Conservation genetics and population surveys of the critically endangered wild camel *Camelus ferus* in Mongolia.

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Covid-19 Impact Statement

I kindly request the examiners to note how the Covid-19 pandemic impacted this thesis. The onset of the pandemic in March 2020 resulted in the following:

1- Immediate closure of the Sheffield lab at which I was conducting the genetic lab work. The lab remained closed for 11 months.

2- Immediate cancellation of 2020 Mongolia fieldwork which aimed to:

   2a) Collect more samples in the GGASPA including in the East of the park.

   2b) Conduct camera trap fieldwork.

   2c) Collect samples from the ex-situ camels.

3- Cancellation of travel to the University of Veterinary Medicine, Vienna for the analysis of SNP data. This work would have provided data for 1 chapter worth of genetic analysis.

The closure of the Sheffield lab meant that my genetic analysis was delayed until January 2021, which led to me working into my continuation year and using Covid extensions for the lab work of Chapter 4. I was never able to collect more wild samples, but collected ex-situ camel ear tissue samples in April 2022, which also led to completion delays. I was not able to travel to Vienna, so I had to change my proposed data chapter (Chapter 5).

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Abstract

The critically endangered wild camel, *Camelus ferus*, is a separate species to the domestic Bactrian camel, *Camelus bactrianus*. Surviving only in Mongolia and China, it has a narrow range of specialised Gobi Desert habitat. Added to this, its presumed small population and reduced genetic diversity leaves it threatened with extinction. Like many threatened species, conservation action has led to the creation of an ex-situ insurance population in Mongolia. The aim of this thesis was to use genetic research and timelapse camera trap distance sampling methods to improve our understanding of the species – both in the wild (in the Great Gobi A Special Protected Area in Mongolia), and in the ex-situ herd. Using a novel method of camera trap distance sampling using timelapse images we determined one of the first precise abundance estimates for the wild camel in the GGASPA, provisionally estimating the population to be 664 individuals (95% confidence intervals 400-1100). Despite the two species being separate, for *Camelus ferus* and *Camelus bactrianus*, common naming often confuses them. This is of importance when considering conservation implications. We used non-invasive genetic samples to determine that prevalence of introgression of DNA from domestic Bactrian camels *Camelus bactrianus* is extensive across the GGASPA, whilst also confirming that genetic diversity and inbreeding levels are comparable in both the in-situ and ex-situ wild camel populations. Finally, we looked at other ex-situ populations of critically endangered mammals, determining that they provide widely variable contributions to mitigating a taxon's extinction risk, which is inadequately assessed in conservation. All of this used together has improved our understanding of the threats that face the wild camel in Mongolia, both in the wild and in captivity. The data will continue to be used to inform further conservation management and to increase scientific and public awareness of the plight of the wild camel, with the overall aim of saving it from extinction.

Key words: Wild Camel, Camelus ferus, conservation genetics, non- invasive survey, introgression, camera trap distance sampling, abundance estimates.

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Author Declaration

Anna Jemmett wrote all the chapters, with editorial suggestions made by PhD supervisors. Chapters 2-5 have been written as manuscripts for publication and so are written in plural to demonstrate the collaborative effort of each.

Chapter 2 was conceived and written by A.M Jemmett with input from co-authors. This chapter has been published as: Jemmett, Anna M., Jim J. Groombridge, John Hare, Adiya Yadamsuren, Pamela A. Burger, and John G. Ewen. 2023. "What's in a Name? Common Name Misuse Potentially Confounds the Conservation of the Wild Camel Camelus Ferus." *Oryx* 57 (2): 175–79.

Chapter 3 Originated in discussions between M Rowcliffe, A.M Jemmett, J.G Ewen, S. Merson and A Yadamsuren. The methodology was developed by all the co-authors listed above with GIS assistance from A Huggins. A.M Jemmett, M Tserendorj, S Merson and A Yadamsuren led initial fieldwork. After initial deployment of cameras fieldwork was led by M Tserendorj and A Yadamsurem. Tagging of the images was conducted by M Tserendorj and A.M Jemmett. Data analysis was performed by A.M Jemmett, with assistance from M. Rowcliffe. A.M Jemmett wrote the chapter for publication as first author.

Chapter 4 Originated in discussions between A.M Jemmett, J.J Groombridge, J.G Ewen and P. A Burger. The methodology was developed by all the co-authors listed above. A.M Jemmett led fieldwork with J.G Ewen A Yadamsuren, B Johnson and J Quayle providing sample collection assistance with P.A Burger providing additional samples and scored genotype data. Lab work was conducted by A.M Jemmett with input from J.J Groombridge, P.A Burger and D. Dawson. All data analysis was performed by A.M Jemmett with input from J.J Groombridge, P.A Burger and D Dawson and with GIS assistance from A Huggins. A.M Jemmett wrote the chapter for publication as first author.

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Chapter 1 Introduction

1.1 Global biodiversity loss and climate change impacts on endangered species

We are in a time of unprecedented anthropogenic climate change and human mediated biodiversity loss. The scale at which we have lost, and are continuing to lose, species indicates that we are in the 6th period of mass extinction (Ceballos et al. 2015). In the last 500 years, over 300 vertebrate species have gone extinct (Dirzo et al. 2014; IUCN RedList 2022). Over 42,100 (28%) species assessed by the IUCN RedList are threatened with extinction and 20% of all vertebrates (Hoffmann et al. 2010) have been shown to be at risk of extinction in the wild. This number is increasing (Hoffmann et al. 2010) and the magnitude of these losses has led to the use of the term defaunation (Dirzo et al. 2014). Extinction of fauna is a cost in itself, but species loss changes biodiversity which can lead to subsequent loss of ecosystem function (Dirzo et al. 2014). Loss of ecosystem function or services such as pollination, water quality, pest control, scavenging and disease management have very detrimental impacts, which threaten the survival of all of life on Earth. Climate change and anthropogenic disturbances exacerbates these species declines, leading to international concern and targets being set. In 2022, COP15 globally committed to halting human-induced extinctions (COP15 2022). Despite these worrying trends in defaunation, research has shown that overall declines would have been significantly worse without conservation management (Hoffmann et al. 2010). Between 28 and 48 bird and mammal species would have gone extinct between 1993 and 2020 without conservation management (Bolam et al. 2021). Landscape scale conservation and global climate mitigation conservation are necessary to mediate for these losses, but for many species, more immediate single species conservation is necessary.

1.2 Ex-situ management of threatened species.

In-situ conservation, defined as the protection of species in their natural surroundings is crucial to global biodiversity protection (Pritchard et al. 2012). But as extinction rates are exacerbated by climate change, those species closest to extinction may require more direct action. As populations get closer

to extinction, threats to individuals, and so to overall populations or species, become even more dangerous. As populations get smaller, they can become increasingly vulnerable and less resilient to demographic, environmental and genetic threats. Any one issue, which alone may not lead to extinction, could reduce population size. These reduced populations are then vulnerable to further threats, this is termed the extinction vortex.

For these small, threatened, populations increased management may be necessary, either to protect individuals from threats or to increase population sizes, to prevent overall extinction. One such management technique used is ex-situ management where "individuals are maintained in artificial conditions under different selection pressures than those in natural conditions in a natural habitat" (IUCN RedList 2022). These populations are numerous with: 25% of all described bird species; 20 % of all known mammal species and 15% of all threatened species, being held in ex-situ care (Conde et al. 2011).

Ex-situ populations have varied aims which include entertainment, education, financial incentives and conservation (Conde 2013). Those that are used for conservation also vary in their function; as a way of reducing the primary threats to the species, to offset the effects of those threats, to buy time for other conservation efforts taking place in-situ and finally to restore populations. They can be used as a form of rescuing injured or displaced animals (F. Zhang et al. 2017), as a source population for translocations, restorations or introductions (IUCN 2014) and for an insurance against extinction in the wild. Ex-situ insurance populations are defined as populations that are used to recover wild populations and prevent extinction by buying time: "When wild population decline is steep and the chance of sufficiently rapid reduction of primary threats is slim or uncertain or has been inadequately successful to date" (IUCN RedList 2022). Insurance populations are those that keep individuals in captivity with the hope of saving the species from extinction and for future, successful recovery and release into a safer world. One such example being the conservation success of the Scimitar horned Oryx, which in

2023 was the first species to be downlisted to Endangered, after previously being classed as Extinct in the wild (ZSL 2023; IUCN RedList 2022)

Ex-situ conservation can be very successful, with its use being shown to both restore species to the wild (Smith et al. 2023) and prevent extinction (Bolam et al. 2021; Conde et al. 2011). But it can also be a risk, with such small, threatened populations many factors can impact overall success. Both financial and socio-political factors can determine success or failure (Conde et al. 2011) as well as the management of the animals themselves. There are also risks to being held in ex-situ long term, these include: adaptation to captivity (Conde et al. 2011), stress, disease and genetic risks. Genetic risks, in these small, closed ex-situ populations include: genetic drift, which may cause the loss of beneficial alleles or causing fixation of deleterious alleles; inbreeding depression and genetic adaptation to captivity, all of which reduce the potential for survival post release (Colléony et al. 2017).

Ex-situ populations can be impactful, they allow for the preservation of a species on the very brink of extinction, but they need to be carefully monitored and managed for success. This includes decision making on starting an ex-situ population, as well as subsequent effective future planning of that population and integrated management with in-situ populations and habitats where possible for recovery. This is not always possible, with limitations to successfully using an ex-situ population as an insurance, with practicalities and constraints on managers impacting success. But with so many threatened species being held in captivity these individuals should be considered critical.

1.3 The Wild camel *Camelus ferus.*

Background and Threats.

The wild camel, *Camelus ferus*, was first described by Przewalski in 1878 (IUCN RedList 2022). Unknown to the western world until this point, Przewalski presumed it to be either a feral Bactrian camel, *Camelus bactrianus*, or the wild animal from which the Bactrian was domesticated, leading to its initial scientific

designation of *Camelus bactrianus ferus*. This has now been superseded, both by changes in nomenclature legislation (Gentry, Clutton-Brock, and Groves 2004) and by scientific research agreeing with indigenous knowledge - the two species are indeed separate. Throughout its range the wild camel was known locally, and named as, a separate species to the domestic Bactrian camel (Hare 1997). Named Khavtgai- Хавтгай- in Mongolia and 野骆驼 -Ye Luo Tuo or "wild camel" in China. There are behavioural and morphological differences between the two species with the wild camel exhibiting increased "wildness" and different features such as smaller, pyramid shaped humps, smaller body, slimmer legs (Ji et al. 2009) and a notably flatter skull shape, from which it gets its name in Mongolian. There are also genetic differences, these seen across nuclear, mitochondrial and sex linked markers (Zhang et al. 2019; Felkel et al. 2019; Jirimutu et al. 2012; Silbermayr et al. 2010). Throughout this work I will refer to *Camelus ferus* as the wild camel, to reflect both scientific accuracy and cultural implications.

The wild camel inhabits the desert ecosystems of Central Asia. The historic range is thought to be from central Kazakhstan through southern Mongolia and into Xinjiang and the great bend of the Yellow River in China (Tulgat and Schaller 1992). It is now extant in only 2 Countries and four locations of true desert and semi-arid desert habitats (Yadamsuren, Daria, and Liu 2019): in China in the Gashun Gobi, Lop Nur and the Taklamakan deserts, and in Mongolia in The Great Gobi A Special Protected area (GGASPA) (Figure 1.1). The GGASPA covers over 45,000 square kilometres of Gobi Desert habitat. This stronghold for the wild camel is landlocked, with a continental climate of four distinct seasons. Although the average annual temperature is 5^{o}C, this is only because summer highs reach 40^{o}C, and winter lows can drop to below -35^{o}C. As well as extremes in temperature the area also receives very low precipitation of less than 50mm annually (Yadamsuren, Daria, and Liu 2019). Open water in the GGASPA is limited to approximately 40 springs, many of which are salt water, and not all of which are always active. Springs are located primarily in the mountainous areas of the park (Yadamsuren, Daria, and Liu 2019). The vegetation across the Gobi is scarce, drought adapted and in some cases precipitation driven. All of this

impact's wild camel movement and the need to migrate large distances to access food and water. The wild camel shows one of the largest on-land annual migrations on the planet at 2821km (Joly et al. 2019). When collared, some individual wild camels had an annual home range of > 12,000 km^2 and covered average straight-line distances of between 3.0-6.4 km per day (Kaczensky et al. 2014). This highly adapted ecosystem is threatened by climate change. Desertification of the Gobi is increasing and water points are drying, making habitat less suitable for the wild camel. Predictions show that up to 44% of the current suitable habitat in China could be lost to climate change by 2050 (Xue et al. 2021).

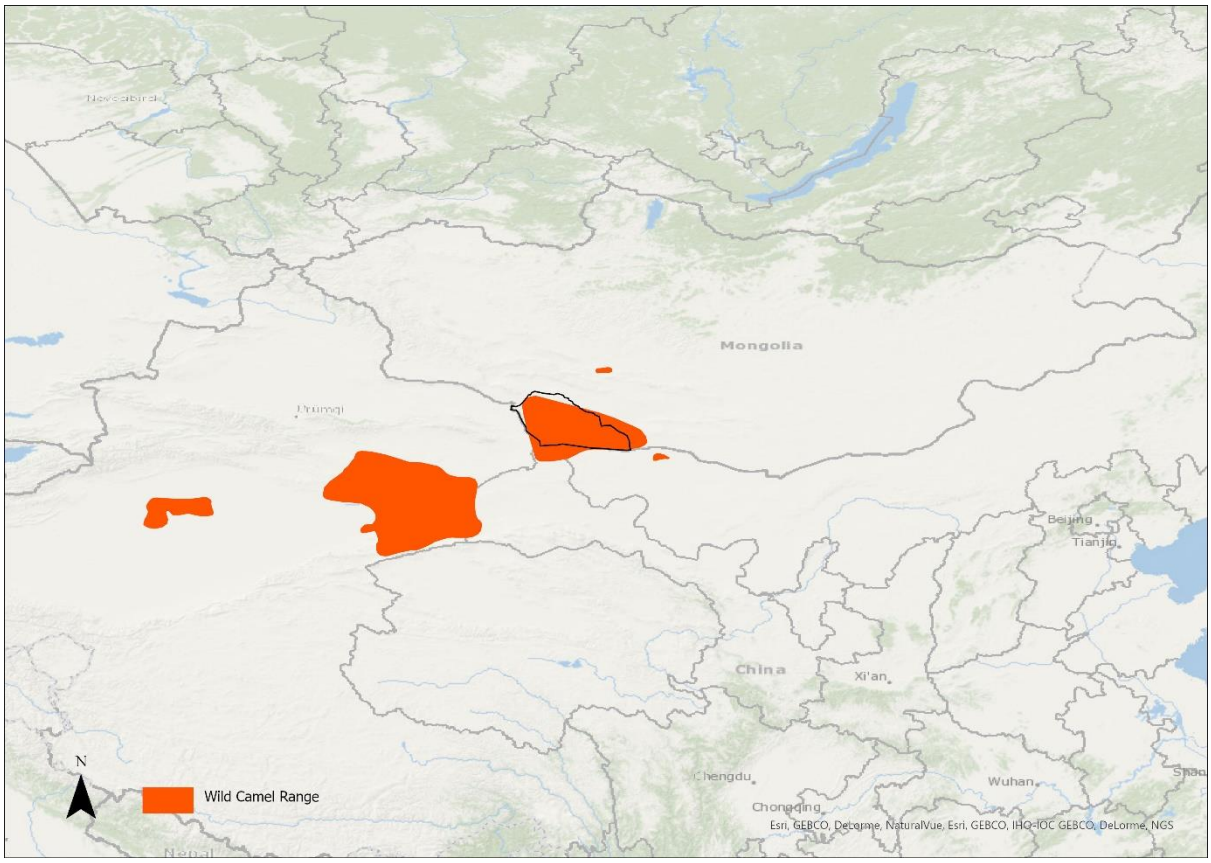

Figure 1.1: *Wild camel range (IUCN RedList 2022) in orange, with GGASPA boundary outlined.*

There are an estimated 950 wild camels left, with approximately 600 in China and 350 in Mongolia (IUCN RedList 2022; Hare 2004). This population estimate comes from a culmination of historic surveys and observation data (Hare 1997; Reading et al. 1999; Tulgat and Schaller 1992; Bannikov 1975; Zhirnov et al. 1986; Dash et al. 1977). With available data and in-country reports, the wild camel population is

presumed extremely low, giving it the critically endangered status (IUCN RedList 2022). Many factors threaten the survival of the wild camel and these, along with the presumed low population number, could be important to the future viability of the species. Common threats in both Mongolia and China include: habitat loss and degradation, including impacts caused by both legal and illegal mining (Yadamsuren, Daria, and Liu 2019); increased desertification due to climate change (Xue et al. 2021); human encroachment and disturbance (Kaczensky et al. 2014) and both competition and hybridisation with the domestic Bactrian camel, *Camelus bactrianus* (Silbermayr et al. 2010). Human infrastructure such as fences, roads and mines, are not only threats themselves, but they can also act as barriers to migration, with loss of migration itself considered a threat to large herbivores (Joly et al. 2019). Migration for the wild camel is a necessity to survival. A combination of lack of scientific information about this species and remoteness of its habitat has led to a lack of international interest in scientific research and conservation action (Ji et al. 2009).

Figure 1.2: *Morphological differences between the Wild camel, Camelus ferus, 1 individual in the foreground, and Bactrian camel, Camelus bactrianus, 2 individuals in the background. Image captured by camera traps in Chapter 3.*

Genetic research

Early genetic research suggested the wild camel to be a distinct species from the domestic Bactrian camel (Han et al. 2002), yet these studies were not considered resolved and were taken with caution (Burger, Ciani, and Faye 2019). The first reliable study to prove the wild camel to be a separate species to the domestic Bactrian camel was in 2009 (Ji et al. 2009). Since then, further genetic research has shown high sequence divergence in both mitochondrial DNA (Ji et al. 2009; Silbermayr et al. 2010; Mohandesan et al. 2017) and nuclear loci (Chuluunbat et al. 2014; Jirimutu et al. 2012; Wu et al. 2014; Y. Zhang et al. 2019). The wild camel is a genetically distinct species (Ji et al. 2009; Silbermayr et al. 2010; Mohandesan et al. 2017). The domestic Bactrian camel and the wild camel shared a common ancestor an estimated 0.7 million years ago, whereas domestication of the Bactrian occurred approximately 6000 years ago (Burger, Ciani, and Faye 2019), long after the two species diverged. The wild ancestors of both the domestic Bactrian camel and the domestic dromedary are extinct, making wild camel the very last species of extant *Camelini* left on the planet.

Initial genetic studies to determine the uniqueness of the wild camel were mitochondrial, comparing Cytochrome B genes to find two divergent clades, with an average distance of 2.8 +/- 0.5% and with 26-33 substitutions (Ji et al. 2009). This led the authors to suggest that "the domestic camel and the wild camel may belong to two different lineages". This work was conducted on samples from Mongolia and China and so they also showed these populations as coming from the same lineage. Further work on the CytB gene gave a mitochondrial sequence divergence 1.9% (Silbermayr et al. 2010) and later an estimated a divergence of 1.8% (Mohandesan et al. 2017). These divergence estimates are comparable to divergence seen in other extant and wild populations, including that of the new world camelids, the lama and guanaco (Burger, Ciani, and Faye 2019). ATP genes also supported the theory that the wild camel and the domestic Bactrian camel have evolved from two distinct matrilineal lines. This work gave 16 haplotypes of which there were two haplogroups, one domestic and one wild (Ming et al. 2017). Further backing up this conclusion of sister taxa, was work on the paternal line (Felkel et al. 2019). The

Y chromosome can suitably compare male and female genealogies as the male-specific region of the Y chromosome (MSY) is also passed directly from parent (father) to offspring (son) without recombination. This study was the first Y phylogeny for the wild camel and it also showed two distinct haplotypes- one wild, one domestic. Genomic work on the wild camel was started in 2000 with the use of 20 new world microsatellites for the cross amplification of loci in old world camelids- including wild camels (Jianlin et al. 2000). This study used two Chinese wild camel samples to test markers. Further microsatellite work concluded that across all 20 loci there was a significant degree of differentiation between the wild and the domestic Bactrian camel (Silbermayr et al. 2010; Silbermayr K et al. 2010). In the first study to sequence the entire wild camel genome, it was estimated to be 2.38Gb (Jirimutu et al. 2012). In comparison, the domestic Bactrian camel genome size is 2.45Gb (Wu et al. 2014). They identified 244,141 microsatellite loci and found rates of heterozygosity of 1.0x10-3 across the whole genome. Further full genome sequencing for all three old world camelids was conducted in 2014 (Fitak 2014). When using genomic data to determine wild camel species uniqueness it was shown that the wild camel formed a distinct clade, separated from domestic Bactrian camel with a bootstrap value of 71% (Burger 2016).

The wild camel is critically endangered and although a robust population estimate is lacking, it is presumed to have both a low population number (N), and a low effective population (Ne). Reduced diversity has been seen in the mtDNA haplotype (Mohandesan et al. 2017; Felkel et al. 2019; Silbermayr et al. 2006), in the Y chromosome (Felkel et al. 2019) and genome-wide (Fitak et al. 2020). For the wild camel, Low Ne is thought to have first occurred between 200 thousand (k) to 20 k years ago (Fitak 2014). This was followed by rapid population expansion after the last glacial maximum and then a second population bottleneck of which todays apparent low N^e is caused (Fitak 2014).

Although hybridisation is often considered a major threat to the wild camel, levels of introgression with the domestic Bactrian camel are currently unknown. Understanding the risk of hybridisation between the Bactrian camel and the wild camel to overall species survival is important as the GGASPA lacks both

geographical and reproductive barriers (Silbermayr et al. 2006). Introgression has been seen in other camelids with hybridisation between domestic Bactrian camel and dromedary camel, *Camelus dromedarius,* for animal husbandry purposes being common (Burger 2016) and with the dromedary, after initial domestication, introgression followed from the now extinct wild populations (Almathen et al. 2016). Whilst we know that hybridisation occurs between Bactrian camels and wild camels (Silbermayr K et al. 2010) we do not know its extent across either the wild population or in captivity. The first work on this used PCR-RFLP to determine hybridisation in mtDNA (Silbermayr K et al. 2010). This was then followed by work on paternal hybridisation on the MSY (Felkel et al. 2019). In which one wild caught individual had a maternal wild haplotype and a paternal domestic haplotype (Felkel et al. 2019). RAD sequencing has also been used to determine hybridisation in captive bred wild camels in China (Zhang et al. 2019). It is important that introgression is monitored in the wild camel, as knowing what level it is may impact conservation objectives. Introgression may be relevant as it could reduce the genetic diversity of populations, leading them to be less able to adapt to change- further threatening species survival. Understanding levels of introgression is the first step in determining if this is indeed a threat to the wild camel.

1.4 Wild Camels Ex-situ.

There are only two known captive populations of wild camel. One in Mongolia and one in China. There is little available information on the Chinese population (Zhang et al. 2019) so the research in this PhD will focus only on the Mongolian population. The captive population was founded in 1995, originally as a collection of rescued or captured animals, managed by local herdsmen. The population was taken on by the Wild Camel Protection Foundation (WCPF) in 2001 as an insurance population **"**With so few captive animals, the whole species could be wiped out if their natural habitats in China and Mongolia are destroyed. It is therefore important to breed enough animals in captivity to insure against this

possible disaster" (WCPF 2023). The current holding facility at Zakhyn Us, Govi-Altai, Mongolia, was completed in 2004 and the first 13 animals were installed then. Of these 13, 10 animals were mother/offspring pairs, so only 8 can be considered founders. Females can breed from approximately 4 to 35 years old and some of the original founders are still breeding. The herd is currently at approximately 36 animals. A studbook has been partially maintained since the captive population was founded, yet some parentage is uncertain. Furthermore, as founders were not chosen, but obtained, we do not know how much of the available genetic diversity in the remnant wild herd in Mongolia is captured in the captive stock. Despite this, this herd has been successful both in terms of longevity and breeding success. It is also the most well understood captive population of this species. These ex-situ individuals contribute to extensive scientific research on the species, such as access to samples for genetic and disease analysis, veterinary studies and behavioural studies. All of which is research which may not be possible in wild populations(Zhang et al. 2019; WCPF 2023). The individuals in this ex-situ population are vital as they may constitute a significant proportion of overall species population. Extensive attempts are being made to improve the captive management of the species and to make the herd a much more effective insurance population.

Genetic studies on the Mongolian captive population have so far been used as a way of obtaining good quality genetic samples for initial wild camel genetic monitoring, such as genomic data (Jirimutu et al. 2012) and mitochondrial data (Silbermayr et al. 2010). Whereas the one published study on the Chinese population focuses on using RAD sequencing data to determine breeding suitability for conservation purposes. Of the 13 individuals at the Gansu captive breeding centre in China 11 individuals, did not show recent admixture, suggesting no recent hybridisation. Two showed genetic admixture of approximately 10%, which roughly corresponds to a 3rd generation cross back. Three individuals also had lower than expected heterozygosity values, caused by continuous inbreeding. These three individuals showed decreased heterozygosity in sites that are previously reported as highly discrepant

regions between wild and domestic, those that are essential in surviving extreme environments (Zhang et al. 2019).

Reduced diversity, increased inbreeding and hybridisation are all detrimental to captive management as they can lead to reduced fitness and compromise adaptability. Management of the captive insurance population of wild camels should aim to focus on maximising genetic diversity. It is of great importance to understand the genetic health of the captive population, to understand relatedness, to determine hybridisation risk and to determine if the diversity of the remnant wild populations is captured in the genetic stock. All of this will allow for improved management of the herd.

1.5 Conclusions

In this changing and threatened world, some species and habitats are especially threatened, with some teetering on the very brink of extinction. Larger bodied herbivorous mammals are one of the trophic groups at greatest risk, with 25% of all herbivores threatened with extinction (Atwood et al. 2020). With a narrow range of specialised habitat, small isolated populations and reduced genetic diversity (Yadamsuren, Daria, and Liu 2019; Y. Zhang et al. 2019; Xue et al. 2015), the wild camel is one of them. But effort is being made to save this species, both in-situ and ex-situ. Evidence is needed to provide some of the basic information to inform conservation management and decision making. This includes both a wild population abundance estimate and a greater understanding of the extent of hybridisation and diversity in both the wild population and the captive herd. The aim of this PhD research was to gain this evidence and to support the Wild Camel Protection Foundation (WCPF) in both its overall charitable aims; of reducing the probability of extinction of the critically endangered wild camel, *Camelus ferus,* and in its current goal of producing a species survival plan. By producing a research-based survival plan WCPF can provide evidence for the management of the species in both its range in the Great Gobi A Special Protected Area and in captivity.

1.6 PhD Data chapter outline

My data chapters follow:

Chapter 2: Common name misuse potentially confounds the conservation of the wild camel Camelus ferus. (Jemmett et al. 2023)

Published: Jemmett, Anna M., Jim J. Groombridge, John Hare, Adiya Yadamsuren, Pamela A. Burger, and John G. Ewen. 2023. "What's in a Name? Common Name Misuse Potentially Confounds the Conservation of the Wild Camel Camelus Ferus." *Oryx* 57 (2): 175–79.

Common naming of the wild camel, Camelus ferus, should reflect that is a separate species to the Bactrian camel, Camelus bactrianus, to reflect biological and cultural purposes.

Abstract*:* Common names allow species diversity to be acknowledged by experts and non-specialists alike. They are descriptors with both scientific and cultural implications, and lack of clarity when using a common name can risk altering perceptions of a threatened species. This is true for the Critically Endangered wild camel *Camelus ferus*, which, despite extensive scientific proof of its species status, is frequently referred to in English as "wild Bactrian camel". However, the wild camel (Mongolian: Khavtgai- Хавтгай and Chinese: 野骆驼 Ye Luo Tuo) is not a wild version of the domestic Bactrian camel *Camelus bactrianus* but a separate species in its own right, at the very edge of extinction, with an estimated population of c.950. Failure to clearly separate Bactrian and wild camels in name risks masking the plight of the few remaining wild camels with the visible abundance of the domesticated species. Here we advocate the use of an accurate English common name for *C. ferus* – the wild camel – ideally alongside its Indigenous names to correctly represent its cultural and conservation importance.

Chapter 3: Estimating Wild Camel, *Camelus ferus,* Abundance Using a Large-Scale Time-Lapse Camera Trap Design.

Using Camera Trap Distance Sampling in timelapse mode, the wild camel population in Mongolia is estimated to be 664 individuals (95% confidence intervals 400-1100).

Abstract: The Great Gobi A Special Protected Area (GGASPA), a 45,000 square kilometre protected area, is the final stronghold for the wild camel, *Camelus ferus*, in Mongolia. This area of the Gobi Desert is vast and remote, meaning that gaining information on this critically endangered species has been difficult. The last robust wild camel abundance estimate in the GGASPA was conducted over 25 years ago. We successfully used a novel method of distance sampling, using camera traps in timelapse function, to estimate wild camel abundance in the GGASPA. Our study is the first wildlife abundance estimation that used timelapse camera traps anywhere globally. This technique allowed for more efficient data collection from across the entirety of the GGASPA. We preliminarily estimate the wild camel population in the GGASPA to be 664 (95% confidence intervals 400-1100) which provides the most precise estimate of wild camel population in the GGASPA to date. It also emphasizes the continued low wild camel population size in Mongolia. This camera trap approach may be suitable for estimating abundance of species inhabiting remote locations at low densities.

Chapter 4: Surveillance of genetic diversity and introgression using non-invasive sampling of both in-situ and ex-situ Populations of Wild Camel, *Camelus ferus* in Mongolia.

Prevalence in the wild camel of introgression of DNA from domestic Bactrian camels is extensive, whilst genetic diversity and inbreeding levels are comparable in both the in-situ and ex-situ wild camel population.

Abstract: One of the main threats to extinction risk of the critically endangered wild camel, *Camelus ferus*, is hybridisation with the Bactrian camel, *Camelus bactrianus.* The last remaining stronghold of the wild camel in Mongolia is the 45,000 square kilometre Great Gobi A Special Protected Area (GGASPA), where this range-restricted threatened species comes into contact with the globally-

distributed domesticated Bactrian camel. Non-invasive sampling combined with genetic monitoring, using a combination of nuclear and mitochondrial DNA markers, has allowed us to gain a greater understanding of the extent and source of introgression and levels of genetic diversity in *Camelus ferus*, in both the wild population and the captive insurance population in Mongolia. Our results show evidence of both nuclear, mitochondrial and historic introgression of Bactrian camel genes in the *C. ferus* population across the GGASPA, and in some individuals within the captive herd. We also show that heterozygosity is reduced and inbreeding is increased in the wild population, and show that these levels are represented in the captive herd. Our findings illustrate that, whilst an acceptable level of introgression is largely determined by thresholds adopted by the global conservation community, a detailed genetic perspective is crucial in increasing our understanding of the hybrid problem and is an important first step towards identifying options for conservation management.

Chapter 5: Does our insurance cover extinction? Ex-situ populations of highly threatened mammals

Ex-situ populations provide widely variable contributions to mitigating a taxon's extinction risk which is inadequately assessed in conservation.

Abstract: The future of all critically endangered species is precarious, and any individuals maintained in ex-situ captivity are potentially crucial to the species' persistence. In these cases, if an extinction event were to happen in the wild, it is important for managers to be confident that ex-situ populations represent adequate insurance against outright extinction. Available guidance on best practices in population management, population size targets and conservation planning could help ensure they can provide this. We characterised these critical factors to assess the ex-situ populations of the world's most threatened mammal taxa as determined by the IUCN Red List as either critically endangered (N=291) or extinct in the wild (N=2). We found that of these 293 mammal taxa, approximately a quarter (69) are represented in ex-situ care, almost double the number reported on the Red List. Worryingly, almost all (91%) of these are held at population sizes below 500, with 44% (29) falling below 50

individuals. Although 67% show genetic monitoring through pedigree analyses, only 10% are monitored demographically. We conclude that despite their proven conservation potential, ex-situ populations constitute inadequate insurance policies against extinction for many of the most threatened mammals.

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Chapter 2 What is in a name? Common name misuse potentially confounds the conservation of the wild camel *Camelus ferus.*

Common naming of the wild camel, Camelus ferus, should reflect that it is a separate species to the Bactrian camel, Camelus bactrianus, to reflect biological and cultural purposes.

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There are some edits to the published manuscript in this thesis chapter.

Abstract:

Common names allow species diversity to be acknowledged by experts and non-specialists alike. They are descriptors with both scientific and cultural implications, and lack of clarity when using a common name can risk altering perceptions of a threatened species. This is true for the Critically Endangered wild camel *Camelus ferus*, which, despite extensive scientific proof of its species status, is frequently referred to in English as "wild Bactrian camel". However, the wild camel (Mongolian: Khavtgai- Хавтгай and Chinese: 野骆驼 Ye Luo Tuo) is not a wild version of the domestic Bactrian camel *Camelus bactrianus* but a separate species in its own right, at the very edge of extinction, with an estimated population of c.950. Failure to clearly separate Bactrian and wild camels in name risks masking the plight of the few remaining wild camels with the visible abundance of the domesticated species. Here we advocate the use of an accurate English common name for *C. ferus* – the wild camel – ideally alongside its Indigenous names to correctly represent its cultural and conservation importance.

2.1. Introduction

The Roman Empire's camel-riding armed forces were named the "Dromedarii". Although both dromedary *Camelus dromedaries* and Bactrian camels *Camelus bactrianus* were present across the Roman Empire (Vuković-Bogdanović and Blažić 2014) and used by these armies, the Romans didn't deem it necessary to distinguish between the two species (Tomczyk 2016; Nefedkin 2012). It may be that the Romans did not need to distinguish between a one-humped and a two-humped camel as they performed similarly in war. Descriptions of camels by Pliny the Elder, and previously Aristotle (Bostock and Riley 1855) portray their temperament and endurance as one, contrasting to that of the horse (of which one role was to counter enemy cavalry). Even today, the global database for livestock (FAOSTAT 2023) does not distinguish between domesticated one- and two-humped camels, instead grouping them together (Faye 2020). However, failing to distinguish the two separate species of double humped camels may have conservation ramifications given one is at risk of extinction.

2.2 Camel evolution and distribution

After dispersing from the North American continent to Eurasia, the ancestors of modern camelids diverged into the *Lamini*, which include the llama *Lama glama*, alpaca *Vicugna pacos*, vicuña *Vicugna vicugna* and guanaco *Lama guanicoe*, and *Camelini* (Burger, Ciani, and Faye 2019). There are three species of *Camelini* ; these are the one-humped domestic dromedary *Camelus dromedarius*, the two-humped domestic Bactrian camel *Camelus bactrianus* (the species which the Romans came into contact with first (Nefedkin 2012)) and the Critically Endangered (IUCN RedList 2022) two-humped wild camel *Camelus ferus,* Mongolian: *Хаѳмгаӣ Mongolian, Chinese: **野**骆驼* (Figure 2.1). The one- and two-humped camels are estimated to have diverged around 4.4 [1.9-7.2] Million Years Ago (MYA) (Wu et al. 2014). Divergence estimates for the wild camel and Bactrian camel vary depending on whether maternal or paternal DNA are used (reflecting the different size of these sex chromosomes) but range from 0.7 MYA (Ji et al. 2009) and 1.1 [0.58-1.8] MYA (Mohandesan et al. 2017) from mitochondrial studies to approximately 27,000 YA on the male-specific region of the Y chromosome (Felkel et al., 2019). The Bactrian camel is monophyletic (Ji et al. 2009) and so originates from one wild population with a single domestication process around 4000-6000 YA (Burger, Ciani, and Faye 2019). The direct ancestors of the Bactrian camel either became extinct or all were completely domesticated, leaving no wild *Camelus bactrianus* population behind- this is similar to the domestication process of the dromedary (Almathen et al. 2016) or the horse (Gaunitz et al. 2018). Bactrian domestication occurred long after divergence estimates with wild camel, such that the wild camel is neither the direct progenitor of the Bactrian camel, nor is it a feral version of this species, but rather a sister species.

In this paper, we argue for the use of an accurate English common name for *Camelus ferus* – the wild camel – ideally alongside its indigenous name to correctly differentiate these Critically Endangered (IUCN RedList 2022) wild animals from their domesticated congeners.

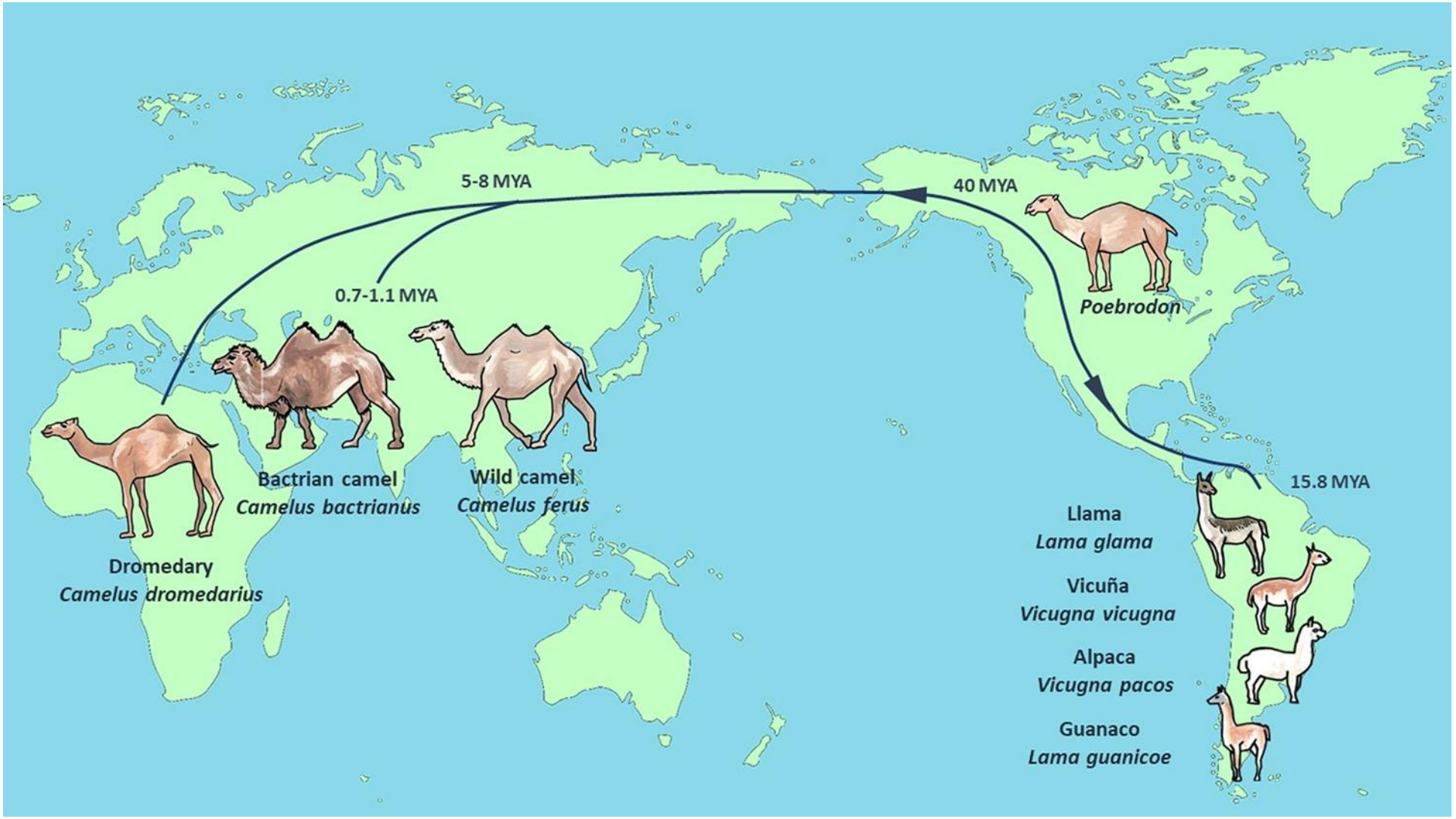

Figure 2.1(a): *The evolution of the three Camelini species (dromedary Camelus dromedarius, Bactrian camel Camelus bactrianus and wild camel Camelus ferus) and the Lamini species (guanaco Lama guanicoe, llama Lama glama, alpaca Vicugna pacos and vicuña Vicugna vicugna) from the ancestral Poebrodon.*

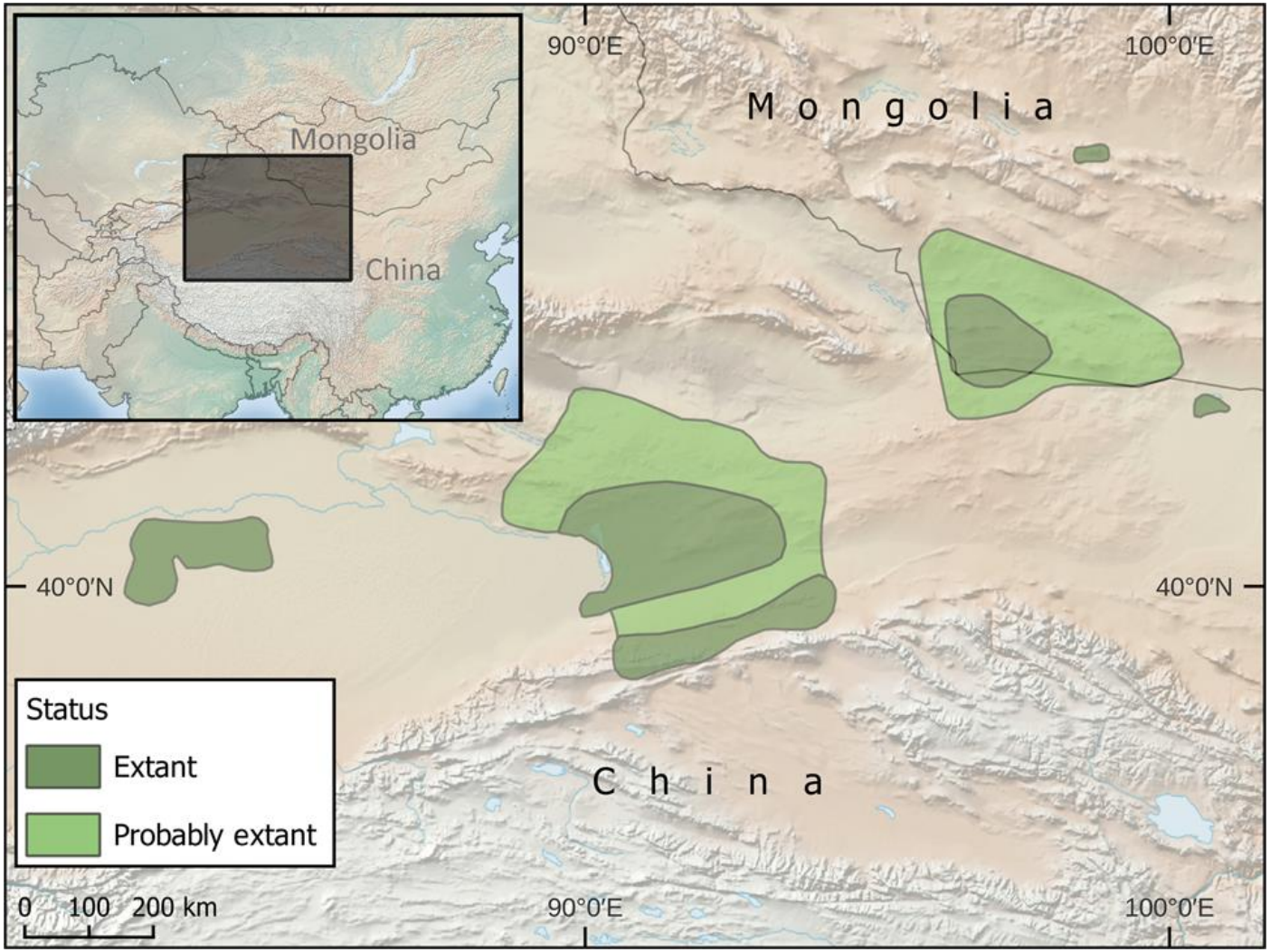

Figure 2.2(b): *Current range of the wild camel Camelus ferus (data from the IUCN Red List)*

2.3 Camel names

Despite the extensive scientific proof that supports a species-level distinction between the wild camel *Camelus ferus* and the Bactrian camel *Camelus bactrianus* (Fitak et al. 2020; Ming et al. 2017; Felkel et al. 2019; Han et al. 2002; Ji et al. 2009; Jirimutu et al. 2012; Mohandesan et al. 2017; Silbermayr et al. 2010), the English text common names currently used for *Camelus ferus* are "wild Bactrian camel", "wild two-humped camel" and "wild camel". As the name "Bactrian camel" refers to the place of potential domestication in the ancient region of "Bactria" (modern-day Afghanistan), we believe that the use of "Bactrian" should not be applied when describing the wild species, as it is inaccurate and confuses the clear distinctions between these species. Throughout this text we will use the English common name "wild camel" to describe *Camelus ferus* and "Bactrian camel" to describe *Camelus bactrianus*. We are not proposing this name but rather reporting a position taken by most wild camel researchers who, writing in English, now use wild camel or wild two-humped camel exclusively in research publications (Burger, Ciani, and Faye 2019; Farnworth, Campbell, and Adams 2011; Lado et al. 2020).

The wild camel, originally given the scientific name *Camelus bactrianus ferus*, reverted to the first available specific name based on a wild population (as a naming standard change for presumed progenitor species, not due to species distinction), *Camelus ferus* (Gentry, Clutton-Brock, and Groves 2004). Wild camels were first described by the renowned Imperial Russian explorer and geographer, Nikolaj Przwalski in 1878 (IUCN RedList 2022). Unknown to the western world until this point, it was presumed to be either a feral version of the Bactrian camel or the wild ancestor from which the Bactrian camel was domesticated. For this reason, the species was named *Camelus bactrianus ferus* meaning "wild/ feral Bactrian camel". Throughout its range across Mongolia and China, the wild camel was locally thought of and consequently named a separate species to the Bactrian camel (Hare 1997). This attitude was based on both behavioural characteristics of "wildness" and distinct morphological differences (Figure 2.2). These differences include a smaller, pyramid-shaped hump, smaller body, and slimmer legs

in the wild camel (Ji et al. 2009), and most notably a flatter skull. The name for the wild camel in Mongolia is Khavtgai- Хавтгай- translating to “flat head”. In China the animal is called 野骆驼-Ye Luo Tuo, which means “wild camel”.

***Figure 2.3**: Morphological differences. Bactrian camel, Camelus bactrianus: Left (both top and bottom) and Wild camel **Camelus** ferus: Right (both top and bottom). Morphological differences include smaller, pyramid-shaped humps, a smaller body, slimmer legs and a flatter skull in C. ferus. Top images: Anna Jemmett. Bottom image: Pauline Charruau*

2.4 Confusion of scientific names and implications

Scientific naming is determined by taxonomy which itself ought to be underpinned by evolutionary genetic, morphological and ecological evidence of distinction. It allows for the accurate identification and classification of a species (Suren 2018) which is an important component for determining conservation status. Scientific names are vital for scientists and practitioners who work in species conservation, they allow global understanding and provide consistency irrespective of the language spoken, but they are not widely used beyond the (conservation) scientific community. This is where a common name is important. A common name allows science experts to communicate with a wider non-specialist audience (Sarasa, Alasaad, and Pérez 2012). Therefore, common names also play a crucial role as a descriptor that allows for the distinction between one species and another, whilst also providing a more emotional connector between humans and other species. Common names mean that everyone can appreciate diversity (Ehmke, Fitzsimons, and Garnett 2018).

Wild camel has always been known as different and distinct from the Bactrian camel in Mongolian: “Temee- Тэмээ” for domestic Bactrian and “Khavtgai- Хавтгай” for wild camel. Something that was not recognised by the Western world until genetic data (Silbermayr et al. 2010; Jirimutu et al. 2012) confirmed this view.

Elsewhere, there have been calls for indigenous names, where possible, be reinstated to decolonise taxonomy (Mabele, Kiwango, and Mwanyoka 2023). Indigenous or local names recognise the societal value systems of the people who interact most with that species (Guedes et al. 2023). These indigenous names both reflect cultural and historic knowledge of species ecology, but also, as is the case here,

indigenous naming is often constant when English common naming changes with taxonomic change (Gillman and Wright 2020). Given that so many cultural values are linked to species, care should be taken when considering naming or renaming. Something as simple, and serious, as a name can have long lasting ramifications both for local people and the conservation of the relevant species. For example, in biodiversity reporting in New Zealand, using Maori species names has been shown to "support the cultural aspirations of Maori, [it] helps to retain the Maori language and implicitly acknowledges indigenous relationships with the environment" (Wehi et al. 2019). Although our focus here is on correcting an inaccuracy in English common naming for wild camels, we also encourage the use of indigenous names alongside these wherever possible.

Critically, common names can affect human perception of a species' value, invoking emotional responses which can have both positive and negative consequences for the conservation of that species. This phenomenon is widespread. In Europe for example, local renaming of ibex to wild goat lowered people's perception of the animal's conservation importance (Sarasa, Alasaad, and Pérez 2012). In New Zealand public attitude towards lethal control was seen as more acceptable for "feral" cats than with "stray" cats (Farnworth, Campbell, and Adams 2011). In Australia, there is an intriguing distinction between the use of "wild dog" in livestock production literature, where messaging is often used for species control, and the use of 'dingo' in conservation-related literature (Kreplins et al. 2018). Elsewhere in Australia, a call to standardize common names for sub-species of threatened birds was made to improve public appeal for conservation, as common names have been shown to reduce or increase conservation appeal (Ehmke, Fitzsimons, and Garnett 2018). In Africa, much like the wild camel, the *Lycaon pictus* has struggled with consistent, accurate nomenclature (a mix of African Wild Dog, Painted Hunting Dog or Painted hunting wolf) which confuses audiences, and some argue alters public perceptions of the species (Blades 2020).

2.5 Conservation status of the wild camel

The wild camel is categorized on the IUCN Red List as Critically Endangered (IUCN RedList 2022). Despite being a large, charismatic mammal (Macdonald et al. 2015), the wild camels' risk of global extinction may not be at the forefront of people's minds because of the inaccurate information available to the public (EDGE, 2021). The widely held image of double-humped camels is the domestic animal- not this rare species. On the Zoological Information Management Software (ZIMS) (Species360 2023), (a web-based system used by zoological and wildlife facilities to hold and share information, including studbooks, both for animal management and conservation) there are currently 934 Bactrian camels in captivity. They are spread across 263 zoos and private collections (Species360 2023). In Mongolia alone, national statistics estimated the 2019 population of Bactrian camels to be 459,400. Although there is no accurate global population estimate, the FAOSTAT database, which does not distinguish between Bactrian camels or dromedary camels, estimates the total global domestic camel population to be over 35 million individuals (Faye 2020). As for the wild camel, there are just 34 individuals in captivity in a single institution (in Mongolia), and less than 1000 remaining in the wild across Mongolia and China (IUCN RedList 2022). It is therefore understandable that the first animal that comes to mind when thinking of a double humped camel is the Bactrian camel. Most people will be aware of the Bactrian camel. They will have seen it on TV, in zoos or private collections, or working as a beast of burden and so will presume, correctly, that this species is safe from risk of extinction.

Zoological institutions are partly responsible for inaccuracies in naming by failing to clearly distinguish wild camels from the Bactrian camels held in their collections. It may be zoological institutions are using the plight of the wild camel to advertise the Bactrian camels they have on display. For example, we searched the websites of all zoological organizations recorded as holding Bactrian camel on ZIMS (searches conducted between the 8th and 12th of March 2021) to assess how they referred to their Bactrian camels. Of 263 institutions, 134 (all in Europe or North America) had some information available on their websites. We found that of 133 institutions reporting the species common name, 2% incorrectly referred to their camels as 'wild camel'. This largely correct use of common name is not

surprising as 'Bactrian' is wrongly linked to both *C.ferus* and *C.bactrianus*, so in common naming zoological institutions may be correct by default. More strikingly however, 16% of 102 institutions who reported the scientific name did so incorrectly, using *Camelus ferus* to advertise *Camelus bactrianus*. 85 institutions reported IUCN Redlist status, of these a substantial 84% reported it incorrectly. A correct Redlist status for *C.bactrianus* would be 'not evaluated' or 'domesticated' rather than the commonly reported 'critically endangered'. Of the 96 institutions containing information on both species, either in naming or extinction threat. Only 21 institutions, 22%, actually state that there are two separate species. By unknowingly or intentionally advertising Bactrian camel as *Camelus ferus* or "critically endangered", institutions are failing to distinguish the two species and their respective conservation value. This risks generating a public perception of the species being held safely and widely in captivity. Something that is not the case. Zoological institutions are not the only places where camel naming inaccuracies occur. The IUCN red list uses *C. bactrianus* as a synonym for *C. ferus* (IUCN RedList 2022) and the Oxford English Dictionary definition of the Bactrian Camel states "The two-humped camel, which has been domesticated but is still found wild in central Asia. *Camelus ferus* (including the domesticated *C. bactrianus*), family Camelidae 'The wild Bactrian camel, a two-humped ancestor of domesticated camels, is now critically endangered in its native habitat in the harsh deserts of Northwest China and Mongolia.' With such abundant misinformation confusion is understandable.

2.6 Conclusions

Inappropriate use of English common names for the wild camel may contribute to the continued confusion in species distinction and risks generating (or reinforcing) a perception of a "critically endangered" species that is at least safe within captivity. In English texts, we advocate not using the word "Bactrian" and using the English common name "wild camel" to describe *Camelus ferus*. Indigenous names should also be used either in place of English common names, or alongside them, wherever possible. As, unlike the Romans, we have good reason to distinguish between camel species.

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Chapter 3 Estimating Wild Camel, *Camelus ferus,* Abundance Using a Large-Scale Time-Lapse Camera Trap Design.

Using Camera Trap Distance Sampling in timelapse mode, the wild camel population in Mongolia is estimated to be 664 individuals (95% confidence intervals 400-1100).

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Abstract

The Great Gobi A Special Protected Area (GGASPA), a 45,000 square kilometre protected area, is the final stronghold for the wild camel, *Camelus ferus*, in Mongolia. This area of the Gobi Desert is vast and remote, meaning that gaining information on this critically endangered species has been difficult. The last robust wild camel abundance estimate in the GGASPA was conducted over 25 years ago. We successfully used a novel method of distance sampling, using camera traps in timelapse function, to estimate wild camel abundance in the GGASPA. Our study is the first wildlife abundance estimation that used timelapse camera traps anywhere globally. This technique allowed for more efficient data collection from across the entirety of the GGASPA. We preliminarily estimate the wild camel population in the GGASPA to be 664 (95% confidence intervals 400-1100) which provides the most precise estimate of wild camel population in the GGASPA to date. It also emphasizes the continued low wild camel population size in Mongolia. This camera trap approach may be suitable for estimating abundance of species inhabiting remote locations at low densities.

3.1. Introduction

Understanding species abundance is crucial in the estimation of extinction risk of threatened species. But these estimates can be difficult to gain, especially for those species which are elusive, have small populations or are distributed across vast ranges. All of these considerations apply to the critically endangered wild camel, *Camelus ferus.* The wild camel survives only in the Gashun Gobi, Lop Nur and the Taklamakan deserts of China and within the Great Gobi A Special Protected area (GGASPA) in Mongolia. The GGASPA covers approximately 45,000km^2 of the Transaltai Gobi (Yadamsuren, Daria, and Liu 2019). Gaining information on the wild camel has been difficult due to the extreme remoteness of these remaining habitats. Many factors are thought to threaten the wild camel's survival, including: habitat loss and degradation; desertification due to climate change; and, hybridisation with the domestic Bactrian camel, *Camelus bactrianus*, (Chapter 4). Further research is needed to better

understand the species, the threats to its survival, including estimates of how many wild camel remain (Kaczensky et al. 2014).

The wild camel population size is presumed to be extremely low but stable (Yadamsuren, Daria, and Liu 2019). The current population estimate on the IUCN RedList (IUCN RedList 2022) is based on expert judgement and suggests a global population of 950, with 600 in China and 350 in Mongolia. Although population estimate studies have been conducted (Reading et al. 1999; Bannikov 1975; Hare 1997; Tulgat and Schaller 1992; Dash et al. 1977; Gu and Gao 1987; Zhirnov et al. 1986; Bannikov 1945), they come from a combination of historic surveys and observation data with low precision (the margin of error of those estimates) and likely poor accuracy (how close the estimate is to the true population value) due to bias in sampling design. A statistically robust approach, using unbiased systematically collected data has eluded wild camel researchers until recent advances in use of camera traps and associated analytical tools.

Population estimates in the GGASPA are highly variable, with a low estimate of 300 individuals (expert judgement without uncertainty) in 1943 (Bannikov 1945), to 1985 (95% CI=413 to 3557) individuals in 1997 (Reading et al. 1999). Surveys were conducted as aerial transects (Reading et al. 1999), vehicle transects (Dash et al. 1977), a combination (Tulgat and Schaller 1992), as well as on foot, or travelling with domestic Bactrian camels (Hare 1997) and counting at water points (Tulgat and Schaller 1992). Only one of these studies report an estimate of precision around the count based on a distance sampling analysis of aerial transects (1985, CI=413 to 3557) (Reading et al. 1999). In most cases the authors conclude that there is a need for a more accurate and precise population estimates to be made.

Camera traps have become increasingly important in species management. Camera traps have been extensively used to estimate density using capture mark recapture models (CMR). CMR depends on individual recognition, for example recognising individual tigers, *Panthera tigris*, by their stripes (Karanth 1995). This is not possible for most species (including the wild camel) as individuals are not recognisable. To overcome this a range of analytical approaches have been developed for estimating

density of unmarked species. These include the Random Encounter Modelling (REM) (Rowcliffe et al. 2008) and Random Encounter and Staying Time (REST) models (Nakashima, Fukasawa, and Samejima 2018) both of which are based on the ideal gas theory. REM uses random encounters between animals and static camera traps to estimate abundance. REST is similar but requires knowing the amount of time an animal remains in the field of view of the camera. Detections can be variable in both of these methods, so alternatives that use cameras in time-lapse mode to take photographs at regular intervals, to decrease uncertainty in detection, can be used. Two such models are the Time To Event (TTE), and Space To Event (STE) methods (Moeller, Lukacs, and Horne 2018). TTE estimates abundance from the trapping rate, STE is the same but replaces time with space.

Distance sampling, which uses the measurement of distances to detections, is a standard, well-described, and widely-used method in the estimation of animal abundance, including of unmarked animals (Thomas et al. 2010). Distance sampling methods often use observers to record detections, but using camera traps can be advantageous. They can be more cost effective, can survey for a greater period of time, can be less prone to observer bias, and can cause less disturbance to animals (Fleming et al. 2014). Distance sampling assumes that not every animal in each survey point is detected. It is a two-step process (Buckland et al. 2001). The first is fitting a detection function to distances of animals from the observer (used to estimate the proportion of animals missed within the covered area); the second to estimate density based on the number of animals seen, the size of the covered area, and the proportion of animals missed within the covered area. The density estimate can then be used to estimate abundance across the entire survey area.

Here we used camera traps and distance sampling to estimate the abundance of wild camel with a novel application of CTDS using timelapse rather than triggered cameras. Time lapse, at a moderately high frequency, can survey a larger area at preset intervals than trigger images, which are constrained by the trigger distance (typically much shorter than the full field of view). We calculated this would be more appropriate for a species likely found at extremely low density within a large area. This chapter

therefore has two aims: (i) to trial a novel method of CTDS using timelapse function and (ii) to provide a precise abundance estimate of wild camel population size in the GGASPA.

3.2. Methods

3.2.1 Camera trap specification.

We used L-Shine LS 987 and Crenova wide angle camera traps, with a field of view of 130 degrees (typically camera traps have a 35-55 degree field of view), helping to maximise detectability of wildlife. All of the Crenova cameras failed in the 2019 trial (N=20) (Appendix 3.5.5) and were subsequently replaced with L-shines. Prior to the survey, we showed it to be possible to detect large mammal species (horse/sheep/camel) greater than 150 meters away (Appendix 3.5.1). Cameras were set to take timelapse images at a resolution of 5 megapixels, every 20 minutes during daylight hours (between 5 am and 8 pm). This frequency was chosen to generate sufficient images to maximise the detection of wildlife while allowing continuous operation over six-month deployments.

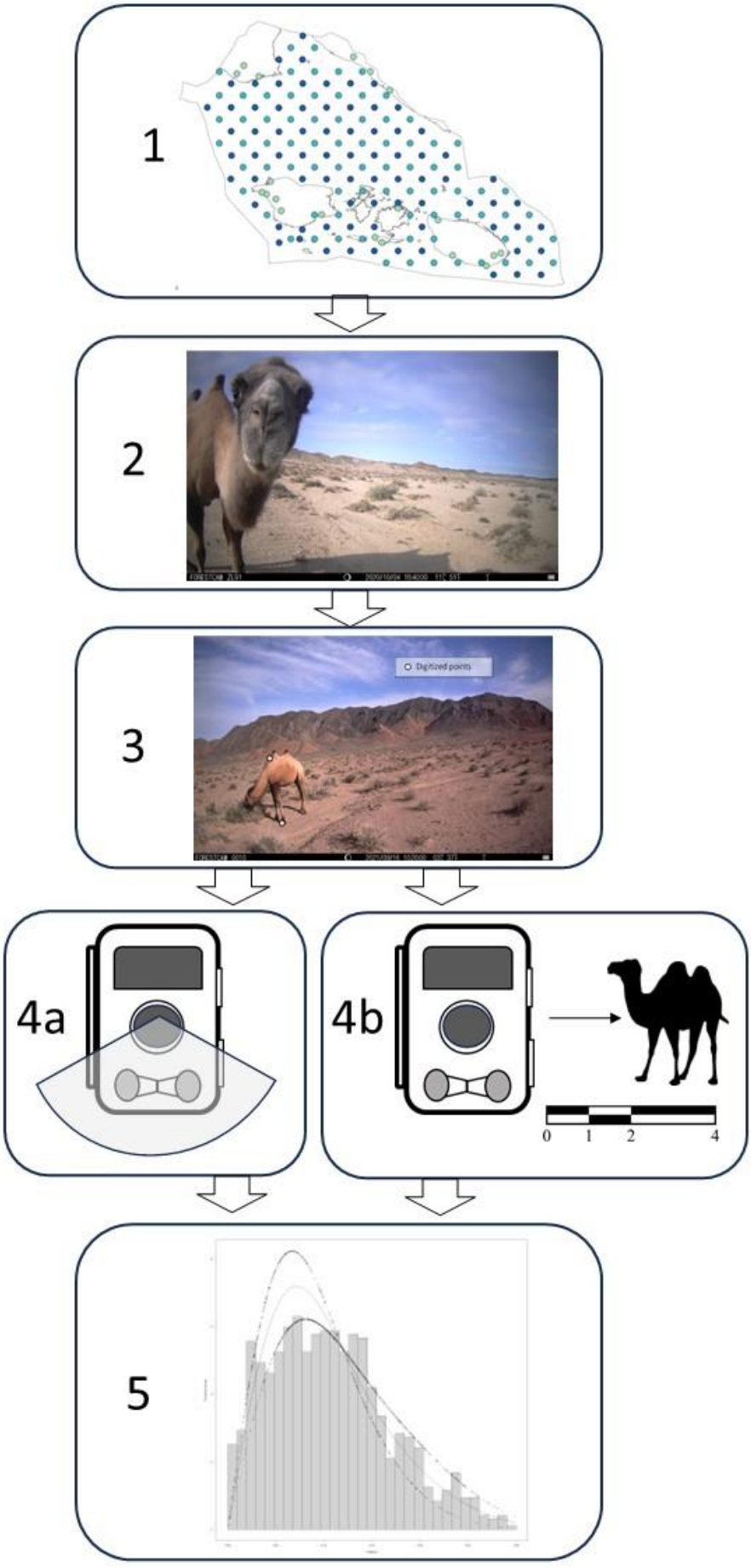
1
2
3
Digitized points
4a
4b
0 1 2 4
5

Figure 3.0: *Framework detailing the 5 main stages of this distance sampling methodology. Stage 1 outlines the survey design process. Stage 2 outlines camera trap deployment and image collection. Stage 3 looks at image processing, which includes image sorting, data cleaning and digitisation. Stage 4 uses the data extracted from digitised images to produce the distance data, 4a=Effort data, 4b=distance data. The final Stage 5 fits the detection functions to distance date to produce a density estimate.*

3.2.2 Stage 1: Survey design

Wild camels are known to use the entirety of the GGASPA, excluding the high mountains (Kaczensky et al. 2014; Yadamsuren, Daria, and Liu 2019). To ensure the camera deployment was as comprehensive and representative as possible, a form of stratified systematic survey design was used to predetermine locations for cameras. The GGASPA is mostly covered by low-lying plains that are easily accessible by vehicle, but also contains mountainous areas that are difficult for either wild camels or humans to access. However, wild camels do access canyons that traverse mountainous areas, possibly favouring them for crossings, water, food and shelter (Yadamsuren, Daria, and Liu 2019). Two zones were created (Appendix 3.5.2) by using a 1500-meter contour line as a boundary. Above the 1500-meter contour was the "mountain" zone and below the contour was considered the "flat" zone. The "flat" zone was treated as a single polygon in ArcGIS (ESRI 2023) and a grid overlaid using the Fishnet data management tool (ESRI 2023) We used the centre point of each grid cell as a random location for a camera. In the "mountainous" zone we mapped the canyons by hand in ArcGIS using Basemaps and the polyline tool (Appendix 3.5.2). The canyons were then ground-truthed by researchers and rangers in Mongolia. Final polylines were treated as one continuous transect, with points allocated equidistantly.

In 2019 we deployed 80 cameras at a spacing of 22 km on the plains, and 10 cameras at a spacing of 46 km in canyons (Figure 3.1), running for two six-month deployments. Preliminary analysis of this data (Appendix 3.5.5) suggested that increasing the density of cameras could improve precision in our abundance estimates. Therefore in 2020 we doubled the number of cameras to 160 in the plains (spacing 11 km) and 20 in canyons (spacing 23 km) (Figure 3.1). The 2019 locations were re-used, and new locations created equidistant from those, in the case of the plains grid locating new points at the vertices of the original to maintain a systematic grid structure. This larger array was then run for a further three six-month deployments (Table 3.1).

3.2.3 Stage 2: Camera deployment

Camera deployment and subsequent checks and collection were conducted by authors and Ranger teams from the GGASPA Park Authority. After initial training in camera methods, teams travelled to pre-determined grid co-ordinates. Camera traps were placed within 500 meters of that pre- determined grid co-ordinate. Within this area, where possible, placement choice took the following into consideration:

1- Direction. Cameras were placed facing North (N). If N was not possible then they were placed facing either North-East (NE) or North-West (NW). Placing the cameras to face N avoids glare from the rising and setting sun. NE placement was also considered where prevailing northerly or westerly winds may blow sand into the camera lens.

2- Visibility. Cameras were placed with the maximum possible field of view. They were not placed directly behind anything that would obstruct the image such as cliff faces or thick vegetation.

Height. Where possible cameras were placed at a higher elevation to the surrounding land (e.g. on top of a hill or rise). This was to gain maximum visibility for the camera. All cameras were also set on a post at 1.5 meters high (full protocol can be found in Appendix 3.5.3).

Survey	Start	End	Number of cameras
A	April 2019	October 2019	90
B	October 2019	May 2020	90
C	May 2020	October 2020	180
D	October 2020	May 2021	180
E	May 2021	November 2021	180

Table 3.1*: Camera survey information detailing the start (camera placement/SD card change) and the end (camera/SD card collection) period and the number of cameras included.*

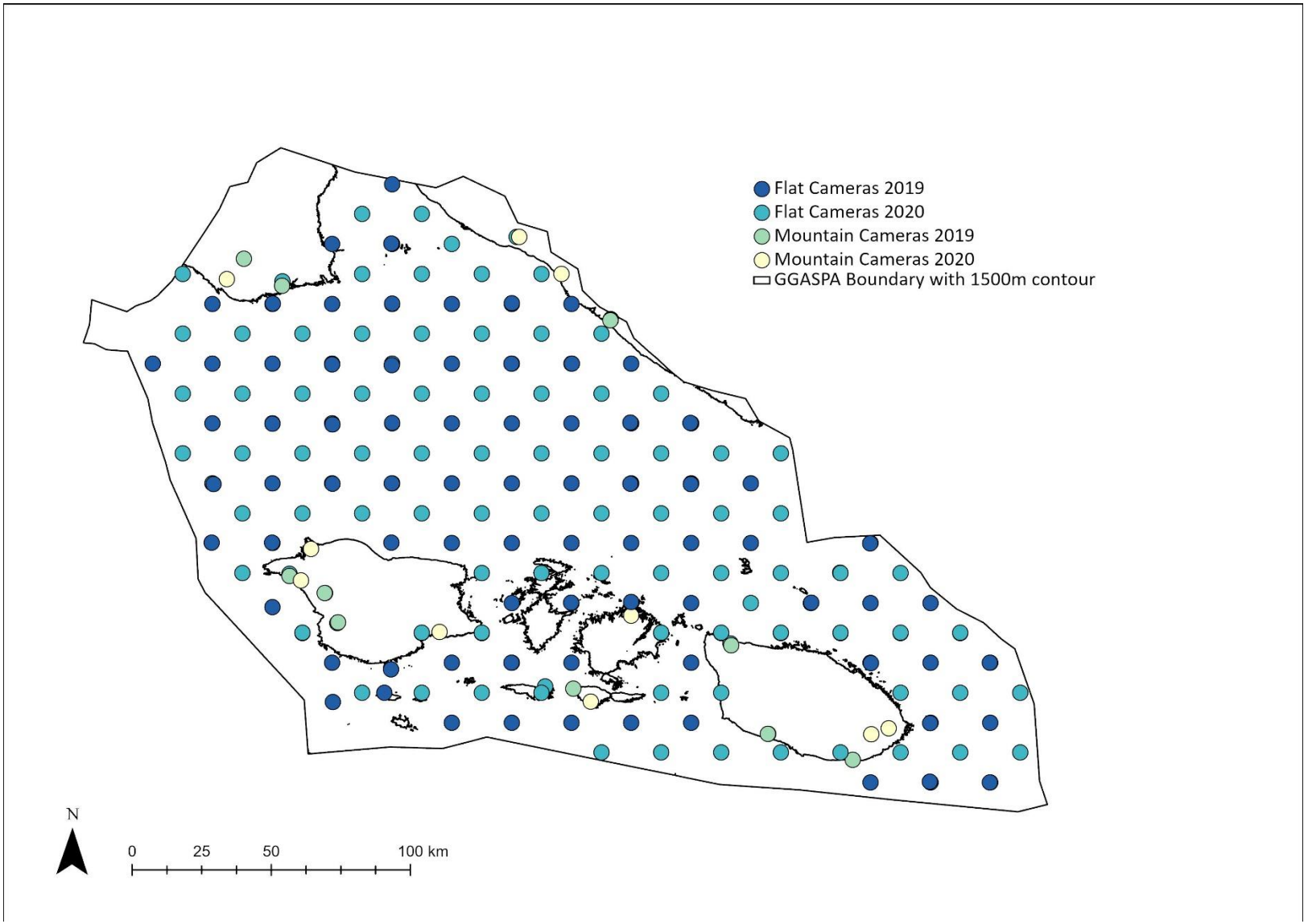

Figure 3.1: Camera trap locations including 2019 Flat cameras and Mountain cameras and, after project extension, 2020 Flat cameras and Mountain cameras.

3.2.4 Stage 3: Image processing.

Every image was tagged using either the Exifpro (ExifPro 2023) or the XnView (Pierre 2023) software. Tags covered three core categories: 1) Placement - used to indicate camera placement ID where the image was taken; 2) Species - used to indicate the species present in images; 3) Count - used to indicate the number of individual animals of a given species in each image. A further fourth 'Comment' category allowed for unusable images to be removed from analysis (e.g. "Poor quality images" and "Camera knocked over"). Any images that were not set to timelapse, either due to mis-setting or malfunctioning, were removed from further analysis, as were poor quality images, images from cameras that were knocked over, night images and all images from day 1, to avoid disturbance errors. We performed data cleaning and sorting in R (R Core Team 2023) using functions provided in (Rowcliffe 2023a)

In order calculate distances from a camera to the wild camel, the camera's parameters (specifically focal length to sensor size ratio) were first estimated using images of a pole of known size at several known distances from an LS987 camera with a 5 M resolution Camel captures were extracted from usable camera trap images (Table 3.2) and digitized using Animal Tracker open software (Varzco 2023). Each animal detected was digitized with 2 points, one at the ground/foot and one at the shoulder (Figure 3.2). Camel shoulder height was estimated as the minimum average measured on Bactrian camels (Lamo et al. 2020) given body measurement data is not available for wild camels. Camera calibration and camel distance calculations were conducted in R Studio (R Core Team 2023) using package CTtracking (Rowcliffe 2023b).

***Figure 3.2**: Example of camera trap image digitization. Points were digitized by hand using Animal Tracker open software (Varzco 2023). Each animal detected was digitized with 2 points, one at the ground/foot and one at the shoulder.*

3.2.5 Stage 4: Distance data

Distance sampling analysis was conducted in R (R Core Team 2023) using package Distance (Miller et al. 2019). Effort data (determined as number of timelapse images per camera trap per survey season, multiplied by the camera field of view, 130/360 - stage 4a), was then combined with distance data (distance from camera to animal – stage 4b) to give a "flatfile" for subsequent analysis. Analysis was conducted with pooled data from all 5 survey periods.

3.2.6 Stage 5: Fitting detection functions

Detection functions were fitted to camel distance data, describing the relationship between probability of detection and distance, from which probability of detection within the covered region can be calculated. First, distance distributions were visualized to assess outliers, suggesting a reasonable truncation distance of 0.3 km, beyond which data were discarded. Alternative point transect detection

function distributions were then fitted to the truncated data and compared. The detection function was estimated by fitting the following models to the data:

1. Half-normal distribution with cosine adjustment terms of order 2. Order 2 was chosen as best fit after trialling 0-5;
2. Half-normal distribution (with parameters set as above), but separating effort into summer and winter seasons to determine if there was seasonal variation in camel detection;
3. Hazard rate distribution with 3 cosine adjustment terms; and
4. 4: Uniform distribution with 3 cosine adjustment terms.

Akaike Information Criteria (AIC, Akaike 1998) were then used to select the best supported model. The distribution function with the best fit was further tested for goodness of fit with visual inspection of Q-Q plots and Cramer-Von Mises (Chiu and Liu 2009). The best fitting model was used to estimate average density across the entirety of the GGASPA, excluding areas above 1500m which camels cannot access, and population size was estimated by multiplying density by the area of this study area (38,000 km^2). Standard errors (SE) were calculated empirically based on variation in encounter rate between camera locations and the variance in detection probability. Coefficients of variance (CV – SE expressed as a proportion of the mean) were calculated to measure uncertainty in the abundance estimate.

3.3 Results

Eighty four percent of cameras placed produced useable data for at least part of their deployment. From these, a total of 3,777,494 images were used in analysis. Across the six-month survey periods A-E (Table 3.1), 1034 wild camel captures were recorded. For cameras with captures, the mean number of wild camel captures per camera was 19 (range 1 to 373).

Across survey periods, the mean percentage of cameras with at least one camel capture in that period was 13% (range 6-17%) (Table 3.2, Figure 3.3). 56 cameras (31%) in total had at least one capture across all survey periods (Table 3.2). Of these, the majority (77%, 43 cameras) had captures in just one survey

period. 16% (9 cameras) had captures in 2 survey periods, 2% (1 camera) had captures in 3 survey periods, 5% (3 cameras) had captures in 4 survey periods and 0 had captures across all 5. One camera had 35% of total captures, 29% (N=304) of which were in survey D alone.

23% of the total cameras with captures (13) were from camera placements within the "mountain" zone, but only 11% of cameras were "mountain" placements. Overall, 65% of the total "mountain" cameras captured camels, while only 26% of "flat" cameras had captures. Captures were focused around the central mountainous areas of the park, and this spatial pattern was reasonably consistent across seasons (Figures 3.3 and 3.4), showing greatest captures along the mountains of the central and Northern GGASPA.

Deployment	Analysed cameras	% of grid working	Effort	Cameras with captures	%	Images with captures	Camels tagged	Camel capture rates (per mil)
A	77	86%	497078	11	14%	32	61	123
B	70	78%	486485	11	16%	49	120	247
C	163	91%	1010172	9	6%	39	104	103
D	145	81%	1122078	24	17%	163	527	470
E	149	83%	661681	21	14%	135	235	355

Table 3.2: *Camera survey deployment information and initial analysis. Analysed cameras show the number of cameras that produced useable timelapse images. % of grid working is the overall grid that worked in that survey. Effort shows the number of useable timelapse images produced. Cameras with captures is the number of cameras that had at least one camel capture, % is the percentage of overall cameras in that survey that had captures. Images with captures is the number of images in that survey that captured camels. Camels tagged are the total number of camels tagged in images. Camel capture rates (per mil) is the rate of camel captures across that survey period per million images.*

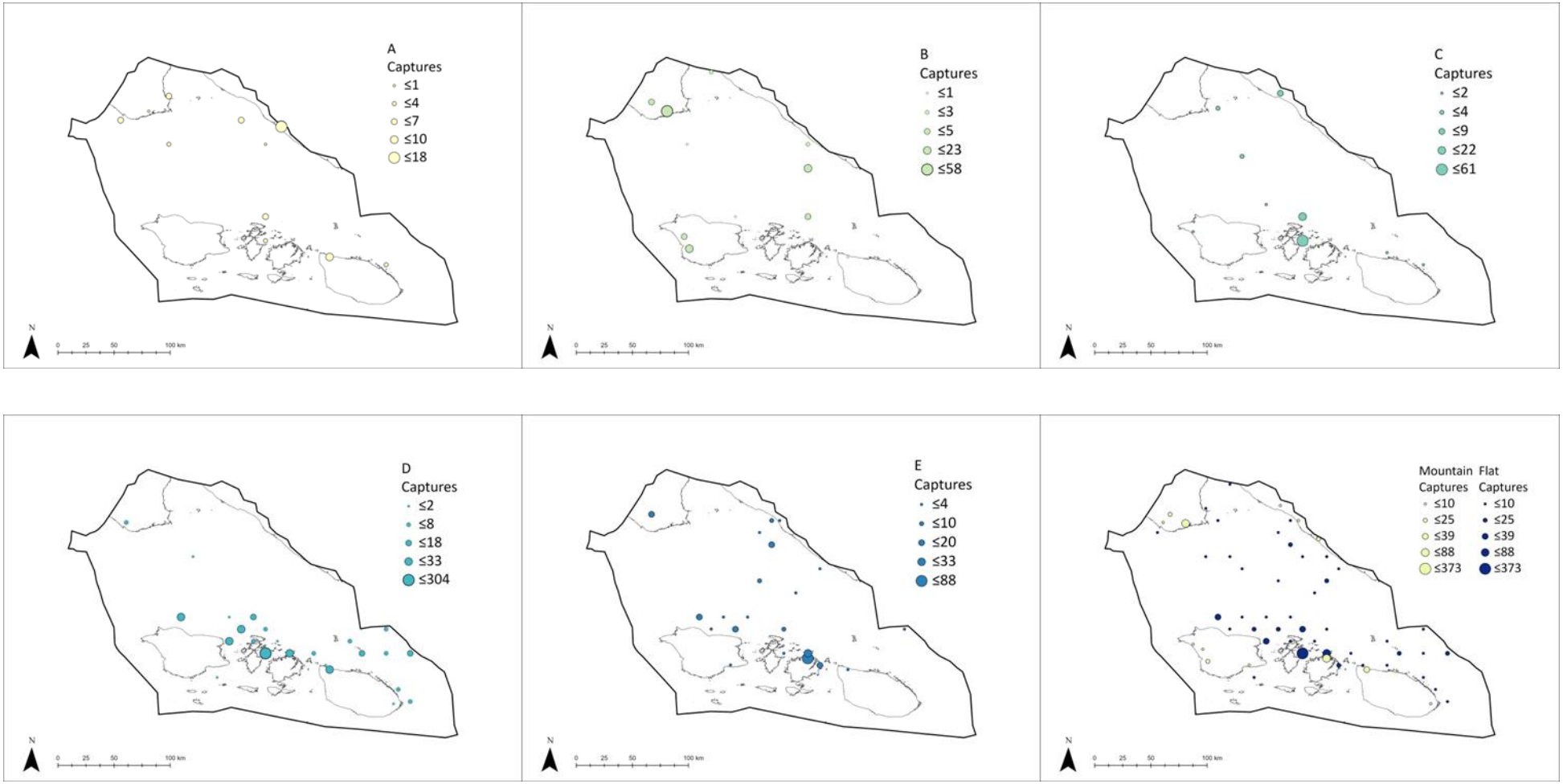

Figure 3.3(a): *Variation in encounter rates and locations across the 5 survey periods: (a)-(e) encounter rates for each of survey periods A-E; each marker represents a location where at least 1 encounter occurred within that period; marker size is proportionate to number of encounters. Bottom right- Variation in total capture rates across all cameras for all survey periods (A:E) with captures split into "flat" and "Mountain" areas.. Each marker represents a camera placement where at least 1 camel capture(s) were recorded across all survey periods; marker area is proportionate to number of captures at that placement.*

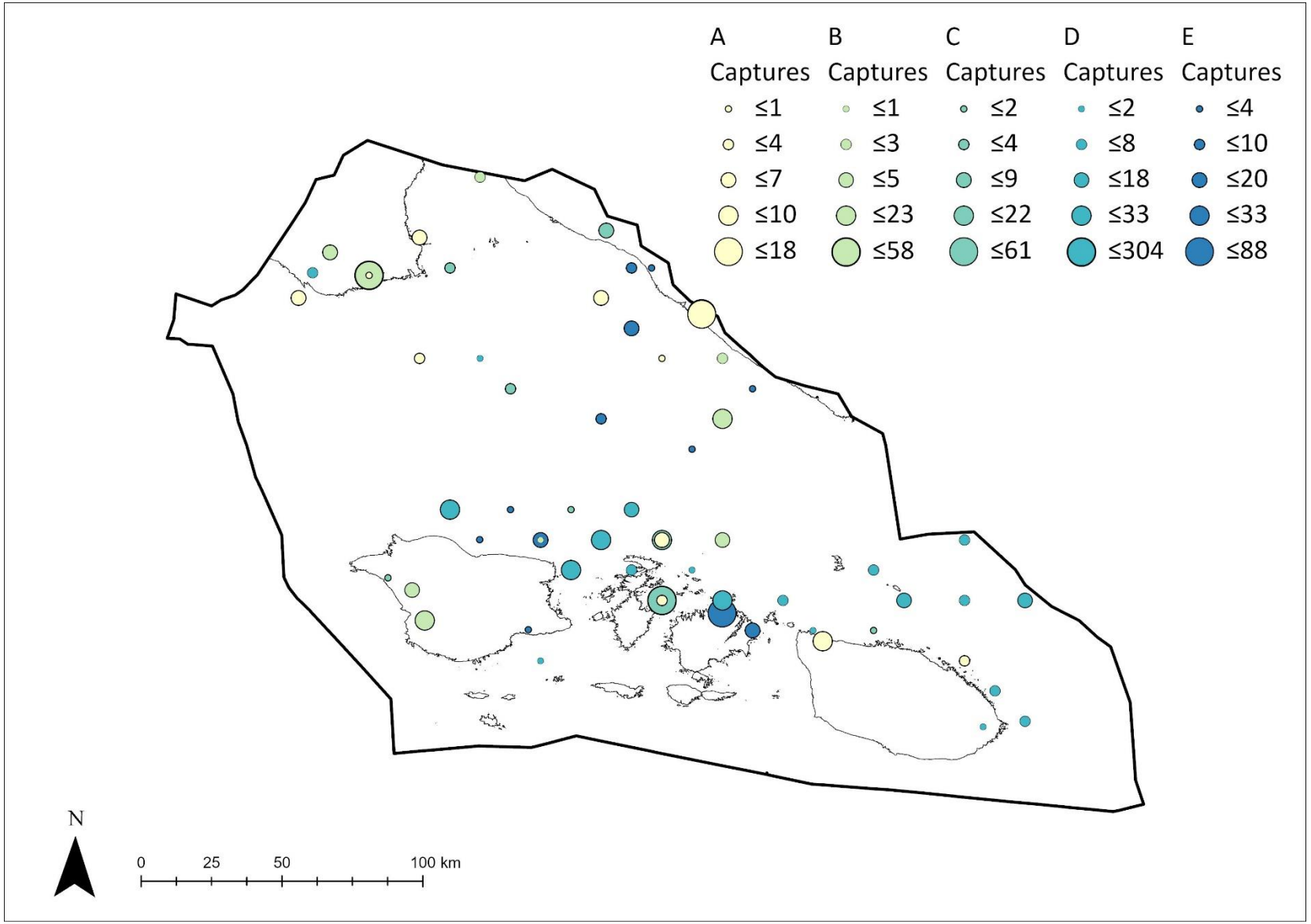

Figure 3.3(b): Summary of all encounters across the 5 survey periods: (a)-(e) Each marker represents a location where at least 1 encounter occurred; marker size is proportionate to number of encounters. 1500m contour line included, below the line are "flat" cameras, above the line are "mountain" cameras.

		CV		
	AIC	Average P	N	EDR
Half normal	-2752	0.05	0.06	0.03
Hazard rate	-2693	0.05	0.06	0.02
Uniform	-2744	0.04	0.05	0.02

Table 3.3: *AIC and CV values for detection models: Half-normal key function, truncation = 0.3 to and with cosine adjustment term of order 2. Hazard rate, truncation=0.3 and Uniform truncation=0.3. Model with the best fit is Half Normal, with both the lowest AIC value and largest effective detection radius. AIC = Akaike information criterion. CV =coefficient of variation P= the proportion of wild camel captures missed in full 360^{o} from camera. N = proportion of the region covered. EDR = proportion of the effective detection radius.*

The half normal detection function was clearly the best supported of the three shapes tested (Table 3.3) half normal, hazard rate and uniform AIC values respectively -2752, -2693, -2744). This is supported by the evidence ratio (Burnham, Anderson, and Huyvaert 2011; Howe et al. 2019) in that the half normal compared to the next best supported model (Uniform) is 54.6, whereas the evidence ratio of the half normal against hazard rate is 6.48×10^{12}. The half-normal detection function showed no significant lack of fit to the data (Figure 3.4), and yielded an effective detection radius estimate of 111 m (SE 2.8).

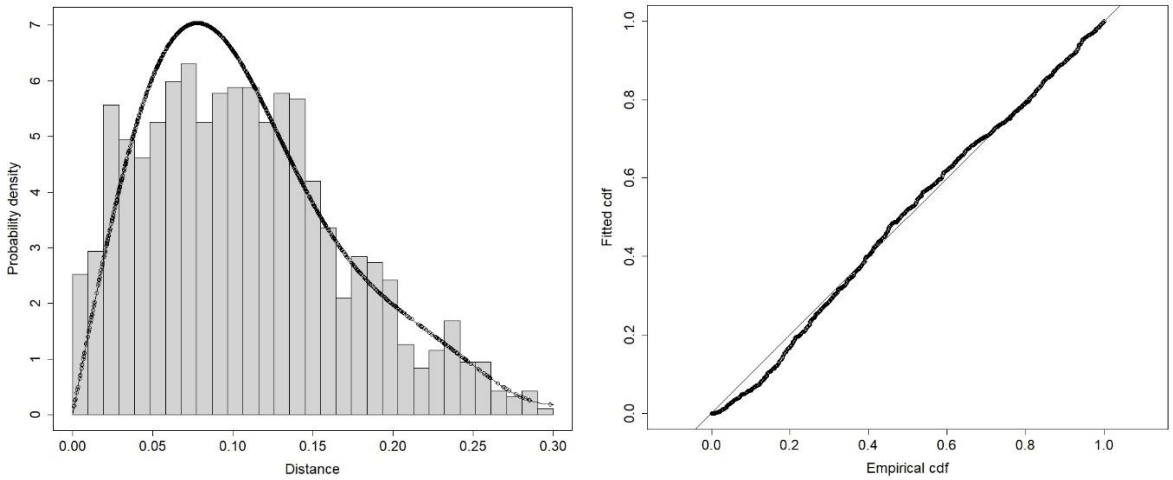

Figure 3.4: *Left: Detection function of calculated distances for wild camel (Camelus ferus) with Half Normal function with a truncation of 0.3 km and with cosine adjustment term of order 2. Right: Q-Q plot of empirical against fitted cumulative distribution functions for the half-normal detection function. Goodness-of-fit testing for the half-normal model showed that the empirical half-normal distribution did not differ significantly from the model distribution (Cramer-von Mises test, T = 0.38, p = 0.08).*

As we were able to determine that the half normal detection function with two cosine adjustment terms provided an acceptable fit to the empirical distribution, we proceeded to estimation of abundance using this model. Across all survey periods, we estimate mean wild camel abundance in the GGASPA as 664 (CV = 0.26, 95% confidence interval 400-1100). Abundance estimates per survey ranged from 246 (CV = 0.71; 95% CI 79-867) to 1157 (CV = 0.61; 95% CI 381-3508) (Table 3.4, Figure 3.5).

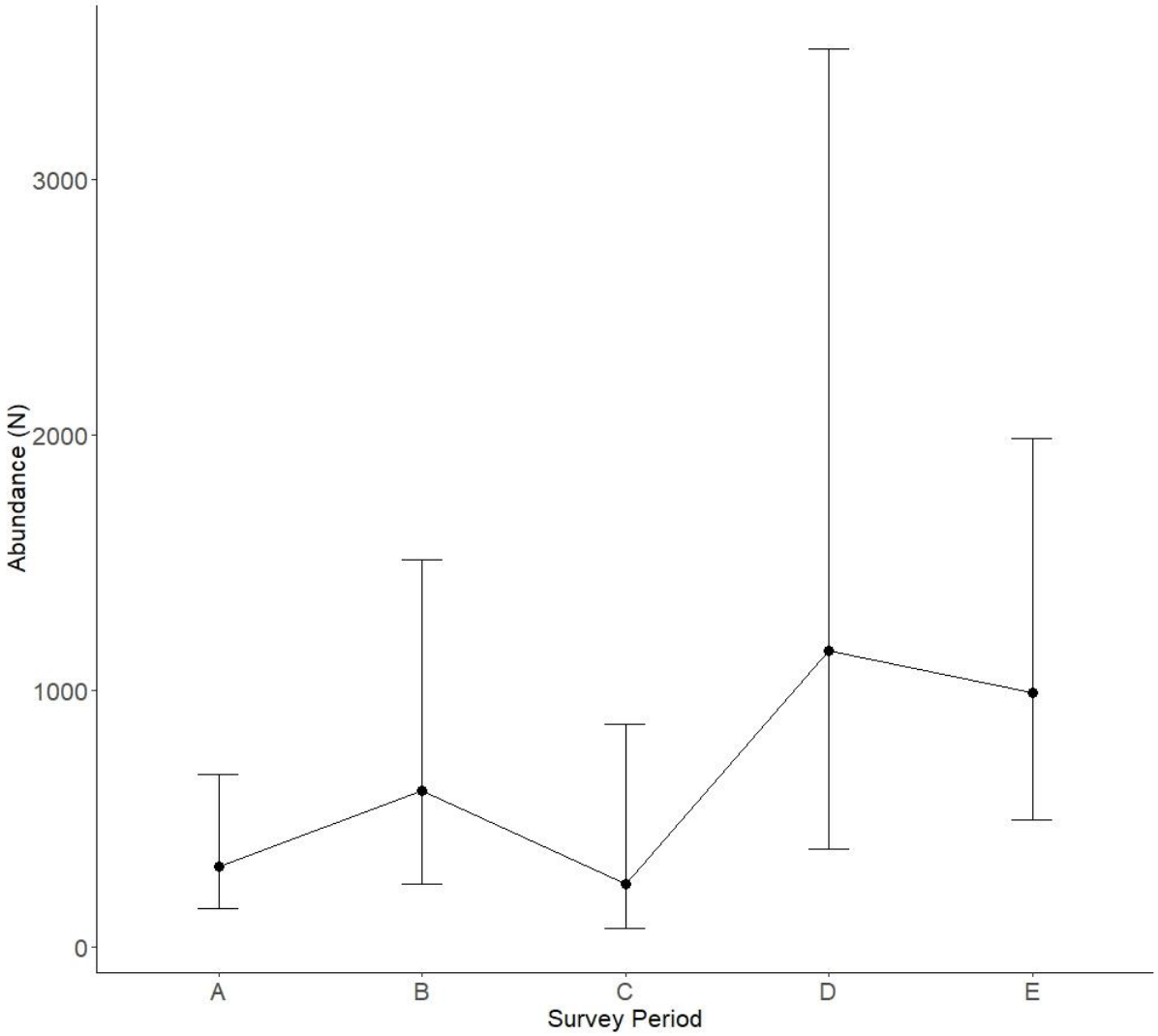

Figure 3.5: *Abundance estimates per survey period including 95% confidence intervals.*

Survey	N	CV	95% CI
A	315	0.4	147-675
B	610	0.48	246-1512
C	246	0.71	79-867
D	1157	0.61	381-3508
E	991	0.36	495-1986
mean	664	0.26	400-1100

Table 3.4: *Abundance estimates (N), Coefficient of variations (CV) and 95% confidence intervals (95% CI) survey period A-E and mean across pooled data set.*

3.4 Discussion

3.4.1 Wild camel abundance in the GGASPA.

We used camera traps to estimate wild camel abundance in the GGASPA, obtaining a final estimate of 664 individuals (95% confidence intervals 400-1100). This is the most precise estimate of wild camel abundance in the GGASPA to date and provides a comparison to the only previous estimate, 1985 (95% CI=413 to 3557) (Reading et al. 1999), done in 1997. Our updated population estimate, with improved precision, provides a measure of abundance essential to understanding extinction risk faced by this critically-endangered species, and to serve as another point estimate against which future conservation efforts can be measured.

3.4.2 CTDS Timelapse.

We used a novel method of distance sampling using camera traps in timelapse function. To our knowledge, this was the first CTDS wildlife abundance estimation that used timelapse camera traps anywhere globally. Not only does the abundance estimate produced allow for improved conservation management planning for the wild camel, but trialing the method provides opportunities for other remote, low density and threatened species in open habitat. Despite there being a number of available methods for determining species abundance, including traditional sampling approaches like aerial counts or capture mark recapture (Harris et al. 2020), these can be expensive, logistically difficult, generate bias if model assumptions are not met (Harris et al. 2020), and cause increased disturbance. For the wild camel it was important that any estimate obtained was non-invasive, both due to the low presumed population number (meaning every individual may be necessary for species survival), and because the species is known to be very sensitive to human disturbance (Kaczensky et al. 2014). Camera traps offered a non-invasive method for wild camel abundance estimation. Timelapse function allowed for the detection of camels at greater distances than would be detected by triggered cameras, as shown by our peak of captures at around 100 meters. This was particularly advantageous in the open terrain

of the GGASPA, as it allowed for a far greater detection range than would have been achievable using triggered camera traps (in this study we were able reliably to detect camels as far as 300m distant, whereas trigger mode is generally <20m).

3.4.3 Distance sampling

Distance sampling is a 2-stage process with assumptions that need to be met at both stages in the model to accurately estimate abundance (Buckland et al. 2001). Stage 1 aims to estimate the number of camels in an observed area, namely the camera trap field of view. Stage 1 assumes that the observation process is a snapshot in time and camels are detected at their initial location, prior to any movement in response to the observer (Buckland et al. 2001; Thomas et al. 2010). We have minimized the violation of this assumption as we used camera traps, not observers. We also used the timelapse images, so the observation process indeed presents a snapshot in time. Stage 1 also assumes that detections are independent. This is not the case in our data set, as some camels stay in site of the camera for multiple captures. This is considered a less important assumption as: it is violated in most studies (Howe et al. 2017), especially using camera traps (Thomas et al. 2010); it can be mitigated against by bootstrapping (Howe et al. 2017); and its violation has been shown to have little impact on the accuracy of abundance estimates (Buckland et al. 2001; Thomas et al. 2010). We were able to maximise the precision of our estimated detection probability within the covered area by pooling data across survey periods to maximise the number of captures used to model the detection function. We also minimized the risk of bias at this stage by finding a robust detection function model with no significant lack of fit to the data.

Stage 2 of distance sampling combines the estimated number of animals in the effectively-covered area with the size of that area to estimate density representative of the entire study area. Stage 2 assumes that cameras are located according to a systematic or randomized survey design, so that the entire GGASPA is represented, independent of camel location (Howe et al. 2017). If camera placement was not representative but focused on areas of the GGASPA where camels were more likely to occur, then

estimates would be biased. We placed points independent of camel locations by predetermining camera placement locations to ensure systematic coverage that was comprehensive, representative and random. Added to this was the accurate placement by rangers in the GGASPA to those pre-determined points (Figure 3.1). For a successful abundance estimate, stage 2 also requires a large number of camera points (N=151) and a minimum of 60 animal observations (N=1034), both of which we achieved.

3.4.4 Improving abundance estimates: survey design.

As this is the first robust population estimate gained since 1997, being able to repeat the survey in the future will allow for comparable abundance estimates to follow population trends. The cameras and methods have been trialed and have been proven to work, even in the extremes of the Gobi. If the survey were to be repeated, some improvements could be made to reduce variance in the estimate. At times batteries were the limiting factor, running out before SD card collection. This can be improved by only using quality batteries and replacing batteries at each collection. Placement of cameras should be stricter in terms of placement direction as some cameras placed into the setting or rising sun produced images with too much glare and consequently had to be excluded from analysis. Some cameras had errors in settings, such as being set to trigger mode, time and date errors or timelapse time scale error. This all led to a decrease in the number of images available for analysis, reducing the efficiency of surveys. Increased training and improved dissemination of methods for field staff could reduce these errors and so improve efficiency. There are some issues in camera placement that cannot be mitigated for, such as theft, damage by animals or failure of individual cameras. Further improvements can be made in the image analysis stage by either image tagging being conducted by one member of staff (as this will keep image tags consistent throughout the survey period, though would be subject to systematic bias of a single rater's opinion) or by independent multiple-rater tagging with overlap to determine inter-rater reliability in tagging (which would permit assessment of reliability of single-user visual inspection as a means of identifying target species in camera trap data).

3.4.5 Improving abundance estimates: analysis

Although this method has produced a sensible and reasonably precise abundance estimate overall, the survey specific estimates (Table 3.3) are extremely variable and imprecise. Abundance estimates range from 246 (95% CI= 79-867) to 1157 (95% CI=381-3508) depending on survey period. The CV value, which depicts the amount of uncertainty (standard error expressed as a proportion of the estimate), was 0.26 for the overall density, close to the target value 0.2 often recommended for acceptably precise estimation of wildlife abundance (Cappelle et al. 2021). However, individual survey CVs ranged from 0.36 to 0.71. Clustering of camels at cameras could be the cause of this, as clustering gives high variance. We see camels clustering at some cameras. All but one camera (373 captures) had fewer than 100 captures, with 66% having fewer than 10 captures. Lowering the timelapse rate (and so allowing animals sufficient time to move away from a camera's field of view before the next capture) could potentially reduce clustered captures.

Some additional analysis could be conducted to potentially reduce variance, such as adding spatial information to the model. As we know where each camera is, we know habitat type, ruggedness, altitude, distance to water and distance to human settlement. All of these factors may influence how camels are using the GGASPA. Our results show that the majority of the captures are in the central area of the park, which confirms previous indications that this is the core area for the species in Mongolia (Yadamsuren, Daria, and Liu 2019). Modelling spatial patterns of abundance would give a better understanding of habitat preferences and might allow us to improve the precision of the density estimates. Given the apparent preference camels showed for mountain canyons, and the vastly lower coverage of these canyons across the study site, precision and accuracy of the abundance estimates might also be improved by stratifying the analysis according to zone ("flat" vs "mountains"). For this reason, the current results should be seen as preliminary until this stratified analysis is in place, as they may improve accuracy, and give a different result in so doing.

Abundance estimates could also be impacted by mis-identification of camel species in the GGASPA. Wild camel and Bactrian camel are morphologically similar so misidentification (Chapters 2 and 4), especially at distance or in poor quality images, is possible. We were confidently able to identify wild camels from Bactrian camels in clear images to approximately 100 to 200 meters. Truncation of the data, by removing those captures over 300 meters, should have helped remove many of those that were clearly "camel" but not identifiable to species. Further work could estimate the sensitivity of our estimate to species misidentification through multiple independent raters tagging of images and evaluation of inter-rater reliability.

3.4.6 RedList Assessment

The wild camel is classed as critically endangered under the IUCN RedList assessment (IUCN RedList 2022), on the grounds of population reduction (RedList criteria A3de+4ade). This requires "a population size reduction of at least 80% over three generations (estimated at 45 to 50 years)". The abundance estimate we have produced for Mongolia is 664 (95% CI: 440- 1100). When we compare this to the last robust estimate of 1985 (95% CI=413 to 3557) (Reading et al. 1999), in the intervening 26 years, our mean estimate, relative to the mean of the previous estimate, while falling within 95% confidence intervals, could represent a population decrease of 67%. These preliminary results suggest a population size low enough population to warrant concern and an update of the RedList assessment to determine extinction risk is vital.

3.4.7 Conclusions

This is the global first CTDS study to use camera traps in timelapse function to estimate wildlife abundance. This novel technique allowed for data to be captured across the entirety of the GGASPA and we believe the estimate produced to have relatively high precision and has the potential to be accurate given our efforts to avoid assumption violations. The vast dataset collected in this study could

be utilised to estimate abundance of other important and endangered species within the GGASPA, such as the khulan (Asiatic wild ass) *Equus hemionus* and the goitred gazelle *Gazella subgutturosa*. Such estimates would support landscape-scale conservation efforts, complementing our single-species focus. We can also extend the use of this method to estimate the abundance and distribution of the Bactrian camel*, Camelus bactrianus* in the GGASPA, in order to inform conservation management of hybrids (Chapter 4.). Finally, our abundance estimate highlights the continued low wild camel population size, something that will be of concern for conservation managers.

3.5 Appendices

Appendix 3.5.1 Camera trap test images in time-lapse mode.

Acorn cameras, tested in timelapse mode in the UK, detected horses to more than 200 meters and sheep to 150 meters.

Primos and Spypoint cameras tested in timelapse mode at the wild camel breeding centre. It was not possible to mark distances accurately for safety, but it is thought that camels can be detected to 150 meters.

Wide angle scouting camera tested in time lapse mode in Mongolia. Building at 56 meters away.

Appendix 3.5.2 Mapping of mountainous areas.

Mapping gullies using the 1500 boundary line gives 461197 meters.

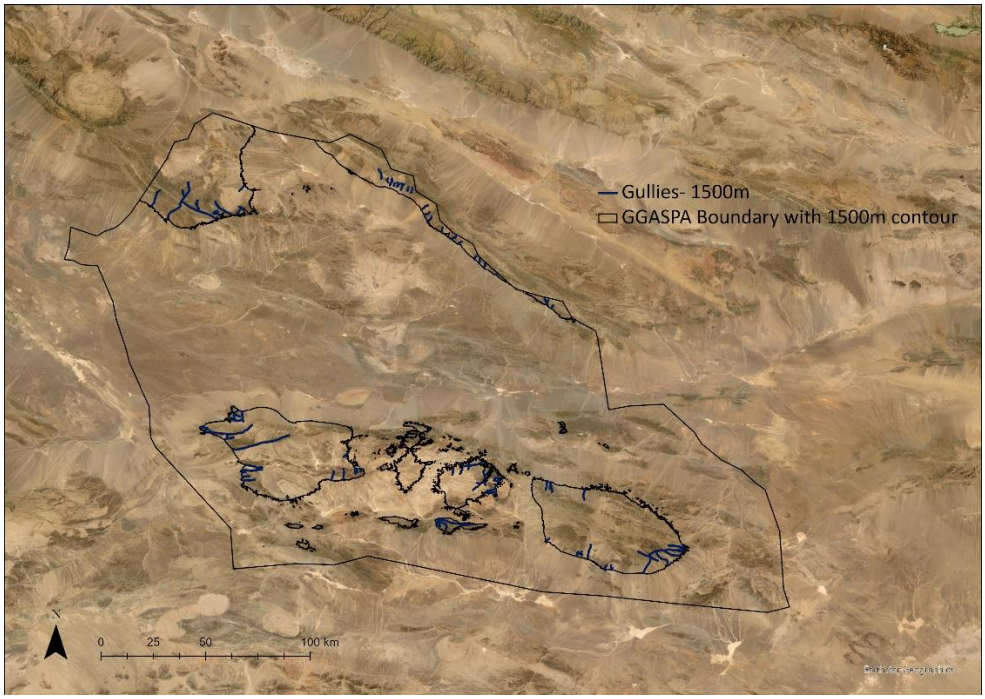

Figure 3.6: *All canyons mapped by hand in ArcGIS using Basemaps and the polyline tool.*

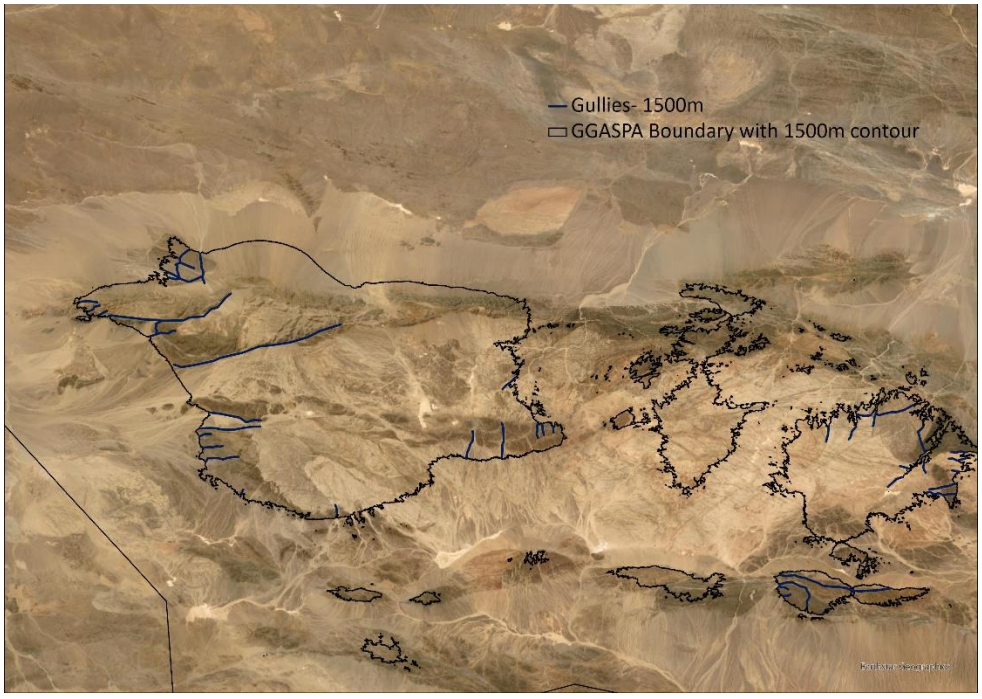

Figure 3.7: *Close up of central mountains to show canyons mapped by hand in ArcGIS using Basemaps and the polyline tool.*

Appendix 3.5.3 Camera trap placement protocol

Camera traps should be placed within 500 meters from the pre- determined grid co-ordinate. Within this area, where possible, placement choice should take the following into consideration:

- Direction. Cameras should be placed where possible facing North. If north is not possible then place facing either North East or North West. Placing the cameras to face north avoids glare from the rising and setting sun.
- Visibility. Cameras should be placed with the maximum possible field of view. They should not be placed directly behind anything that would obstruct the image such as cliff faces or thick vegetation.
- Height. Where possible cameras should be placed at a higher elevation to the surrounding land eg on top of a hill or rise. This is to gain maximum visibility from the image.

All cameras are to be set on a post at 1.5 meters high.

Posts should be placed securely so that they do not move in the wind. Cameras should be strapped to the post tightly so that they do not move in the wind. The strap should also be tied so that there are no loose ends.

Cameras should be set to time-lapse mode. They should take 1 image every 20 minutes from 5am to 8pm.

Make sure the date and time settings are correct.

Camera trap patrol protocol

Both when cameras are placed and on subsequent visits to cameras the following data should be collected:

- Are there visible camel signs (such as scat, hair or footprints)

- Does the location look to be suitable camel habitat.
- Were any animals were spotted in the area.
- Collect behavioural data (e.g., group size, GPS location, age class, sex, feeding behaviour, mortality) of camels encountered.
- Collect faecal samples- *See protocol for sample collection.*

A data form will be provided to fill in.

Appendix 3.5.4 Method design.

Survey design and decision making

To determine wild camel density in the GGASPA we considered three options: 1) using camera traps in time-lapse mode, 2) using camera traps in triggered mode and 3) using drones to capture images. Calculations comparing these methods (Table 3.5.1) determined that time lapse gave the greatest number of camel records. Added to increased captures, timelapse was also chosen because of:

- Cost- The monetary cost of purchasing camera traps and equipment was estimated as less than either the use of drones or conducting an aerial survey. It also allows for the use of cameras in future projects.
- Effort- The human effort involved in setting traps and collecting SD cards is less than that of a drone survey.
- Reduced disturbance- Camera traps are currently used widely in the GGASPA, especially at water points, therefore animals in the GGASPA have already come into contact with them. There was potential disturbance when cameras were initially placed, but as SD card collection was conducted during ranger patrols- by trained and experienced rangers, excess disturbance was reduced.
- Additional data collection – Using camera traps allowed for the collection of additional data. This includes population demographic and movement data of wild camels in the GGASPA. In placing cameras in a grid across the entirety of the GGASPA it allowed for the collection of genetic samples widely across the park, with reduced effort. The time-lapse method also allows for population estimates to be made on other species, such as the goitred gazelle *Gazella subgutturosa* (IUCN Vulnerable)(IUCN RedList 2022) and the khulan or Asiatic wild ass, *Equus hemionus* (IUCN Near threatened)(IUCN RedList 2022).
- Increased ranger patrols – By funding the collection of SD cards it allowed for increased number of patrols by rangers into the GGASPA. This itself is positive to the park management as increased patrols may reduce illegal movement and destruction of the park.
- Ranger training - The project allowed for increased ranger training.

TIME LAPSE		
Input	Unit	Value
Survey area	km2	45,000.00
Camels	n	350
Camera radius	km	0.15
Camera angle	radians (degrees)	2.268928028
Placements	n	250
Photo rate	per day	72
Days	n	180
Photo size	Mb	3
Card size	Gb	32
Intermediate calculations		
Camel density	per km2	0.007777778
camera zone area	km2	0.02552544
Total photos	n	3240000
CAMEL RECORDS	n	643.2410958
TRIGGERED (REM)		
angle		2.268928
radius		0.008
day range		20
camera days		45000
density		0.007778
CAMEL RECORDS		76.09515
DRONE		
Area	km2	45000
Camels	n	350
Density	per km2	0.007778
flight range	km	50
camera angle	radians (degrees)	2.268928
height		0.2
strip width	km	0.857803
flight rate	n/day	2
days	n	200
total flights	n	400
total area covered	km2	17156.06
% area covered	%	0.381246
CAMEL RECORDS	n	133.436

Table 3.5.1 *Example calculations comparing the time-lapse, triggered and drone image collection for number of camel "captures". Time lapse gives the greatest number of camel records.*

Survey design

Wild camels occur at a low density with large home ranges (Kaczensky et al. 2014). The survey was designed to optimise use of cameras and camera trapping days. Initial survey design considered the following:

- Number of cameras available.
- Type/model of cameras available.
- Area covered. This is estimated using camera range of sight and field of view.
- Area of the GGASPA to be covered.
- Time lapse specifications including multi photo, field of view, detection, model, time between traps, distance between traps and length of study.

Camera specifications

Time lapse function was used in this study as it allowed for greater capture of animals at distance and does not rely on the sensitivity of the camera sensors.

This range of sight (timelapse) and the field of view of the cameras (130 degrees) give the area in which each camera monitors. This gives the area of the park monitored and so this estimate is then used in the model.

Tests were made on both camera batteries and SD cards previous to study. These tests showed that 25000 frames filled a 32Gb SD card. Both the filling of an SD card and the lifespan of batteries at this capacity is approximately 6 months. Suggesting that rangers were required to change batteries and SD cards once every 6-month period.

Calculation requirements for camera placements.

Input	Unit	Value
Survey area	Km2	X
Camels (n)	N	350
Camera radius	Km	0.15
Camera angle	Radians (degrees)	2.268928028
Placements	N	X
Photo rate	Per day	72
Days	N	180
Photo size	Mb	3
Card size	Gb	32

Table 3.5.2*- Calculation requirements to determine camera trap number. These are: Survey area- area of open landscape. Wild camel population estimate uses last population estimate of 350 animals (Tulgat 2002). Camera radius and angle taken from the ZSL camera. 1 image every 10 minutes for 12 hours = 72 images per day. Photo rate was decreased in deployment to 45 images per day (Every 20 min from 5am to 8pm) after advice from rangers on camel activity.*

Placement method considerations

Monitoring within the mountainous areas of the GGASPA is difficult, due to the inaccessibility of the terrain to both the animals and the rangers. But as species access the mountainous areas; to cross them, for water, food and shelter, it is necessary that they were monitored. Camera placement was split into two methods: “flat” areas and “mountainous” areas. These initial zones were created using a contour line as a boundary. This boundary was either above either the 1400m or the 1500 meter contour being the “mountain” zone and below the 1400 meter or 1500 meter contour being the “flat” zone. Calculations (Table 3.5.3) determined that the 1500 meter contour line was chosen as the boundary.

In the “flat” area camera placement was determined prior to fieldwork using a grid system placed across the GGASPA (Figure 3.1). In the “mountainous” areas a grid would lead to the placement of cameras in inaccessible places. Instead, gullies were mapped as a polyline by hand using ArcGIS and then ground truther by rangers who know the terrain. This line was then used as a line transect in placing the cameras.

Captures	Camera number:			
Altitude line	150	200	250	300
1400	542	723	904	1085
1500	457	609	761	914

***Table 3.5.3**- Estimated camel captures with different numbers of camera used compared against the two options for splitting the GGASPA into “flat” and “mountainous” areas. By using the 1400-meter line, 32000km2 would be considered flat and surveyed using the grid. The 1500-meter line requires a grid that covers 38000km2.*

Grid (km2)	Camera number:			
Altitude line	150	200	250	300
1400	14.7	12.7	11.4	10.4
1500	15.9	13.8	12.3	11.2

***Table 3.5.4**-Estimated grid spacing (the size of the grid in which cameras are placed) required when comparing the two altitude options for splitting the GGASPA into “flat” and “mountainous” areas. By using the 1400-meter line, 32000km2 would be considered flat and surveyed using the grid. The 1500-meter line requires a grid that covers 38000km2.*

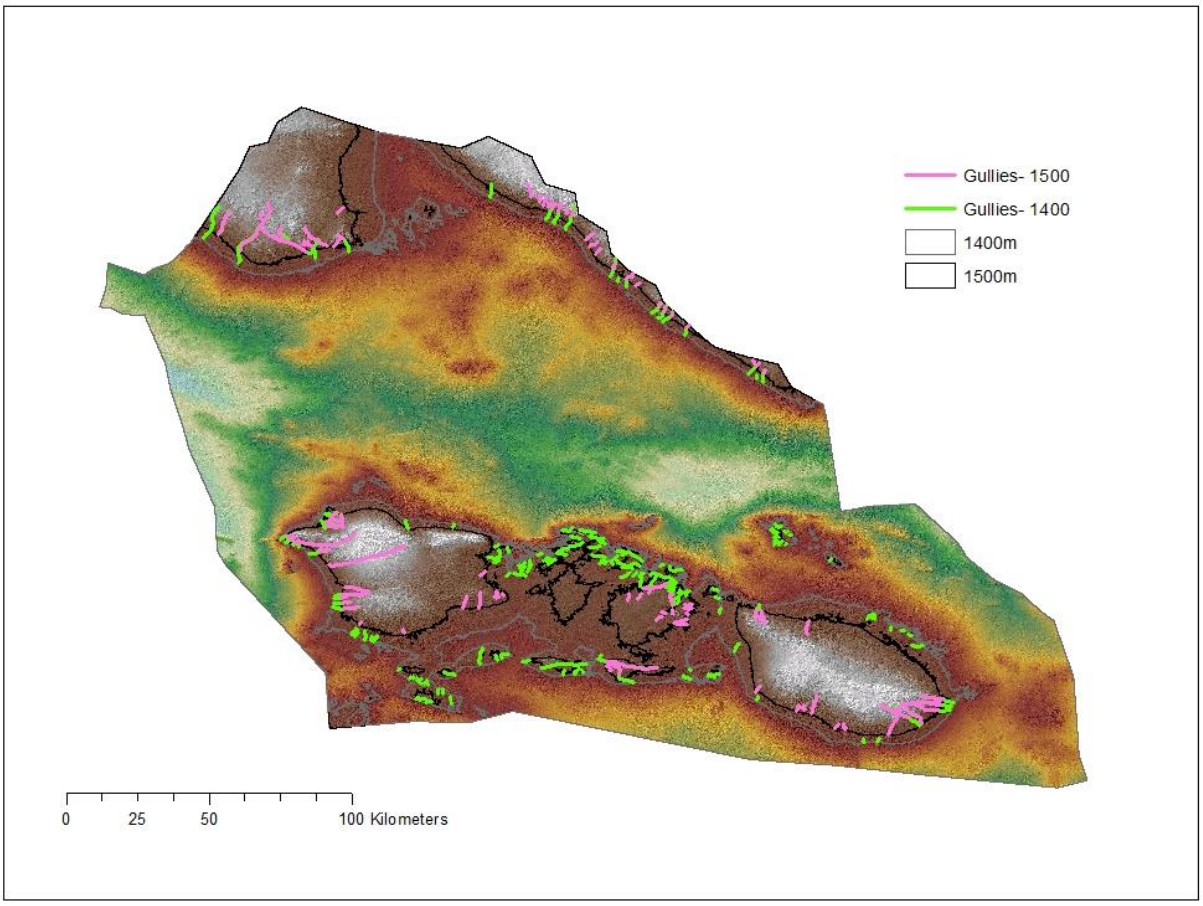

Figure 3.8- *Mapping of the gullies using ArcGIS polyline tool. The 1400 meter altitude line (grey) gives a combined transect length (green) of 754168 meters. The 1500 meter contour line (black) gives a combined transect length (pink) of 461197 meters.*

Appendix 3.5.5 Camera Trap Pilot study

From April 2019 to October 2019 a pilot study was initiated that aimed to use camera traps to study the wild camel (*Camelus ferus*) population in the GGASPA. The initial findings from this first six months of monitoring was used to determine method viability and to determine for improvements for project continuation. Ninety cameras were used, split between the two different methods of systematic random placement- 80 cameras in the "Flat" areas and 10 in valleys connected to the plains within the "Mountain" areas (Figure 3.9).

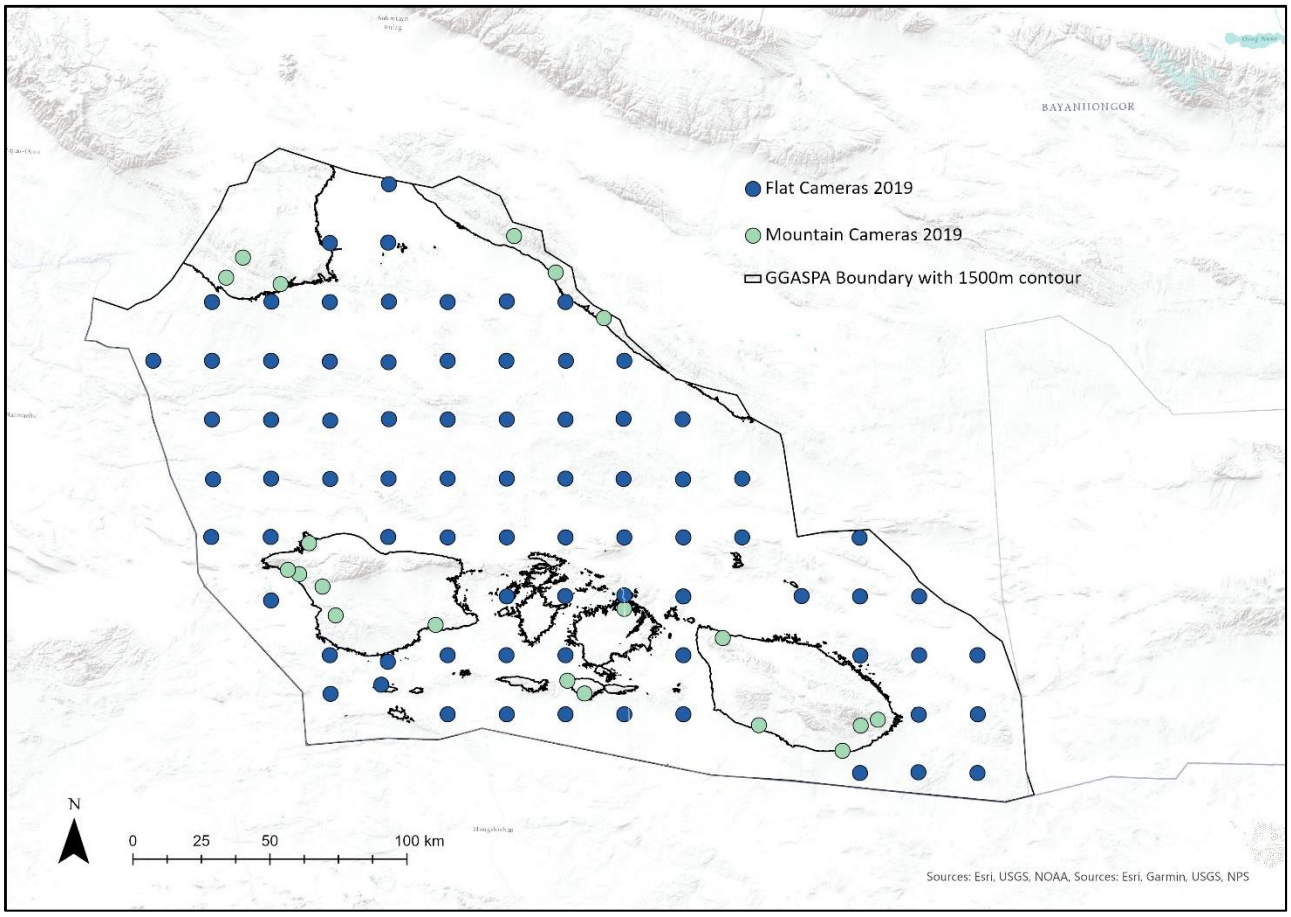

Figure 3.9 *Camera trap placement in 2019. Stratified to "flat" and "mountain" placements.*

Camera model	Image angle (degrees)	Number used 2019	Number to be used in 2020
Crenova	120	20	0
L-SHINE	130	70	180

Table 3.5.5: *Camera trap specifications. Two camera models were used: Crenova Model and L-SHINE Forestcam 987 as both had time-lapse function and wide angle.*

Initial Results

From the 90 cameras set, 78 produced images suitable for analysis. Images were checked for presence of animals by a student in Mongolia. From these 78 cameras there were 221 wild camel detections, 50 khulan and 90 goitred gazelle. From the 78 cameras that worked only 15% contained camel captures and of these 79% were captured at just 2 cameras. This proved the method to be valid, but results show high variance and therefore any density estimate made with this data will have a high level of uncertainty.

Trap rate variance

In order to evaluate the degree of statistical error likely to arise in ultimate density estimates, trap rates were calculated for each point. Then the mean, standard error (SE), coefficient of variation (CV), and 95% confidence intervals were calculated, using bootstrapping either with or without stratification by habitat to estimate errors (Table 3.5.6). Trap rate was calculated as the number of animals counted per 10,000 time-lapse images taken. Coefficients of variation were around 50% for all species, with only marginal gains from stratification. There were accordingly wide confidence intervals.

					Confidence interval (95%)	
Species	Stratified	Mean	SE	% CV	Lower	Upper
Wild camel	No	4.17	2.4	57.6	0.55	9.65
	Yes		2.28	54.6	0.57	9.15
Gazelle	No	1.7	0.8	47.3	0.38	3.51
	Yes		0.81	47.5	0.38	3.52
Khulan	No	0.94	0.54	57.6	0.14	2.18
	Yes		0.54	57	0.15	2.17

Table 3.5.6 *Initial statistical results for camera trap pilot study*

Improving Study Design- 2020

The 2019 trial showed the survey design to be valid, but due to high variation between captures across points, any density estimate produced using this data would have high variance. Substantial improvement in precision was required to increase in the number of points sampled. In part this could be achieved by increasing the sampling effort from 78 cameras back to the intended 90. The reasons for failures at planned points include faulty cameras (almost all 20 Crenova models had issues in resetting. Approximately 20% of images were lost in these cameras due to these problems) and human error in programming and placing cameras. Precision could also be increased by increasing sampling effort- increasing the number of cameras used.

A smoother-running set up of the 2019 study design would help to some extent, but increasing the number of cameras set would be necessary to achieve acceptable precision. To explore the potential impact of increasing sampling effort on precision, analysis was repeated with increasing numbers of points sampled, simulated by replicating the existing camel records up to ten times (780 points). Trends in coefficient of variation and confidence interval against number of points (Figure 3.10) shows a gain in precision up to approximately 300 points, with limited improvement thereafter. It takes approximately 250 points to get CV below 30%, and 500 points to approach 20%.

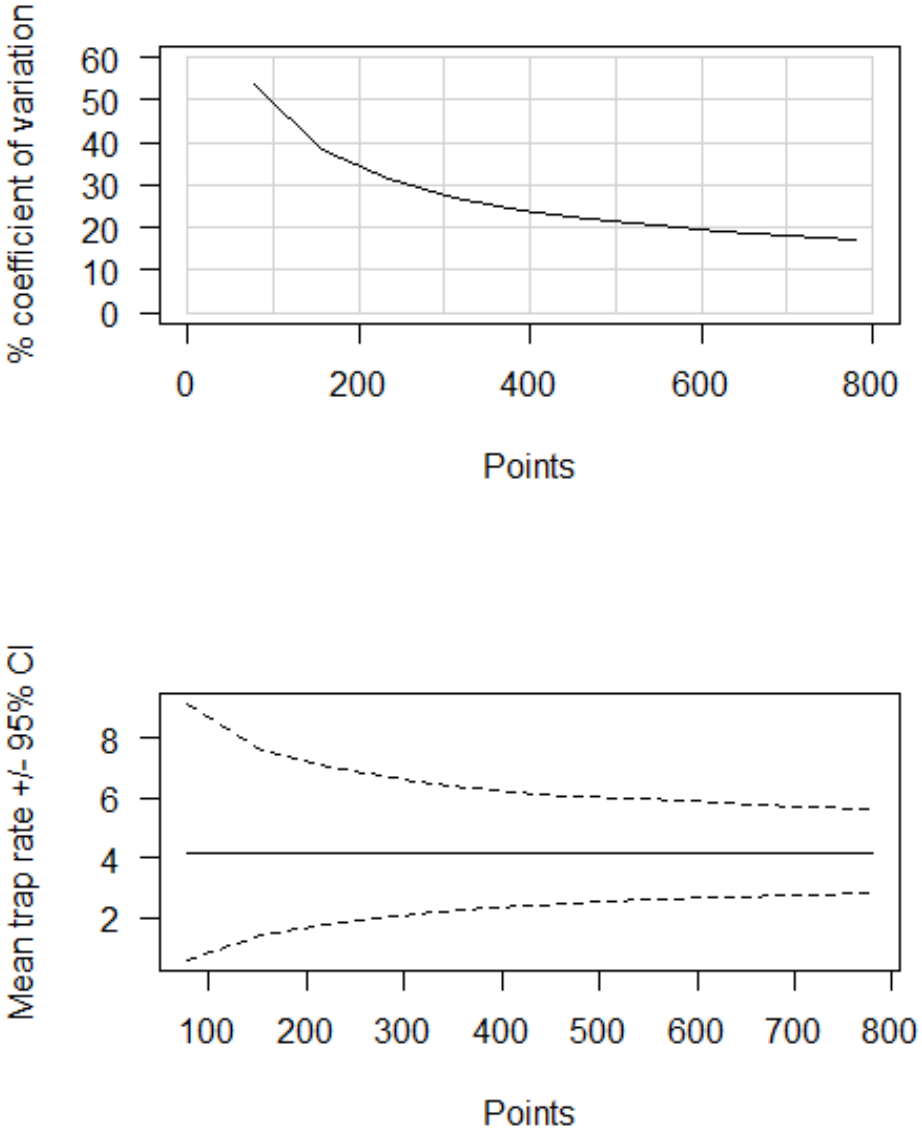

Figure 3.10 *Sampling effort simulations. Top = percentage co-efficient of variation against number of camera points used. Bottom = mean trap rate against number of camera points used.*

The 2019 pilot survey demonstrated that the survey design had essentially worked and that with an increase in effort, the precision of a density estimate could be increased. There are two ways in which this improvement was conducted. The first was to improve on errors in the 90 cameras to increase 78 working cameras to the full 90 by; replacing the 20 Crenova cameras with the L-SHINE models, improvements in the setup of the camera grid and training of rangers. The second strategy

used was to increase the number of cameras in the survey grid by doubling the number of cameras used from 90 to 180.

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Chapter 4 Surveillance of genetic diversity and Introgression using non-invasive sampling of both in-situ and ex-situ Populations of Wild Camel, *Camelus ferus* in Mongolia.

Prevalence in the wild camel of introgression of DNA from domestic Bactrian camels is extensive, whilst genetic diversity and inbreeding levels are comparable in both the in-situ and ex-situ wild camel population.

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Abstract

One of the main threats to extinction risk of the critically endangered wild camel, *Camelus ferus*, is hybridisation with Bactrian camel, *Camelus bactrianus.* The last remaining stronghold of the wild camel in Mongolia is the 45,000 square kilometre Great Gobi A Special Protected Area (GGASPA), where this range-restricted threatened species comes into contact with the globally-distributed domesticated Bactrian camel. Non-invasive sampling combined with genetic monitoring, using a combination of nuclear and mitochondrial DNA markers, has allowed us to gain a greater understanding of the extent and source of introgression and levels of genetic diversity in *Camelus ferus*, in both the wild population and the captive insurance population in Mongolia. Our results show evidence of both nuclear, mitochondrial and historic introgression of Bactrian camel genes in the *C. ferus* population across the GGASPA, and in some individuals within the captive herd. We also show that heterozygosity is reduced and inbreeding is increased in the wild population, and show that these levels are represented in the captive herd. Our findings illustrate that, whilst an acceptable level of introgression is largely determined by thresholds adopted by the global conservation community, a detailed genetic perspective is crucial in increasing our understanding of the hybrid problem and is an important first step towards identifying options for conservation management.

4.1 Introduction

Hybridisation is "*the process of breeding together animals or plants of different species or varieties to produce a hybrid*" (Oxford Dictionary 2023). If this breeding causes "*the movement of genetic material from one unit into the gene pool of another*" (Bohling 2016) then it is classed as introgression and if it can occur between both units, then it is classed as admixture. Whilst hybridisation is a naturally occurring evolutionary process, important in the facilitation of both adaptation and speciation (Allendorf et al. 2001), it can also be an extinction threat for endangered species (Todesco et al. 2016).

Anthropogenic hybridisation is caused by changes in species' distributions brought about by human activity (McFarlane and Pemberton 2019). These novel distributions, exacerbated by habitat destruction and climate change, accelerate the rate, frequency, and extent to which species or populations interact that would not otherwise do so, which in turn increases opportunities for hybridisation (McFarlane and Pemberton 2019).

Hybridisation can cause extinction - if not directly then as a contributing factor (Rhymer and Simberloff 1996), and in as few as five generations by outbreeding depression and genetic swamping (Wolf, Takebayashi, and Rieseberg 2001). Outbreeding depression, in which hybrid offspring show reduced fitness compared to either parental species, can further increase extinction risk by demographic swamping (Wolf, Takebayashi, and Rieseberg 2001) when outbreeding depression causes reduction in population growth rates. Genetic swamping occurs when hybrid individuals, with higher rates of population-level fitness, exceed the replacement rate of the 'pure' non-hybrid population, and can have devastating consequences for a threatened species, such as loss of localised adaptation (Todesco et al. 2016). Both outbreeding depression and genetic swamping are especially problematic for those species with very small populations, which are already threatened with extinction from other genetic and ecological drivers (Willi et al. 2022). Conversely, hybridisation, by increasing genetic diversity of small, isolated or threatened populations, may aid in adaption and survival in this period of biodiversity loss and rapid environmental change. Outbreeding depression too can be a positive, with the increase in phenotypic variability allowing for population adaptation. Hybridisation or outbreeding depression could allow for genetic rescue of populations via the increase of heterozygosity and subsequent enhancement of adaptive potential (Chan, Hoffmann, and van Oppen 2019). Hybrids could allow for the conservation of parental genetic material, they can fill important niches and now with modern genetic techniques allow for the eventual recovery of the threatened parental species (Lawson et al. 2023).

Within a conservation context, hybridisation is challenging. In highly threatened species, endemic genetic diversity may already be reduced and hybridisation could reduce diversity further by risking loss of locally adapted or specialised traits and potentially contributing to population extinction (Rhymer and Simberloff 1996). However, hybridisation also has the capacity to conserve unique genetic diversity by increasing heterozygosity and contributing to hybrid vigour (heterosis), thereby enhancing overall population fitness (Taylor and Larson 2019; Draper, Laguna, and Marques 2021). Genetic diversity underpins adaptive potential in populations, which is increasingly important in many systems undergoing rapid environmental change. Threatened populations are often small, isolated, genetically impoverished or inbred, and therefore improving future population trajectory with other genetic conspecifics is not always possible; in these instances, hybridisation might be considered beneficial, as a form of genetic rescue: an increase in population fitness due to the contribution of genetic material from immigrants. As well as using hybrids to increase diversity, hybrids themselves can be conserved as a reservoir for the genetic material of a threatened parental species (as has been proposed in grey wolf/dog admixture (Pilot et al. 2018)) or for performing a necessary ecological function (Steiner et al. 2017). It may also now be possible to use captive breeding programmes and whole genome sequencing to identify and remove hybrid segments of DNA from populations (Lawson et al. 2023).

Detection of hybrids can be difficult. Until the 1960s, hybrids were identified through morphological phenotypes alone (Allendorf et al. 2001), but hybrids can express a range of phenotypes that are not necessarily intermediate between parent species, especially after several generations of backcrossing (Rhymer and Simberloff 1996). Phenotypes of hybrids are not easily predictable: they can display morphological characteristics intermediate between parents, as in Indo-pacific coral species (in which F1 hybrids show intermediate morphology relative to their parent species (Fukami et al. 2019)); alternatively hybrids can be cryptic, like the pine species *Pinus uncinata* and *sylvestris*, whose hybrids are morphologically similar to the maternal species (Jasińska et al. 2010); or they may express phenotypes unlike either parent, illustrated by the hybrid backcross offspring of hummingbird species *Heliodoxa gularis* and *branickii,* both of which have pink throat feathers but which together produce a

hybrid with a golden throat (Eliason et al. 2023). Therefore, phenotype can be a poor predictor of hybridisation which creates challenges for conservation, as has been shown for the Scottish wild cat (for which pelagic scores alone are insufficient, requiring molecular techniques for more accurate assessment (Senn et al. 2019).)

Genetic analysis allows for the monitoring of hybridisation across populations through time and space, regardless of phenotype, and is an important tool for managing populations of threatened species. There are a variety of methods available to monitor introgression, using both nuclear (Senn et al. 2019) and mitochondrial DNA (Silbermayr et al. 2010). However, like phenotypic traits, using molecular data is also not straightforward. Most often, extent of introgression within a population of individuals varies across a hybrid spectrum, meaning there is no consensus as to what is an acceptable level of genetic admixture that does not compromise evolutionary integrity and what threshold is considered "pure" (Allendorf et al. 2001). Much of this uncertainty is because hybridisation occurs most often between closely-related species and so the reliability of any comparison is dictated by the quality of the reference genome and the markers used. Furthermore, evolutionarily distant hybrid ancestry can be difficult to detect with traditional methods (Taylor and Larson 2019) such as phenotype or fragment analysis. Despite these challenges, it is important for conservation practitioners to monitor hybridisation in populations to understand whether threats posed by introgression are chronic or manageable.

The critically endangered (IUCN RedList 2022) wild camel, *Camelus ferus* (Mongolian: хавтай, *khavtgai*; Chinese: 野骆驼, *ye luo tuo*) is the last remaining truly wild species of *Camelini* (Burger 2016). The extreme Gobi Desert environment in which they live is threatened by climate change (Han, Dai, and Gu 2021). The wild population is estimated to be c.950 animals (IUCN RedList 2022) restricted to four fragmented populations, three in northwest China (Burger 2016) (Taklamakan desert, Gashun Gobi Desert and Arjin Mountains in the Lop Nur Lake region) and one in the 45,000 square kilometre Great Gobi A Special Protected area in Mongolia (GGASPA) (IUCN RedList 2022) (Chapter 3). A single captive population of approximately 36 individuals is managed by the Wild Camel Protection Foundation

(WCPF) in Mongolia (WCPF 2023) (Chapter 5). The wild camel is a separate species to the globally common Bactrian camel *Camelus bactrianus*, having diverged from a common ancestor approximately 1.1 MYA (Mohandesan et al. 2017) (Chapter 2), but the two species can hybridise. The Bactrian camel, a domestic species, is abundant, with over 450,000 in Mongolia alone (Jemmett et al. 2023, Chapter 2). Due to the close relatedness of the two species, hybrid offspring are viable and back crossing is possible, leaving *C. ferus* at risk of genetic swamping. Introgression from *C. bactrianus* has previously been documented in the *C. ferus* population (Burger, Ciani, and Faye 2019) using mitochondrial (Silbermayr et al. 2010), nuclear (Burger 2016) and Y chromosome DNA (Felkel et al. 2019), but its population-wide extent and conservation implications are unknown.

C.ferus and *C. bactrianus* exemplify the hybrid problem: one is a range-restricted, highly adapted critically-endangered species on the brink of extinction; the other is a globally-distributed domestic species. Although there are morphological differences between the two species, hybrids are often cryptic and the small population size of *C. ferus* and remoteness of the wild camel habitat means that identifying hybrids phenotypically is difficult. In this study we applied non-invasive sampling across the 45,000 square kilometre GGASPA (Kaczensky et al. 2014) and a combination of nuclear and mitochondrial DNA markers to (i) determine extent of nuclear introgression in both the captive and wild populations; (ii) evaluate the prevalence of this introgression based on hybrid threshold estimates currently adopted by the conservation community; and (iii) identify hybridisation patterns in mitochondrial DNA and compare them to patterns of nuclear introgression to identify drivers of hybridisation; and (iv) quantify population genetic diversity and structure across the wild population distributed throughout the GGASPA and the captive herd in Mongolia.

4.2 Methods

4.2.1 Sampling and DNA extraction

Prior to sample collection, we obtained ethics approval from the University of Kent's School of Anthropology and Conservation Research Ethics Advisory Board. From September 2018 to October 2019, AJ, AY, JE and National Park Rangers collected samples from across the Great Gobi A Special Protected Area (GGASPA), Mongolia (Figure 4.1). To ensure minimal disturbance to wildlife and the ecosystem we employed a non-invasive sampling protocol; samples included faeces, hair caught in vegetation and tissue taken from carcasses. Samples were collected during systematic camera trap placement (Chapter 3). For each sample, we recorded collection location on a Garmin GPS using the Universal Transverse Mercator (UTM) coordinate grid system. We used this location data for spatial analysis of genetic diversity using ESRI ArcGIS map and ArcGIS pro (ESRI 2023). We stored samples dry with silica beads, as required by the UK Animal and Plant Health Agency (APHA) (APHA 2023) before authorized transportation to the UK (import permits from APHA Authorisation number ITIMP21.1842 and export permits from Customs General Admission of Mongolia (No 02231060279). A registered veterinarian collected tissue samples from 34 captive-bred wild camels (*C. ferus*) in October 2022 (permission granted by the Wild Camel Protection Foundation (WCPF 2023)) during standard veterinary ear tagging procedures (using DALTON flexo-DNA ear tags (Dalton 2023)). Further faecal samples were collected from known domestic Bactrian camels (*C. bactrianus*) in herder inhabited areas surrounding the GGASPA. Combined with both DNA extractions from samples previously collected from known domestic, wild and hybrid Bactrian camels (Silbermayr K et al. 2010; Silbermayr et al. 2010) and genotype data (n=59) scored PB at the University of Veterinary Medicine Vienna, Austria, this resulted in a total of 416 samples.

Depending on sample type, we extracted DNA using either a QIAGEN DNeasy blood and tissue kit (tissue, blood, hair) or QIAGEN Qiamp fast DNA Stool Mini kit (faeces) (QIAGEN 2023). We included extraction controls in each batch of extractions, replacing samples with double distilled water (ddH2O), to monitor for the detection of cross contamination.

4.2.2 Analysis of nuclear genetic diversity

We used extracted DNA to amplify 16 autosomal polymorphic microsatellite markers (Appendix 4.1, Table 4.4) previously shown to amplify in both *C. ferus* and *C. bactrianus* (Silbermayr et al. 2010; Silbermayr K et al. 2010) (Appendix 4.5.1); and four sex-linked markers; three of which were previously validated (Felkel et al. 2019) but sequenced in this study, whilst one was newly validated (Appendix 4.5.2). Of the four sex-linked markers, three used polymorphisms for sexing and one was designed only to amplify in males. We amplified all 20 markers using the multitube approach to minimize error caused by allelic dropout (potentially more likely in lower-quality samples obtained non-invasively) (Taberlet et al. 1996), with each PCR repeated 3 times and a negative control (ddH20) included in each plate. We performed 5 μl volume PCRs with the following reagents: 1 μl DNA, 1 μl of primer mix (23 μl Low TE (10mM Tris-HCl (pH 8.0) and 0.1mM EDTA (pH 8.0)), 1 μl forward and 1 μl reverse primers (both at 5 uM) and 1 μl QIAGEN Multiplex PCR Master Mix (supplied with the QIAGEN Multiplex PCR Kit, Cat. No. / ID: 206145). PCR protocols are described in Appendix 4.5.1.

We scored genotypes using GENEMAPPER v5.0. Of the 416 samples, 198 (48%) (5 blood, 105 faecal, 45 hair and 43 tissue) amplified successfully at a minimum of 75% of the microsatellite DNA loci and so were included in downstream analysis. Microchecker (Van Oosterhout et al. 2004) was used to confirm absence of null alleles across the 16 loci. We combined this data set with previous microsatellite genotyping data that used the same loci (Silbermayr et al. 2010; Chuluunbat et al. 2014), with three samples included in both datasets for harmonizing allele lengths between laboratories. The final data set used for downstream analysis comprised 260 individuals genotyped at 16 microsatellite loci (Appendix 4.1, Table 4.4). These 260 genotype profiles were assumed to be from different individuals, but when probability of identity P(ID) (Waits, Luikart, and Taberlet 2001) was calculated using CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski, Taper, and Marshall 2010) (with a minimum loci for match=2, mismatch=0), three pairs of individuals were closely matched (P(ID)=<0.0001). On checking these individuals, one pair was from a repeatedly sampled captive camel (individual “Saran”) so the match

was confirmed, whilst the other two pairs were all collected from the same family group (Figure 4.7). After accounting for these matches, the final data set comprised 257 individuals. Before analysis we presumed the full data set of 257 individuals to comprise: 47 samples from known Bactrian camels (*C. bactrianus*)*;* 188 samples from wild camels (*C. ferus*) (from the WCPF captive breeding center (n=47) and presumed wild camels (n=141) from the GGASPA); and 22 samples from known Bactrian X wild camel hybrids, based on verified breeding history, collected from camel herders both in Mongolia and China

4.2.3 Analysis of mitochondrial DNA

Using mtDNA, we performed a PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) (Silbermayr et al. 2010) to determine whether hybridisation had occurred on the maternal line. We redesigned a mitochondrial primer developed by Silbermyer et al 2010 (Silbermayr et al. 2010) to amplify a shorter length fragment that would be more suitable for amplifying mtDNA from degraded faecal samples. We used the "HYB 185 bp" primer pair (forward 5'- GTT CAT CGT AAT CGG CCA AGC -3' and reverse 5'- GGC CTC TTC CCT GAG TCT TAG -3') in 20µL volume PCRs to amplify a 185bp mtDNA fragment containing the single nucleotide polymorphism that is diagnostic for either *C. ferus* or *C. bactrianus*. Reactions used the following reagents: 3µL DNA, 0.4µL forward primer, 0.4µL Reverse primer, 10µL Red Taq, 2.2µL double distilled water and 4µL diluted Bovine Serum Albumin (BSA) (1µL BSA - Acetylated; 99µL double distilled water). Negative controls, where DNA was replaced with ddH_2O, were included in each plate. After a PCR protocol (92°C for 8 min, 45 cycles of: 95°C for 30 seconds, 52°C for 45 seconds, 72°C for 45 seconds and a final extension of 72°C for 10 min), PCR product was digested with: 1 unit of *Xmil*, 10x Buffer B (with BSA) and double distilled water at 37°C for 1hr. This digestion was either analysed by gel electrophoresis or sequenced following the Macrogen EZ-Seq protocol for purified PCR product of less than 300bp. For gel electrophoresis, the presence of two bands at 60bp and 120bp indicated *C. bactrianus* maternal lineage, whereas an uncut single band of 185bp indicated a *C. ferus* maternal

lineage. Using DNA sequence to verify maternal lineage, the base motifs differ between species, with "CATATGAT" for *C. bactrianus* and "TATATGAT" for *C.ferus.* This mitochondrial analysis was successfully amplified in 184 (93%) of the 198 available samples used in this analysis (the full microsatellite data set of 257 samples included genotype data from 59 individuals. that we were unable to access the for mtDNA analysis). 14 samples did not amplify with the mtDNA marker.

4.2.4 Population summary statistics

We used CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski, Taper, and Marshall 2010) to estimate the following measures of genetic diversity: number of alleles per locus; deviations from Hardy Weinberg equilibrium; estimation of null allele frequencies; observed (H_O) and expected (H_E) heterozygosity (Table 4.3). The presence of heterozygotes in known males and females of both species confirmed these markers as autosomal. We tested for normality using the Shapiro-wilk test, and then tested for significant differences between H_O and H_E with a Student's *t* Test in R (R Studio Team 2022) and the Hardy Weinberg test (Table 4.3), to determine that any difference between H_O and H_E was not due to chance alone.

We used Bayesian clustering methods to determine shared ancestry proportions, identify potential wild and domestic Bactrian camel hybrids, and determine population structure (Pritchard, Stephens, and Donnelly 2000). This method is appropriate as it uses multi-locus genotypes (93% of the 16 loci were successfully amplified across all 257 samples) both to infer population structure and assign individuals to populations using allele frequencies, without the need for prior classification. As our samples were predominantly non-invasive, it was important that classifications didn't require individual species visualization. We ran STRUCTURE 2.3.4 (Pritchard, Stephens, and Donnelly 2000) software, with correlated allele frequencies to detect distinct populations that are closely related, for 1000000

iterations, with a burn-in of 500000 and 10 iterations, varying number *K* of clusters between 2 and 10. We then used STRUCTURE HARVESTER (Earl and vonHoldt 2012) and CLUMPAK (Kopelman et al. 2015) to infer the optimal clustering solution (*K*) and to plot the results.

We assessed levels of introgression of domestic Bactrian camel DNA into wild camels using a predetermined *K* = 2, clusters representing each species. As known hybrids are present, admixture was assumed. Due to the close relatedness of the two species (Silbermayr et al. 2010) we initially allowed for up to 5% of ancestral alleles (qi>0.95) shared between the two species, and considered individuals with more than 5% (qi<0.95) as hybrids. However, threshold values in other comparable hybrid studies (Kingston and Gwilliam 2007) use a q-value of 0.10 (Vähä and Primmer 2006) as a conventional threshold for distinguishing between pure and hybrid individuals. Given that our genotype data does not include associated phenotype values for individuals, and in the absence of a standard threshold used by previous studies for camels, we report both threshold values (Table 4.1, Figure 4.2).

We used a threshold value of qi=0.10 to identify hybrids from parental species (Vähä and Primmer 2006). We removed all pure domestic (qi> 0.95) and hybrid camels (qi>0.90) (Vähä and Primmer 2006) for the subsequent analysis of wild camel population structure and summary statistics. We used the R package "Adegenet" (ADEGENET 2023b; 2023a) for principal component analysis (PCA) and to generate inbreeding coefficient (F_{IS}) values.

Analysis of Molecular Variance (AMOVA) was used to partition proportions of genetic variation: between populations (populations determined as: 1- pure (qi>0.95) Wild *Camelus ferus*, 2- pure (qi>0.95) Bactrian *Camelus bactrianus*, 3- hybrids (qi >0.95) and 4- captive wild camels), between samples within populations, and within samples. AMOVA and FST were measured using GenAlEx (Peakall and Smouse 2006).

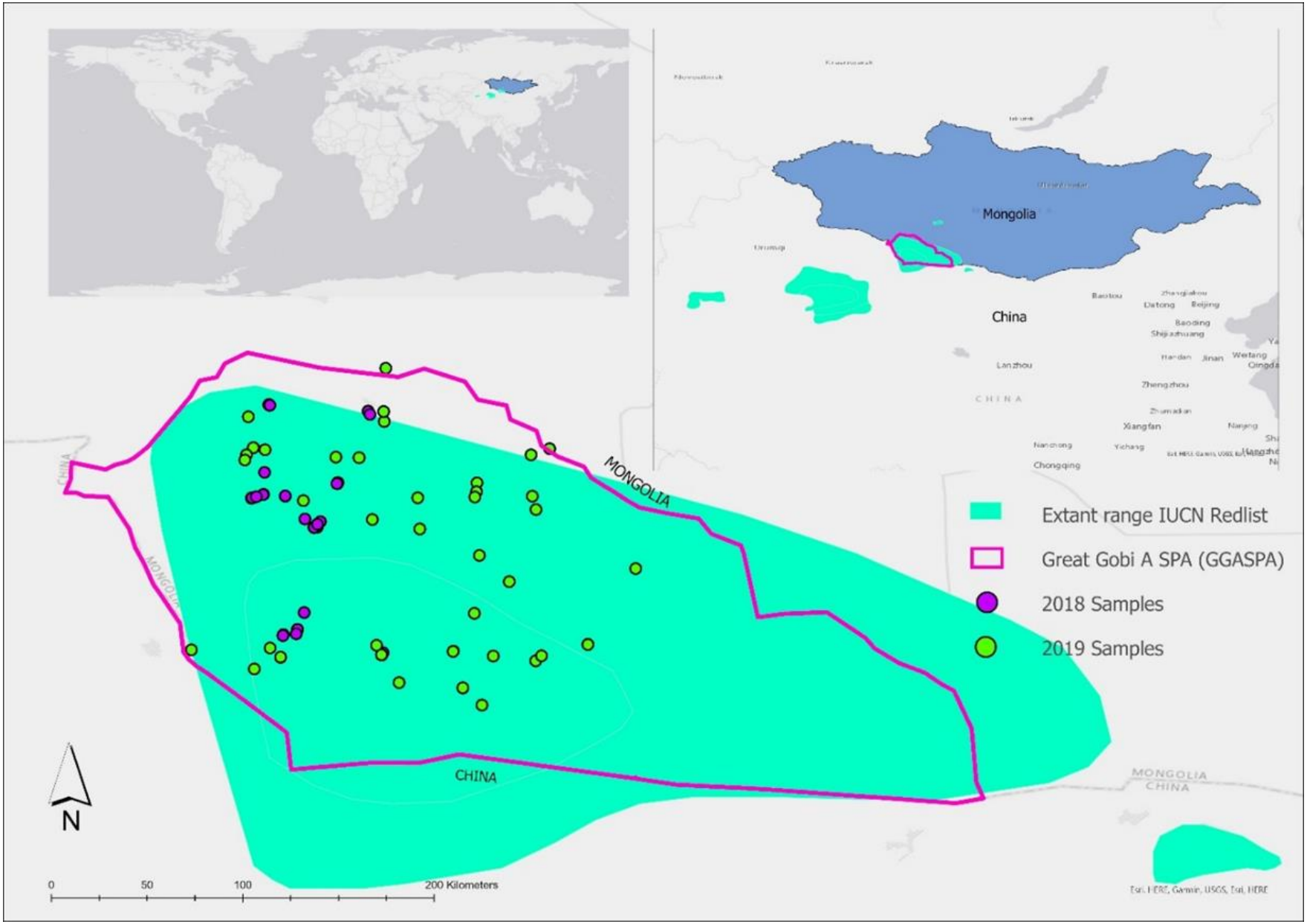

***Figure 4.1**: Location of GGASPA and locations of samples collected in the Great Gobi A Special Protected Area in 2018 and 2019, with reference to wild camel (Camelus ferus) range and global location (IUCN Redlist).*

4.3 Results

4.3.1 Population structure and introgression in wild camels

Initial STRUCTURE analysis of the full genotype data set suggested a predominant structuring comprising the two species (K=2): wild camel, *Camelus ferus* and domestic Bactrian camel, *Camelus bactrianus*. When Bactrians and hybrids were removed the analysis indicated comparatively weak structure of K=3, but this structuring could not be visualized when mapped across the GGASPA (Appendix 4.5.4).

Population structure with an assumed *K*=2 allowed for classification of individuals of each of the two pure parental species and of the hybrid individuals along a continuum of introgression (Figure 4.3). At a threshold for introgression of qi=0.95, 58 (28%) of the 204 wild camel samples are shown to be hybrids; across the full sample set (n=257), 146 are wild (57%), 53 are Bactrian (20%) and 55 are hybrid (22%). At a threshold for introgression of qi=0.90, 20% of the wild camel (202) samples are hybrids; across the full data (n=257) 161 (63%) are wild, 55 (21%) are Bactrian and 41 (16%) are hybrids. To gain a better representation of the wild population of the wild camel, we removed known hybrids (samples of known hybrids collected from domestic herds) (n=22) and Bactrian camels (n=47), leaving a total of 189 wild and captive animals, which on sample collection were of unknown “purity”. Of these 189, at qi=0.95 19% are classified as hybrid and at qi=0.90 10% are classified as hybrid.

Of the 47 samples from captive-bred wild camels used in this analysis (34 of which are current surviving members, which comprises 5% of the Mongolian population of *C. ferus,* chapter 5), two sampled individuals (of which one is a current herd member) show introgression at qi=0.90 and five (of which three are current herd members) show introgression at qi=0.95.

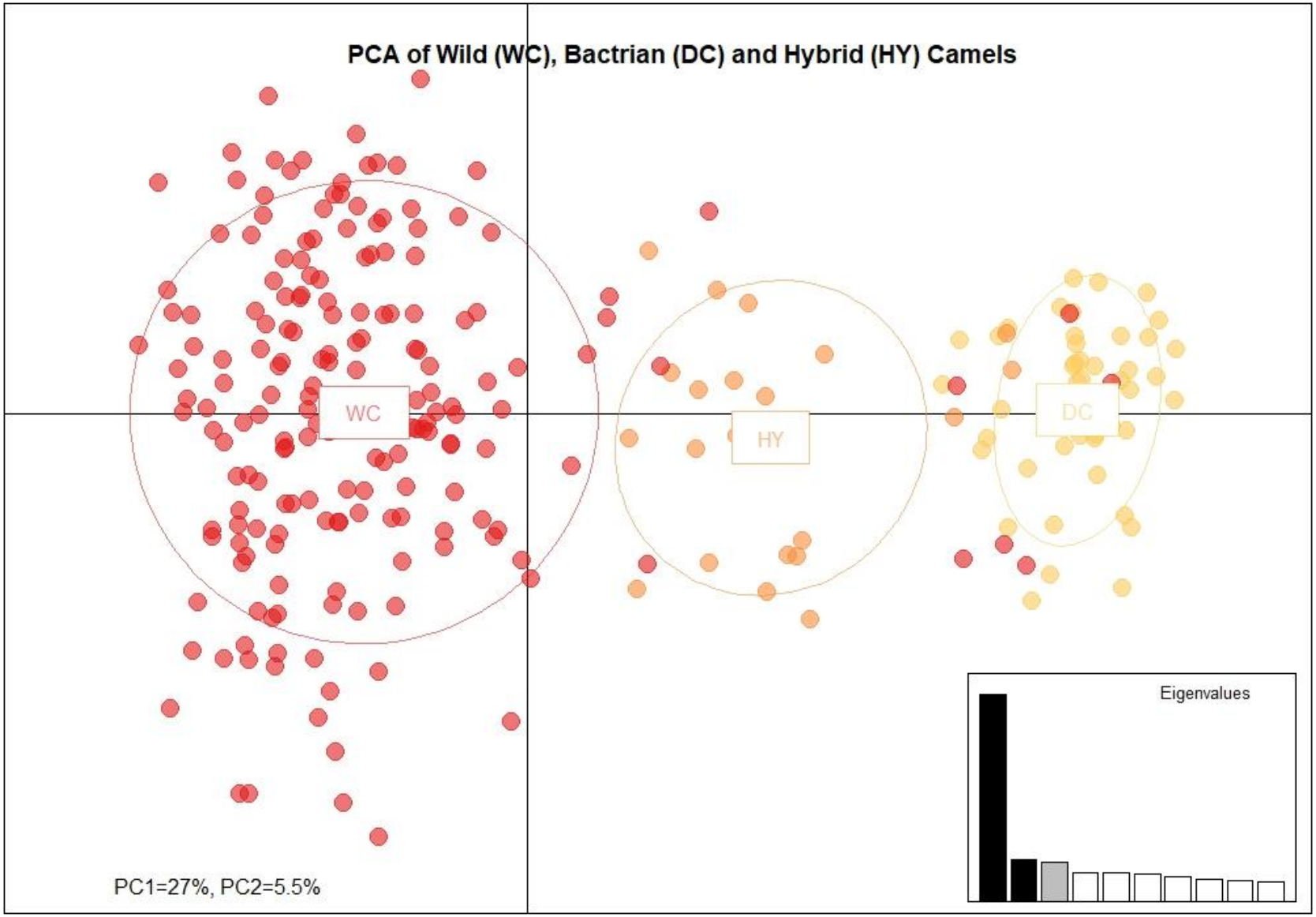

Figure 4.2(a): *Principal component analysis including all 257 samples. Discrete colours relate to presumed species on sample collection: WC= wild camel, Camelus ferus, DC= Bactrian camel, Camelus bactrianus and HY= hybrids. PC1 represents 27% of variance and PC2 represents 5.5% of variance. Figure produced in Adegenet R.*

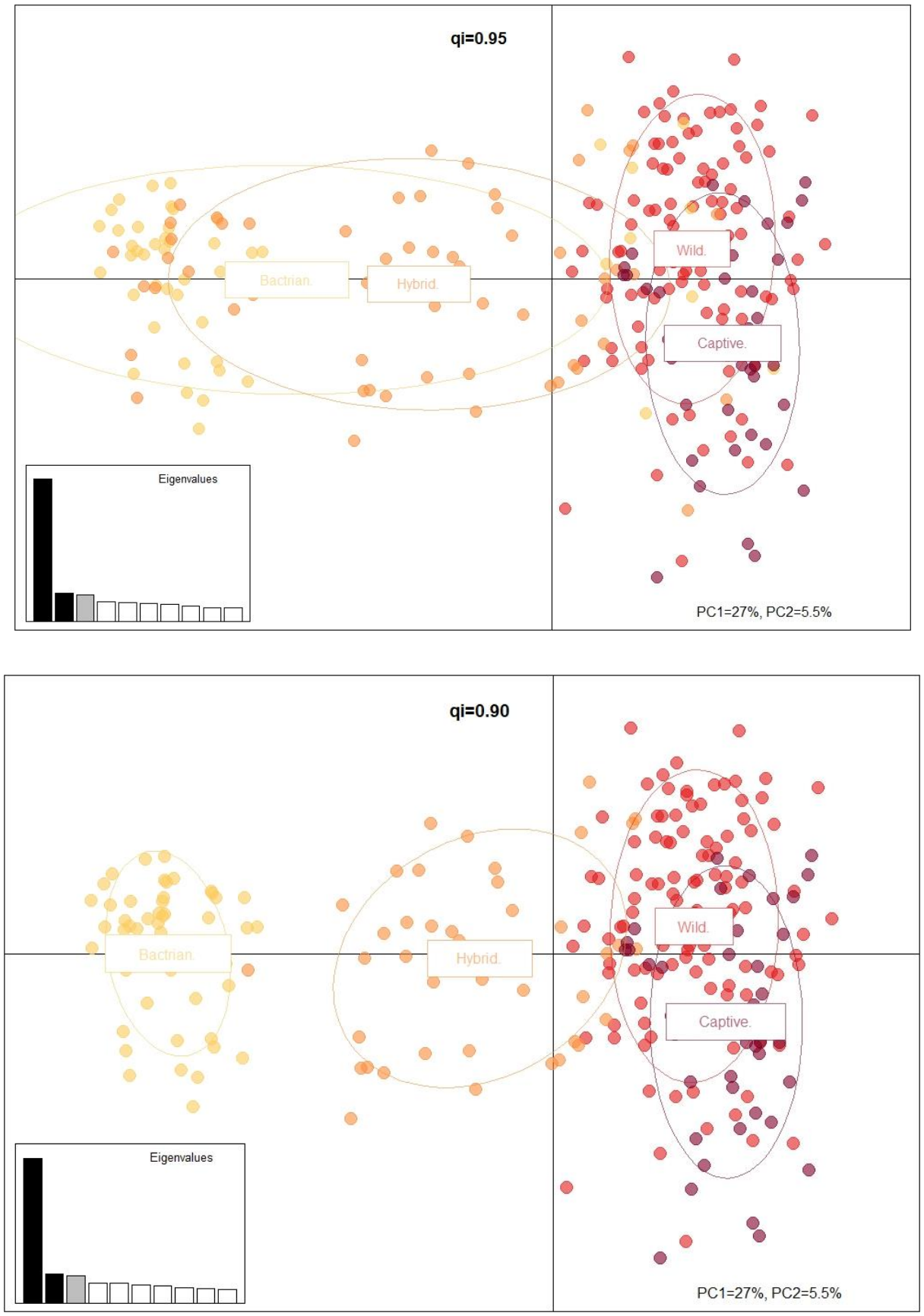

Figure 4.2(b): *Top: Principal component analysis (PCA) including all 257 samples. Discrete colours relate to known captive population (Captive) and populations determined after STRUCTURE analysis using a Qi value of 0.95 to determine hybrids. Wild camel, Camelus ferus (Wild); Bactrian camel, Camelus bactrianus (Bactrian) hybrids (Hybrid). Bottom: Principal component analysis (PCA) including all 257 samples. Discrete colours relate to known captive population (Captive) and populations determined after STRUCTURE analysis using a Qi value of 0.90 to determine hybrids. Wild camel, Camelus ferus (Wild); Bactrian camel, Camelus bactrianus (Bactrian) hybrids (Hybrid). PC1 represents 27% of variance and PC2 represents 5.5% of variance Figure produced in Adegenet R.*

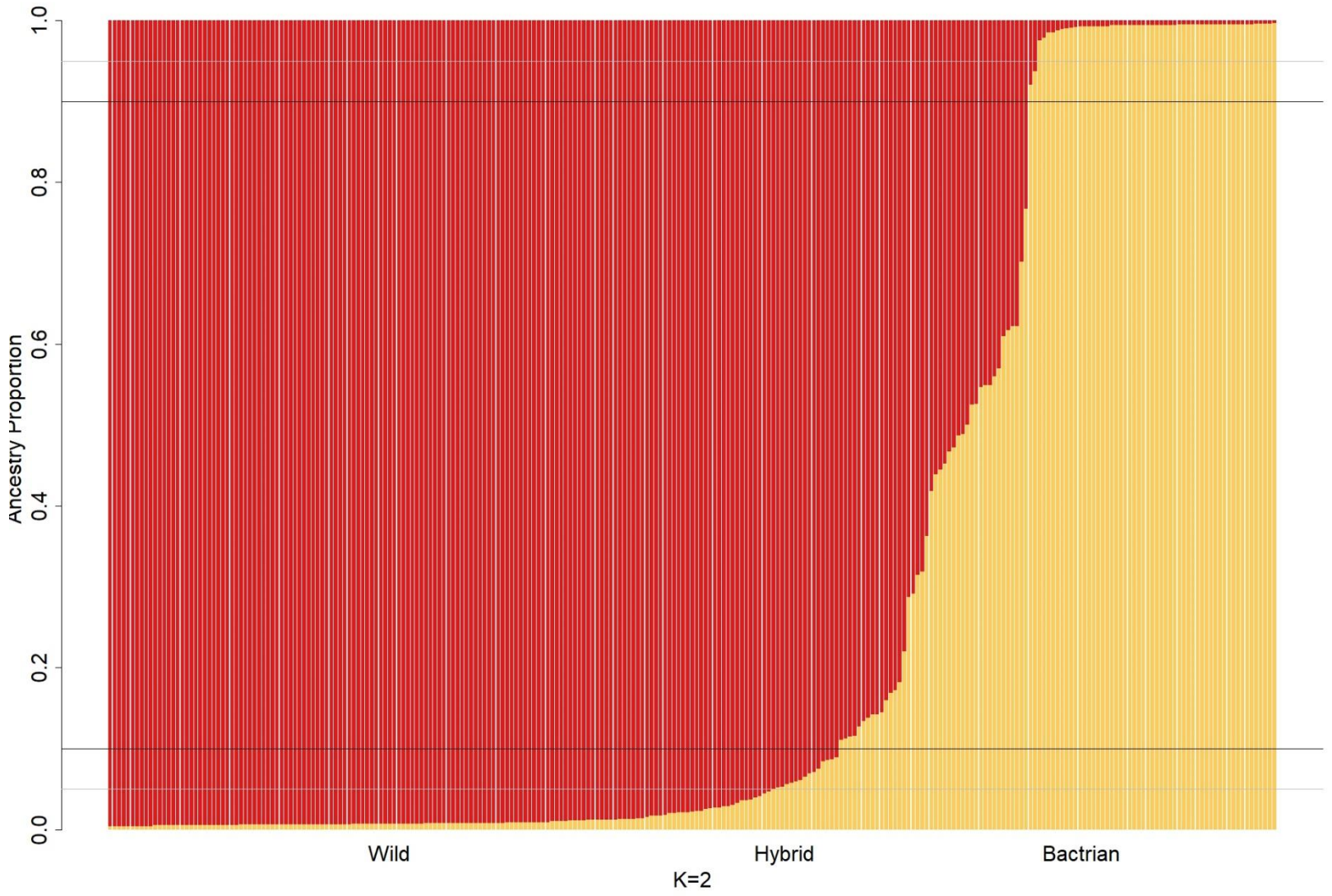

Figure 4.3: *STRUCTURE analysis of K=2. Each vertical line represents the ancestry proportion of one individual. Ancestry proportion is identified as Wild - Camelus. ferus in red, with Bactrian- Camelus bactrianus in yellow. Black horizontal lines indicate qi=0.10 and 0.90, grey horizontal lines identify qi= 0.05 and 0.95. 5%, or less, of shared ancestral alleles (qi>0.95) or 10%, or less, shared ancestral alleles (qi=0.90) is considered "pure". An individual with more than 5% (qi<0.95) or 10% (qi<0.90) is considered as an introgressed or hybrid individual. These are the thresholds used for distinguishing between pure and hybrid species. Figure produced in Adegenet.*

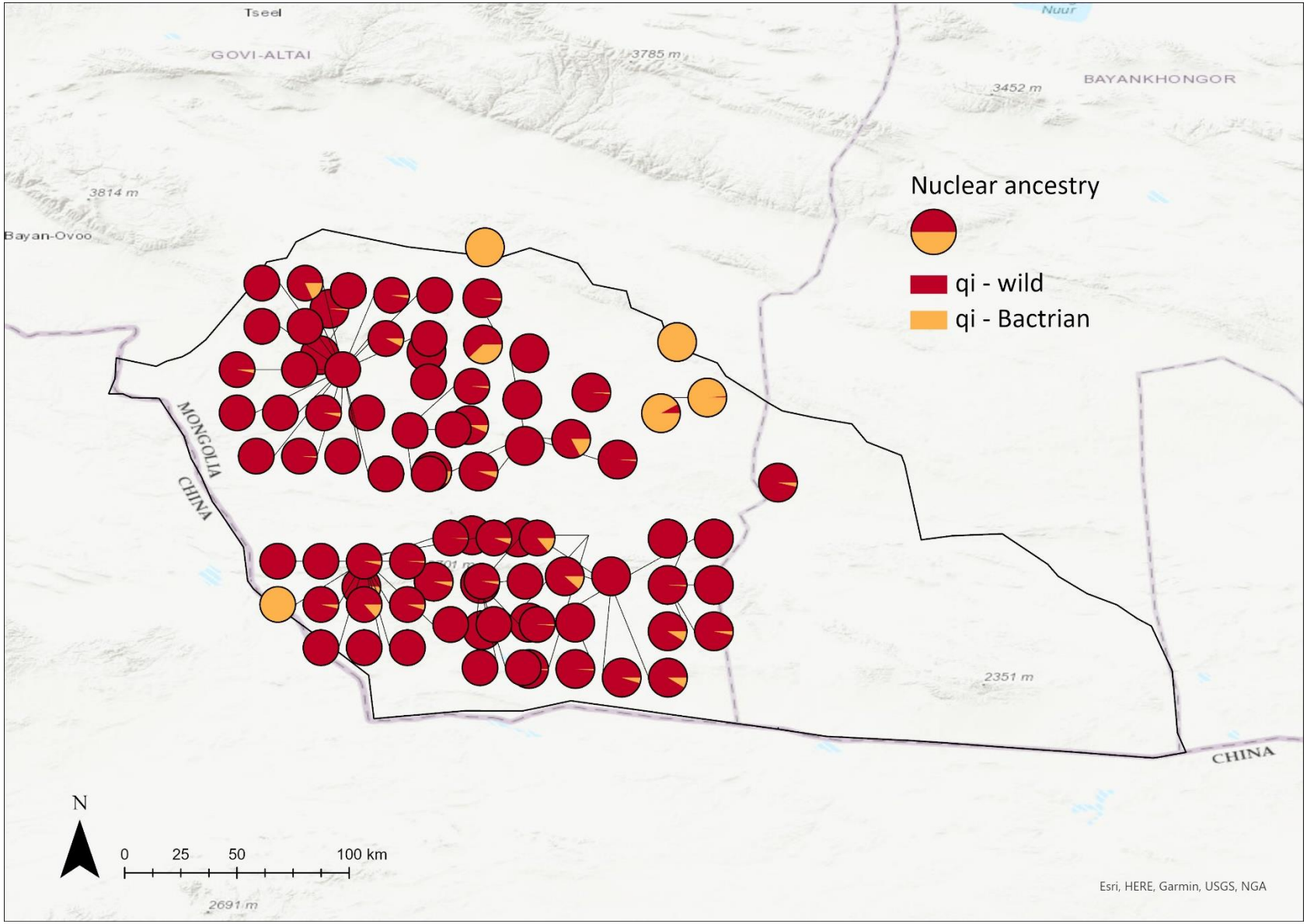

Figure 4.4*: STRUCTURE results (K=2). Each pie chart represents an individual sample, plotting the proportions of nuclear ancestry (qi) from wild Camelus ferus alleles and Bactrian Camelus bactrianus alleles. Pie charts are plotted at the location where the sample were collected. This allows for monitoring of introgression geographically across the GGASPA.*

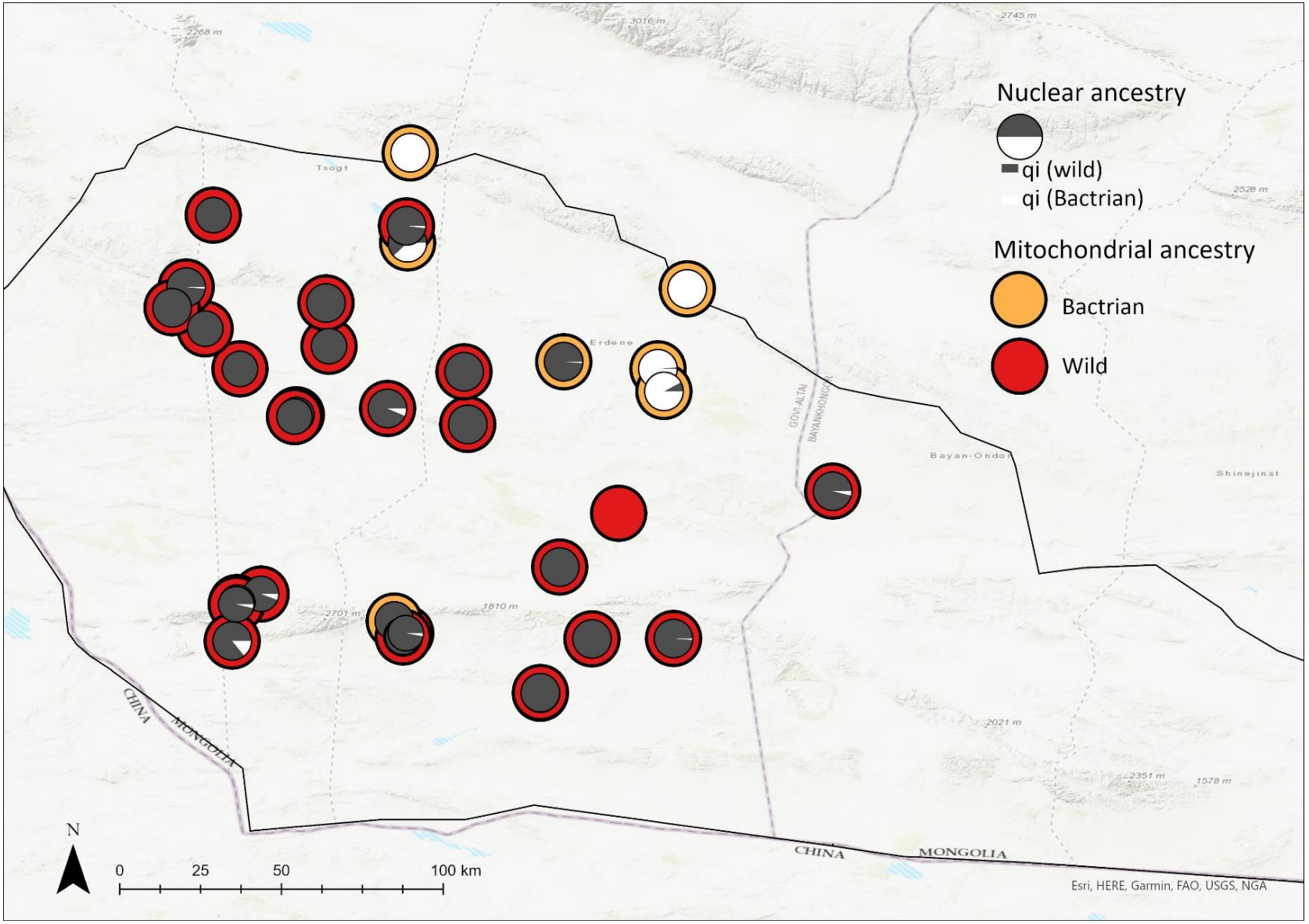

Figure 4.5: *Mitochondrial introgression across the GGASPA. Each pie chart represents an individual sample, showing the nuclear DNA-derived proportions of ancestry (qi) from wild Camelus ferus and Bactrian Camelus bactrianus alleles. The outline colour of each pie chart represents mtDNA evidence for maternal introgression. Pie charts are plotted at the location where the sample were collected.*

	Presumed	Results qi=0.95	Results qi=0.90
Wild (incl. captive)	191	146	161
Bactrian	47	53	55
Hybrid	22	58	41
Total	260	257	

Table 4.1: *Total number of samples in the data set according to presumed species identification on collection and genetically confirmed species identity after structure analysis with a threshold of qi=0.95 and qi=0.90. When q=0.95 the presumed sample species show an increase of 6 Bactrians and 36 hybrids. When qi=0.90 results show an increase of 8 Bactrian and 19 hybrids. Of the 22 presumed hybrids, 20 were hybrid, 1 (cHyb17) was above both our thresholds and so is Bactrian, one was above our qi=0.90 threshold (Chyb45) so is Bactrian. Results show that genotype STRUCTURE data can determine species and hybrids from non-invasive, unidentified animals. This can be visualized in figure 4.4*

4.3.2 Mitochondrial introgression

Combining both nuclear DNA genotypes with mitochondrial DNA can help identify the source of introgression. If a microsatellite genotype profile suggests a sample comes from a hybrid specimen but mtDNA indicates *C. ferus*, this implies the *C. bactrianus* genetic material is of paternal origin (paternal introgression). Conversely, if a microsatellite genotype profile suggests hybrid and mtDNA assay indicates *C. bactrianus*, it implies maternal introgression.

Of 41 hybrids initially identified using microsatellite data (qi=0.90), 36 amplified successfully using the mitochondrial technique (Table 4.2, Figure 4.5). The mtDNA results indicated that 17 were maternally inherited, and 19 paternally. Sex information, obtained using the sex-linked markers, identified that of the 17 hybrids that showed maternally-inherited introgression, 10 are female and can therefore further pass on Bactrian mtDNA. Of the 58 hybrids determined using the qi threshold of qi=0.95, 52 amplified

successfully using mtDNA. These results suggested that 32 are paternally inherited hybrids, whereas 20 are maternally inherited; of these 11 are female and so can pass on Bactrian mtDNA.

Eight samples initially considered "pure" *C. ferus* (qi=0.90) on nuclear results and on the basis of their collection location in the GGASPA, show introgression in their mtDNA (Table 4.2, Figure 4.5). The extent of nuclear introgression within these mtDNA-typed individuals ranges from 0.947 to 0.994. Only one of these eight individuals would be considered hybrid at the 0.95 threshold. Given that mtDNA provides a longer-term picture of evolutionary ancestry, in these cases where introgression is observed in the mtDNA genome of nuclear 'pure' individuals they are likely to be the product of historical hybridisation. This result can be visualised in the family group in Figure 4.6.

All of the captive-bred camels showed "wild" mtDNA, including those with nuclear introgression.

Sample ID	qi= 0.90 Spp Result	qi= 0.95 Spp Result	% Wild	% Bactrian	mtDNA Spp Result	Sex
AWC110	Wild	Wild	0.994	0.006	Wild	n/a
AWC111	Wild	Wild	0.99	0.01	Wild	M
AWC112	Wild	Wild	0.996	0.004	n/a	M
AWC113	Wild	Wild	0.995	0.005	n/a	n/a
AWC114	Wild	Wild	0.995	0.005	Wild	M
AWC115_127	Wild	Wild	0.992	0.008	Wild	M
AWC117	Wild	Wild	0.995	0.005	Wild	n/a
AWC118	Wild	Wild	0.993	0.007	Wild	M
AWC119	Wild	Wild	0.969	0.031	Wild	M
AWC120	Wild	Wild	0.986	0.014	Wild	F
AWC123	Wild	Wild	0.992	0.008	Wild	n/a
AWC124	Wild	Wild	0.996	0.004	Wild	M
AWC125	Wild	Wild	0.992	0.008	Wild	F
AWC128	Wild	Wild	0.973	0.027	Wild	F
AWC129_135	Wild	Wild	0.983	0.017	Wild	F
AWC130	Wild	Wild	0.99	0.01	Wild	M
AWC131	Wild	Wild	0.992	0.008	Wild	M
AWC132	Wild	Hybrid	0.924	0.076	Wild	n/a
AWC133	Wild	Wild	0.992	0.008	Wild	F
AWC134	Wild	Wild	0.963	0.037	Bactrian	n/a
AWC136	Wild	Wild	0.991	0.009	Wild	M

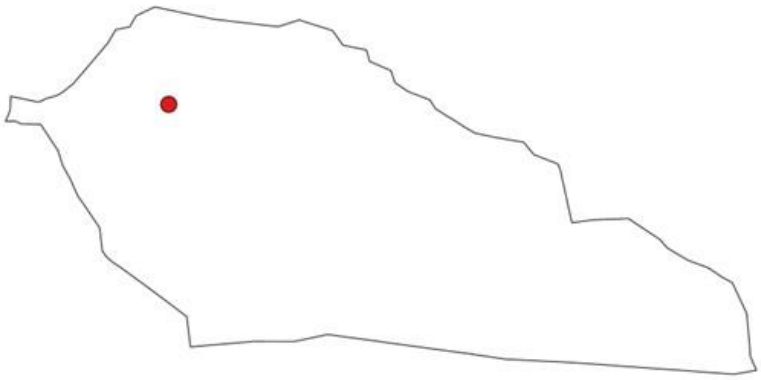

Figure 4.6*: Hybrid analysis wild camel case study. 26 samples were collected from one herd of wild camels (Sample ID= AWC110-AWC136). After observing the herd, without disturbing them we went to the location (map insert) where they had slept. "Beds" were obvious (photo L), with clear areas in the sand where camels had lain and areas wet with urine (photo R). Samples were collected from 26 individual beds. P(ID) determined 2 matches (orange: AWC115/ AWC127 and AWC129/AWC139) suggesting the herd consisted of 24 individuals. 21 individuals successfully amplified in the nuclear markers, allowing for further analysis. STRUCTURE analysis (K=2, % wild and % Bactrian) determined all 21 to be wild with the qi=0.9O threshold (qi=0.90 Spp result). With the qi=0.95 threshold used (qi=0.95 spp result), one individual is considered hybrid (AWC132, yellow). Of the 21, mtDNA amplified in 19 (mtDNA spp result). 18 of which showed wild, 1 showed Bactrian (Red, AWC134). The 1 showing mtDNA of a Bactrian had nuclear value of 0.96, so would not be determined a hybrid in either of our thresholds. Suggesting historic introgression. Of the 21, sex markers amplified in 15 samples (Sex). Of which 10 were male and 5 were female.*

Sample ID	Nuclear (qi= 0.90) Spp Result	mtDNA Spp Result	Sex (sex marker data)	% Wild	% Bactrian	Intogression inheritance	Location
Camelus ferus- historic intogression							
AWC134	Wild	Bactrian		0.963	0.037	Historic	GGASPA
AWC243	Wild	Bactrian	M	0.959	0.041	Historic	GGASPA
AWC275	Wild	Bactrian	F	0.988	0.012	Historic	GGASPA
AWC308	Wild	Bactrian	F	0.968	0.032	Historic	GGASPA
AWC330	Wild	Bactrian	M	0.947	0.053	Historic	GGASPA
AWC72	Wild	Bactrian		0.962	0.038	Historic	GGASPA
AWC74	Wild	Bactrian		0.994	0.006	Historic	GGASPA
WC31	Wild	Bactrian	F	0.971	0.029	Historic	GGASPA
Hybrids, paternally inherited							
AWC122	Hybrid	Wild	F	0.819	0.181	Paternal	GGASPA
AWC248	Hybrid	Wild	M	0.859	0.141	Paternal	GGASPA
AWC300	Hybrid	Wild	F	0.839	0.161	Paternal	GGASPA
AWC305	Hybrid	Wild	M	0.884	0.116	Paternal	GGASPA
AWC35	Hybrid	Wild	F	0.86	0.14	Paternal	GGASPA
AWC80	Hybrid	Wild	F	0.866	0.134	Paternal	GGASPA
CHYB41	Hybrid	Wild	M	0.451	0.549	Paternal	China
CHYB57	Hybrid	Wild	M	0.45	0.55	Paternal	Herder China
CWC2	Hybrid	Wild	M	0.581	0.419	Paternal	China
E3	Hybrid	Wild	F	0.684	0.316	Paternal	Captive bred
Hyb17	Hybrid	Wild	M	0.389	0.611	Paternal	Captive bred
wc117	Hybrid	Wild	M	0.709	0.291	Paternal	GGASPA
WC121	Hybrid	Wild	M	0.855	0.145	Paternal	GGASPA
WC205	Hybrid	Wild	M	0.889	0.111	Paternal	China
WC207	Hybrid	Wild	M	0.555	0.445	Paternal	China
WC208	Hybrid	Wild	M	0.782	0.218	Paternal	China
WC225	Hybrid	Wild		0.83	0.17	Paternal	GGASPA
WC249	Hybrid	Wild	M	0.858	0.142	Paternal	Mongolia
WC251	Hybrid	Wild	M	0.874	0.126	Paternal	GGASPA
Hybrid, maternally inherited							
AWC327	Hybrid	Bactrian	M	0.889	0.111	Maternal	GGASPA
AWC331	Hybrid	Bactrian	M	0.828	0.172	Maternal	GGASPA
AWC334	Hybrid	Bactrian	F	0.62	0.38	Maternal	GGASPA
CHYB14	Hybrid	Bactrian	F	0.452	0.548	Maternal	Herder China
CHYB33	Hybrid	Bactrian	M	0.499	0.501	Maternal	Herder China
CHYB34	Hybrid	Bactrian	M	0.532	0.468	Maternal	Herder China
CHYB36	Hybrid	Bactrian	M	0.232	0.768	Maternal	Herder China
CHYB37	Hybrid	Bactrian	F	0.439	0.561	Maternal	Herder China
CHYB38	Hybrid	Bactrian	M	0.475	0.525	Maternal	Herder China
CHYB39	Hybrid	Bactrian	F	0.378	0.622	Maternal	Herder China
CHYB40	Hybrid	Bactrian	F	0.297	0.703	Maternal	Herder China
CHYB42	Hybrid	Bactrian	F	0.636	0.364	Maternal	Herder China
CHYB44	Hybrid	Bactrian	M	0.511	0.489	Maternal	Herder China
CHYB46	Hybrid	Bactrian	F	0.378	0.622	Maternal	Herder China
CHYB47	Hybrid	Bactrian	F	0.474	0.526	Maternal	Herder China
CHYB48	Hybrid	Bactrian	F	0.68	0.32	Maternal	Herder China
CHYB53	Hybrid	Bactrian	F	0.429	0.571	Maternal	Herder China
Camelus bactrianus							
AWC272	Bactrian	Bactrian	M	0.006	0.994	n/a	GGASPA
AWC278	Bactrian	Bactrian	M	0.012	0.988	n/a	GGASPA
AWC279	Bactrian	Bactrian	M	0.079	0.921	n/a	GGASPA
AWC336	Bactrian	Bactrian	M	0.007	0.993	n/a	GGASPA
AWC345	Bactrian	Bactrian	F	0.024	0.976	n/a	GGASPA
AWC84	Bactrian	Bactrian	F	0.008	0.992	n/a	GGASPA
B1	Bactrian	Bactrian		0.005	0.995	n/a	ZU Bactrian
B2	Bactrian	Bactrian	F	0.003	0.997	n/a	ZU Bactrian
B5	Bactrian	Bactrian	F	0.005	0.995	n/a	ZU Bactrian
CHYB45	Bactrian	Bactrian	F	0.063	0.937	n/a	Herder China
Khara	Bactrian	Bactrian	F	0.005	0.995	n/a	Austria
Camelus ferus							
AWC110	Wild	Wild		0.994	0.006	n/a	GGASPA
AWC111	Wild	Wild	M	0.99	0.01	n/a	GGASPA
AWC114	Wild	Wild	M	0.995	0.005	n/a	GGASPA
AWC115_127	Wild	Wild	M	0.992	0.008	n/a	GGASPA
AWC117	Wild	Wild		0.995	0.005	n/a	GGASPA
AWC118	Wild	Wild	M	0.993	0.007	n/a	GGASPA
AWC119	Wild	Wild	M	0.969	0.031	n/a	GGASPA
AWC120	Wild	Wild	F	0.986	0.014	n/a	GGASPA
AWC123	Wild	Wild		0.992	0.008	n/a	GGASPA
AWC124	Wild	Wild	M	0.996	0.004	n/a	GGASPA
AWC125	Wild	Wild	F	0.992	0.008	n/a	GGASPA
AWC128	Wild	Wild	F	0.973	0.027	n/a	GGASPA
AWC129_135	Wild	Wild	F	0.983	0.017	n/a	GGASPA
AWC130	Wild	Wild	M	0.99	0.01	n/a	GGASPA
AWC131	Wild	Wild	M	0.992	0.008	n/a	GGASPA
AWC132	Wild	Wild		0.924	0.076	n/a	GGASPA
AWC133	Wild	Wild	F	0.992	0.008	n/a	GGASPA
AWC136	Wild	Wild	M	0.991	0.009	n/a	GGASPA

Table 4.2: MtDNA Results. Table includes all available data for Bactrians, hybrids and historic introgressed C.ferus, plus example selection of Camelus ferus AWC110-136. Species determined by qi=0.90. Nuclear species result determined my microsatellite testing (qi=0.90), mitochondrial DNA species result determined by mitochondrial PCR-RFLP or sequencing

analysis, Sex determined by the sex-linked markers and introgression inheritance being the direction of inheritance presumed from maternal mtDNA.

4.3.3 Heterozygosity and inbreeding

Across the 16 autosomal microsatellite loci, H_O ranged from 0.03 to 0.85, mean=0.39 standard deviation= 0.21 (Appendix 4.5.1, Table 4.8). Null alleles were not present in any of the 16 loci. The estimated null allele frequency (*F*) ranged from -0.0016 to 0.179 calculated in CERVUS (Appendix 4.5.1, Table 4.8). We calculated population summary statistics separately for the 'pure' wild camel individuals sampled in the strictly protected area of the Gobi A (n = 106-116, depending on hybrid threshold) after removing the data derived from individuals from the WCPF captive breeding center and the detectable hybrids (>10% domestic Bactrian camel alleles). This partitioning of the data ensured that analysis of genetic composition was not distorted by either mixing data from individuals of two different species or by inadvertently including data from closely related captive animals, thereby enabling more appropriate comparisons of genetic diversity in *C. ferus.* Alleles per locus ranged from 3 to 16 (Appendix 4.5.1, Table 4.8).

Analyses of genetic diversity revealed that the only population to deviate significantly from HWE was the full data set (unsurprising given that it contains both parent species and hybrids). All other populations did not deviate significantly from HWE (Table 4.3). However, when a threshold of 10% shared ancestry was applied, this resulted in a significant difference between the H_O and H_E in the wild population (*t*-test *p* value: 0.03). This result suggests that although in HWE, *C. ferus* is showing a difference in observed heterozygosity that is not due to chance alone, and is showing lower levels of heterozygosity than expected. This result is supported by F_{is} values suggesting more inbreeding than expected at random in all populations other than the captive population. The captive population shows less inbreeding than we predicted for a captive ex-situ population (F_{is}=-0.04). That the inbreeding value was highest in the Bactrians (Fis= 0.13 95% CI= 0.01-0.24) could be explained by the small number of related Bactrian individuals used in this study (70% coming from a single herd). Indeed, the wider

Mongolian population of Bactrian camels has been shown to have evidence of inbreeding (Chuluunbat et al. 2014). Given that we included the Bactrian camel in this analysis only to identify hybrid individuals amongst the *C. ferus* population, the small data set of related Bactrian camel individuals does not impact further analysis. Allelic richness is greater in the wild population (median 10) compared to the captive population (median = 6) (Unpaired Mann Whitney p-value = 0.0005, Figure 4.7), a result likely due to both the larger-sized wild population that is spread over a vast geographic area in comparison to the captive herd which originates from a small number of related founders, and is therefore likely to have been impacted by effects of random genetic drift. Results also suggest there is no significant difference between the inbreeding coefficients (*F*, TrioML) for the captive herd when compared to the wild (Unpaired Mann Whitney: Wild/Captive p value=0.5158p Figure 4.7). There is however a significant difference between the inbreeding coefficients (*F*, TrioML Unpaired Mann Whitney: Wild/Hybrid p-value = <0.0001, Captive/hybrid p-value = 0.0007) of all other groups which suggests that despite allelic richness being greater in the wild compared to in captivity, the genetic composition and extent of inbreeding observed in the wild population is no different to that in the captive population.

Analysis of molecular variance AMOVA showed that the greatest variance was within individuals (75%), then among populations (21%) and finally among individuals (1%) (Appendix 4.5.5). This reflects the overlap between all these populations. As expected, the largest FST values are between the two species *C. bactrianus* and the captive population (FST=0.397), with wild and Bactrian showing similar (FST=0.372). Hybrids show higher FST when compared to Bactrian (FST=0.165) than wild (FST=0.074) or captive (FST=0.105).

qi=0.95							
Population	N	Mean Hobs	Mean Hexp	hw mean p value	t. test p value	FIS	Mean *F* (TrioML) Comb
Full data set	260	0.45	0.57	0.0013	<0.0001	0.21 (0.13-0.27)	0.23
Wild (including historic captives)	118	0.39	0.42	0.45	0.05	0.08 (-0.01-0.16)	0.24
Wild only	106	0.39	0.42	0.42	0.05	0.09 (0.01-0.18)	0.25
Wild and captive	149	0.39	0.41	0.52	0.09	0.06 (<0.01-0.15)	0.24
Bactrian	53	0.5	0.56	0.26	0.06	0.13 (0.03-0.24)	0.29
Captive (2022)	34	0.38	0.35	0.63	0.96	-0.08 (-0.16-<0.01)	0.2
Captive no hybrids (2022)	31	0.38	0.34	0.64	0.97	-0.11 (-0.20- -0.02)	0.2
Captives- total	48	0.39	0.37	0.64	0.92	-0.04 (-0.09- 0.03)	0.2
Captive no hybrid total	43	0.39	0.36	0.63	0.96	-0.08 (-0.15- 0.02)	0.21
Hybrids	58	0.57	0.60	0.39	0.1	0.06 (<-0.01- 0.13)	0.14
qi=0.90							
Population	N	Mean Hobs	Mean Hexp	hw mean p value	t. test p value	FIS	Mean *F* (TrioML) Comb
Full data set	257	0.46	0.58	0.001	<0.0001	0.21 (0.13-0.27)	0.23
Wild (including all captive)	161	0.39	0.42	0.43	0.04	0.07 (0.007-0.15)	0.23
Wild only (no cap, no hyb)	116	0.4	0.44	0.38	0.03	0.07 (0.01-0.15)	0.24
Bactrian	55	0.5	0.56	0.18	0.06	0.13 (0.01-0.24)	0.29
Captive total	47	0.39	0.37	0.65	0.91	-0.04 (-0.1-0.04)	0.21
Captive no hybrids total	45	0.38	0.36	0.61	0.94	-0.04 (-0.09-0.02)	0.21
Hybrids	41	0.62	0.61	0.46	0.56	0.0075 (-0.1-0.08)	0.1

Table 4.3: *Populations determined using STRUCTURE data as well as sample collection data. Mean and expected heterozygosity of populations, HW mean (Null hypothesis is that the population is not deviating from the HWE), t.test and FIS including 95% confidence intervals (Deviation of Hobs of an individual relative to the expected heterozygosity under random mating- FIS>0 more, FIS=0 No inbreeding, FIS<0 Less) gained all gained in R Studio. Inbreeding co-efficient ((F)Probability of 2 homologous genes in an individual being identical by descent)) gained using Coancestry (TrioML).*

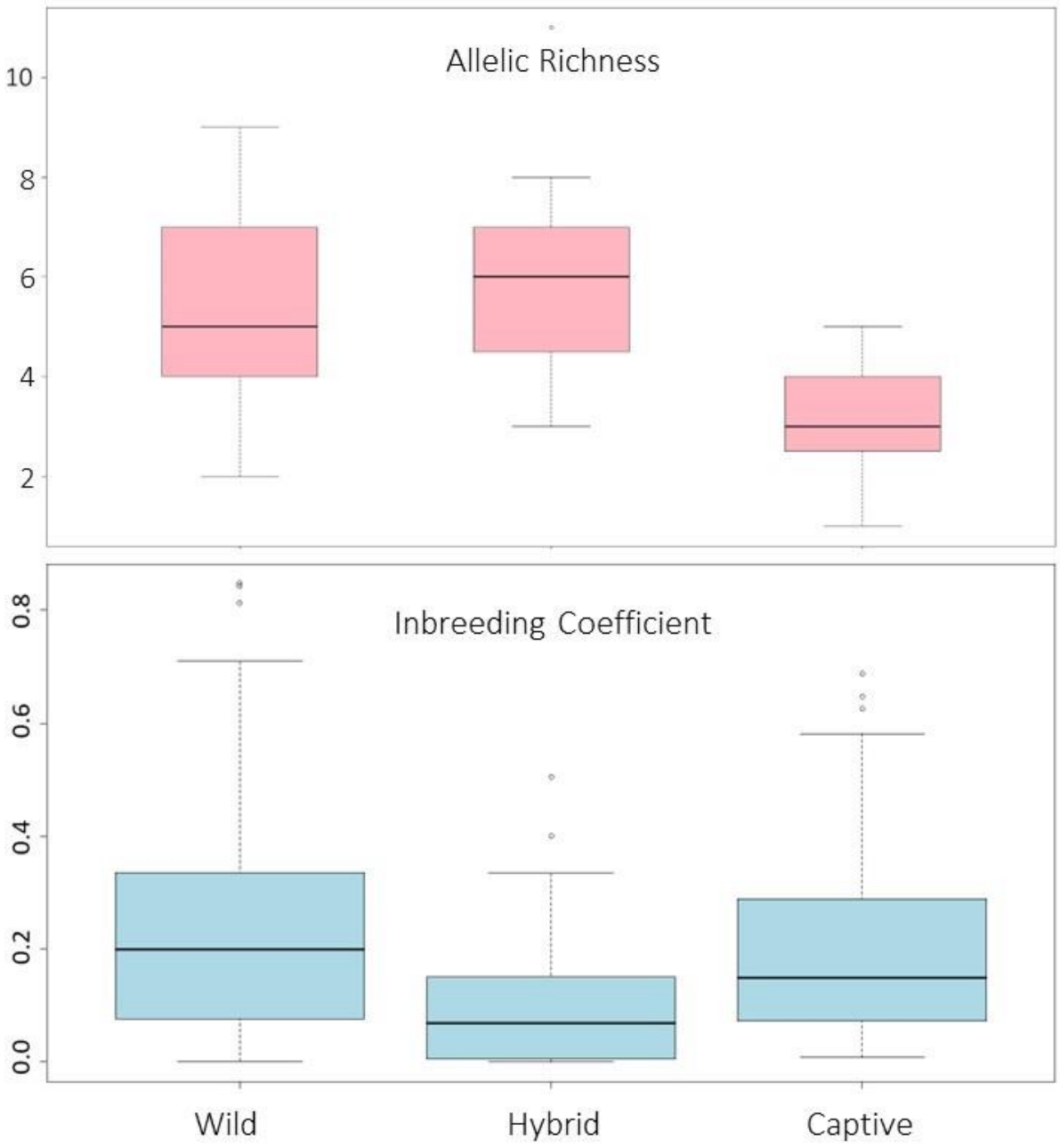

Figure 4.7: *Boxplots. Top: Comparing allelic richness between populations. Kruskal-Wallis chi-squared = 20.097, df = 2, p-value = <0.0001, suggesting significant difference between populations. Unpaired Mann Whitney: Wild/Captive p-value = 0.0005378, Wild/Hybrid p-value = 0.479, Captive/hybrid p-value = <0.0001. Bottom: comparing population (F) inbreeding coefficients (TrioML). Kruskal-Wallis chi-squared = 35.975, df = 3, p-value = <0.0001, suggesting significant difference between populations. Unpaired Mann Whitney: Wild/Captive p value=0.5158, Wild/Hybrid p-value = <0.0001, Captive/hybrid p-value = 0.0007. Plot and statistics R studio*

4.4 Discussion

Non-invasive sampling combined with genetic monitoring has enabled a greater understanding of the extent and source of introgression and levels of genetic diversity in *Camelus ferus* in Mongolia. This genetic perspective is crucial to understand the hybridisation problem and is an important first step towards identifying options for conservation management. We show evidence of both nuclear, mitochondrial, and historic introgression of Bactrian camel genes in the *C. ferus* population across the GGASPA and in some individuals within the captive herd, the true extent of which is determined by which thresholds of introgression are applied. Our results have also revealed reduced heterozygosity and increased inbreeding in the wild population of wild camel in the GGASPA, and shown that these levels of heterozygosity are present in the captive herd.

Introgression in nature is a common occurrence, with at least 25% of known plant species and 10% of known animal species (Mallet 2005) showing some level of hybridisation. Detection of hybrids depends on monitoring, and monitoring is difficult if hybrids are cryptic, widespread or are associated with highly elusive threatened species. If hybrid traits are maladaptive, cause decreased survival rates or remove traits important for survival in the wild, then hybridisation can be detrimental to the survival of a threatened species. Fundamentally, diversity is required for adaptation, but genes important for survival can be lost due to swamping from a domestic species, so hybridisation is of particular concern when domestic animals come into contact with rare species (Iacolina et al. 2019). This scenario may be that facing the wild camel. In Mongolia the wild camel is range-restricted to the GGASPA, a 45000km^2 closed National Park established to protect this species. Despite the vast size of this un-fenced park and the estimated 600 wild camels that live in it, we see introgression throughout. Introgression in threatened species from domestic species is usually due to range overlap (Iacolina et al. 2019). Climate change is causing increased desertification and drought in the Gobi, and this combined with overgrazing and an increase in human activities such as mining (Han, Dai, and Gu 2021) is increasing competition for resources and therefore range overlap between *C. ferus* and *C. bactrianus*. Local nomadic Bactrian

herding practices also increase frequency of contact, and therefore probability of mating is more likely. Exacerbating this problem is the small population size of *C. ferus* that restricts mate choice, and the compatible breeding behaviours between the two species. For these reasons in-situ hybridisation was anticipated in the wild camel population. Furthermore, we confirmed Bactrian camel presence across the GGASPA by capturing images of them in approximately 9% of our cameras in chapter 3. This study confirms that hybridisation could be a risk to the in-situ population of wild camel if introgression erodes evolutionary traits important for this species' long-term survival in the wild.

Ex-situ populations are often managed with the aim of preserving taxonomic integrity and providing a potential future source of individuals for reintroduction (Milián-García et al. 2015), so understanding the true conservation value of these populations is important. Prior to this study, genetic diversity, relatedness and hybridisation values for the captive herd of wild camels were unknown. We have shown that allelic richness is greater in the wild than in the captive population, a result likely due to a larger-sized wild population that is spread over a vast geographic area in comparison to the captive herd which has come from a small number of founders and has captivity-associated problems such as random genetic drift. Our AMOVA results show a slightly higher variance between the Bactrians and the captive individuals, than Bactrian and wild. This could be due to the captive population showing recent genetic drift, with allelic differences being more pronounced in the captive population when compared to the wild population. This is supported by the allelic richness and inbreeding results previously reported. Initial results suggest there is little population structuring in the Gobi other than between species, but more samples from the Chinese populations would be required to determine this result definitively. Encouragingly, our results suggest that the extent of genetic diversity and level of inbreeding observed in the wild population is reflected in the captive population.

Our analysis also allowed us to identify three individuals showing introgression in the captive herd. As none of the captive herd show evidence of introgression from their maternal mitochondrial DNA, this introgression has come from the paternal line (i.e. a domestic bull breeding with a captive female). The

wild camel ex-situ population is closed; breeding is determined by animals being added to or removed from the population This allows for breeding managing that maintains diversity and hybrids can be easily isolated from breeding. As this captive population is considered an insurance population (Chapter 5), monitoring hybridisation and genetic diversity in the ex-situ herd could help to evaluate population integrity and inform species recovery.

Defining an individual as a "hybrid" by proportion of introgression or admixture is not straightforward. The proportion of admixture present in a population considered to represent 'purity' is ultimately arbitrary, set by the perspective and interests of those managing the population; furthermore, accuracy of admixture measurement will depend on the quality of DNA extracted, sensitivity and diagnostic ability of markers used, and other species information available. In some cases, previous work helps determine threshold, like with the sika, *Cervus nippon*, and wapiti deer, *Cervus canadensis,* that compares to putatively pure populations and uses diagnostic mitochondrial regions to confirm species (Smith et al. 2014), whilst in other cases phenotype data is used (Schrey et al. 2007). In the wild camel we have neither previous data to work with nor phenotypic data. Therefore, we set both a 10% (qi=0.90) threshold and 5% (qi=0.95) threshold. We included the 10% threshold as this a frequently-cited modelled threshold (Vähä and Primmer 2006) that is widely used in other studies (Barilani et al. 2007). The 5% threshold as a lower estimate that we considered more realistic. These differing thresholds generate different extents of hybridisation, ranging from 10% to 22% of the wild population. Further complicating the definition of "pure" when describing an individual's provenance is the possibility of historic introgression which we were able to infer using mitochondrial DNA. When we compare our nuclear results to mitochondrial results, eight of our samples show no introgression at a nuclear level, but do show introgression in mitochondrial DNA, suggesting that this introgression could be historic. This finding has been observed in other species including other camelids (Almathen et al. 2016) and in the Canadian brook trout, *Salvelinus fontinalis,* where nuclear haplotypes were "pure" whereas mitochondrial haplotypes were shown to be associated with Arctic char, *Salvelinus alpinus,*

(Allendorf et al. 2001). It has also been seen in the wild camel that the mtDNA haplotype is wild, but the Y haplotype was Bactrian (Felkel et al. 2019).

Mitochondrial data gained in this study allowed us to observe hybridisation patterns and the potential drivers producing them. Of the paternally inherited hybrids (n=19), the majority (79%) were from "wild" samples collected from either the GGASPA or China. This finding suggests bull Bactrian camels are entering the GGASPA and mating with female wild camels. Our F_{ST} values back this up, with results showing that hybrids are closer in variance to the wild population than the Bactrian. Conversely, the majority (82%) of the maternal hybrids are from Bactrian herds in China, suggesting a wild camel bull mating with captive Bactrian females. Camels are a rutting species, with one bull holding and fighting for a number of females, so these results make both ecological and circumstantial sense: the introgression that we detected could be originating from bulls travelling to find females: Bactrian bulls into the park and Wild bulls out of the park. This is a scenario exacerbated by: a park that is unfenced, and unlikely to be fenced due to its scale, local practices of nomadic herding methods, compatible mating behaviours and increased competition for resources (Silbermayr et al. 2010).

Hybridisation is considered an extinction risk, via loss of parental species and outbreeding depression. Yet of the 939 extinct species or extinct in the wild reported on the IUCN RedList, only 11 mention hybridisation in their reports (Draper, Laguna, and Marques 2021). If populations are already small, then other issues may cause extinction before outbreeding depression can have an impact; there is in fact more empirical evidence of inbreeding depression than of outbreeding depression (Chan, Hoffmann, and van Oppen 2019). Genetic diversity is unlikely to be maintained without gene flow; and without diversity, populations are unlikely to be able to adapt and survive to changing environments. Current conservation practices need to be able to conserve species during this period of extensive biodiversity loss and rapid environmental change, and biodiversity managers need to understand whether or not their conservation efforts facilitate the rate of adaptation to keep up with these changes. Genetic diversity can be lost via hybridisation, but it can also be gained. In some small or

isolated threatened populations, with low genetic diversity and inbreeding depression, successful restoration of the original population genetic variation may not be possible using traditional population recovery methods, in these cases, genetic rescue of populations may be required. As we have shown for the wild camel, hybridisation can increase heterozygosity and it is possible that this may enhance adaptive potential of the population (Chan, Hoffmann, and van Oppen 2019). While extinction is forever, hybridisation – if it allows a species' genetic diversity to be preserved in some capacity – can conserve part of the parent genome. It has therefore been argued that, as "reservoirs of the parent's genetic material," (Chan, Hoffmann, and van Oppen 2019) hybrids should be protected. These hybrids could be of value themselves, but full genome sequencing techniques could be used to inform captive breeding programmes to remove hybrid segments of DNA, with the aim of eventually recovering a species threatened with genetic swamping (Lawson et al. 2023).

As well as safeguarding a genetic reservoir, hybridised species can also fill an important ecological function. The Takhi, *Equus ferus przewalskii* (Pzwalski's wild horse) is known to have up to four domestic horses in its founding population (Der Sarkissian et al. 2015). Saved from extinction by captive breeding and release, this species now survives in Mongolia as an introgressed conservation success story. In the case of the New Zealand grey duck, *Anas superciliosa*, the population shows complete admixture so conservation of hybrids is the only option (Allendorf et al. 2001). The American Chestnut*, Castanea dentate,* was pushed to near extinction by blight, but now following a system of hybridizing with a blight-resistant Asian chestnut species and repeated backcrossing, the American type is almost recovered and the restoration of this keystone species is within reach (Steiner et al. 2017). Hybridisation can also lead to new adaptive traits, which may allow for expansion into new niches and ranges (Chan, Hoffmann, and van Oppen 2019), an ability that will become increasingly important as environments change.

Managing hybridisation is a challenge. As climate change, habitat loss and degradation increase, the opportunity for anthropogenic hybridisation also increases. Considerable time and financial investment

are required to monitor for and then manage this hybridisation. The red wolf, *Canis rufus* is now considered "conservation reliant"; hybridisation with coyotes, *Canis latrans,* is the biggest threat to this species. Considerable resources have been invested since the 1970s to prevent this extinction; these efforts have successfully limited introgression, enhancing the recovery programme (Gese et al. 2015). In species for which outbreeding depression threatens their genetic integrity and adaptive potential, limiting introgression is especially important. In threatened species with small populations, management is especially difficult when a hybridising species (including domestic species) outnumbers the rare species. This scenario is true for both the wild camel and the Scottish wild cat, *Felis silvestris,* (Senn et al. 2019), in that introgression from an abundant domestic species risks swamping the wild genome. The wild cat population in Scotland is now considered a hybrid swarm, the population being made up of genetically intermediate types (Senn et al. 2019), making effective conservation management even more complex. Management of hybrids can also negatively impact the conservation of a species. In dingos, *Canis lupus dingo*, hybridisation is both considered a threat to the species and also a justification for their control (van Eeden et al. 2019); lethal management has been shown to exacerbate the hybridization problem. This scenario is also true when monitoring admixture between grey wolves, *Canis lupus,* and dogs, *Canis lupus familiaris*. Determining genetically "pure" wolves is ambiguous as admixture is widespread, long term and hard to monitor. Removing backcrosses could remove too many "wolves" and lead to greater hybridization with dogs (Pilot et al. 2018).

Of course, there is a difference between deciding to not actively manage hybrids due to various capacity problems such as access to resources, staff or funds, versus actively using hybridisation as a conservation management tool. But as we learn more about hybridisation, species thresholds and environmental threats, we are better able to make informed decisions. Hybrids can be considered a threat, an advantage or a reservoir for threatened genomes. The majority of conservation laws either neglect hybrids in legislature or consider them a threat, with even the IUCN recommending that hybrids should not be protected (Draper, Laguna, and Marques 2021). Discussion around the conservation of hybrids remains controversial, but if a species, showing introgression or admixture, holds the role of

fulfilling an ecological function, is it not an evolutionary entity, worthy of protection? We know that genetic diversity is reduced in the wild camel (Lado et al. 2020) and our results confirm this to be true in the GGASPA population. We have increased our understanding of the extent of introgression across the GGASPA, in nuclear and mitochondrial genomes, and have shown that there are no geographic barriers stopping this introgression. The wild camel remains critically endangered; whilst we do not fully understand whether hybridisation poses a threat or an opportunity for conserving this species, managers will be better informed to make key decisions.

4.5 Appendices

Appendix 4.5.1 Primer Information

Name	Dye	Primer F	Obs. Allele range (bp)	No. Alleles (Full data)	Temp	PCR	Multiplex	Repeat	Ho/He (wild only 118)	F (null) (wild 118)	GenBank No./Reference	Species designed from
VOLP08	6FAM	f: CCATTCACCCCATCTCTC	148-178	8	58	Qmix58	1			-0.0083	GI:10719682	Vicuna pacos- Alpaca
		r: TCGCCAGTGACCTTATTTAGA						(TG)	0.64 0.63		AF305230	
VOLP10	6FAM	f: CTTTCTCCTTTCCTCCCTACT	238-268	12	58	Qmix58	1			0.1434	GI:10719683	Vicuna pacos- Alpaca
		r: CGTCCACTTCCTTCATTTC						(TG)	0.48 0.65		AF305231	
VOLP59	HEX	f: CCTTCCTCAGAATCCGCCACC	101-119	11	58	Qmix58	1			0.0151	GI:10719688	Vicuna pacos- Alpaca
		r: CCCGCGCACCAAGCAG							0.49 0.51		AF305236	
YWLL36	6FAM	f: AGTCTTGGTGTGGTGGTAGAA	141-179	8	58	Qmix58	2			-0.0288	Lang et al 1996	New world camelid. Used in old world- Jainlin et al 2002
		r: TGCCAGGATACTGACAGTGAT						(CA)22	0.46 0.44		Jianlin et al 2002	
VOLP32	6FAM	f: GTGATCGGAATGGCTTGAAA	254-268	3	56	Qmix56	6	(TG)		0.1874	GI:10719686	Vicuna pacos- Alpaca
		r: CAGCGAGCACCTGAAAGAA							0.37 0.54		(Obreque et al, 1998) AF305234	
LCA65	6FAM	f: TTTTTCCCCTGTGGTTGAAT	171-191	7	56	Qmix56	6	(TG)12		0.0451	Penedo et al 1998	
		r: AACTCAGCTGTTGTCAGGGG							0.29 0.32		AF091124	Llama glama- Llama
CVRL07	HEX	f: AATACCCTAGTTGAAGCTCTGTCCT	272-306	6	56	Qmix56	6	(GT)14, (AT)14		0.0063	Mariasegaram et al 2002	
		r: GAGTGCCTTTATAAATATGGGTCTG							0.48 0.49		AF217607	Camelus dromedarius- Dromedary
KS01	HEX	f: TGGCATTTCCTGCAACTGA	125-157	11	58	Qmix58	3	(TG)25		0.0768	GU138968	Camelus bactrianus- Bactrian
		r: AGGAAAGGGCTGAATTGCTC							0.49 0.57		Silbermayr et al 2009	
KS02	HEX	f: TCTCTGCACCACCAGTATTCC	155-197	14	58	Qmix58	4	(AC) 16		0.2735	GU138969	Camelus bactrianus- Bactrian
		r: TCAGATATGGGGAGCCTTACA							0.08 0.14		Silbermayr et al 2009	
KS03	6FAM	f: TCTCCACTGGCTCCTCAAAT	176-222	16	58	Qmix58	3	(CA)24		0.1799	GU138970	Camelus bactrianus- Bactrian
		r: CTTGGGATATCTGCCATTGTC							0.11 0.16		Silbermayr et al 2009	
KS04	HEX	f: AGGGTCAGGTTTCCTCCAAT	240-246	4	58	Qmix58	2	(AC)14		-0.0016	GU138971	Camelus bactrianus- Bactrian
		r: GGGTTTGCACCATCTCAGTT							0.03 0.03		Silbermayr et al 2009	
KS05	HEX	f: GGTCCTAGGAGAGAGGAAAAAGA	229-241	7	58	Qmix58	5	(GT)11		0.0437	GU138972	Camelus bactrianus- Bactrian
		r: GGACTAAGGAACCCCAAAGG							0.35 0.38		Silbermayr et al 2009	
KS06	6FAM	f: GGCATTATGATTAGTGGGGTAAGTA	182-192	6	58	Qmix58	4	(GT)9		0.0706	GU138973	Camelus bactrianus- Bactrian
		r: GAACCCCAAAGGAAGATGCT							0.34 0.39		Silbermayr et al 2009	
KS07	HEX	f: TCTGCTCTGTATGAGTTTATGCTG	91-127	5	60	Qmix60	n/a	(GGAT)5GGA		-0.0869	GU138974	Camelus bactrianus- Bactrian
		r: GCGCCAATCCACTTATTTATG							0.85 0.73		Silbermyar et al 2009	
KS08	HEX	f: ATTCAGCCCCATTCTTTCCT	127-153	9	58	Qmix58	5	(CA)16		0.0707	GU138975	Camelus bactrianus- Bactrian
		r: TTCCTACCCTCCACATGGTC							0.23 0.28		Silbermayr et al 2009	
KS09	6FAM	f: CTGCCCACTTTTCAATTGGT	198-223	5	58	Qmix58	5			-0.0384	GU138976	Camelus bactrianus- Bactrian
		r: AAACAGTGCACAGCAAAGGA							0.58 0.55		Silbermayr et al 2009	

Table 4.4 *Primer information: Primer name. Fluoro dye used in genotyping. Primer sequences. Observed allele size ranges in base pairs. Number of alleles from the full data set (n=260). Annealing temperature of the PCR cycle. PCR cycles:* ***QMix56****: Incubate at 95 degrees for 15 min, 45 cycles of 94 degrees for 30 secs, 56 degrees for 90 sec, 72 degrees for 60 sec; Incubate at 60 degrees for 30 min.* ***QMix60****: Incubate at 95 degrees for 15 min, 35 cycles of 94 degrees for 30 seconds, 60 degrees for 30 seconds, 72 degrees for 45 seconds. Incubate at 72 degrees for 10 min.* ***Qmix58****: Incubate at 95 degrees for 15 min, 46 cycles of 94 degrees for 30 seconds, 58 degrees for 90 seconds, 72 degrees for 90 seconds. Incubate at 60 degrees for 30 min. Multiplex primer is included in. Repeat motif. Observed and expected heterozygosity, calculated using the wild only samples with captives removed N=118, to avoid the different species and possible hybrids impacting values and to avoid including related individuals. Estimated null allele frequencies, using the wild individuals only N=118, to the different species impacting values to avoid including related individuals. GenBank sequence accession numbers and references for primer sets.*

Appendix 4.5.2 Sex linked marker development and validation and sexing results

Sex Marker	Fluro-dye	Primer Sequences	Allele sizes of the X and Y chr homologs (bp)	Sequence from which marker was designed (GenBank sequence accession number /Reference)
COO	Hex	F: TAGTCTGCAGCTCCTGGTCA	Y = 170; No amplification in X chr	CBacY1775_contig157:1502 (170bp)
		R: ATTTGCCAGGCTAACAATGG		
CJT	Hex	F:ATATCCCAGGCACTGCTGAA	X = 146, y=144	CBacY1775_contig185:3779 (146bp)
		R: ATTAGCGGATTTCCCTCTGC		
CJE	6Fam	F: GTCTTGGTCAGGGATTGCAT	X=181; Y=178	CBacY1775_contig57:6691 (178 bp)
		R: CTCTTAGCCCTTGCATCTGG		
C11	6Fam	F: CACAGACATGTGTGCCATC	No amplificaton in Y chr; In X chr. ranges from 198 – 222	Unpublished
		R: AAAGCAAATGGAAGATGCTC		

Table 4.5: *Sex linked marker information. PCR protocol: Incubate at 95 degrees for 15 min, 35 cycles of 95 degrees for 30 seconds, 60 degrees for 30 seconds, 72 degrees for 40 seconds. Incubate at 72 degrees for 4 mins.*

Three Y linked primers (COO, CJT, CJE) were used for sexing camel species (*Camelus ferus* and *Camelus bactrianus*). Three primer sets used were taken from Felkel et al 2019 (Felkel et al. 2019), but individuals were genotyped with fluorescently labelled primers on an ABI3730 DNA Analyzer rather than running on an agarose gel, as was done by Felkel et al 2019. The use of QIAGEN multiplex master mix also enable additional previously un detected homologs to be amplified on both sex chromosomes increasing the accuracy and utility of these primer sets for sexing (Table 4.6) ABI genotyping revealed that 2 markers (CJT and CJE) amplify in males and females indicating they have homologs on the X and Y chromosomes. One new sex marker (C11) was designed from an X-linked contig isolated from the camel genome. It was found to only amplify the X chromosome but was variable so able to identify some females (those that were heterozygous) allowing individuals to be sexed when combined with other Y linked markers. C11 does not amplify a Y chromosome homolog (Table 4.6)

These 4 primers were used initially for testing on known sex individuals. These were 34 captive animals (19 Female, 15 male). Of these 85% agree with sex based on morphology and none disagreed. After successful testing on known sex individuals, this method was then used across the full available sample set (N=163).

4.5.2.1 Known sex samples

Methods

34 tissue samples collected from known sex individuals a captive herd of wild camels (*Camelus ferus*) in Mongolia. Tissue samples collected by a registered veterinarian using Dalton flexo-DNA ear tags during standard veterinary ear tagging procedures. DNA extracted using Qiagen DNeasy blood and tissue kits. Extracted DNA was used to amplify the 4 sex linked markers using the multitube approach (Pritchard, Stephens, and Donnelly 2000), with each PCR repeated 3 times and a negative control (ddH20) included in each plate. 3ul volume PCRs were performed with the following: 1 μl DNA, 1 μl of primer mix (23 μl Low TE (10mM Tris-HCl (pH 8.0) and 0.1mM EDTA (pH 8.0).), 1 μl forward and 1 μl reverse primers (both at 0.2 uM) and 1 μl QIAGEN Multiplex PCR Master Mix (supplied with the QIAGEN Multiplex PCR Kit, Cat. No. / ID: 206145). The PCR was incubated at 95 degrees for 15 min, 35 cycles of 95 degrees for 30 seconds, 60 degrees for 30 seconds, 72 degrees for 40 seconds. Incubate at 72 degrees for 4 mins. Genotypes were scored using the Genemapper software. Each sample was tested with each marker 3 times. For that sample to be scored it must show at least 2 matching repeats and no mismatches.

- COO- a 170 bp amplicon indicates the presence of a Y chromosome, indicating the individual is male (the X chromosome does not amplify).
- CTJ- shows a 146 bp amplicon in the presence of an X chromosome, indicating the individual is female and shows a 144 bp amplicon in the presence of an Y chromosome, indicating the individual is male.
- CJE- shows a 181 bp amplicon in the presence of an X chromosome, indicating the individual is female and shows a 178 bp amplicon in the presence of an Y chromosome, indicating the individual is male.
- C11- is X-linked (Y chromosome doesn't not amplify) and so considered female (XX) if heterozygote. Males (XY) are always homozygotes and cannot be heterozygotes since males

have only one X chromosome. Females can also be homozygotes, but any heterozygotes must be female for marker C11. Homozygotes were not sexed, since homozygotes could be female or male for this marker. All heterozygotes were scored as female.

Results

Used across the 34 known sex individuals, no DNA sex-typing marker results disagreed with morphology. Only 2 markers are polymorphic in detecting each sex (CTJ and CJE). Of the 4 markers, one only amplifies in males (COO) and another marker only detects females (C11). To be confident we only assigned sex if a minimum of 2 markers amplified. 29 individuals (85%) agree with morphometric sex for a minimum of 2 sex markers.

ID	Known sex (based on morphology)	COO_ Y=170 X-no amp.	CTJ_ X=146 Y=144	CJE_ X=181 Y=178	C11_ X-linked Y=no amp.	Sex based on sex markers combined	How many markers agree with sex based on morphology*	How many markers disagree
E1	Male	Y	Y	Y	Homo	Male	3	0
E2	Female			X		Female	1	0
E3	Female		X	X	Het=Female	Female	3	0
E4	Female		X	X	Het=Female	Female	3	0
E5	Male	Y	Y		Homo	Male	2	0
E6	Female		X	X	Het=Female	Female	3	0
E7	Male	Y	Y	Y	Homo	Male	3	0
E8	Female		X	X	Homo	Female	2	0
E9	Male				Homo	Unknown		

E10	Female		X	X	Het=Female	Female	3	0
E11	Female		X	X	Het=Female	Female	3	0
E12	Male	Y	Y	Y	Homo	Male	3	0
E13	Female		X	X		Female	2	0
E14	Male	Y	Y	Y	Homo	Male	3	0
E15	Female		X	X	Het=Female	Female	3	0
E16	Male				Homo	Unknown		
E17	Female		X	X	Homo	Female	2	0
E18	Female		X	X	Het=Female	Female	3	0
E19	Female		X	X	Het=Female	Female	3	0
E20	Male					Unknown		
E21	Female		X	X		Female	2	0
E22	Male	Y	Y	Y	Homo	Male	3	0
E23	Female		X	X	Homo	Female	2	0
E24	Male	Y	Y	Y	Homo	Male	3	0
E25	Male	Y	Y	Y	Homo	Male	3	0
E26	Male	Y			Homo	Male	1	0
E27	Female		X	X	Het=Female	Female	3	0
E28	Male	Y	Y	Y	Homo	Male	3	0
E29	Female		X	X	Het=Female	Female	3	0
E30	Female		X	X	Het=Female	Female	3	0
E31	Female		X	X	Het=Female	Female	3	0
E32	Male	Y	Y	Y	Homo	Male	3	0
E33	Female		X	X	Het=Female	Female	3	0

E34	Male		Y	Y	Homo	Male	2	0

***Table 4.6**: Results of sex marker testing on known sex individuals from the captive bred wild camel herd*

4.5.2.2 Full data set

Methods

163 samples (faecal, hair and tissue) were collected from both *Camelus bactrianus* and *Camelus ferus* in Mongolia and China (see main text). DNA was extracted, depending on sample type, using either Qiagen DNeasy blood and tissue kits (Tissue, blood or hair) or the Qiamp fast DNA Stool Mini kit (faeces). Extraction controls were included in each batch of extractions, replacing samples with ddH2O. Extracted DNA was used to amplify the 4 sex linked markers using the multitube approach (Pritchard, Stephens, and Donnelly 2000), with each PCR repeated 3 times and a negative control (ddH20) included in each plate. 3ul volume PCRs were performed with the following: 1 μl DNA, 1 μl of primer mix (23 μl Low TE (10mM Tris-HCl (pH 8.0) and 0.1mM EDTA (pH 8.0).), 1 μl forward and 1 μl reverse primers (both at 0.2 uM) and 1 μl QIAGEN Multiplex PCR Master Mix (supplied with the QIAGEN Multiplex PCR Kit, Cat. No. / ID: 206145). The PCR was incubated at 95 degrees for 15 min, 35 cycles of 95 degrees for 30 seconds, 60 degrees for 30 seconds, 72 degrees for 40 seconds. Incubate at 72 degrees for 4 mins. Genotypes were scored using the Genemapper software. Each sample was tested with each marker 3 times. For that sample to be scored it must show at least 2 matching repeats and no mismatches.

- COO- a 170 bp amplicon indicates the presence of a Y chromosome, indicating the individual is male (the X chromosome does not amplify).
- CTJ- shows a 146 bp amplicon in the presence of an X chromosome, indicating the individual is female and shows a 144 bp amplicon in the presence of an Y chromosome, indicating the individual is male.

- CJE- shows a 181 bp amplicon in the presence of an X chromosome, indicating the individual is female and shows a 178 bp amplicon in the presence of an Y chromosome, indicating the individual is male.
- C11- is X-linked (Y chromosome doesn't not amplify) and so considered female (XX) if heterozygote. Males (XY) are always homozygotes and cannot be heterozygotes since males have only one X chromosome. Females can also be homozygotes, but any heterozygotes must be female for marker C11. Homozygotes were not sexed, since homozygotes could be female or male for this marker. All heterozygotes were scored as female.

Results

ID	Sample type	COO_Y	CTJ_Y	CJE_Y	C11_X	Sex	No markers that agree	No markers that disagree
Camelus bactrianus								
Khara	Blood		X		HOM	F	3	0
B1	Faecal					U		
B2	Faecal		X		HET-FEMALE	F	3	0
B3	Faecal		X		HOM	F	2	0
B4	Faecal		X		HET-FEMALE	F	3	0
B5	Faecal		X		HOM	F	2	0
Hybrid- *Camelus ferus x Camelus bactrianus*								
AWC122	Faecal		X		HET-FEMALE	F	3	0

AWC132	Faecal					U		
AWC134	Faecal				HOM	U		
AWC179	Faecal	Y		Y		M	2	0
AWC248	Faecal	Y	Y	Y	HOM	M	4	0
AWC272	Faecal	Y	Y	Y	HOM	M	4	0
AWC278	Faecal	Y	Y	Y	HOM	M	4	0
AWC279	Faecal		Y	Y	HOM	M	3	0
AWC282	Faecal	Y	Y	Y		M	3	0
AWC299	Faecal	Y	Y	Y	HOM	M	4	0
AWC300	Faecal		X		HET-FEMALE	F	3	0
AWC305	Faecal	Y	Y	Y	HOM	M	4	0
AWC308	Faecal		X		HET-FEMALE	F	3	0
AWC310	Faecal		X		HET-FEMALE	F	3	0
AWC314	Faecal	Y	Y	Y	HOM	M	4	0
AWC327	Faecal	Y	Y	Y	HOM	M	4	0
AWC328	Faecal		X		HOM	F	2	0
AWC33	Faecal		Y			M	1	0
AWC330	Faecal	Y	Y	Y	HOM	M	4	0
AWC331	Faecal	Y	Y	Y	HOM	M	4	0
AWC334	Faecal		X		HOM	F	2	0
AWC335	Tissue	Y	Y	Y	HOM	M	4	0
AWC336	Faecal	Y		Y	HOM	M	3	0

AWC345	Tissue		X		HOM	F	2	0
AWC35	Faecal		X			F	2	0
AWC38	Faecal		X			F	2	0
AWC50	Faecal					U		
AWC55	Faecal	Y		Y	HOM	M	3	0
AWC64	Faecal		X		HET-FEMALE	F	3	0
AWC73	Faecal	Y	Y	Y	HOM	M	4	0
AWC80	Faecal		X		HOM	F	2	0
CHYB14	Hair		X		HOM	F	2	0
CHYB33	Hair	Y	Y	Y	HOM	M	4	0
CHYB34	Hair	Y	Y	Y	HOM	M	4	0
CHYB36	Hair	Y	Y	Y	HOM	M	4	0
CHYB37	Hair		X		HET-FEMALE	F	3	0
CHYB38	Hair	Y	Y	Y	HOM	M	4	0
CHYB39	Hair		X		HET-FEMALE	F	3	0
CHYB40	Hair		X		HET-FEMALE	F	3	0
CHYB41	Hair	Y	Y	Y	HOM	M	4	0
CHYB42	Hair		X	X	HET-FEMALE	F	4	0
CHYB43	Hair		X		HET-FEMALE	F	3	0

CHYB44	Hair	Y	Y	Y	HOM	M	4	0
CHYB45	Hair		X		HOM	F	2	0
CHYB46	Hair		X		HOM	F	2	0
CHYB47	Hair				HET-FEMALE	F	2	0
CHYB48	Hair		X	X	HOM	F	2	0
CHYB53	Hair		X		HET-FEMALE	F	3	0
CWC2	Hair	Y	Y	Y	HOM	M	4	0
CWC9	Hair	Y	Y	Y		M	3	0
HYB17	Blood	Y			HOM	M	2	0
HYB57	Hair	Y	Y	Y	HOM	M	4	0
wc117	Hair	Y	Y	Y	HOM	M	4	0
WC119	Hair	Y	Y	Y	HOM	M	4	0
WC121	Hair	Y	Y	Y	HOM	M	4	0
WC205	Hair	Y	Y	Y	HOM	M	4	0
WC207	Tissue		Y			M	1	0
WC208	Hair	Y			HOM	M	2	0
WC210	Tissue	Y	Y	Y	HOM	M	4	0
WC225	Hair					U		
WC242	Hair	Y	Y	Y	HOM	M	4	0
WC246	Hair	Y	Y	Y		M	3	0
WC249	Hair		Y	Y		M	2	0
WC251	Hair		Y		HOM	M	2	0
Camelus ferus								

AWC11	Faecal	Y	Y	Y	HOM	M	4	0
AWC111	Faecal		Y	Y	HOM	M	3	0
AWC112	Faecal		Y		HOM	M	2	0
AWC113	Faecal				HET-FEMALE	F	2	0
AWC114	Faecal	Y	Y	Y	HOM	M	4	0
AWC115	Faecal	Y	Y	Y	HOM	M	4	0
AWC118	Faecal	Y	Y	Y	HOM	M	4	0
AWC119	Faecal	Y	Y	Y		M	3	0
AWC120	Faecal		X		HET-FEMALE	F	3	0
AWC123	Faecal				HOM	U		
AWC124	Faecal	Y	Y	Y	HOM	M	4	0
AWC125	Faecal		X		HET-FEMALE	F	3	0
AWC127	Faecal	Y	Y	Y	HOM	M	4	0
AWC128	Faecal		X		HET-FEMALE	F	3	0
AWC129	Faecal				HET-FEMALE	F	2	0
AWC13	Faecal					U		
AWC130	Faecal		Y	Y	HOM	M	3	0
AWC131	Faecal	Y	Y	Y	HOM	M	4	0
AWC133	Faecal		X		HOM	F	3	0

AWC135	Faecal		X		HET-FEMALE	F	3	0
AWC136	Faecal	Y	Y	Y	HOM	M	4	0
AWC156	Hair					U		
AWC166	Hair					U		
AWC184	Faecal		X		HOM	F	2	0
AWC20	Faecal		X			F	2	0
AWC200	Faecal	Y	Y	Y	HOM	M	4	0
AWC21	Faecal				HET-FEMALE	F	2	0
AWC215	Faecal		X		HOM	F	2	0
AWC22	Faecal		X		HOM	F	2	0
AWC243	Faecal	Y	Y	Y	HOM	M	4	0
AWC245	Faecal		Y		HOM	M	2	0
AWC252	Faecal				HET-FEMALE	F	2	0
AWC256	Faecal	Y	Y	Y	HOM	M	4	0
AWC27	Faecal		Y			M	1	0
AWC275	Faecal		X		HOM	F	2	0
AWC283	Faecal	Y		Y	HOM	M	3	0
AWC286	Faecal		X		HET-FEMALE	F	3	0
AWC289	Faecal		Y	Y	HOM	M	3	0
AWC29	Faecal					U		
AWC294	Faecal		X		HOM	F	2	0

AWC298	Faecal		X		HOM	F	2	0
AWC302	Faecal	Y	Y	Y	HOM	M	4	0
AWC303	Faecal		X		HET-FEMALE	F	3	0
AWC306	Faecal		X		HOM	F	2	0
AWC309	Faecal		X		HET-FEMALE	F	3	0
AWC311	Faecal	Y	Y	Y	HOM	M	4	0
AWC317	Faecal	Y	Y	Y	HOM	M	4	0
AWC319	Faecal				HET-FEMALE	F	2	0
AWC32	Faecal		X			F	2	0
AWC329	Faecal	Y	Y	Y	HOM	M	4	0
AWC332	Faecal	Y	Y	Y	HOM	M	4	0
AWC333	Faecal	Y	Y	Y	HOM	M	4	0
AWC337	tissue	Y	Y	Y	HOM	M	4	0
AWC34	Faecal					U		
AWC341	tissue		Y		HOM	M	2	0
AWC347	Faecal	Y	Y	Y	HOM	M	4	0
AWC36	Faecal	Y	Y	Y	HOM	M	4	0
AWC37	Faecal		X			F	2	0
AWC49	Faecal		X		HET-FEMALE	F	3	0
AWC61	Faecal	Y	Y	Y	HOM	M	4	0
AWC63	Faecal		X		HOM	F	2	0

AWC65	Hair				HOM	U		
AWC67	Faecal	Y	Y	Y	HOM	M	4	0
AWC68	Faecal		Y			M	1	0
AWC70	Faecal				HET-FEMALE	F	2	0
AWC71	Faecal	Y	Y	Y	HOM	M	4	0
AWC72	Faecal					U		
AWC74	Faecal					U		
AWC82	Faecal	Y	Y	Y	HOM	M	4	0
AWC86	Faecal		X		HOM	F	2	0
CWC5	Hair	Y	Y	Y	HOM	M	4	0
R120	Faecal					U		
SK	tissue		X		HET-FEMALE	F	3	0
WC115	Hair	Y	Y	Y	HOM	M	4	0
WC118	Hair		X		HOM	F	2	0
WC120	Hair	Y	Y	Y	HOM	M	4	0
WC18	Blood		Y	Y	HOM	M	3	0
wc20	Blood		X		HOM	F	3	0
WC202	Hair		X		HET-FEMALE	F	3	0
WC221	Hair		X		HOM	F	2	0
WC243	Hair	Y	Y	Y	HOM	M	4	0
WC254	Hair		Y	Y	HOM	M	3	0
WC257	Blood	Y	Y	Y		M	3	0

WC261	tissue		X	X	HET-FEMALE	F	4	0
WC264	Hair		Y		HOM	M	2	0
WC31	tissue		X			F	2	0
WC41	tissue	Y	Y	Y	HOM	M	4	0
Disagree								
WC162	Hair	Y	Y	Y	HET-FEMALE	M/U	3	1
AWC117	Faecal	Y	Y	Y	HET-FEMALE	M/U	3	1
WC164	Hair	Y	Y	Y	HET-FEMALE	M/U	3	1
AWC209	Faecal	Y	X		HOM	U	1	1
AWC110	Faecal		X	Y	HET-FEMALE	F/U	3	1
WC165	Hair	Y	Y	Y	HET-FEMALE	M/U	3	1

Table 4.7: *Results from marker testing across full available samples (N=163). Samples include Wild camel Camelus ferus, Bactrian camels Camelus bactrianus, and hybrids. Samples are a mixture of faecal, hair and tissue.*

Of the 163 samples, 142 (87%) were successfully scored to suggest a sex (minimum 1 marker with 2 successful repeats in each, with no markers disagreeing). Of those 142, 61 (42%) are female and 81 (57%) are males. 15 were not possible to score as they did not amplify to the required minimum repeats. 6 had disagreeing scores between markers, which may be a result of what contamination during the PCR process. Therefore 21 (13%) cannot be used to determine sex of that sample.

If we consider accurate determination of sex requiring a minimum of 2 agreeing markers then 94 (57%) samples can be sexed.

Considering those samples only from the GGASPA (N=115) 45 are female (39%) and 70 are male (61%). This result, showing more male than female in the GGASPA, is probably an artifact of poor amplification of the polymorphic markers in poor quality samples. When used on DNA extracted from tissue samples in known sex individuals of the captive herd this sex discrepancy is not seen. In the 163 samples, the 21 samples that didn't amplify were all either faecal or hair. All 15 blood and tissue samples amplified. 14% of the faecal samples didn't amplify and 15% of the hair samples didn't amplify. Of the 6 samples that disagreed, 3 were faecal and 3 were hair. Faecal (63%) and hair (28%) samples make up the majority of the sample set (91 %) See table 4.8.

Sample type	No. Samples	No that didn't amplify	%
Blood	5	0	0
Faecal	103	14	14
Hair	45	7	15
Tissue	10	0	0

***Table 4.8**: Amplification of markers used in different sample types. Includes type of sample, the number of each type tested, of those, the number that didn't amplify and then that number as a percentage.*

Appendix 4.5.3 Sex ratio demographics

To determine the sex ratio in the wild population we used only the 2 markers with fixed size X and fixed size Y homolog that differ in size: CJE and CTJ. This allows a comparison as both markers can determine either male or female, whereas COO and C11 determine only male (COO) or female (C11).

Population	Sex determined (%)	Sex determined using both CJE/CJT (%)	Sex determined using either CJE/CJT (%)	Overall % Male	Overall % Female
Captive herd N=34	88	81	6	37	63
Full available sample set N=163	81	42	39	59	41

Table 4.9: *Sex determined in each population in either one or both polymorphic markers. In the captive population, 30 of the 34 were scored with at least one sex marker (CJE or CTJ). 28 were scored in both- 18 female (64%), 10 male (36%) and 2 were scored in only sex marker. In the full available data set 133 out of the 163 were scored in either one or both markers. 69 were scored in both CJE and CTJ, 3 female (4%), 66 male (96%) and 64 in only sex marker- 51 female (80%), 13 male (20%). In the captive herd, overall, 19 were scored as female (63%) 11 scored as male (37%)- which is true when compared to known sex. When looking at the full available data set overall 41% female, 59% male. Considering only those samples from the GGASPA N=107, 38 are female 36%, 69 are male 64%.*

Appendix 4.5.4 Population Structure K=3

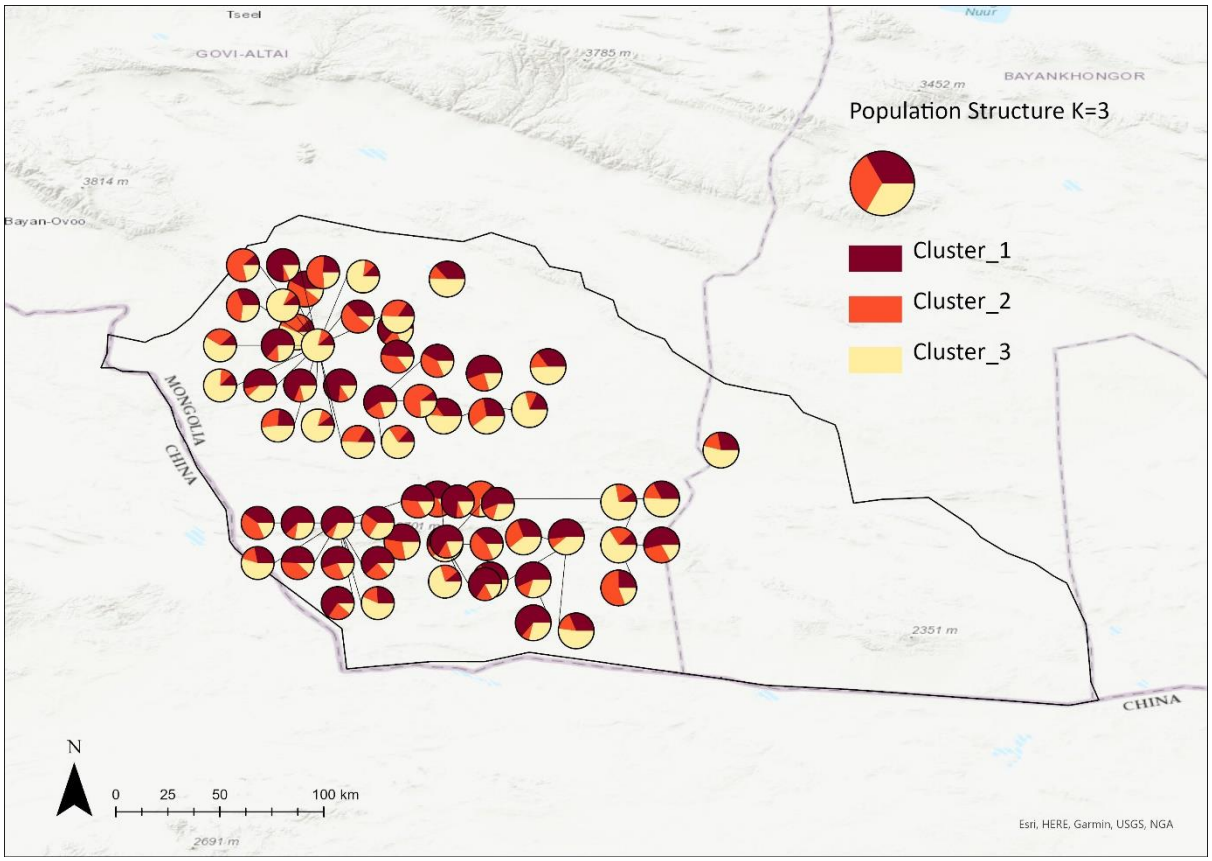

***Figure 4.7**: Population structure (K=3). Clusters (K) mapped geographically across the GGASPA.*

Appendix 4.5.5 AMOVA and FST Tables

Source	df	SS	MS	Est. Var.	%
Among Populations	3	394.857	131.619	1.039	21%
Among Individuals	253	1040.930	4.114	0.217	4%
Within Individuals	257	945.736	3.680	3.680	75%
Total	513	2381.522		4.936	100%

***Table 4.10**: Summary AMOVA table with degrees of freedom (df), Sum of squared deviation (SS), Mean sum of squares (MS), Estimated Variation (Est.Var) and percentage variance (%).*

	Wild	Bactrian	Hybrids	Captive
Wild	0.000	0.001	0.001	0.001
Bactrian	0.372	0.000	0.001	0.001
Hybrids	0.074	0.165	0.000	0.001
Captive	0.043	0.397	0.105	0.000

***Table 4.11**: Pairwise FST Analysis. FST Values below the diagonal, p values above.*

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Chapter 5 Does our insurance cover extinction? Ex-situ populations of highly threatened mammals.

Ex-situ populations provide widely variable contributions to mitigating a taxon's extinction risk which is inadequately assessed in conservation.

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Abstract:

An *ex-situ* captive population forms a key component of present efforts to maintain species survival of the wild camel. For many other critically endangered species too, individuals maintained in ex-situ captivity are potentially crucial to the species' persistence. In these cases, if an extinction event were to happen in the wild, it is important for managers to be confident that ex-situ populations represent adequate insurance against outright extinction. Available guidance on best practices in population management, population size targets and conservation planning could help ensure they can provide this. To compare the ex-situ wild camel population both to existing practice for other critically-endangered species, and against normative 'best practice' standards for insurance populations, we characterised critical factors to assess the ex-situ populations of the world's most threatened mammal taxa as determined by the IUCN Red List as either critically endangered (N=291) or extinct in the wild (N=2). We found that of these 293 mammal taxa, approximately a quarter (69) are represented in ex-situ care, almost double the number reported on the Red List. Worryingly, almost all (91%) of these are held at population sizes below 500, with 44% (29)- including the wild camel- falling below 50 individuals. Although 67% show genetic monitoring through pedigree analyses, only 10% are monitored demographically. We conclude that despite their proven conservation potential, ex-situ populations constitute inadequate insurance policies against extinction for many of the most threatened mammals.

5.1 Introduction

We are in the midst of a largely human caused global biodiversity crisis (Ceballos et al. 2015). Mammalian decline is a well-understood illustration; we have lost approximately 77 to 111 species since the 1500s, between 35-69 since 1900 (Ceballos et al. 2015). Of the 5973 mammal species assessed by the IUCN's Red List (IUCN RedList 2022), 1342 (22%) are threatened with extinction and 4% are classed as either critically endangered (CR) or extinct in the wild (EW). In 2022, COP15 (COP15 2022) committed

the global conservation community to halting human-induced extinctions and to maintaining and restoring genetic diversity. However, a sole reliance on in-situ management could be insufficient or too late for species on the very brink of extinction (Smith et al. 2023). For some species, there may be increased need for ex-situ management (Pritchard et al. 2012) in institutions like zoos and captive breeding facilities to act as 'insurance' against extinction (Gippoliti 2012). In light of this, conservation efforts for the wild camel in Mongolia have sought to include management of an ex-situ population, the stated rationale being that **"**with so few captive animals, the whole species could be wiped out if their natural habitats in China and Mongolia are destroyed. It is therefore important to breed enough animals in captivity to insure against this possible disaster" (WCPF 2023).

The wild camel is far from the only ex-situ population of critically endangered mammal; ex-situ populations are diverse, designed to meet a range of objectives including entertainment, education, financial incentives and conservation (Conde 2013). Some ex-situ populations were founded by removing individuals from the wild purely to save the species from extinction (e.g., red wolf, *Canis rufus* (Gese et al. 2015)), while others were historically collected for fascination, with these populations becoming critical long after individuals were first taken into ex-situ care (e.g., Dama gazelle, *Nanger dama* (Senn et al. 2014)). Other conservation-motivated ex-situ populations comprise animals rescued from the pet or food trade (e.g., Sunda pangolin, *Manis javanica* (Zhang et al. 2017)) or that were established for educational and entertainment purposes (e.g., grey shanked douc langur, *Pygathrix cinerea* (ASAP 2023)). Some are important in the study of individuals in ex-situ populations to inform conservation of wild counterparts (Miranda et al. 2023). These motivations in part follow societal trends (Carr and Cohen 2015) and often species charisma is more important to visitors than extinction threat (Colléony et al. 2017). Collectively, these drivers have resulted in a varied and fascinating collection of ex-situ management of species.

Regardless of why ex-situ populations were founded, their existence may provide important insurance against extinction for species highly threatened in the wild. For example, ex-situ care was a component

in the management of 9 out of the 16 (56%) mammals whose extinction was prevented by conservation action since 1993 (Bolam et al. 2021). In the extreme, ex-situ populations have prevented overall extinction following the total loss of wild populations for seven mammal species since 1950, five of which subsequently regained wild status following conservation action, with black footed ferret, *Mustela nigripes,* (Santymire et al. 2014), red wolf (Gese et al. 2015) and European bison, *Bison bonasus*, (Smith et al. 2023) providing iconic examples.

However, despite their obvious importance in enhancing species persistence, ex-situ populations are not considered in RedList assessments of extinction risk. How much they might influence extinction risk is also variable given ex-situ population management is not a panacea with variation in their 'insurance' utility. Success is determined by management of both the ex-situ populations themselves and the wild places and/or populations into which they could be returned. Outright failure is not the only risk to ex-situ populations. Holding species ex-situ is precarious, with risks including: physiological risks such as stress and disease; behavioural risks such as habituation to ex-situ care (Conde et al. 2011) and genetic risks such as inbreeding depression, loss of genetic diversity through genetic drift, and genetic adaptation to ex-situ environments making individuals less suitable for survival in the wild (Robert 2009). These risks have led to the development of a range of management and monitoring practices, such as studbooks, survival plans or population viability analyses, to ensure ex-situ populations remain viable. Despite this, there is no unified protocol with which to assess the conservation potential of these populations, so we have limited understanding of how comprehensive the insurance is that ex-situ populations provide.

Following from the work by Smith et al 2023, that analysed data on extinct-in-the-wild plant and animal species and assessed their risk and recovery potential (Smith et al. 2023) we use similar methods to assess the insurance potential of the ex-situ wild camel population, and compare it to other ex-situ populations focussing on CR and EW mammal taxa. Here we focus on mammals, not only because it is the class to which our focal species, the wild camel *Camelus ferus,* belongs, but also because it is a class

especially well represented in conservation research and where ex-situ populations exist for many species (Conde et al. 2011; Martin et al. 2014; Miranda et al. 2023)). We use this class to assess the use of ex-situ management as a conservation insurance tool against outright extinction. To do this we (i) identified which CR/EW mammals have ex-situ populations, (ii) assessed the status of their ex-situ populations in terms of size, number of holders and number of founders, and (iii) characterised how these are being monitored, managed and planned for future recovery. Overall, we show that there is substantial variation in how current ex-situ populations cover the risk of extinction.

5.2 Materials and Methods

5.2.1 Identification of ex-situ populations.

We extracted the assessments of all CR (235 species, 56 subspecies) and EW (2 species) mammal taxa from IUCN Red List version 2022-2 (IUCN Red List 2022). Each species on the resulting list of 293 taxa was checked for whether or not an ex-situ population existed using a variety of publicly available sources. Systematic checks (Table 5.1) for ex-situ populations of these 293 taxa were done within:

- The IUCN Red List (IUCN Red List 2022). The page for each species was searched for mention of ex-situ populations and the "Conservation Actions" section was checked for the "Subject to ex-situ conservation" indicators.
- The ZIMS database on Species360 (Species360 2023), is a database which gives real time information on the holdings and studbooks of over 1300 zoo and aquarium institutions. We crosschecked against this database to identify any taxa with reported ex-situ care.
- EAZA's (European Association of Zoos and Aquariums) ex-situ programme overview (EAZA 2023).
- AZA's (Association of Zoos and Aquariums) (AZA 2023) animal programme database.
- Internet searches using Google, Google scholar (Google Scholar 2023) and Web of Science (Web of Science 2023) and with the search terms including each taxa's scientific name,

common name and “Ex-situ captive”. We restricted the review to the first page returned from each search following and reading relevant links.

Searches were conducted in the UK in June 2020, with 2023 updates, for species up-listed to critically endangered (IUCN Red List 2022), conducted in February 2023.

To avoid double counting, we only included subspecies in which the parental species was not itself CR or EW. If it was, we only assessed at the species level, this removed 6 subspecies (but retained species level assessments) from analysis (Black and white ruffed lemur, *Varecia variegata variegata;* Eastern black rhino, *Diceros bicornis ssp. michaeli;* Eastern lowland gorilla, *Gorilla beringei graueri;* Western lowland gorilla, *Gorilla gorilla gorilla*; Northwest Bornean orangutan, *Pongo pygmaeus pygmaeus* and Southeastern black rhino, *Diceros bicornis minor).* We also removed Van der Decken’s sifaka *Propithecus deckenii* from our analysis as we could not verify the captive population mentioned in the “Conservation Actions in Detail” section of the Red List assessment (IUCN Red List 2022). Lastly, we removed the African forest elephant *Loxodonta cyclotis* from our assessment because a recent reclassification meant that available data have not yet been updated against this new species classification.

5.2.2 Population sizes and number of holders.

We determined wild population size using the IUCN Red List (IUCN Red List 2022). There are wild population estimates available for 31 species. We estimated the number of institutions holding each species and total ex-situ population size using the ZIMS database in Species 360 (Species360 2023). Although ZIMS is the most comprehensive and widely used database of zoological institutions, we acknowledge that there could be further populations, or individuals held ex-situ that are not monitored by Species360. We looked for information on number of founders for each population using internet searches (Table 5.1) using Google, Google scholar (Google Scholar 2023) and Web of Science (Web of

Science 2023) and with the search terms including each taxa's scientific name, common name and "Founder number". We restricted the review to the first page returned from each search following and reading relevant links.

5.2.3 Management of ex-situ populations.

To determine how well ex-situ mammal populations are meeting recommendations that will better allow for species recovery, we assessed if global ex-situ populations are monitored and managed both genetically via a pedigree, using a studbook or survival plan and demographically using a Population Viability Analysis (PVA). Studbooks are used to monitor individual animals and their genetic relatedness across institutions (WAZA 2023). A survival plan or action recovery plan, are programmes which act cooperatively across institutions to manage populations for conservation, AZA uses "Species Survival Plans" (SSP's) and EAZA uses Ex-situ programmes EEPs (SSP AZA 2023; EAZA 2023). PVAs use current data and models to assess the likelihood a population will go extinct over a certain time frame. They allow for the use of current species knowledge to project future population trajectories and identify conservation priorities (Beissinger and McCullough 2002).

We also attempted to determine if these populations are used for planning future recovery in the wild using an action recovery plan and whether there has been releases of ex-situ individuals, as evidence of release suggests that ex-situ populations are on a recovery pathway.

	Data source	Search Criteria
2.2 Population size and number of holders.		
Ex-situ Population (N)	ZIMS Species360	Population search by species name
	Internet searches	Scientific name, common name and "ex-situ captive". Search restricted to the first page.
Number of Holders (N)	ZIMS Species360	Population search by species name
Wild Population (Mature individuals)	IUCN Red List	Advanced search by species name
Founders (N)	Internet searches	Scientific name, common name and "Founder number" criteria. Search restricted to the first page.
2.3 Management of ex-situ populations.		
Studbook	IUCN Red List	Advanced search by species name
	ZIMS Species360	Population search by species name
	AZA	By species name
	EAZA	By species name
	Internet searches	Scientific name, common name and "studbook". Search restricted to the first page.
Species/action plan	Internet searches	Scientific name, common name and "Species Survival Plan" criteria. Search restricted to the first page.
	AZA	By species name
	EAZA	By species name
	IUCN Red List	IUCN Redlist category "Action Recovery Plan"
PVA	AZA	Population search by species name
	EAZA	By species name
	Internet searches	Scientific name, common name and "Population Viability Analysis". Search restricted to the first page.
Releases	Internet searches	Scientific name, common name and "Reinforcement/ Reintroduction" criteria. Search restricted to the first page.
	IUCN Red List	IUCN Redlist category "Successfully Introduced"
	ZIMS Species360	Releases- ZIMS

Table 5.1*: Sources and search criteria used to assess ex-situ populations. The IUCN RedList is the standard resource used to categorise risk of species extinction (IUCN Red List 2022). The ZIMS database by Species360 is a global database used by animal collections to monitor ex-situ populations (Species360 2023). This database contains stud books and information on ex-situ populations. It allows the monitoring of species across multiple countries and institutions. It also maintains genetic information across institutions so creates a global population. AZA (Association of Zoos and Aquariums) have an "Animal Programme Database" which holds all species held in collections (AZA 2023). EAZA (European Association of Zoos and Aquariums) produced a "Ex-situ programme overview" listing all ex-situ programmes (EEPs) and populations in EAZA collections (EAZA 2023). Internet searches were conducted using the following search engines: Google, Google scholar (Google Scholar 2023) and Web of Science (Web of Science 2023). For internet searches, the search indicators used were: Scientific name, common name and "Ex-situ/ captive". Search was restricted to the first page.*

5.3 Results

5.3.1 Population size and number of holders.

Of the 293 CR mammal taxa, 26% (77) currently have ex-situ populations. Of the 77 taxa with ex-situ populations, 6 subspecies (as they are reported at species level) and 2 species were removed from analysis. Only living populations are included in this analysis, biobanks are not included. This gives a final data set of 69 taxa (55 species and 14 subspecies) which includes 23% of all CR mammal taxa plus two species which are EW.

We find that the number of CR and EW mammal taxa in ex-situ care (69) is almost double that reported on the Red List (36). 66 of the 69 (95%) are on the ZIMS database (Species360 2023). The 3 species not on ZIMS are the; Wild camel, *Camelus ferus*; the Western Giant Eland, *Tragelaphus derbianus derbianus* and the Northern White Rhino, *Ceratotherium simum cottoni*. The wild camel (Silbermayr K et al. 2010) and the Western Giant Eland (Koláčková et al. 2011) are known to have captive populations but at the time of writing were not included in Species360. The entire Northern white rhino population consists of 2 females and so is functionally extinct (Ryder et al. 2020). We included only the wild camel as additional in our analysis given it is the focus of this PhD. The other 2 species are not included in further analysis and main results and figures are restricted to the wild camel and species on Species360.

The 67 species included in this analysis cover 12 orders (Figure 5.2 and 5.2). Of the 12 orders, the number of species representatives range from 1-37, with a mean of 5.5. The IUCN Redlist divides mammal species into 25 orders (IUCN RedList 2022), therefore 48% of mammal orders have representatives in insurance populations. The most commonly held order is *Primates* (55%), followed by *Artiodactyla (14%). All other orders included have under 6% each.*

There is variation in number of individuals held in ex-situ populations, from 1 individual (3 species; Eastern gorilla, *Gorilla beringei*; Western ringtail possum, *Pseudocheirus occidentalis* and Western long beaked echidna, *Zaglossus bruijnii*) to 6967 individuals for the EW Scimitar horned oryx (*Oryx dammah)*.

The median size of an ex-situ population (n) is 73, double that of the wild camel (36). A total of 44% of populations (29) – including the wild camel- have fewer than 50 individuals and 91% (60) have fewer than 500. Only 3 populations have over 1000, (Figure 5.3) and only the scimitar horned oryx exceeds 1500 individuals (Figure 5.1).

The number of holding institutions varies (Figure 5.2); while 14 species (21%) are held in only a single institution, the cotton headed tamarin, *Saguinus oedipus,* population is held across 294 institutions, the mean number of holders is 35, the median is 9. Population sizes increase with the number of holding institutions (Figure 5.2).

Where data exist for comparison (29 species), our results show that the ex-situ population can make up a substantial proportion of the estimated global population. For the African Wild Ass, *Equus africanus* the ex-situ population is 90% of the global population (Figure 5.3). Furthermore, the availability and size of the wild populations are not correlated with the size of the ex-situ population, such that there are numerous cases where critically small wild populations have substantially larger ex-situ population sizes. Of the 15 species with wild populations under 250 individuals, 8 show an overall population increase of over 50%, when ex-situ populations are considered. For some the size difference is considerably larger (e.g., the addax, *Addax nasomaculatus* overall population increases by 3427% when ex-situ individuals are considered). These findings are not directly comparable with each other as RedList assessments only consider “mature individuals” while numbers from ZIMS are total population size, but despite this the numbers reveal the substantial potential of ex-situ populations in global conservation of some taxa.

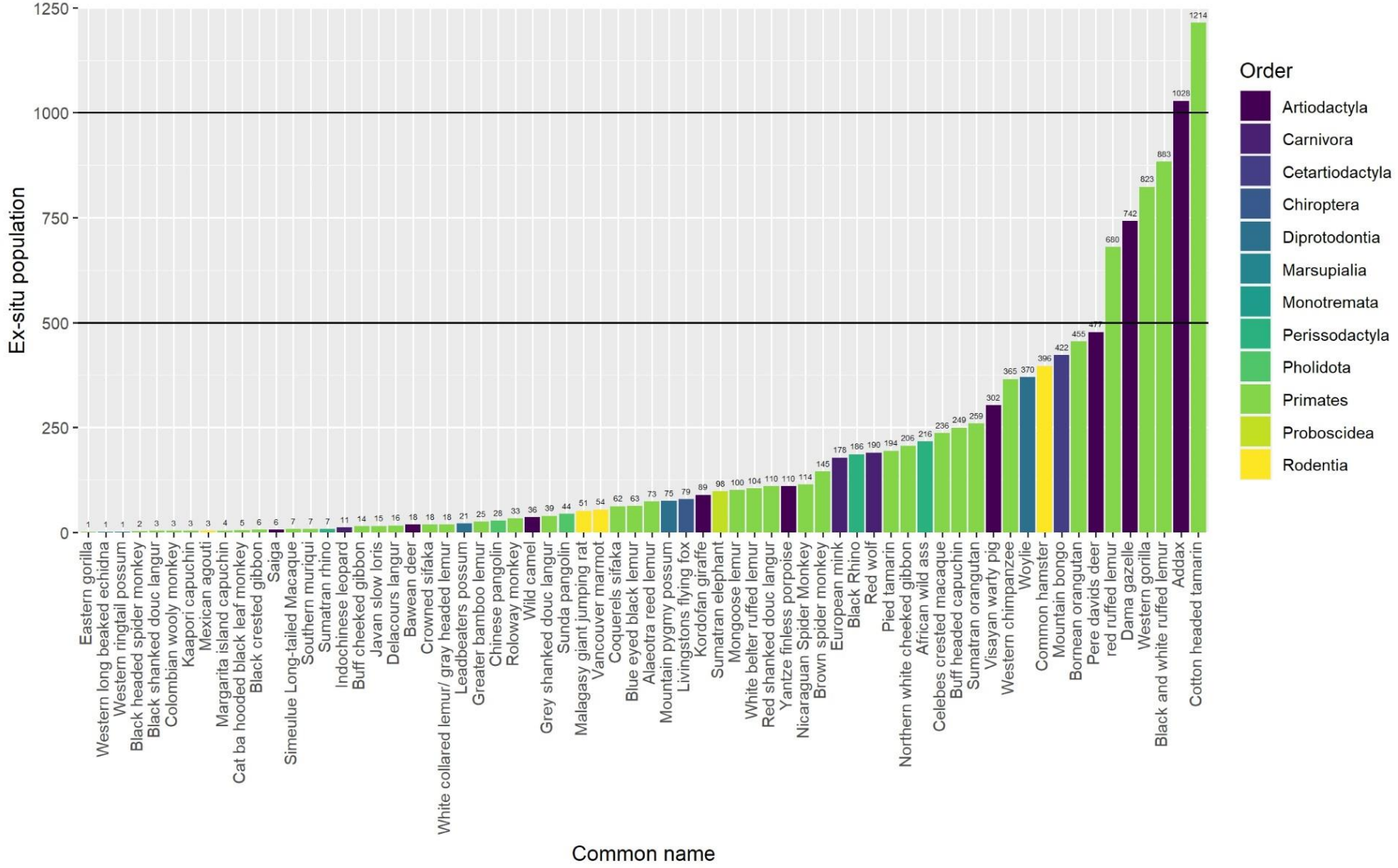

Figure 5.1*: Ex-situ absolute population sizes ZIMS (N=66 EW Oryx removed for scale) with the 500 and 1000 thresholds marked. 44% of populations (29) have fewer than 50 individuals and (60) 91% have fewer than 500. Only 3 population have over 1000. Order in which the species belongs is visualised by colour. The wild camel has an ex-situ population of 36.*

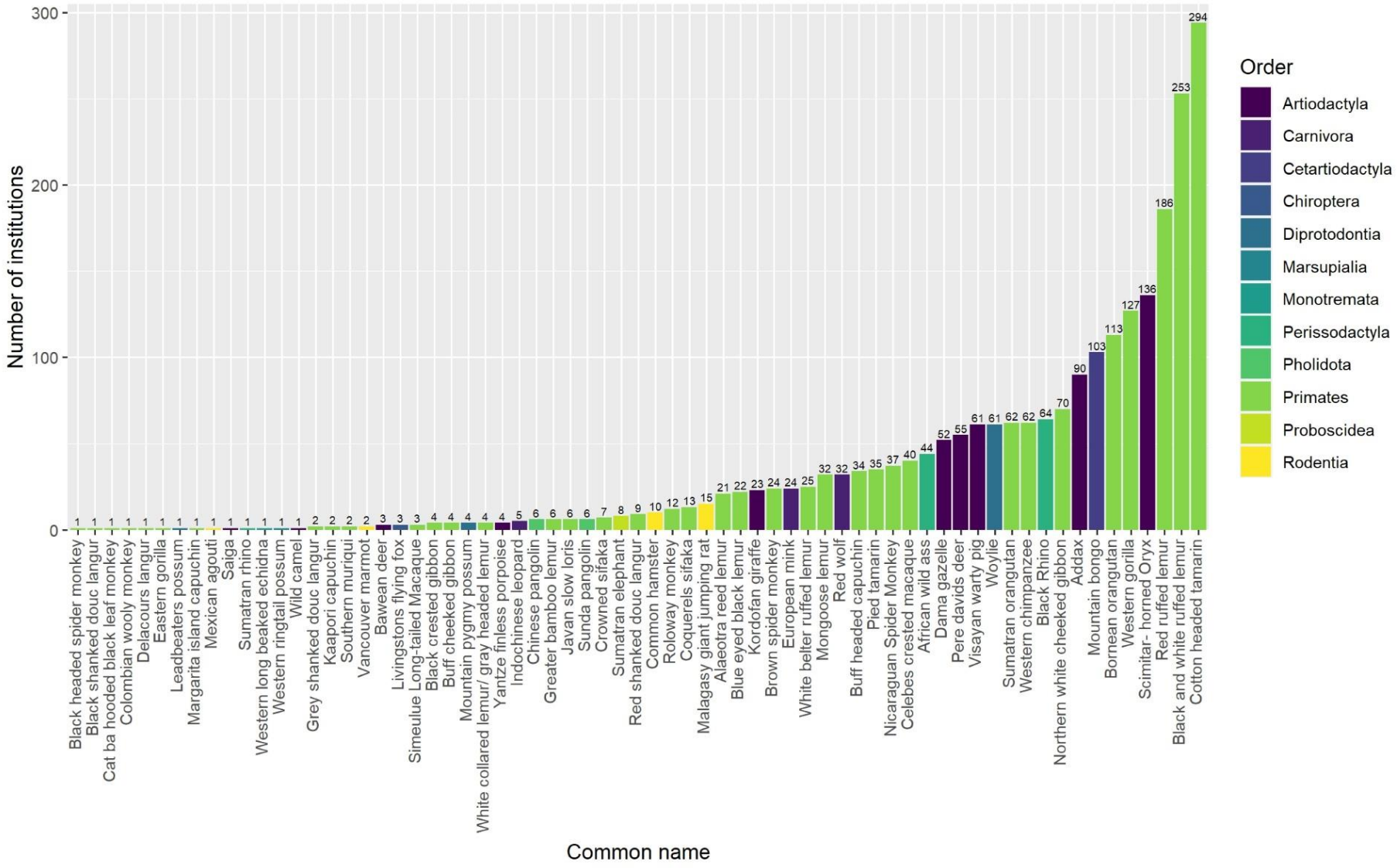

***Figure 5.2**: Number of ZIMS institutions holding each species (*N=67*). Order in which the species belongs visualised by colour. The 67 species are across 12 orders. 55% of the species are in the order Primates, 14% are Artiodactyla. All other orders included have under 6% each. The wild camel is held in only 1 institution.*

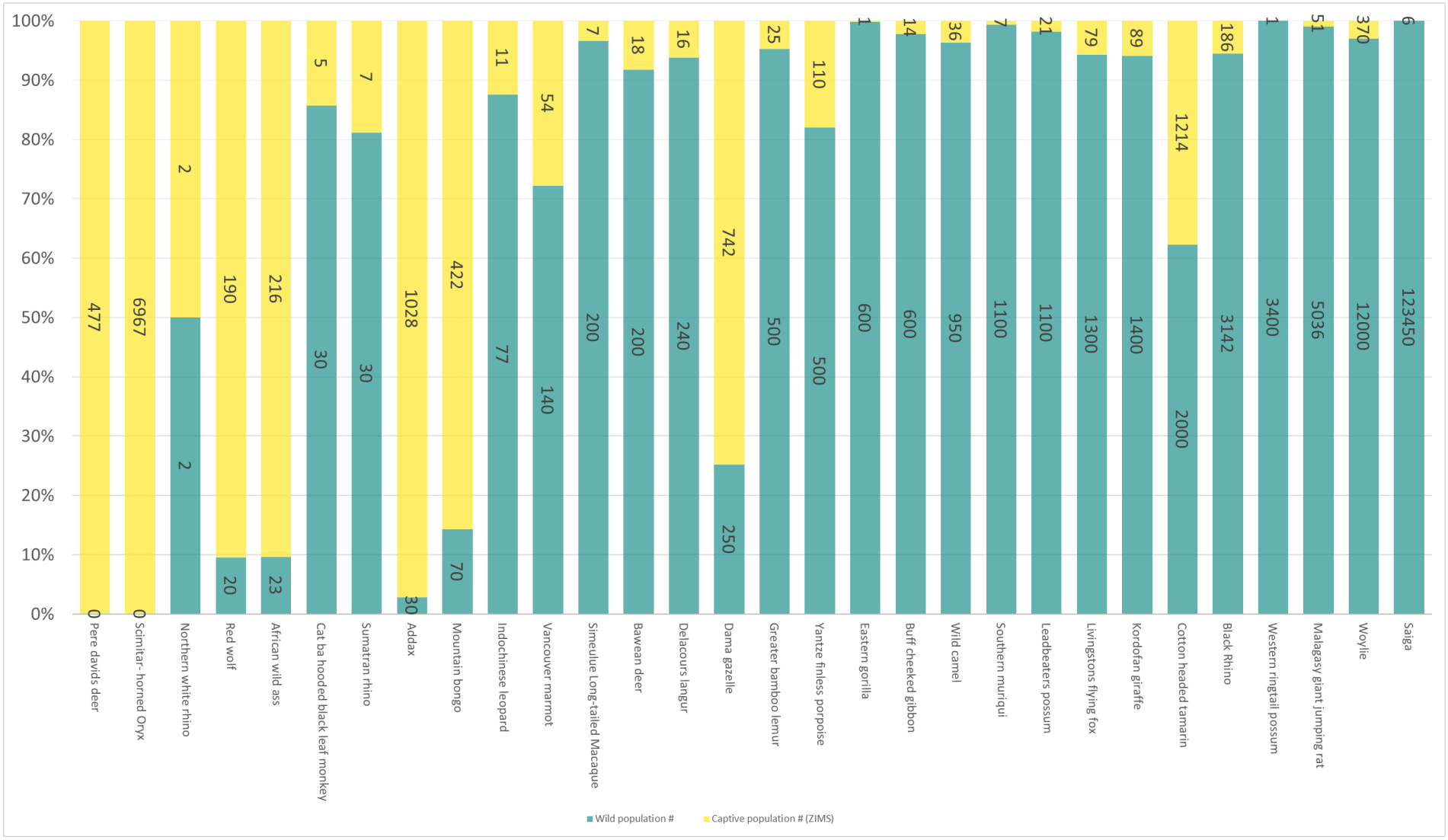

Figure 5.3: *Proportion of overall populations in wild and ex-situ populations. Population numbers (N) included on bars, for ex-situ populations this is total recorded population on ZIMS (Species360 2023, 360). For wild population this is mature individuals, as recorded by the IUCN Red List (IUCN Red List 2022). Populations are ordered from left to right by increasing total wild population size. The ex-situ population of wild camel represents 4% of the global population.*

5.3.2 Management of ex-situ populations.

We find that genetic considerations are managed better than demographic considerations with 67% showing genetic management. 64% (43) use a studbook in management (although only 49% (33) are recorded on ZIMS) and 37% (25) have survival plans for ex-situ populations managed by zoological associations (AZA= SSPs, WAZA, EAZA= EEPs). In contrast, only 10% of taxa have had PVAS developed to help manage the ex-situ population. 34% (23) do have some record of a PVA, either for the ex-situ or in-situ population. 7 of the 23 (11% of the 67 ex-situ species) are managed by zoological associations (AZA, WAZ, EAZA) and so focus is on the ex-situ population. Only 4 of these were conducted on in-situ populations in preparation for collection of animals for ex-situ, of these 2 also have PVAs after collection and one is still collecting animals.

For planning future recovery and release, 61% (41) have some form of recovery plan at species level, only 16 of which are reported as having “action recovery plan” on Red List assessments. 30% (20) have a record of animals being released, reintroduced or reinforced, only nine of which are reported on Red List assessments.

Common name	Monitored demographically	Monitored genetically
Addax	✓	✓
Bornean orangutan	✓	✓
Northern white cheeked gibbon	✓	✓
Red wolf	✓	✓
Scimitar- horned Oryx	✓	✓
Visayan warty pig	✓	✓
African wild ass	✓	✓
Black and white ruffed lemur	×	✓
Blue eyed black lemur	×	✓
Brown spider monkey	×	✓
Celebes crested macaque	×	✓
Coquerels sifaka	×	✓
Cotton headed tamarin	×	✓
Crowned sifaka	×	✓
Dama gazelle	×	✓
European mink	×	✓
Greater bamboo lemur	×	✓
Livingstons flying fox	×	✓
Malagasy Giant Jumping Rat	×	✓
Mongoose lemur	×	✓
Mountain bongo	×	✓
Pere davids deer	×	✓
Pied tamarin	×	✓
Red ruffed lemur	×	✓
Roloway monkey	×	✓
Sumatran orangutan	×	✓
Alaeotra reed lemur	×	✓
Bawean deer	×	✓
Black Rhino	×	✓
Buff headed capuchin	×	✓
Cat ba hooded black leaf monkey	×	✓
Colombian wooly monkey	×	✓
Common hamster	×	✓
Leadbeaters possum	×	✓
Mountain pygmy possum	×	✓
Saiga	×	✓
Southern muriqui	×	✓
Sumatran rhino	×	✓
Sunda pangolin	×	✓
Vancouver marmot	×	✓
Western chimpanzee	×	✓
Western gorilla	×	✓
White belter ruffed lemur	×	✓
Wild camel	×	✓
Woylie	×	✓
Black crested gibbon	×	×
Black headed spider monkey	×	×
Black shanked douc langur	×	×
Buff cheeked gibbon	×	×
Chinese pangolin	×	×
Delacours langur	×	×
Eastern gorilla	×	×
Grey shanked douc langur	×	×
Indochinese leopard	×	×
Javan slow loris	×	×
Kaapori capuchin	×	×
Kordofan giraffe	×	×
Margarita island capuchin	×	×
Mexican agouti	×	×
Nicaraguan Spider Monkey	×	×
Red shanked douc langur	×	×
Simeulue Long-tailed Macaque	×	×
Sumatran elephant	×	×
Western long beaked echidna	×	×
Western ringtail possum	×	×
White collared lemur/ gray headed lemur	×	×
Yantze finless porpoise	×	×

***Table 5.2**: Monitoring and management of ex-situ critically endangered populations (N=67). Monitored demographically= if the ex-situ population has a record of a population viability analysis (7/67 10%). Genetically monitored= if the ex-situ population has a record of having a studbook or survival plan (45/67 67%). The wild camel is not monitored demographically. A studbook is held, so it is monitored genetically.*

5.4 Discussion

5.4.1 Global population proportions.

We have shown that those ex-situ species with the smallest wild populations (under 250) have a disproportionate share of very large ex-situ populations. Some of those species, most threatened with extinction in the wild, are being held in highest numbers ex-situ, potentially making the insurance value of great importance. For some, they hold a significant proportion of the overall global population. Every ex-situ population of CR species could either be considered as an insurance, or has the potential to become an insurance, against extinction. EW species are an insurance against extinction by definition. In those highly-threatened species, every individual could be important.

5.4.2 Global population risk assessments.

We revealed that of the 293 mammal taxa, approximately a quarter (69) are represented in ex-situ care, almost double the number reported on the RedList. Despite the potential importance of these ex-situ populations, the RedList doesn't include quantification of ex-situ populations as part of the assessment process. Not only that, RedList assessments have missed species in ex-situ care, with only 36 of the 69 species being reported as "Subject to ex-situ conservation". Because the RedList doesn't focus on this it misses a potential large and important source of animals which will influence extinction risk. We find that this is substantial, and certainly higher than what the red list has recognised, as almost a quarter (23%, 291 CR on IUCN RedList, 66 on ZIMS) of our CR and EW mammal species are currently held ex-situ, an absolute increase of 5% since 2011 (187 CR on IUCN, 36 in ZIMS) (Conde et al. 2011). RedList assessments also miss other species information, although 31% (21 species) have a record of animals being translocated to wild conditions only 42% of released species were marked as "Successfully

introduced". Of those with a survival plan, only 39% are captured under "action recovery plan" on RedList assessments.

5.4.3 Ex-situ population sizes.

The majority of the ex-situ populations of CR/EW species we considered in this analysis are held at low population sizes. Viability of a population, determined by population size, is difficult to define and can depend on; the species biology, management, environment and demography (Smith et al. 2023). In CR or EW species it may not be possible to supplement populations with in-situ individuals, making retention of genetic diversity of great importance. If these populations are acting as an insurance against extinction, they need to have the capacity to adapt. In these cases, to avoid loss of genetic diversity, an effective population size (N_e) of 500 individuals is advised, but an N_e of 1000 may actually be needed to maintain evolutionary potential (Frankham, Bradshaw, and Brook 2014). In our analysis only 3 species have a population (N) > 1000; 91% have populations lower than 500, 44% below 50 individuals. Furthermore, census population size (*N*) is larger than effective population size (Ne) making this contrast in recommended and realized ex situ population sizes starker. Ideally, an ex-situ population should be founded by between 30 and 50 individuals to better represent the genetic diversity of any remnant in-situ population (Segelbacher 2022; Smith et al. 2023; Frankham et al 2007). Our analysis revealed only 9 taxa had founder representation that met these requirements. Whilst these estimates do not determine whether an ex-situ population will be viable or not, they do indicate the risks of further loss of genetic diversity.

To improve potential population viability these ex-situ populations could aim to increase population size. This can be done by increasing the number of holders. 19% of the species in our analysis were held in only one institution. Increasing the number of institutions holding species allows for an increase in population number, but it also has benefits in population management including mitigating for localised disease outbreaks and localised institutional problems (Smith et al. 2023). On the other hand, there is

a limit of space available in institutions for ex-situ populations for conservation as well as zoos needing to balance conservation need and the requirement for enigmatic species, usually of large body size (Miranda et al. 2023; Martin et al. 2014), to bring in visitors (Alroy 2015). Much of the available space in zoos is already taken and effort would be required to make more (Alroy 2015). Furthermore, splitting already-small populations could lead to further risks of fragmentation and isolation, but these issues are mitigated against by using studbooks and survival plans to allow for breeding and movement of individuals between institutions.

5.4.4 Ex-situ population management.

Good ex-situ management should consider strategic breeding of individuals to maximise genetic diversity and ensure that animals are moved, held and bred in ways that ensure demographic security. We found that many of ex-situ CR and EW species are not using these tools with only 67% monitored genetically, using a studbook or a survival plan in management, and only 10% monitored demographically using a PVA. When used together, these tools can improve genetic management by increasing the link between individuals and institutions, which overall allows for informing ongoing conservation management, so their lack of use could be a concern.

The CR/EW problem is urgent, prolonged periods as small ex-situ populations leads to genetic drift (Frankham et al 2007) whereas prolonged periods as large ex-situ populations can lead to adaption to captivity (Witzenberger and Hochkirch 2011; Gilligan and Frankham 2003; Frankham 2008). Either of these scenarios can lead to loss in genetic diversity and subsequent inability to adapt. Diversity can be increased by translocating individuals, but in CR species source populations may not be viable for harvesting (Conde et al. 2011), and in EW species ex-situ populations are the only source available for translocations (Smith et al. 2023). During this time, ecosystems are changing and both ex-situ and in-situ issues need to be addressed to reduce extinction threat. Reducing the time in which species are held ex-situ is therefore imperative. We used release as an indicator of species recovery as its use

suggests that ex-situ populations are on a recovery pathway. Our results show that only 31% (21 species) have a record of animals being translocated to wild conditions.

5.4.5 Ex-situ population of wild camel *Camelus ferus* in Mongolia.

The above data helps to illustrate the adequacy for insurance, and comparison to current ex-situ population management, of the wild camel, *Camelus ferus*. The species is critically endangered, with a current Global population of 950 (IUCN RedList 2022) and a Mongolian population of under 700, (Chapter 4). The combination of little available information on the species, a presumed low population and a restricted range has all led to concern over its extinction risk. This concern led to the creation of an ex-situ population in Mongolia, the purpose of which was to act as an insurance against extinction (WCPF 2023).

The ex-situ population was founded in 2004. These founding members were not chosen, but were made up of 13 rescued, herded and captured wild camels from the buffer zone of the GGASPA. These 13 animals consisted of 8 adults and 5 offspring, so the true number of founders is 8. As females can breed from approximately 4 to 35 years old, some of these original foundering members are still breeding now. The current population is 36 animals. A studbook has been partially maintained since the captive population was founded, but it is inaccurate and parentage is uncertain.

If we were to consider the recommendations discussed in this chapter, then the ex-situ population of wild camel could have been considered an inadequate insurance against extinction. In terms of conservation planning, up until now the genetic management could be considered inadequate as both the ex-situ population size (N=36) and the number of founders (N=8) are far below those thresholds required to avoid loss of genetic diversity (Figure 5.1). Although a studbook has been maintained in this one, small, population it may have inaccuracies. No SSP has been conducted, nor has a PVA, so the population is also not monitored demographically (Table 5.2). Finally, although releases of captive

individuals into the GGASPA have taken place, the aim of these was predominantly for the management of aggressive bulls, not necessarily for conservation management, so the recovery pathway is unclear.

Although this insurance population may not appear to be adequate now, extensive efforts are being made to improve the functionality of the ex-situ wild camel population. Genetic monitoring and management are being improved. The building of a secondary breeding centre is ongoing, the aim of which is to increase breeding capacity and so number of animals. The research conducted during this PhD will allow us to: update the studbook (which is held in the offline version of ZIMS (SPARKS)), create a SSP and for demographic monitoring, the production of a PVA, all of which prove the commitment to working towards best practice.

To disregard the insurance potential of this population would be an omission. The ex-situ population of wild camel in Mongolia breeds successfully, it is a long standing in-country project with local support and active work is ongoing to improve it. This ex-situ populations constitutes 4% of the current Global population (IUCN RedList 2022) and it makes up approximately 5% of the total wild Mongolian population (Chapter 4). This is significant not only in the overall proportion of the population it holds, but also, as we have shown it to hold equal genetic diversity and to be less inbred than that wild population, (Chapter 4) in genetic potential. This is an important population and should be considered critical when considering wild camel conservation.

5.4.6 Conclusions.

We show variation in the size, monitoring and management of ex-situ populations of critically endangered mammals, and the limitations of current ex-situ populations – including that of the wild camel – to serve as insurance against extinction. With both practicalities of population function and further constraints that institutions will have as businesses and education facilities (Conde et al. 2011), there will always be competing objectives that might constrain monitoring, management and function

of ex-situ populations. There is a clear difference between knowingly choosing not to meet best practice because of these constraints and not meeting best practice out of lack of strategic thinking or not having the capacity or tools to do so. But for those species closest to extinction, ex-situ populations could be a fundamental insurance against outright extinction. The variation in functionality of these ex-situ populations can be improved or mitigated for with best practice, and further used to inform conservation management across multiple institutions. In starting this work, we looked for a set of standard best practise guidelines or tools used for effective management of threatened ex-situ populations, but were unable to find a standardised resource. There are guidelines for starting an ex-situ population (IUCN 2014), but not to aid those that are already in place. We therefore advocate for the creation of such guidelines, to be used to guide good managed and integration of ex-situ populations for conservation.

We conclude that there is wide variation in the insurance capacity of ex-situ populations of EW/CR mammals to act against extinction. Many populations are both not large enough to provide security against genetic deterioration nor do they show adequate monitoring of conservation planning. Despite their conservation potential, making up considerable proportions of overall population size, many are also overlooked in global risk assessments. If the ultimate goal is continued persistence of the species, then our ex-situ populations should be considered critical.

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Chapter 6 Discussion

6.1 The importance of *Camelus*

For the last 6000 years the *Camelus* genus has been very important to human life (Burger, Ciani, and Faye 2019). The domestic species have provided sustenance in the form of meat and dairy products. They have clothed people in wool and leather. As the predominant draught animal of the Silk Road, the camel carried the development of global civilisation on its back (UNESCO 2023). They have been important in medicine, with research on camelid nanobodies producing a wealth of important medical information, including using their nanobody antibodies for the development of treatment for Covid-19 (Hong et al. 2022; Bessalah et al. 2021; Arbabi-Ghahroudi 2017). They have also provided entertainment, as racing animals and even as beauty queens (Burger, Ciani, and Faye 2019; Saudi Moments 2023). We owe a great debt to the camels; saving the last wild representative from extinction seems a small price to ask. Of course, this in itself may not be considered a valid reason for investing in the conservation of the only "wild" *Camelus*, but as climate change increases desertification, the need for sustainable meat and dairy production in arid landscapes grows (Burger, Ciani, and Faye 2019). The wild camel survives in a place of extremes, drinking salt water and migrating through nuclear testing zones (WCPF 2023). We can learn from the wild camel. The loss of this species may risk the loss of currently unknown information on extreme survival in a changing world.

The wild camel, *Camelus ferus*, is the last surviving extant species of *Camelini*. Since 2007 the EDGE (Evolutionarily Distinct and Globally Endangered) metric has been one metric used to prioritise practical conservation of threatened species that represent large amounts of evolutionary history, the loss of which would pose particular threats to biodiversity given their distinctive evolutionary histories (Gumbs et al. 2023). Initial EDGE assessments considered the wild camel to be in the top 10 (9^{th}) most threatened species. The 2022 update, which considers closest relatives (two domestic species in the

case of the wild camel) moved it to the top 100 (74th) (EDGE 2023; Gumbs et al. 2023). Despite this update, the EDGE list position of the wild camel displays the importance of its conservation.

Not only is the species evolutionarily distinct; it is also considered an umbrella species. To maintain its migratory behaviour and large range (Joly et al. 2019; Kaczensky et al. 2014; Yadamsuren, Daria, and Liu 2019) requires protecting vast areas of the Mongolian and Chinese Gobi. The Gobi is a key biodiversity site, with a functioning and healthy ecosystem. It has one of the lowest levels of human influence globally (McCarthy et al. 2022), and is home to a host of threatened animals (IUCN RedList 2022), including: Khulan/Asiatic wild ass *Equus hemionus* Vulnerable; Goitered Gazelle *Gazella subgutturosa,* Vulnerable; Gobi bear, *Ursus arctos gobiensis,* Critically Endangered; Snow leopard, *Uncia uncia,* Endangered; Pallas' cat, *Otocolobus manul,* Nr Threatened; Argali, *Ovis ammon,* Endangered; Saker Falcon, *Falco cherrug,* Endangered and Long eared Jerboa, *Euchoreutes naso,* Endangered. The desert habitat also holds a number of endemic and critically endangered plant species such as the desert poplar (*Populus diversifola*) and the desert broomrape (*Cistanche deserticula*). Beyond individual species, the Gobi itself is threatened. Climate change (Han, Dai, and Gu 2021) is increasing desertification, making the Gobi the fastest-growing desert in the world (McCarthy et al. 2022). The rarity and importance of the habitat has led to it being part of a proposed UNESCO World Heritage Site (McCarthy et al. 2022). Protecting the wild camel requires the protection of vast areas of this threatened ecosystem, in which large areas of suitable habitat are already predicted to be lost (Xue et al. 2021).

From a conservation policy perspective, the wild camel is in a positive position: much of the in-situ legislative work is complete, with legal protections for the species being granted in both Mongolia and China, and local protections are in place (WCPF 2023). What is lacking for the species is an in-depth understanding of the threats it faces and an evidence-based plan for species recovery. The aim of this thesis was to provide some of the critical evidence necessary to produce a survival plan for the wild camel in Mongolia, both in-situ and ex-situ. Some basic species information was required to inform

conservation management and decision making. This thesis was able to provide this vital information, which includes: the first accurate and precise in-situ abundance estimate since 1997; a greater understanding of population genetic diversity; the extent of hybridisation in the GGASPA; and a genetic comparison of in-situ and ex-situ populations. Finally, through the work highlighting the correct naming of the wild camel, it has hopefully improved wider understanding of the species and its cause.

6.2 GGASPA Abundance estimates

Often the first question asked when discussing extinction threat is "how many are left?"; until now we have not confidently been able to answer this question. The last robust estimate, of 1985 (95% CI=413 to 3557) was gained in 1997 (Reading et al. 1999). Our mean estimate of 664 (95% CI=440-1100) is less than the mean of the 1997 estimate but it does overlap in 95% confidence intervals. Not only is our estimate more precise, but in 1997 wild and Bactrian camels were not considered separate species; this estimate (though certainly more methodologically sound than other estimates that relied largely on expert judgment) may therefore have overestimated "wild" individuals in counts. Gaining an accurate and precise point estimate of abundance allows for monitoring of trends in population size, which in turn allows for modelling future population trajectories. An accurate and precise point estimate also increases our confidence in updating species assessments such as RedList or EDGE (IUCN RedList 2022; EDGE 2023) or for comparisons such as those in our work in chapter 5 that looked at ex-situ populations. For example, we can now compare the Mongolian in-situ population (664, 95% CI=440-1100)) with the ex-situ population (36), allowing us to determine that the ex-situ population is 5% of the average abundance estimate of the wild Mongolian population.

Furthermore, this abundance estimate allows us to evaluate the adequacy of the sample size for the genetic analysis (Chapter 4). Of the samples we collected in the GGASPA (and successfully amplified), 97 are from either wild or hybrid animals. This means we have genetic data for about 15% of current the wild population. What improves this estimate further is that this data was checked for probability that two samples were from the same individual. As only 3 pairs of individuals matched, it suggests that

all others come from individual animals. This is a significant proportion of the overall wild Mongolian population, raising confidence in the representativeness of this sample, important if the data should be used elsewhere in research.

6.3 Extent of Hybridisation

Hybridisation was considered one of the biggest threats to the wild camel, but the extent of which Bactrian genes were introgressed across the GGASPA and the ex-situ population was unknown. This thesis allowed for a greater understanding of introgression in the Mongolian population of wild camel. By using two methods of monitoring –nuclear and mitochondrial – we were able to get a bigger picture of what introgression looks like for the species.

By combining sample collection with camera trap placement (Chapter 3) we were able to ensure a similarly systematic approach to sampling across the GGASPA. While there were fewer samples collected from the East of the GGASPA, our data provides a good understanding of the geographic spread of hybrids. Our Structure analysis showed that predominant structuring of the population was between the two species, wild and Bactrian camel. Removing Bactrians and hybrids from the analysis (i.e., including only "pure" wild samples) we found a structure of K=3 populations maximised information. To determine if this was valid population structuring (e.g., caused by geographic or movement boundaries in the park), we visualized the structure spatially (Figure 6.1). This population structuring could not be determined by geographic boundaries to camel movement, suggesting the population is well-mixed and the pattern we observed most likely a product of overfitting.

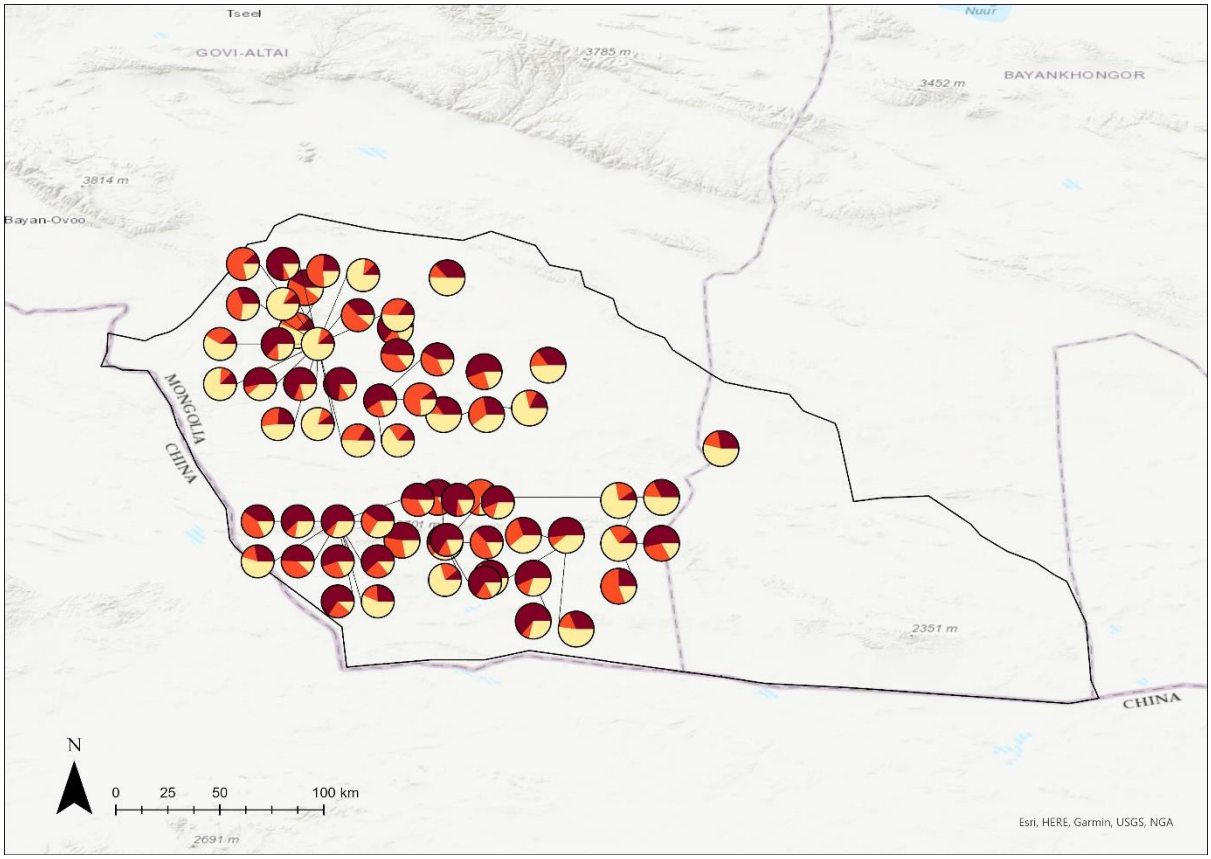

Figure 6.1: *Population structure (K=3), mapped geographically across the GGASPA*

It was previously predicted that Bactrians could access the GGASPA (Kaczensky et al. 2014). Grazing is permitted within the park boundary during times of drought and outwith these times there is no fence to prevent movement. An increase in overall livestock population over the last 20 years combined with the continued climate change-driven desertification of the region has produced a predictable increase in competition for resources between livestock and wildlife (McCarthy et al. 2022; Han, Dai, and Gu 2021). Our systematic collection of data from across the park has allowed us to capture data on "pure" Bactrians accessing the GGASPA. We now know that of the samples collected in the GGASPA, 6% were "pure" Bactrian. Added to this are the captures of images of Bactrians from across the park (Figure 6.2). Bactrians are predictably found predominantly along the northern border, where they are grazed and managed by herders, whereas wild camels tend toward the central mountainous areas of the park. The central mountainous areas of the GGASPA are considered the core for the species (Yadamsuren, Daria, and Liu 2019). Although this core protected area does indeed appear to be the stronghold for the wild

camel in Mongolia, we did capture both hybrids and Bactrian (one photo and one genetic capture) in these areas (Figure 6.2).

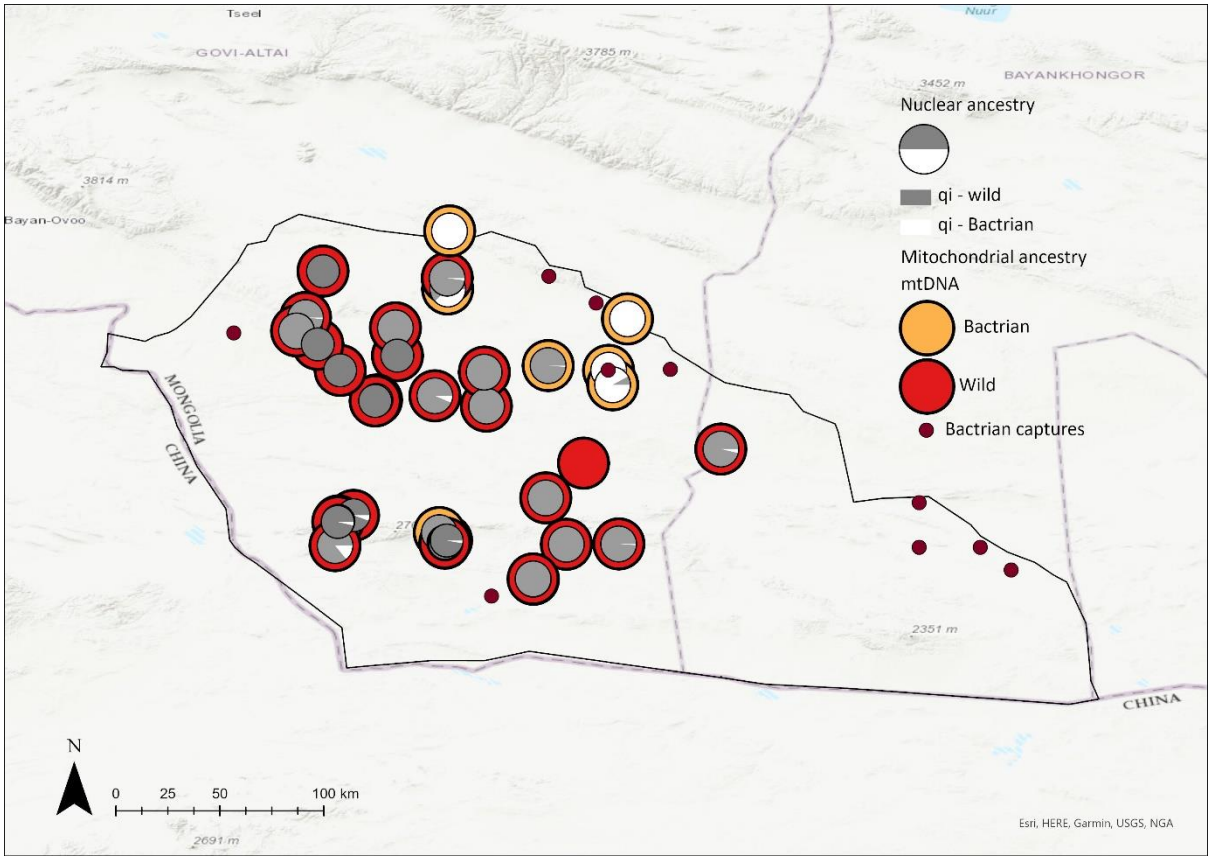

Figure 6.2: *Introgression and Bactrian camel photo captures across the GGASPA. Introgression visualized as pie charts for nuclear ancestry and coloured outline for MtDNA introgression. Locations of cameras that captured Bactrian camel are included (Bactrian captures).*

Our genetic data set used samples from across the GGASPA, from China, from the captive herd and from known Bactrian herders. When considering all our samples used in analysis, between 10% and 22% were considered hybrids, depending on threshold used. If we look at only those samples collected from the GGASPA during field work for this research (and thus have the potential to be from current surviving animals), 103 samples of unique individual camels amplified. On a nuclear level (qi=0.10), of these 103, 6 (6%) were Bactrian, 87 (84%) were wild and 10 (10%) were hybrids. Hybridisation is both considered an extinction threat and an important part of the evolutionary process (Allendorf et al. 2001). Acceptable levels of introgression are determined by thresholds chosen by the conservation

community, so what is considered a hybrid can change depending on the information available. Determining that 10% of the current Mongolian wild camel population could be considered hybrid is an important first step in determining options for conservation management.

6.4 Ex-situ population management

As we have shown in this thesis, even an imperfect ex-situ population could have great conservation importance. The ex-situ wild camel population held in Mongolia could be of significant importance, as it is one of only two known captive populations of wild camels and those individuals in ex-situ care constitute approximately 5% of the total wild population in Mongolia. Management of this herd has until now focused on successful breeding and care of the species in captivity. This has been successful in terms of longevity and breeding success, but improvements can be made to make the herd a much more effective insurance population. When we consider the requirements made in chapter 5 for successful ex-situ management, then this population could be considered an inadequate insurance against extinction. The overall ex-situ population (N=36) is far below the minimum of 500 individuals required to avoid loss of genetic diversity, as is the number of founders (8). It is only held in 1 institution, although this is currently being remedied with the building of a second site in Mongolia. Releases have taken place, but these were for captive management of aggressive bulls, not necessarily for conservation management. There has been neither a SSP nor a PVA conducted for the population. Despite all of this, extensive attempts are being made to improve the management of the species and much of the work conducted in this thesis will aid the transition. The studbook, currently held on the offline version of ZIMS, SPARKS (Species360 2023), is being improved with genetic analysis conducted in chapter 4. Not only that, we now know that the level of genetic diversity of the wild population is indeed captured in the ex-situ stock and surprisingly our results suggest that the ex-situ population is less inbred than the wild population (Chapter 4). Our results allow us to better understand the genetic health of the individuals in our population, including those that show introgression, so we are better able to manage it accordingly. This herd is a long standing, in-country project that has proven breeding

success. Reduced diversity, increased inbreeding and hybridisation can all cause reduced fitness and compromise adaptability, therefore it was important that these levels were understood in the captive herd. This understanding will allow for management that maximises genetic diversity. By continuing to improve the programme using standard monitoring and management tools this population will become a better insurance against extinction.

6.5 Insurance populations of critically endangered mammals

A focus of this work was to look at the insurance utility of the ex-situ population of wild camel in Mongolia. We have found that the wild camel is far from the only case of a critically endangered mammal with a suboptimal ex-situ insurance. Despite a quarter of our CR mammal species being held in ex-situ care almost all of them (91%) are held at population sizes too low to avoid loss of genetic diversity. One reason cited for not being able to increase an ex-situ population is space (Miranda et al. 2023; Martin et al. 2014). Large bodied mammals can indeed take up significant space in a zoological institution, but this pattern is seen across taxa, with even only 28% of the tiny but vital, plant seeds from extinct in the wild species, being stored in seed banks (Smith et al. 2023). For the Bactrian camel, sister species to the wild camel, space does not seem to be an issue, with 934 individuals held across 263 institutions (Chapter 2). For those extinct in the wild or critically endangered, and of greatest threat to extinction, perhaps some alternatives in animal holding can be made. Our ex-situ populations of CR mammals could be of huge importance to overall survival of the species, as these populations can make up a substantial proportion of the estimated global population. Of those with ex-situ held CR mammals with wild populations under 250 individuals, more than half showed an overall population increase of over 50%, when ex-situ populations are considered. That we have more than half of the global population of some critically endangered species in ex-situ care should be considered a conservation opportunity, and better monitoring and maintaining them should be considered critical.

6.6 Future practical conservation application and direction.

The CTDS method used in chapter 3 to estimate wild camel abundance was accurate and precise. It is repeatable and has been proven to work in the GGASPA, so to monitor population trends over time it can be conducted again. In future work – and in repeat studies – this abundance estimate could be improved to demonstrate spatial and seasonal distribution, adding finer-grained information on how camels are using the GGASPA.

The extensive dataset of images collected has already yielded results beyond the intended study. Camera captures provide conclusive evidence of Bactrian camels entering the GGASPA, complementary to the genetic evidence (Chapter 4). One capture (Figure 6.3B) from August 2021 showed a previously-released captive wild camel (estimated release 2015), showing the potential for successful survival of translocated individuals from the ex-situ population. Another image (Figure 6.3A) captured a wild camel with twins, only the second recorded instance of successful twin pregnancy in this species.

The extensive placement of cameras across the GGASPA captured photos every day for two and a half years. It therefore offers a valuable ready resource for study of other species beyond the wild camel in the GGASPA (e.g., khulan, Asiatic wild ass, *Equus hemionus*, goitred gazelle, *Gazella subgutturosa* and Bactrian camel, *Camelus bactrianus*). In particular, by using this analysis method for Bactrian camels, we can estimate their abundance in the GGASPA and also confirm where they are using the park, which could aid future hybridization management.

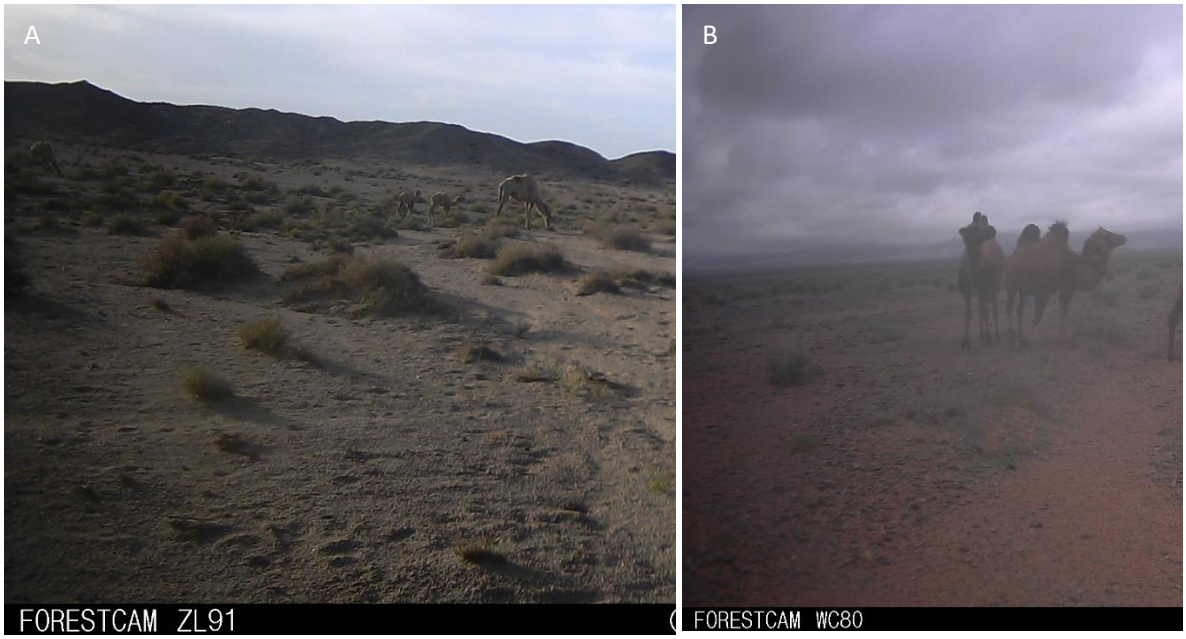

Figure 6.3: *Images captured during CTDS. A) Wild camel with twins, the second only recording of twins. B) Released wild camel showing collar. This camel was estimated to be released in 2015.*

Although chapter 4 extensively monitored introgression across much of the GGASPA, there is a gap in the east of the park. Extending the monitoring to this area will further increase our understanding of hybrid extent in the GGASPA. Monitoring of known hybrids in the buffer zone of the GGASPA could increase the information we have on both nuclear introgression and phenotypic characteristics of hybrids. If we know exactly what level a hybrid is (e.g., F1, F2), it could aid our determination (genetically and phenotypically) of a threshold of what is considered an acceptable level of introgression. Using morphology alongside genetic data is often used in determining hybrid thresholds in other species (Schrey et al. 2007; Williams et al. 2005). However, presently the morphological characteristics of hybrid wild camels are not well described. Not being able to distinguish a hybrid visually, combined with the remoteness of the GGASPA, means that management of hybrids could be very difficult. Given that hybrids may be cryptic (Jasińska et al. 2010) and can hold important genetic material (Hoffmann et al. 2010) management decisions made could have a strong impact on an already reduced population and so should be taken with care. Hybridization has been known to be encouraged in areas around the GGASPA as the hybrid offspring are good racing camels. Increasing surveillance and interaction with

local herdsmen may be a first step in reducing the problem, or at least of understanding it further. This underlines the need for a structured decision-making process that includes local stakeholders in the development of a species survival plan.

The most direct practical application of the work conducted in this thesis will be for the improved management of the captive breeding centre in Mongolia. A second breeding centre will be complete in Spring of 2024, at which time individuals will need to be selected for moving. The information gained from the microsatellite genotyping will be directly used to determine individuals which are the most unrelated. This will also be used to improve the studbook and then move it onto ZIMS (Species360 2023), for a more transparent monitoring system. We used microsatellite analysis because they allowed for inclusion of previous data sets to the analysis and they could be used in degraded DNA, such as that extracted from non-invasive samples. But microsatellite analysis provides only a basic understanding of inbreeding, and so we should use full genome sequencing on the herd to fully understand the gaps in the studbook and to look at inbreeding and genetic load in more depth. The captive population represents just one potential strand in an overall species survival plan, provision of information for which was the overall aim of this thesis.

The RedList assessment for the wild camel has not been updated since 2008; since that time there has been a significant increase in data gathered on the species, including that produced in this thesis, so updating the assessment should be a priority to gain a better understanding of extinction threat. The wild camel was RedList 'critically endangered' status according to criteria of population reduction. This criterion requires "a population size reduction of at least 80% within the next three generations, estimated at 45-50 years". We do not present an overall population estimate, but an abundance estimate for Mongolia, which is the same as the the last robust estimate, of 1985 (95% CI=413 to 3557) produced in 1997 (Reading et al. 1999). Our mean estimate of 664 (95% CI=440-1100), while falling within 95% confidence intervals of the 1997 estimate, could represent a 67% population decrease relative to the mean of the previous estimate, in the intervening 26 years. This is a low enough

population size to warrant the concern of conservation managers; as an effective population size of 1000 may be required to maintain evolutionary potential (Frankham, Bradshaw, and Brook 2014). The preliminary abundance estimate results suggests the population size is low enough to warrant concern, as does our introgression data, so an update of the wild camel RedList assessment is vital to determine extinction risk.

6.7 Novel use of CTDS in timelapse.

This thesis is not solely of value for the information it has yielded regarding the wild camel and the GGASPA; it also represents a methodological innovation, with the first use of timelapse sampling in Camera Trap Distance Sampling to estimate wildlife abundance. Time lapse was chosen as it was thought that, at a moderately high frequency (in our case every twenty minutes during daylight hours), it could survey a larger area at preset intervals than trigger images. In timelapse, a photo is taken a pre-determined interval, whereas triggered mode is constrained by animals triggering the sensors. Trigger distance typically has a field of view of less than 20m. We believed timelapse would be more appropriate as on testing we could "see" animals in images over 150 meters away. For a species at extremely low density, within a large area of an open habitat (like the desert), we predicted timelapse sampling would increase the yield of our camera traps. Our results support this conclusion; the majority of our captures were at approximately 100 meters and camels could reliably be identified at 300 meters, far past the detection range of a triggered camera. This novel technique allowed for data to be captured across the entirety of the GGASPA, and provided a robust estimate with relatively high precision. This method could be used for monitoring abundance in other species that live in low densities over a large range of open habitat.

6.8 Conclusions

The wild camel, with its small population, reduced genetic diversity and narrow range of specialised Gobi Desert habitat, is threatened with extinction. This thesis aimed to use genetic research and

timelapse CTDS methods to improve our understanding of the species in-situ and ex-situ. Working with an in-country team with expertise in the GGASPA environment, we were able to spread data collection systematically across the entire GGASPA, ensuring spatially-representative sampling of both camera trap and non-invasive genetic sample data. Using these data, we were able to determine one of the first precise abundance estimates for wild camel in the GGASPA. We were also able to determine the extent of hybridisation across the national park. All of which has improved our understanding of the threats facing the wild camel in Mongolia, both in the wild and in captivity. The data will continue to be used to inform further conservation management and to increase scientific and public awareness of the plight of the wild camel, with the overall aim of saving it from extinction.

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A wild camel bull in the GGASPA, retreating from AJ, JE and YA in the search of faecal samples.