'subclade' model was developed from the phylogroup predictions to summarize predictions of parakeet occurrence across all phylogroups. Because phylogroup models differ in prevalence, to construct the subclade model, we first made the phylogroup models comparable by standardizing the average probabilities of occurrence for each phylogroup along the environmental gradients considered. Then, we calculated the mean probability of occurrence of at least one of the related phylogroups for grid cells using the multiplicative probability method described in Pearman *et al.* (2010). Clade and subclade models were fitted with and without human footprint, resulting in four different ensemble predictions of parakeet invasion risk in Europe. To exclude the possibility that differences in model performance are solely due to the mere adding of one predictor variable (human footprint) to the models, we also fitted models with a randomised version of the human footprint variable. To further assess the importance of the human footprint, models described above were also run with the human footprint as sole predictor variable. Model transferability was assessed using European parakeet occurrence data (n=513), applying the full range of evaluation statistics available in biomod2, plus two statistics specifically designed for presence-only models (the 10-fold and the continuous Boyce index, Hirzel *et al.*, 2006). Lastly, we applied a TSS-value based on European parakeet occurrences as threshold value for converting the continuous clade and subclade ensemble predictions of invasion risk into discrete predictions of parakeet presence and absence across Europe (Pearman *et al.*, 2010).

Results

Phylogenetic analysis

Mitochondrial DNA sequences comprising 868 bp (cytochrome *b*: 346 bp, control region: 522 bp) were sampled from 98 parakeet specimens (Africa: 38, Asia: 60). In total, 44 unique haplotypes were identified (Africa: 16, Asia: 26). A Bayesian phylogenetic tree provides support for 17 haplotype clades (Africa: 6, Asia: 11; posterior probabilities > 50, i.e. the 'phylogroups', Appendix S1). The 6 African phylogroups correspond to 6 largely parapatric groupings arranged longitudinally along the Sahel region, whereby only the most eastern phylogroups show some range overlap. The 11 Asian phylogroups, in contrast, show a much more complex spatial pattern with varying levels of range overlap between phylogroups (Appendix S2).

Niche analyses

Assessing the climatic niche position of the different phylogroups reveals that significant within-taxon niche structure is present within both Africa and Asia (Appendix S3). Phylogroups occupy partially overlapping but distinct portions of the climate space available in the native range, and climatic niches are not more similar to each other than expected by chance (multiple niche similarity test P-values > 0.05; within Africa: niche overlap D between phylogroups equals 0.30 ± 0.19 (mean and standard deviation), range: 0.07-0.69; within Asia: 0.11 ± 0.0.17, range: 0.00-0.72, Appendix S3). Niche overlap between African and Asian phylogroups is low (D: 0.059), and while the African niche is more similar to the Asian niche than expected by chance (niche similarity P-value: 0.0099), the reverse is not true (niche similarity P-value: 0.14). African ring-necked parakeet populations have only 1% of their niche outside the niche of the Asian populations, and the African niche is thus largely a subset of the Asian niche (Appendix S3). Niche overlap between native (i.e. Africa and Asia) and invasive (i.e. Europe) ring-necked parakeet populations is low (D: 0.003). Native and invasive niches are more similar to each other than expected by chance (niche similarity P-value: 0.0099), yet parakeets in Europe show significant niche expansion as they have 87% of their invasive distribution outside their native climatic niche (Fig. 1). Niche differences between the native and invasive range are largely attributable to a shift along the first PCA-axis of the climate space, indicating that in Europe, ring-necked parakeets have colonised areas far colder than their native range (Fig. 1). Of the 44 native-range mtDNA haplotypes, 14 (11 Asian, 3 African) were also detected in Europe. The small European sample size (i.e. feathers collected at 13 roost sites only) precluded meaningful tests of niche conservatism per haplotype (i.e. sensu Broennimann *et al.*, 2012). Yet, given the shift towards colder climates in Europe, we hypothesised that parakeet haplotypes with a lower native cold tolerance limit should have a higher probability of persisting in Europe. We therefore, for each haplotype, in the native-range climate space, derived its cold native niche limit (i.e. minimum value along the temperature-dominated x-axis of the climate space, Fig. 1) and found that haplotypes present in Europe have significantly lower native cold niche limits than haplotypes not retrieved in Europe (t-test: t = -4.14, d.f. = 15.8, P-value = 0.00079, Appendix S4).

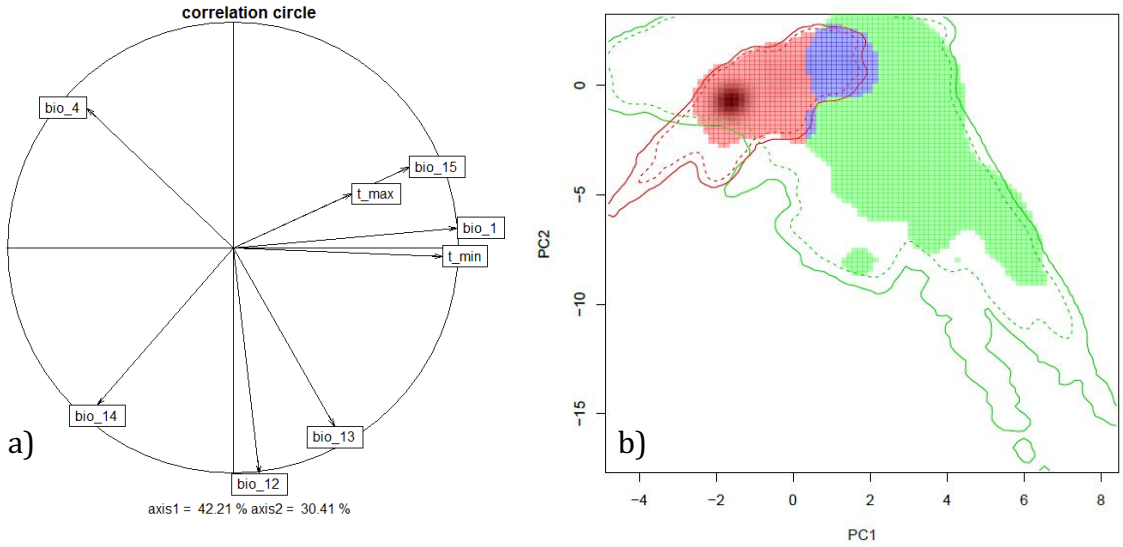

Figure 1. Climate niche dynamics between native and invaded ring-necked parakeet ranges. Fig. 1a shows the contribution of the climatic variables on the two axes of the PCA and the percentage of inertia explained by the two axes. Fig. 1b depicts ring-necked parakeet native and invasive niches. The solid and dashed contour lines illustrate, respectively, 100% and 50% of the available environment in the native range (green lines: Africa + Asia, background defined as all biomes occupied across the native range) and in the invasive range (red lines: Europe, using the ecologically realistic definition of the background, see text). Green areas represent climates only occupied in the native range, blue indicates climates occupied in both the native and non-native range while red areas indicate niche expansion in the invaded range. Shading indicates the density of occurrences of the species by cell in the invaded range. The first PCA-axis (x-axis, 42.4% of the variation) is mainly determined by temperature gradients, the second axis (y-axis, 30.4%) chiefly represent precipitation patterns (Appendix S3).

Bioclimatic envelope models

When considering climatic variables only, bioclimatic envelope models that take the contribution of within-taxon niche structure (i.e. the 17 phylogroups) into account (the 'subclade' model) and those that do not (the 'clade' model) both fail to accurately predict the current invaded distribution, although they accurately predict parakeet occurrence across the native range (Europe: Boyce-index: -0.87 for the clade model vs. -0.60 for the subclade model; native range: Boyce-index: 0.96 and 1.00, respectively; results are similar across a wide range of model evaluation statistics, Appendix S5). The clade model was not successful in discriminating between failed and successful parakeet introductions to Europe (logistic regression between climatic suitability and outcome of introduction, P = 0.914) whereas the subclade model explains a modest part of the variation in introduction outcomes (P: 0.018,

Nagelkerke R^2: 0.09, Appendix S6), mainly because it correctly predicts a higher introduction success in the Mediterranean. When we included human footprint as a variable into the bioclimatic envelope models, transferability of both the clade and subclade models increased dramatically. Clade and subclade models that include human modification of habitats in the native range perform equally well at forecasting parakeet occurrence in Europe (Boyce index: 0.93 and 0.94, respectively). This increase in model performance is not merely due to the adding of an extra environmental variable, as models fitted with a randomised human footprint do not perform any better in predicting parakeet occurrence across Europe than climate-only models do: Boyce index -0.86 and -0.72, respectively (Appendix S5). Models built with human footprint as sole predictor variable could not adequately model ring-necked parakeet distribution across the native range (i.e. TSS of all models < 0.7 criterion, see above), precluding ensemble forecasts of invasion risk for Europe based on human footprint only. Although clade and subclade models combining human footprint and climate produced similar evaluation statistics, there are marked differences in the actual areas predicted to be suitable for parakeets (Fig. 2, Fig. 3). Whereas both models predict that parakeets will occur mainly in parts of the Mediterranean and in major human population centres in north-west Europe (designating 11% of Europe as suitable, Fig. 3), the clade model considers larger parts of central and eastern Europe as suitable for parakeets (19% of Europe, Fig. 3). The subclade model, in contrast, indicates that more extensive areas in southern Spain, Greece, Romania and parts of Turkey and the Middle East are at risk of parakeet invasion (16%, Fig. 3). After including human footprint into the models, both clade and subclade models can accurately discriminate between failed and successful parakeet introductions, although the subclade model performs better at discriminating failed introductions (clade model AIC: 126, Nagelkerke R^2: 0.37, $P < 0.0001$, false negative rate: 0.37 vs. subclade model AIC: 121, Nagelkerke R^2: 0.41, $P < 0.0001$, false negative rate: 0.05, Appendix S6).

Across the native range, adding human footprint did not further improve the already high accuracy of predictions of parakeet occurrence (clade model Boyce-index: 1.00; subclade: 0.91), but resulted in more pronounced, fine-grained predictions, largely within the distributional limits identified by the climate-only models, Appendix S7. Analysis of variable importance reveals that human footprint is highly important in the clade model for the native range (footprint: 0.64 ± 0.12, temperature variables: 0.17 ± 0.18, range 0.10 - 0.30, precipitation variables: 0.12 ± 0.11, range 0.02-0.26) while the subclade model attributes more weight to temperature and precipitation gradients as well (temperature: 0.32 ± 0.16, range 0.01 - 0.65, precipitation: 0.21 ± 0.16, range 0.02 - 0.85, footprint: 0.47 ± 0.18, range 0.23 - 0.86, Appendix S8).

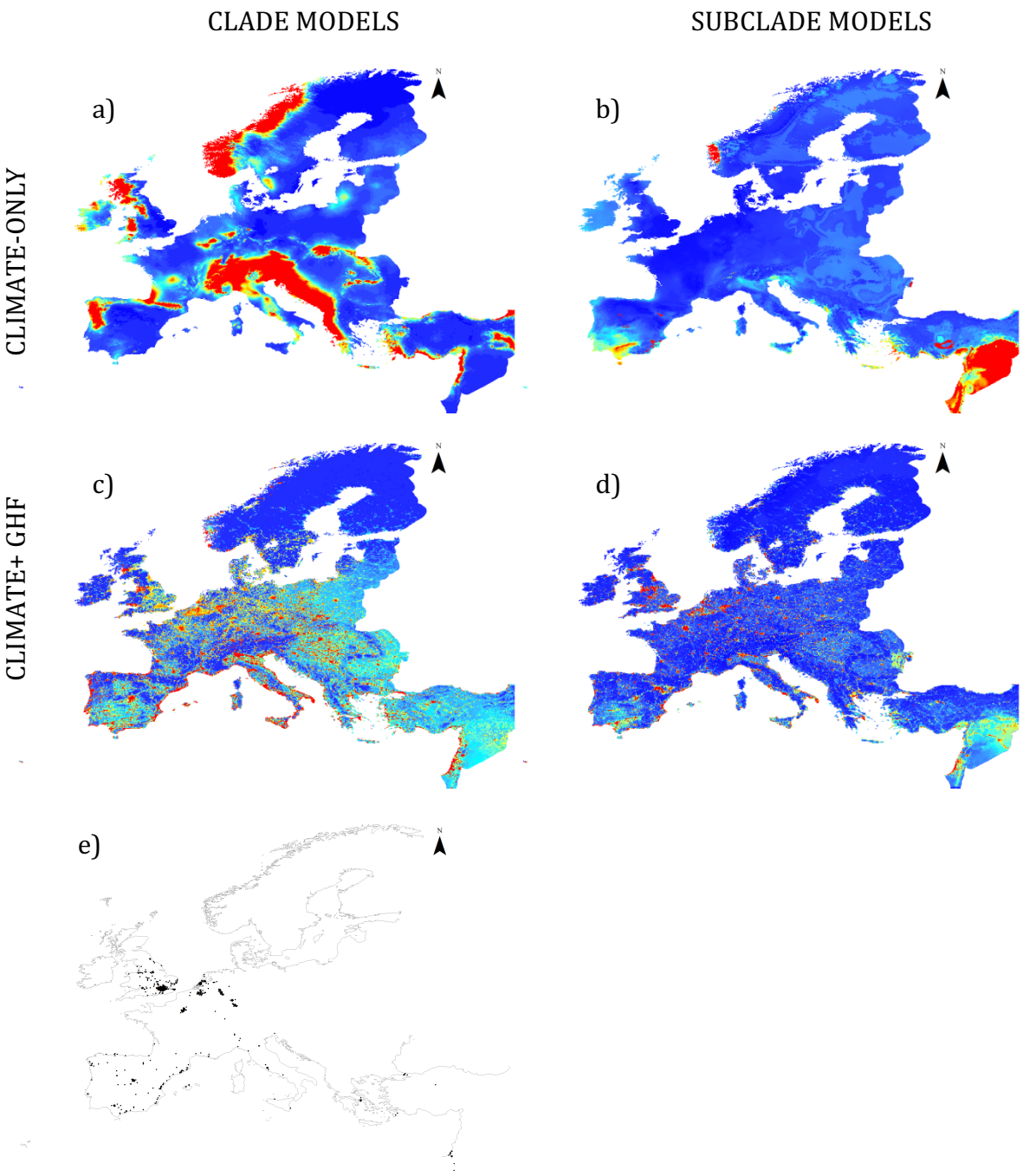

Figure 2. Predictions of invasion risk for ring-necked parakeets derived from native-range based bioclimatic envelope models. Left vs. right panes show models ignoring (Fig. 2a, 2c) and accounting for differing niche requirements of phylogeographic lineages (Fig. 2b, 2d) while upper vs lower panels depict models without (Fig. 2a, 2b) and with (Fig. 2c, 2d) human footprint. Warmer colours indicate a higher predicted risk of parakeet invasion. The black dots in Fig. 2e depict locations with established parakeet populations, used to validate native-range based forecast of invasion risk.

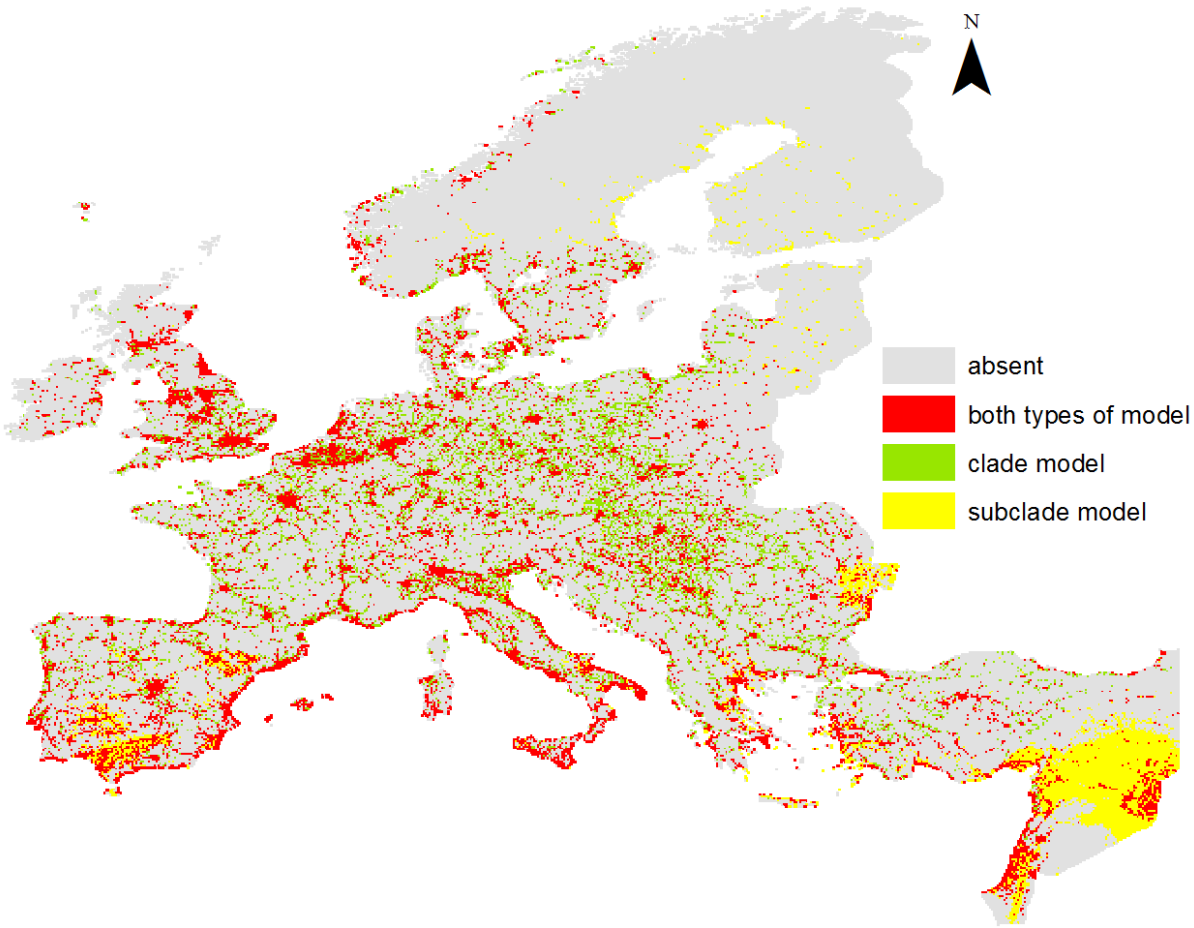

Figure 3. Predictions of invasion risk for ring-necked parakeet in Europe derived from bioclimatic envelope models including association with human-modified habitats in the native range. Continuous model outputs (Fig 2) were converted to binary predictions of invasion risk. Areas at risk according to both models without (see Fig. 2c) and with (see Fig. 2d) phylogeographic structure are indicated in red. Green indicates predicted parakeet presence only by a model without phylogeographic structure. Yellow delineates areas only marked as suitable by a model with phylogeographic structure.

Discussion

Our results support the hypothesis that association with humans in the native range may allow invasive species persistence in areas outside of their native climatic niche, and that accounting for within-taxon niche structure can result in significant changes to predictions of invasion risk. Violating the key model assumptions that climate governs the broad outlines of species distributions and that within-taxon niche structure is insignificant can thus introduce substantial error into predictions of invasion risk derived from bioclimatic envelope models. Information on niche requirements of phylogeographic lineages and association with human-modified habitats in the native range must therefore be integrated into bioclimatic envelope models, if they are to effectively guide invasive species management.

Association with human-modified habitats in the native range may enable ring-necked parakeets to exploit equivalent human-modified landscapes in Europe, allowing them to colonize areas far colder than their native range. Ring-necked parakeets have almost 90% of their invasive distribution outside their native climatic niche (Fig. 1), and this is among the highest values of niche expansion known for vertebrates (Strubbe *et al.*, 2013; Li *et al.*, 2014). Previous studies suggest niche expansion into climates not occupied in the native range is more likely for species with small native ranges (plants, Early & Sax, 2014; amphibians and reptiles, Li *et al.*, 2014), for species introduced longer ago or that have invaded areas located at lower latitudes than the native range (amphibians and reptiles, Early & Sax, 2014). Ring-necked parakeets, however, have a very large native range and have been introduced relatively recent (most European introductions stem from after 1970, Strubbe & Matthysen, 2009b) to much higher latitudes than the native range. Our results thus identify, for the first time, association with humans in the native range as a factor influencing climatic niche expansion during biological invasion. Climate influences species distributions directly through species' physiological tolerances or indirectly through its effect on available habitats, food resources and biotic interactions such as the presence of competitors (Araujo & Peterson, 2012). The fact that ring-necked parakeets thrive in Europe suggests they may be physiologically capable of colonizing colder parts of the climate space in their native range as well. Possibly, a lack of resources and/or competition with congeneric species such as slaty-headed (*P. himalayana*) and Lord Derby's Parakeet (*P. derbiana*) restricts the ring-necked parakeets' native northernmost distribution limits (Wisz *et al.*, 2013). Indeed, endotherms such as birds are often able to tolerate a wide range of environmental conditions but this comes at a potentially high energetic cost (Porter & Kearney, 2009). In Europe, cities present parakeets with abundant food (Clergeau & Vergnes, 2011; Strubbe & Matthysen, 2011) and parakeets have been shown to be behaviourally dominant over native birds during foraging (Peck *et al.*, 2014). Abundant resources and a lack of competitors may underlie the invasion success of ring-necked parakeets in environments far removed from their native (realised) niche. Yet, to elucidate the extent to which thermal and energetic constraints influence ring-necked parakeet distributional limits in their native versus non-native ranges, mechanistic niche models (which use species' functional traits and physiological tolerances for model fitting, Kearney *et al.*, 2010) are required. Furthermore, although little is known about interactions between *Psittacula* species in their native range, the hypothesis of competitive release as an underlying driver of ring-necked parakeet invasion success in Europe may be tested by assessing whether predicted geographic distribution patterns across the native range (derived from bioclimatic models) match expectations under competitive exclusion (sensu Gutiérrez *et al.*, 2014).

The fact that lineages associated with cold climates in the native range have been more successful invaders in Europe, and that the subclade model more accurately discriminates successful from failed parakeet introductions suggests it captures lineage-specific responses to environmental gradients that are undetectable using the clade model (Appendix S8). Incorporating such within-taxon niche structure into bioclimatic envelope models leads to important differences in spatial predictions of invasion risk for Europe (Fig. 2, Fig. 3). The climate-only clade model is strongly influenced by precipitation gradients (Appendix S8), resulting in erroneous predictions of parakeet occurrence for Europe's wetter areas (i.e. parts of the Atlantic and Adriatic coast, and along mountain chains, Fig. 2a). The climate-only subclade model indicates certain phylogeographic lineages indeed respond strongly to precipitation gradients (Appendix S8), although in general, the subclade model is more strongly driven by temperature gradients. The climate-only subclade model accordingly correctly predicts some of the Mediterranean parakeet populations, and except for a high precipitation zone along the coast of Norway, it assigns a low invasion risk to coastal areas and mountain chains (Fig. 2b). Both climate-only models however fail to accurately forecast ring-necked parakeet occurrence across north-west Europe. When including the human footprint, the major difference between the clade and subclade model is that the latter places more weight on temperature and precipitation gradients (Appendix S8) whereas the clade model exhibits a higher dependency on human footprint. Consequently, the clade model predicts a higher invasion risk across human-dominated habitats in colder parts of continental Europe (Fig. 2c, 2d) as well. This becomes especially apparent when converting the predictions of invasion risk into discrete predictions of parakeet presence and absence (Fig. 3), showing that particularly in east and central Europe, the clade model predicts as suitable areas that are geographically peripheral to areas predicted as suitable by the subclade model. In contrast, in southern Europe, the subclade model predicts more extensive areas to be at risk of parakeet invasion, reflecting the different weightings given by the clade and subclade model to climate and human modification of habitats.

Taken together, our results agree with other findings (Strubbe *et al.*, 2013; Early & Sax, 2014; Guisan *et al.*, 2014; Li *et al.*, 2014; Strubbe *et al.*, 2014), suggesting that while rapid post-introduction evolution (i.e. a change in the fundamental Grinnellian niche, Soberon 2007) cannot be ruled out, ecological factors governing the occupancy of the fundamental niche in native versus invaded ranges, such as dispersal, biotic interactions, the ability to exploit human-modified habitats and intraspecific variation in species' niche requirements, are probable drivers of climatic niche differences between native and invasive ranges. This has important ramifications for the use of bioclimatic envelope models as risk assessment tools, as well as, more fundamentally, for understanding how climate and local factors interact to determine species' distributions. Pearson and Dawson (2003) suggested a hierarchical approach to

modelling environment-biota relationships whereby bioclimatic envelope models should form the first step, identifying the broad outlines of species' distributions. Within the area designated as climatically suitable for a species, models including factors such as land-cover and habitat preferences can then be applied to elucidate the fine-grained structure of distributions. We suggest that, at least for invasive species, this framework may not be universally applicable, as association with human-modified habitats in the native range may allow species to overcome their (realised) native-range climatic limitations in human-modified landscapes elsewhere. Trait-based species risk assessments consider association with human-modified habitats in the native range to be a reliable predictor of invasion success (Keller *et al.*, 2011), especially for mammals and birds (Jeschke & Strayer 2006). Our results show that applying a simple and universal variable such as the human footprint can considerably increase the accuracy of predictions of invasion risk, and this finding opens up real perspectives for devising and implementing more robust management strategies for a large number of invasive species. Information about the presence and geographical distribution of phylogeographic lineages may be not be readily available for all invasive species, but subspecies range maps can often be derived from the literature, at least for terrestrial vertebrates. Subspecies are generally based on discontinuities in the geographical distribution of phenotypic traits instead of molecular phylogenies, but can generally be considered useful proxies of patterns of divergence among populations (Phillimore & Owens, 2006). We therefore argue that, in order to provide to policy-makers models that can accurately predict invasion risk, explicit evaluation of within-taxon niche structure and of association with humans in the native range should become standard practice.

Acknowledgments

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Biosketches

Diederik Strubbe is a post-doc working on biological invasions. His research interests include how ecological niche models, evolution and phylogenetic information can be integrated to unravel mechanisms underlying invasion success of non-native species. **Hazel Jackson** is a PhD candidate focussing on phylogeny, biogeography and population genetics of avian invaders. **Jim Groombridge** is Reader in Conservation Biology, primarily interested in population restoration, population ecology, conservation genetics and evolutionary studies involving phylogeny reconstruction. **Erik Matthysen** is a professor studying population dynamics, genetics and behavioural ecology of animals, including birds, mammals and insects. D.S. conceived the project, ran all niche analyses and bioclimatic envelope models and led the writing. H.J. carried out all genetic analyses. All authors contributed substantially to the writing.

Supplementary material

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Phylogenetic analyses on ring-necked parakeet museum specimens (native range) and contemporary feathers (invasive range).

Appendix S2. Native-range data on the distribution of ring-necked parakeets across their native range (haplotypes + occurrence data).

Appendix S3. Analysis of ring-necked parakeet niche dynamics within the native range and between the native and invasive range.

Appendix S4. Invasive range data on the distribution of ring-necked parakeet haplotypes in Europe.

Appendix S5. Predictions of ring-necked parakeet distribution across the native and invasive range: model evaluation statistics.

Appendix S6. Data on ring-necked parakeet introduction success in Europe.

Appendix S7. Predictions of ring-necked parakeet distribution across the native and invasive range: distribution maps.

Appendix S8. Variable importance derived from bioclimatic envelope models.

Appendix S1 – Genetic analysis and Bayesian phylogeny.

DNA isolation, amplification and sequencing

DNA was extracted from 98 historical toepad samples collected from study skins at the Natural History Museum in Tring, using a Bioline Isolate Genomic DNA extraction kit (Bioline, UK). Chopped samples were suspended in 400ul lysis buffer plus 25ul proteinase K and incubated at 55^{o}C overnight (or until the material had completed digested). DNA was washed through a spin column and suspended in 50ul of elution buffer. Negative controls were included to ensure no contamination during the extraction and PCR process. Work on the museum specimens was carried out in a dedicated museum DNA laboratory, under a UV-irradiated fume hood to destroy any contaminants.

Amplification of control region and cytochrome *b* was conducted using a specifically designed suite of short fragment primers (Table 1). Cycle parameters comprised an initial hot start of 95^{o}C for 1 minute followed by 35 cycles of 95^{o}C/15secs, 52^{o}C/15secs and 72^{o}C/10secs followed by a final 10 minutes 72^{o}C incubation period. All amplicons were examined by agarose gel electrophoresis. Amplification volumes of 25ul contained 2ul of template DNA, 12.5ul MyTaq HS redmix, 0.5ul of each primer and 9.5ul of dH20. PCR product was purified and amplified using a 3730xl analyser (Macrogen Inc.). Sequences were edited in 4Peaks (Griekspoor & Groothuis, 2005) and aligned in Clustal (Larkin *et al.*, 2007). Manual edits were made in Jalview (Waterhouse *et al.*, 2009). The two genes were concatenated using Sequence Matrix

(Vaidya *et al.*, 2011), and the entire dataset condensed into haplotypes using TCS (Clement *et al.*, 2000).

Phylogenetic analysis (native museum specimens and contemporary European feather samples)
To identify native phylogroups, phylogenetic tree inferences were computed using Bayesian methods with *Elecus roratus* and *Tanygnathus sumatranus* as outgroups. Additional *Psittacula* species were added to improve resolution, and their topology was constrained. PartitionFinder (Lanfear *et al.*, 2012) was used to identify the best-fit models of nucleotide evolution for each partition (control region 1-521, cytochrome *b* 522-868), according to Bayesian information criteria (BIC). Bayesian inference was implemented in MrBayes v3.2 (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller *et al.*, 2010) with 10 million generations over four parallel Monte Carlow Markov Chains (MCMC**),** under a HKY evolutionary model (Felsenstein, 1981). Tracer v1.6 (Rambaut & Drummond, 2007) was used to assess convergence. After discarding the first 25%, tree topologies were summarised in a 50% consensus tree. Trees were visualised in FigTree v1.4 (Rambaut, 2012) and all nodes with a posterior probability of >50 were used to identify phylogroups.

Extraction of DNA from European contemporary feather samples was conducted using a Bioline Isolate Genomic DNA extraction kit (Bioline, UK). Chopped samples were suspending in 400 µl lysis buffer plus 25 µl proteinase K and incubated at 55^{o}C overnight. DNA was washed through a spin column and suspended in 100 µl of elution buffer. Negative controls were included to ensure no contamination during the extraction and PCR process. Amplification from contemporary feather samples was conducted for control region using CR19f and CR19r, and cytochrome *b* using PKCBf and PKCBr (Table below**)**. PCR cycling conditions were 94^{o}C for 1 minute followed by 35 cycles of 94^{o}C/15 secs, 55^{o}C/15 secs and 72^{o}C/10 secs and a final elongation step of 72^{o}C for 10 minutes. To identify native haplotypes in the invasive range, the combined native and invasive European dataset was condensed into haplotypes using TCS (Clement *et al.*, 2000).

Table 1. Suite of MtDNA primers used to amplify cytochrome *b* and control region in historical and contemporary *Psittacula krameri* specimens.

Primer name	Sequence (5' – 3')
Historical specimen primers	
Cb1f	CTA CCA TTC ATA ATC ACC AGC C
Cb1r	GTG AGG GAG AGG AGT ATG ATA G
Cb2f	CTA TCA TAC TCC TCT CCC TCA C
Cb2r	TAG GAT CAG TAC GGA GGC AG
Cb3f	AAC AAC TCC CCC ACA CAT C
Cb3r	CGG CGA GTG TTC AGA ATA G
CR1f	CGT TCG TGT TTG CTT ACA TTT C
CR1r	GGT CCG TGT TGT TTG TTT TG
CR2f	CAC TGA TGC ACT TTT TCT GAC
CR2r	GGT GAA ATG TAA GCA AAC ACG
MCR2f	GAT GCA CTT TTT CTG ACA TCT G
MCR2r	GTT TCT TGA AAT GAA TCA CAG
CR3f	GAA CAA ACA AAC GTC TCC TTC
CR3r	GGA TAT TTG AGT GCG AGT GAC
Contemporary specimen primers	
PKCBf	CGGCCTACTCCTAGCCGCCC
PKCBr	GGGAAGCAGGCCGGAAGGC
CR19f	CACAGGCTCATTTGGTTCGC
CR19r	TAAGCTACAGGGACATTCGGGG

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