

Biodiversity, Evolution and Conservation of Threatened Desert Ungulates

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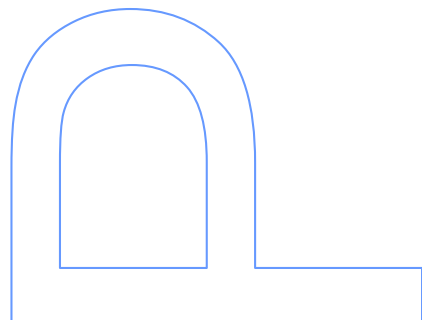
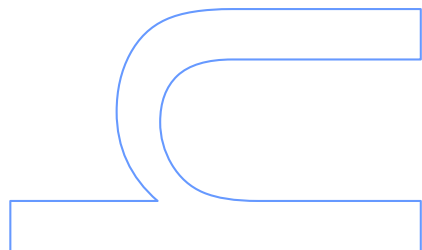
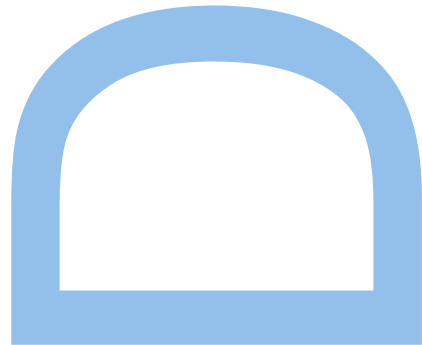
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Para ti, e por ti, meu Zé, vou encerrar este capítulo.*

Resumo

A biodiversidade está a diminuir à escala global como consequência da perda de habitat e das alterações climáticas. As actividades humanas de exploração nos desertos e regiões áridas estão a aumentar, assim como a aridez nessas regiões, as quais afectam negativamente os padrões locais de distribuição da biodiversidade. A maior parte do Norte de África é ocupada pelas ecorregiões Saara-Sael, que compõem o maior deserto quente do mundo e as regiões áridas vizinhas a sul. Múltiplas espécies no Saara-Sael estão listadas como ameaçadas pela Lista Vermelha de Espécies Ameaçadas da UICN (União Internacional para a Conservação da Natureza). Os ungulados desta região são representados por cinco gazelas, *Eudorcas rufifrons*, *Gazella cuvieri*, *G. dorcas*, *G. leptoceros* e *Nanger dama*, e três antílopes de grande porte, *Addax nasomaculatus*, *Ammotragus lervia* e *Oryx dammah*. Duas gazelas ameaçadas, *G. bennettii* e *G. subgutturosa*, estão presentes no Planalto Central Árido do Irão. A megafauna é um dos grupos de animais mais ameaçados na Terra, o qual experimenta fortes declínios no tamanho e distribuição das populações. A principal causa do declínio dos ungulados no deserto é a caça ilegal, mas alterações no coberto do solo/uso do solo, alterações climáticas, e aumento da extracção de recursos naturais, invasão agrícola e competição do gado também são motivos para o declínio.

O objectivo geral desta tese era contribuir para o planeamento da conservação de ungulados ameaçados, particularmente das gazelas do Norte de África. Este objectivo geral foi alcançado delineando quatro objectivos principais: i) aumentar os métodos moleculares disponíveis para a identificação genética de ungulados norte-africanos ameaçados sem a necessidade de amostragem invasiva; ii) esclarecer as relações filogenéticas entre gazelas norte-africanas ameaçadas e identificar áreas de potencial ocorrência; iii) estimar as relações entre as diversidades genéticas de 12 ungulados africanos e os padrões de regressão da distribuição observados e factores intrínsecos e extrínsecos; e iv) avaliar a estrutura genética de uma gazela asiática e os efeitos das características da paisagem nos padrões de fluxo genético.

Os principais resultados obtidos foram:

1. A amostragem não-invasiva constitui uma abordagem útil para obter informações ecológicas e genéticas essenciais para orientar as acções de conservação. O primeiro passo no planeamento da conservação é identificar com precisão as espécies. Foi desenvolvido um método molecular simples, de alta confiança e baixo custo, baseado

em polimorfismos em pequenos fragmentos do citocromo b mitocondrial e no gene nuclear da kappa caseína para identificar ungulados norte-africanos ameaçados de extinção. Estes fragmentos revelaram polimorfismos, que incluem a variação específica de cada espécie. Foi possível a identificação de nove espécies de ungulados que co-ocorrem no Norte de África. O método foi validado em mais de 1000 amostras, incluindo diferentes tipos de amostras não-invasivas colhidas no campo.

2. O planeamento da conservação de espécies ameaçadas depende de dados precisos sobre a sistemática, características ecológicas e habitats adequados. Dentro do género *Gazella*, a taxonomia e a sistemática não são muito claras. Apesar dos esforços de investigação feitos nos últimos anos, ainda há várias incertezas. Usando ferramentas moleculares e ecológicas, foram esclarecidas as relações filogenéticas no género *Gazella* e identificados ecótipos e unidades evolutivas significativas. *Gazella cuvieri* e *G. leptoceros loderi* compõem um único grupo monofilético, as populações destes taxa ocupam áreas geográficas distintas e ambientes específicos, e correspondem a ecótipos de montanha e de planície de *G. cuvieri*, respectivamente.

3. As diversidades genéticas de 12 ungulados africanos são mais baixas nas espécies que sofreram as maiores regressões na distribuição e foram principalmente relacionadas com variação ambiental e variáveis de pressão humana. A sobreposição do nicho ecológico realizado medido em distribuições históricas e presentes de 12 ungulados africanos com base em variáveis ambientais e variáveis de pressão humana foi baixa ou completamente inexistente, indicando fortes mudanças no nicho realizado ao longo do tempo. As populações existentes provavelmente ocupam os últimos redutos de habitat adequado, os quais retêm as mais altas diversidades genéticas observadas entre todos os taxa. As populações existentes ocorrem em condições ambientais mais severas e, portanto, podem ser vulneráveis aos períodos frequentes de seca que caracterizam o Norte de África.

4. No planalto central árido do Irão, a *Gazella subgutturosa* compreende três populações geneticamente homogêneas entre si, e há dispersão restrita entre elas. As distâncias geográficas foram relacionadas com a distância genética e detectaram-se efeitos reduzidos das barreiras antropogénicas na estrutura genética observada. A combinação de distância geográfica, resistência à dispersão pela paisagem e factores antropogénicos afecta a estrutura genética e o fluxo genético entre as populações de gazelas. A construção de estradas no futuro pode afectar a conectividade e o fluxo genético entre populações.

Nesta tese são disponibilizadas ferramentas promissoras para melhorar o conhecimento sobre a biologia de ungulados do Norte de África e, consequentemente, para a implementação de planos de gestão e conservação mais eficientes para estas espécies ameaçadas. No futuro, o planeamento da conservação dos ungulados deve considerar a preservação de ecótipos para ajudar a manter o potencial geral de adaptação das espécies. As medidas de conservação devem considerar também algumas populações isoladas como unidades de gestão separadas.

Palavras-chave: *Addax nasomaculatus*, *Ammotragus lervia*, Análise de datação Bayesiana, conservação, desertos, diversidade genética, ecótipos, *Eudorcas rufifrons*, *Eudorcas thomsonii*, fluxo genético, filogenia, *Gazella bennettii*, *Gazella cuvieri*, *Gazella dorcas*, *Gazella leptoceros*, *Gazella subgutturosa*, genética da paisagem, identificação de espécies, Irão, isolamento à distância, método molecular, modelação baseada no nicho ecológico, *Nanger dama*, *Nanger granti*, *Nanger soemmerringii*, *Oryx beisa*, *Oryx dammah*, regressão da distribuição, Saara, Sael, sistemas de informação geográfica, ungulados.

Abstract

Biodiversity is declining at a global level as a consequence of habitat loss and climate change. Human exploitation activities in deserts and arid regions are increasing, and the progressive aridity conditions experienced in these regions are negatively affecting local biodiversity distribution patterns. Most of North Africa is occupied by the Sahara-Sahel ecoregions that comprise the world's largest warm desert and the neighbouring arid regions to the south. Multiple Sahara-Sahel species are listed as threatened by the IUCN Red List of Threatened Species. In this region, ungulates are represented by five gazelles, *Eudorcas rufifrons*, *Gazella cuvieri*, *G. dorcas*, *G. leptoceros*, and *Nanger dama*, and three large-sized ungulates: *Addax nasomaculatus*, *Ammotragus lervia*, and *Oryx dammah*. Two threatened gazelles, *G. bennettii* and *G. subgutturosa*, are present in the Central Arid Plateaux of Iran. The megafauna is one of the most threatened groups of animals on Earth, experiencing strong declines in population size and range. The main cause for the decline of desert ungulates throughout their ranges is illegal hunting, but changes in land-cover/land-use, climate change, and increasing natural resources extraction, agricultural encroachment and competition from livestock are also reasons for the decline.

The general aim of this thesis was to contribute for the conservation planning of threatened ungulates, particularly of North African gazelles. This aim was achieved by delineating four main objectives: i) increasing the available molecular methods for genetic identification of endangered North African ungulates without the need of invasive sampling; ii) clarifying the phylogenetic relationships between threatened North African gazelles and identifying potential occurrence areas; iii) understading relationships between the genetic diversities of 12 African ungulates and their observed range regression patterns and intrinsic and extrinsic factors; and iv) evaluating the genetic structure of an Asian gazelle and the effects of landscape features on gene flow patterns.

The main results found were:

1. Noninvasive sampling provides a useful approach to obtain ecological and genetic information essential to guide conservation actions. The very first step in conservation planning is to accurately identify species. We developed a simple, high reliable and low cost molecular method based on polymorphisms in small fragments of the mitochondrial cytochrome b and the nuclear kappa casein gene for identifying endangered North African ungulates. These fragments revealed polymorphisms, including species-specific

variation, which allowed species identification of nine ungulate species that co-occur in North Africa. The method was validated across more than 1000 samples, including different types of noninvasive samples collected in the field.

2. Conservation planning of threatened taxa relies upon accurate data on systematics, ecological traits and suitable habitats. Within the genus *Gazella*, taxonomy and systematics are not very clear so far. Despite the research efforts done in the last few years, there are still several uncertainties. Using molecular and ecological tools, we clarified phylogenetic relationships in the genus *Gazella*, and identified ecotypes and evolutionary significant units. *Gazella cuvieri* and *G. leptoceros loderi* comprise a single monophyletic group; the populations of these taxa occupy distinct geographic areas and specific environments, and they correspond to mountain and lowland ecotypes of *G. cuvieri*, respectively.

3. The genetic diversity of 12 African ungulates are the lowest in the species that have suffered the largest range regressions and were mostly related with environmental variation and human pressure variables. The measured realized ecological niche overlap of historical and present distributions of 12 African ungulates based in environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time. Extant populations are likely occupying the last strongholds of suitable habitats, which are retaining the highest observed genetic diversities among all taxa. Extant populations occur under harsher environmental conditions, and thus may be vulnerable to the frequent drought periods that characterise North Africa.

4. *Gazella subgutturosa* in the Central Arid Plateaux of Iran comprises three genetically homogeneous populations and there is restricted dispersal between populations. Geographic distances were found to be related with genetic distance and there were low effects of anthropogenic barriers on observed genetic structure. A combination of geographic distance, landscape resistance, and anthropogenic factors are affecting the genetic structure and gene flow of gazelle populations. Future road construction might impede connectivity and gene exchange of populations.

Evidence of promising tools to improve knowledge on the biology of North African ungulates and consequently, the implementation of more efficient management and conservation plans for these threatened ungulates, are provided in this thesis. In the future, conservation planning of ungulates should consider the preservation of ecotypes

to help maintaining the overall adaptive potential of the species. Conservation measures should consider also some isolated population as separate management units.

Keywords: *Addax nasomaculatus*, *Ammotragus lervia*, Bayesian dating analysis, conservation, deserts, ecological niche-based modelling, ecotypes, genetics, *Eudorcas rufifrons*, *Eudorcas thomsonii*, *Gazella bennettii*, *Gazella cuvieri*, *Gazella dorcas*, *Gazella leptoceros*, *Gazella subgutturosa*, gene flow, genetic diversity, geographical information systems, Iran, isolation by distance, landscape genetics, molecular method, *Nanger dama*, *Nanger granti*, *Nanger soemmerringii*, *Oryx beisa*, *Oryx dammah*, range regression, phylogeny, Sahara, Sahel, species ID, ungulates.

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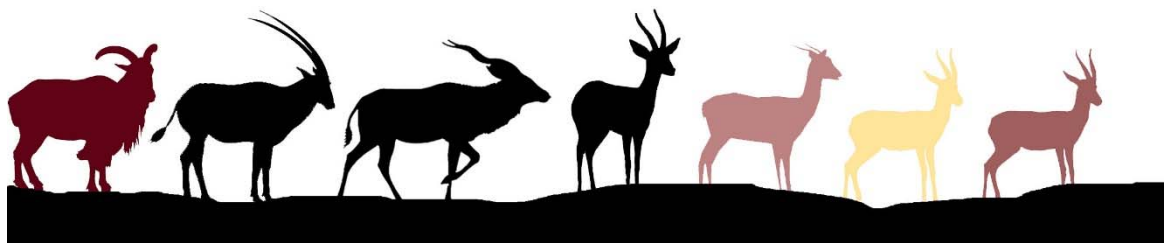
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List of abbreviations (Lista de abreviaturas)

Addax	<i>Addax nasomaculatus</i>
AL	<i>Ammotragus lervia</i>
AUC	Area Under the Curve
BE	Belgium
BI	Bayesian Inference
BIC	Bayesian information criterion
bss	Bootstrap support
COI	Cytochrome c oxidase I
CP	<i>Capra sp</i>
CYTB	Cytochrome b
DE	Germany
DNA	Deoxyribonucleic acid
DZ	Algeria
ENM	Ecological Niche-based Model
ER	<i>Eudorcas rufifrons</i>
ES	Spain
FST	Fixation index FST
GC	<i>Gazella cuvieri</i>
GC_GL	<i>Gazella cuvieri</i> and <i>Gazella leptoceros</i> group
GD	<i>Gazella dorcas</i>
GIS	Geographic Information System
GL	<i>Gazella leptoceros</i>
GPS	Global Positioning System
HWE	Hardy-Weinberg Equilibrium
IUCN	International Union for the Conservation of Nature
KCAS	Kappa casein
LAC	α -lactalbumin intron 2
LD	Linkage Disequilibrium
MA	Morocco
MAXENT	Maximum Entropy
MCMC	Markov-Chain Monte Carlo
ML	Maximum Likelihood
MR	Mauritania
mtDNA	Mitochondrial DNA
ND	<i>Nanger dama</i>
NE	Niger
NN	Nearest Neighbour
Oryx	<i>Oryx dammah</i>
OV	<i>Ovis sp.</i>
PCA	Principal Component Analysis
pp	Posterior probability
PRKCI	Protein kinase C iota
PT	Portugal
ROC	receiver-operating characteristics
SD	Standard Deviation
SDM	Species Distribution Model
SE	Standard Error

SN	Senegal
SPTBN1	b-spectrin nonerythrocytic 1
THYR	Thyroptin beta chain
TD	Chad
TN	Tunisia



CHAPTER 1

Introduction

1.1 Biodiversity in Deserts and Arid Regions

Biodiversity, used in the broad sense as defined in the Convention on Biological Diversity (Leadley et al. 2010), means abundance, distribution and interactions among genotypes, species, communities, ecosystems and biomes. At the global level, biodiversity is declining as a consequence of habitat loss and climate change (Pimm, 2008). The current challenges to halt biodiversity decline are: i) increase knowledge about species diversity and distribution (Whittaker et al., 2005); ii) detect the ecological and evolutionary processes behind the observed biodiversity patterns (Crandall et al., 2000); and iii) systematise biodiversity conservation planning in order to preserve both biodiversity patterns and processes (Margules & Pressey, 2000).

Deserts and arid regions (Figure 1.1) can be defined by the aridity index (average annual precipitation / potential evapo-transpiration): aridity index < 0.05 in deserts, and aridity index between 0.05 and 0.20 in arid and semi-arid regions (Ward, 2016). These regions are generally understood as bare and rather homogeneous areas, exhibiting low diversity levels in comparison to other regions, which results in receiving less scientific attention and funding allocated to conservation (Durant et al., 2012; Waldron et al., 2013).

Deserts and arid regions allow examining the effects of extreme environments on the distribution of biodiversity patterns (Ward, 2016). As a consequence, deserts and arid regions usually exhibit: i) patchily distributed species, whose distribution limits are under strong environmental control; ii) relatively high rates of endemism due to the adaptive processes of organisms to the extreme environmental conditions that characterise these regions; iii) locally endangered micro-hotspots of biodiversity; and iv) climatic extremes that allow generating sharp ecological gradients (Dumont, 1982; Schulz et al., 2009; Davies et al., 2012; Murphy et al., 2012; Wilson & Pitts, 2012).

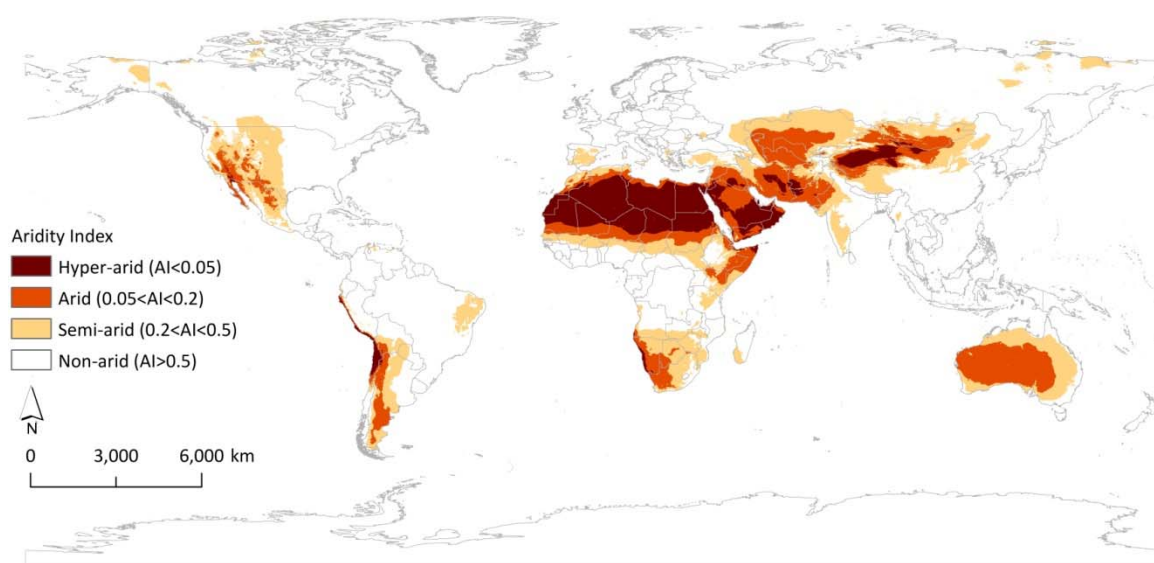


Figure 1.1. Global distribution of deserts (hyper-arid) and arid regions, following the Aridity Index (Ward, 2016).

Human exploitation activities in deserts and arid regions are increasing at the global level, and the progressive aridity conditions experienced in these regions are negatively affecting desert biodiversity patterns and also increasing poverty and the frequency of armed conflicts (McNeely, 2003; UNEP, 2006; Thorton et al., 2008; Trape, 2009; Brito et al., 2014, 2016, 2018).

Climate change is expected to force the distribution of species to higher latitudes, leading to extinctions of species whose future climate potential habitable space passes very small or very isolated from their present geographical boundaries (Chen et al. 2011). The magnitude and velocity of climate change predicted for deserts and arid regions at the global level are strong and fast (Loarie et al., 2009). These predicted changes are the reason of growing international awareness for desert biodiversity (McNeely, 2003; UNEP,

2006; Newby, 2007; Ward, 2016; Davies et al., 2012; Durant et al., 2012; Brito et al., 2014, 2016, 2018).

1.2 The Sahara-Sahel ecoregions of Africa

The Sahara Desert and the neighbouring arid Sahel constitute two major terrestrial ecoregions of the African continent (Dinerstein et al., 2017). Together, they exhibit features that make them unique in comparison with other world deserts and arid regions (Brito et al., 2014; OECD-SWAC, 2014; Figure 1.2). First, the Sahara is the largest warm desert in the world with land coverage of about 11,230,000 km², including the Sahel, which is an area larger than the Australian continent. Secondly, there is high diversity of topographic features in the Sahara-Sahel, from salt pans below sea level to high-altitude peaks (from -155 m at Lake Assal, Djibouti, to 3,415 m at Emi Koussi, Chad). These mountain tops are distributed along a system of “mountain-sky islands” (UNEP, 2006). Thirdly, climatic conditions are heterogeneous and in particular there is significant spatial variability in temperature (average annual temperature ranging from 9.4 to 30.8 °C; Hijmans et al., 2005) and rainfall (average annual total precipitation up to 981 mm; Hijmans et al., 2005). Fourthly, the limit between the Sahara and the Sahel constitutes the transition line between the Palaearctic and Afro-Tropical biogeographic realms (Holt et al., 2013; Dinerstein et al., 2017), which results in latitudinal variations in species distributions and in localised concentrations of species from distinct biogeographic origins that form local biodiversity hotspots (Dumont, 1982; Le Houérou, 1992).

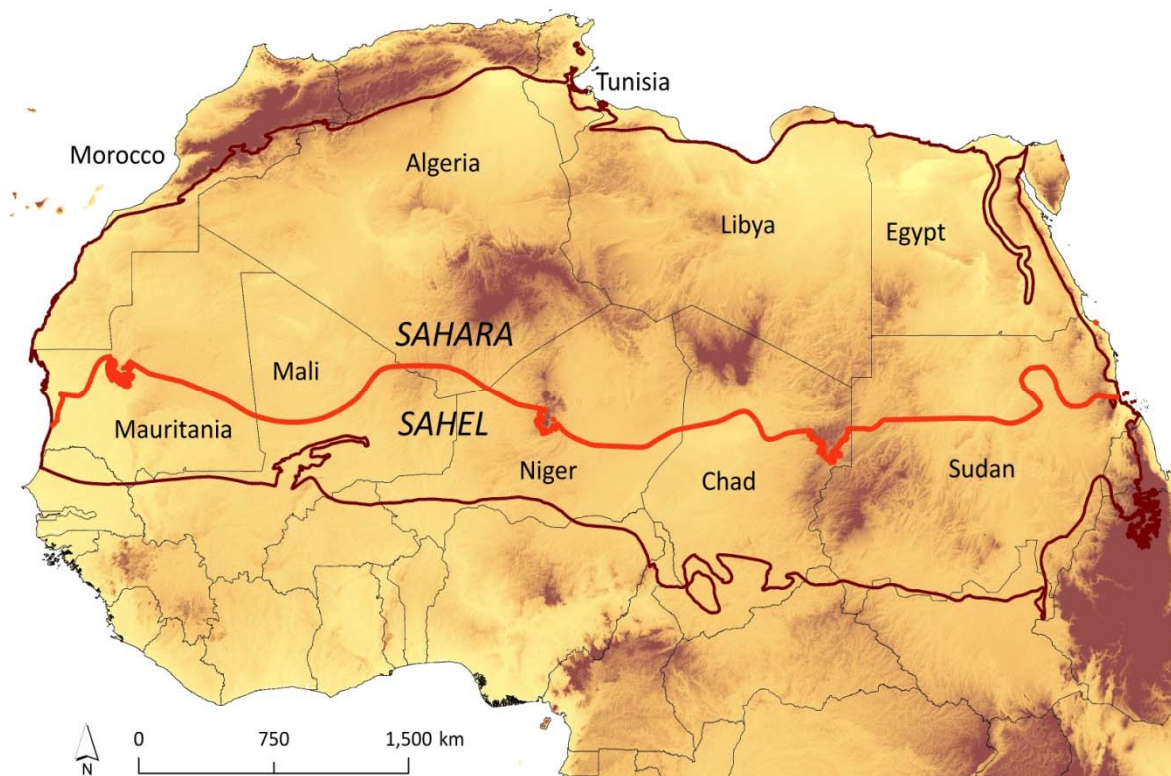


Figure 1.2. The Sahara-Sahel. Limits of the Sahara-Sahel (in red), following Dinerstein et al. (2017). Elevation is represented as a relief shading of brown gradient.

Fifthly, the Sahara-Sahel ranges over 15 countries and many are rated as low development (UNDP, 2010) and usually subjected to long-term political instability (Brito et al., 2014, 2018). Sixthly, the onset of desert conditions in the area currently covered by the Sahara was estimated as rather recently, at about 7 million years ago (Mya) in Chad (Schuster et al., 2006) or around 6–2.5 Mya in western areas (Swezey, 2009). Finally, and perhaps most importantly, there were strong climatic oscillations in the Sahara-Sahel, with feedback mechanisms between decreases in rainfall regimes and vegetation cover (Wang et al., 2008; Claussen, 2009). These climate and land-cover oscillations have greatly shifted the Sahara-Sahel limits, and contributed to the biodiversity distribution patterns observed in the present day (Dumont, 1982; Le Hou  rou, 1992, 1997; Drake et al., 2011).

1.3 The Central Arid Plateaux of Iran

The Central Arid Plateaux of Iran designates in broad sense an area of Central Iran that exhibits hyper-arid and arid conditions. The arid central plateaux encompasses about 363,000 km² and range over three administrative provinces: Esfahan, Kerman, and Yazd (Figure 1.3). The elevation varies from 117 to 4,429 m and the mean annual temperature ranges from 11 to 27°C (Hijmans et al., 2005). The Alborz mountain range from the north and Zagros mountain range from the northwest to southwest form a barrier that prevents air humidity to reach the central plateaux. Consequently, rainfall levels are low and range from 51 to 329 mm/year (Hijmans et al., 2005). Most of the arid central plateaux is covered by three terrestrial ecoregions: the Central Persian desert basins, the South Iran Nubio-Sindian desert and semi-desert, and the Kuh Rud and Eastern Iran montane woodlands (Dinerstein et al., 2017).

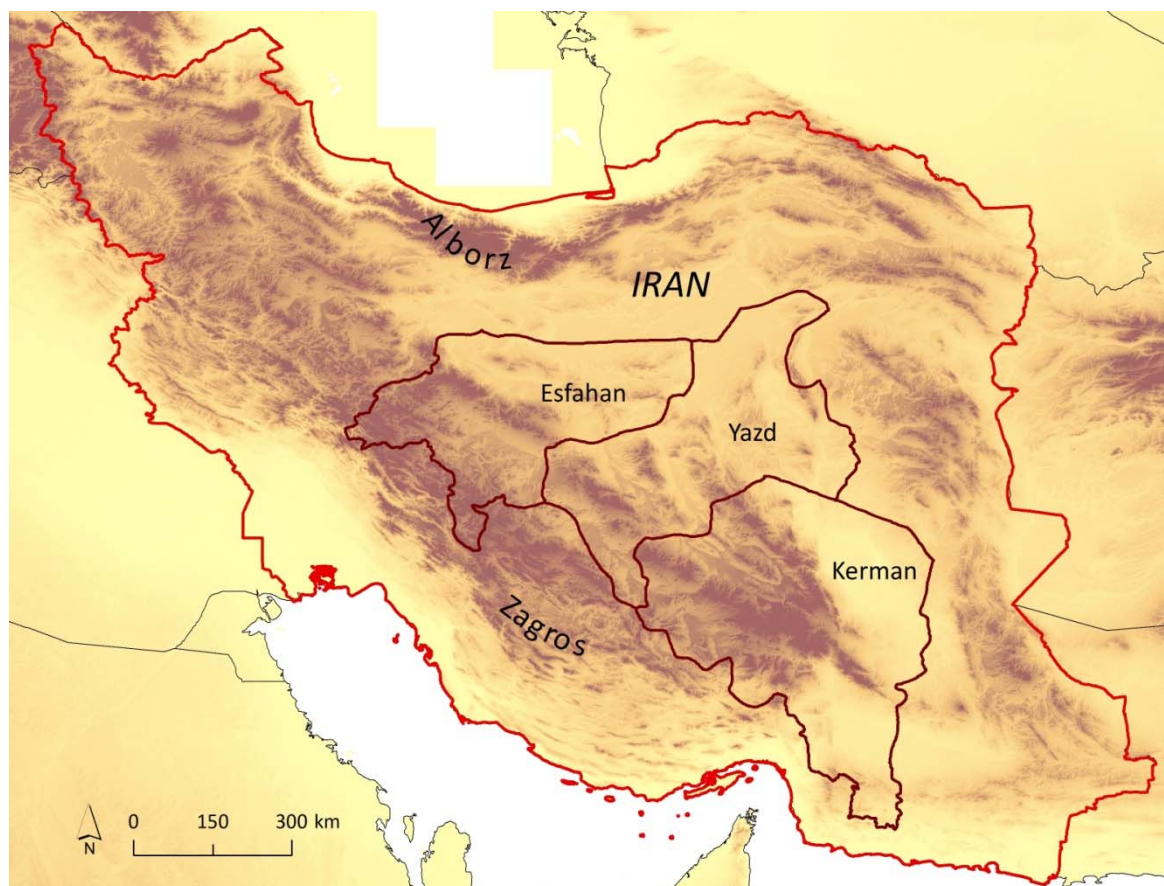


Figure 1.3. The Central Arid Plateaux of Iran. Limits of the plateaux (in red) follow the administrative limits of the provinces of Esfahan, Kerman, and Yazd. Elevation is represented as a relief shading of brown gradient.

1.4 Biodiversity conservation status: the case of ungulates

In the Sahara-Sahel, biodiversity is presently threatened by the synergistic effects of multiple human pressures, land-cover/land-use change, and climate change (Brito et al., 2014, 2016, 2018; Newby et al., 2016). The widespread use of four-wheel-drive vehicles and firearms increased dramatically the extent and impact of hunting activities (Valverde, 1957; Newby, 1980). Over-hunting has driven to local extinction several large mammals, such as *Giraffa camelopardalis* (Ciofolo, 1995), *Acinonyx jubatus* (Saleh et al., 2001), *Oryx dammah* (Beudels et al., 2005) and *Panthera leo* (Barnett et al., 2006). The growing demands for natural resources have stimulated regional mineral exploitation, which in turn has further contributed to greater accessibility and over-hunting, particularly of *Addax nasomaculatus* (Duncan et al. 2014; Newby et al., 2016; Brito et al., 2018). The escalating conflicts observed in the Sahara-Sahel since 2011 are also causing population declines in ungulates, particularly *Addax nasomaculatus* and *Gazella dorcas* (Brito et al., 2018). In a review on the conservation status of large-sized vertebrates (Durant et al. 2014), of the 14 species assessed, 12 have been listed as Extinct in the Wild or as Globally threatened with extinction (Figure 1.4). From the seven ungulates occurring in the Sahara-Sahel, two are Critically Endangered, two are Endangered, and three are Vulnerable (Table 1.1).

To counteract current negative trends, multiple organisations are developing reintroductions and population monitoring of endangered ungulates (*Oryx dammah*, *Addax nasomaculatus*, and *Nanger dama mhorri*) in Algeria, Chad, Niger, Senegal and Tunisia (Abáigar et al., 1997; Newby et al., 2016). Local protected areas were established (e.g. reintroduction facilities for ungulates of Safia, Morocco) aiming to maintain overall genetic diversity of endangered ungulates (Senn et al. 2014).

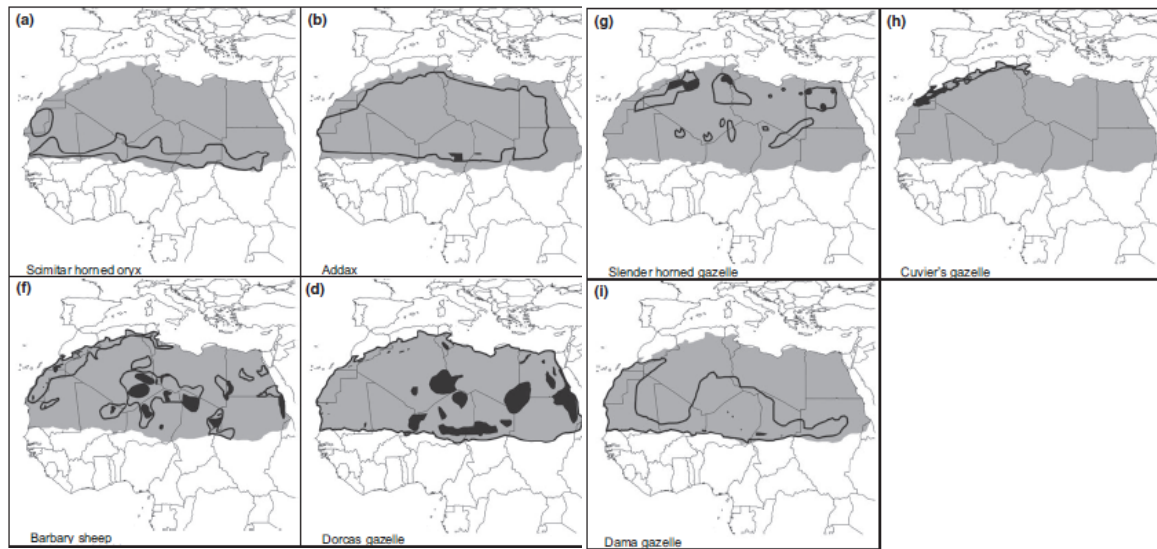


Figure 1.4. Maps of range loss for seven Sahara-Sahel ungulates (adapted from Durant et al., 2014).

Over the last 30 years, the land-cover/land-use change and hunting activities have dramatically reduced the formerly abundant populations of *Gazella bennettii* and *Gazella subgutturosa* in the Central Arid Plateaux of Iran (Mallon, 2008a,b). While the former is considered as Least Concern, the latter categorizes as Vulnerable (Table 1.1). Both are currently mostly restricted to Protected Areas, due to two contemporaneous decline periods: the emergence and dissemination of firearms and off-road vehicles in the late 1940s/early 1950s; and the collapse of Iranian law enforcement agencies after the Islamic revolution of 1979. The following restructuring environmental agencies during the 1980s controlled illegal hunting and some gazelle populations were allowed to recover, mostly within protected areas (Hemami and Groves, 2001). In addition, regular droughts, competition with livestock, and general degradation of habitat quality have also contributed to population decline (Zachos et al., 2010).

Table 1.1. Overview of conservation status and extinction risk of threatened ungulates in the Sahara-Sahel and the Central Arid Plateaux of Iran. IUCN Red List status are: CR -Critically Endangered; EN - Endangered; VU - Vulnerable. Adapted from Mallon (2008b) and Brito et al. (2018).

Species	Scientific name	IUCN status	Conservation status and exposure to extinction risk threats
<i>Sahara-Sahel</i>			
Addax	<i>Addax nasomaculatus</i> (de Blainville, 1816)	CR	Restricted to 1-4 wild populations. Population in Niger currently undergoing major collapse and likely to go extinct due to illegal killing associated to natural resources exploitation activities and human migration routes. Recently rediscovered in Chad (15-30 individuals in Eguey dunes and a larger population in bordering areas with Niger). Unknown status in Mauritania. Suffered extreme range loss in the Sahara (99%)
Dama gazelle	<i>Nanger dama</i> (Pallas, 1766)	CR	250 individuals or less known from three disconnected areas in Niger and Chad. Unknown status in Mali. One subspecies Extinct in the Wild (<i>N. dama mhorri</i>). Suffered extreme range loss in the Sahara (99%). Illegal killing in Niger forced range shifts to inaccessible and low productivity habitats where survival is uncertain
Cuvier's gazelle	<i>Gazella cuvieri</i> (Ogilby, 1841)	VU	The species is threatened by overhunting and habitat degradation, mainly due to the transformation of forests into cropland and pastures for livestock.
Slender-horned gazelle	<i>Gazella leptoceros</i> (Cuvier, 1842)	EN	Patchy distribution restricted to sandy areas. Current population size in Egypt, Libya, Algeria and Tunisia is unknown but there is population decline due to illegal killing. Suffered extensive range loss in the Sahara (86%)
Barbary sheep	<i>Ammotragus lervia</i> (Pallas, 1777)	VU	Isolated in remote mountain areas. Suffered strong decline due to illegal killing and competition from domestic stock. Population status is unclear. Low numbers are reported from Algeria, Chad, Mauritania, Mali and southern Morocco, and population decline is documented in Niger. Status is unknown in Libya, Egypt and Sudan

Red-fronted gazelle	<i>Eudorcas rufifrons</i> (Gray, 1846)	VU	Elusive species with poorly known status in the area. Most of the original range has been affected by human development activities. In Senegal, it is known from small scattered populations (Djoudj N.P., Ferlo Nord Fauna Reserve, Boundou reserve). Extinct from northern Burkina-Faso
Dorcas gazelle	<i>Gazella dorcas</i> (Linnaeus, 1758)	VU	Suffered the most extensive and intensive massacres, providing the most frequent example of illegal killing. It has been extirpated from large areas across Morocco and Mauritania. Conservation status is likely to change to Endangered if current illegal killing levels are maintained. Suffered extensive range loss in the Sahara (86%). The largest densities in the Sahara are found in the Termit & Tin-Toumma National Nature Reserve in Niger and the Ouadi Rimé–Ouadi Achim Game Reserve in Chad
<i>Central Arid Plateaux of Iran</i>			
Goitered gazelle	<i>Gazella subgutturosa</i> (Güldenstädt, 1780)	VU	In Iran, it suffered extensive declines in population size and range. Currently, it is almost entirely restricted to protected areas, surrounded by dense human populated places and road networks. Population was estimated in about 20,000 individuals, virtually all in protected areas. The main threats are illegal hunting and habitat loss

1.5 Conservation genetics

Conservation genetics aims to understand the dynamics of genes in populations with the objective of avoiding extinction (Frankham et al., 2002). Genetic diversity influences both the health and long-term survival of populations, given that decreases have been associated with reduced fitness traits, including high juvenile mortality rates, diminished population growth rates, reduced immunity to pathogens, and ultimately, higher extinction risk (Lande, 1988; Wayne & Morin, 2004; Frankham, 2005). The developments in the fields of molecular biology and ecology provide now a vast collection of tools for genetic and spatial analyses (Carstens, 2008; Thomassen et al., 2010). These tools can assist in resolving a range of contemporary conservation problems, including halting current biodiversity loss, and are briefly addressed in the following subsections.

1.5.1 Non-invasive sampling

Non-invasive genetic sampling was first used in wild animals in the 1990s (Hoss et al., 1992; Taberlet et al., 1997). The main advantage of non-invasive genetics is that it allows studying many individuals and populations without contacting, disturbing, or even seeing the organisms. Samples collected noninvasively are commonly faeces, hairs, sloughed skin, and skulls (Beja-Pereira et al., 2009). The largest contributions of non-invasive approaches are, for example, on studies focusing on the identification of species or individuals, and wildlife populations of species that may already be extinct (Beja-Pereira et al., 2009).

Non-invasive techniques provide good enough DNA and low genotyping error rates that allow addressing nearly all research questions that can be addressed using traditional high-quality samples, such as blood (e.g. Maudet et al., 2004; Perry et al., 2010). Non-invasive techniques also allow analysing more *loci* and more samples in comparison to traditional techniques. For example, it is highly feasible to estimate relatedness, infer parentage and reconstruct pedigrees in natural populations, for which usually many *loci* and low genotyping error rates are required.

The application of non-invasive genetic approaches in molecular ecology and conservation genetics' studies is continuously increasing (Rowe, et al., 2017). Nonetheless, non-invasive genetic studies still require more funding and efforts in the laboratory, compared with traditional genetic studies with high-quality DNA, to ensure

low genotyping error rates. Monitoring the efficacy and error rates associated with each of the multiple steps in a non-invasive approach is crucial to ensure success. One of the greatest needs for additional research is to directly compare the relative performance of new and improved methods in multiple independent laboratories, taxa, and sample types. For instance, the effects of distinct conditions of sample storage, DNA extraction and amplification should be accurately tested. The lack of independent and quantitative comparisons of the efficiency of different techniques makes it difficult to provide advice on which methods are the best for a given species, sample type and sample storage conditions (Waits & Paetkau, 2005). Some techniques might be species-specific and environment dependent.

1.5.2 Phylogenetic studies and DNA barcoding

Because the advances in sequencing and computational technologies, DNA sequences have become the major source of new information for advancing our understanding of evolutionary and genetic relationships (Reuter et al., 2016). Phylogenetics and population genetics are disciplines that have been developing the tools and applications employed to assess biological relationships with DNA sequences (Ajmal et al., 2014). These disciplines focus on different levels of organization. Phylogenetic studies deal with evolutionary relationships among species, whereas those in population genetics target variation within and among populations of a single species.

DNA barcoding provides means to understand local species diversity and evaluate intra-specific variability (Krishnamurthy & Francis, 2012). DNA barcoding uses molecular markers to amplify short and highly variable DNA sequences (Hebert et al., 2003), allowing species identification by comparison of similarities of sequenced barcodes with reference libraries (Hebert & Gregory, 2005). The operational criteria to assure informative taxonomical identification are (CBOL, 2004): 1) a single gene of roughly 600 base pairs, cytochrome c oxidase I (COI) in the 5' end of mitochondrial DNA (mtDNA), is sequenced and used as a barcode; 2) barcode, i.e. the same region of the same gene, is used universally in order to develop standardised protocols; and 3) the obtained sequences are analysed with distance-based approaches to identify specimens and hence their taxon (Savolainen et al., 2005; Rubinoff, 2006).

1.5.3 Population genetics

Population genetics is the discipline studying the genetic diversity and structuring of populations and the processes governing them, including changes in allele frequencies over time, and further embracing the aspects of quantitative genetics (Habel et al., 2015). As an integral part of theoretical and evolutionary biology (Frankham, 2003), classic population genetic data has many limitations. The potential of combining molecular data with additional environmental, ecological and biological datasets in multidisciplinary approaches has been highlighted (Habel et al., 2015). Indeed, the combination of datasets from various fields allows a more comprehensive understanding of extrinsic and intrinsic evolutionary processes affecting populations, such as the distinction between genetic drift and natural selection. The integration of population size estimates and demographic dynamics as well as individual behaviour (embracing aspects like dispersal behaviour) allows testing of adaptations to local environments and understanding time-lags frequently observed in molecular data. Modelling approaches are integrated at increasing rates into population genetic research to identify potential barriers and corridors (Habel et al., 2015).

1.5.4 Ecological niche-based modelling and landscape genetics

The developments in Geographical Information Systems (GIS) and their application to conservation biology allows overcoming the biases and gaps in the current knowledge of biodiversity distribution and available datasets (Yackulic et al., 2013). Inferential procedures that provide robust and reliable predictions of the geographic distributions of species are critical to the planning of biodiversity conservation (Guisan et al., 2013). Models based on the realised ecological niches of species are developed using multiple modelling techniques, including machine learning, neural networks or regression-based modelling (Elith & Leathwick, 2009; Franklin & Miller, 2009; Broennimann et al., 2012). The modelled relationships between the environmental characteristics of localities where target species are present are then projected onto the relevant spatial environmental layers to predict the potential distribution of the target species. Synthetic analyses based on primary point occurrence data (presence–absence) are the ones most frequently used

because they require fewer assumptions and inferences can be made about clearly defined parameters, such as occurrence probability (Peterson, 2001).

The integration of molecular data with GIS-based environmental data and ecological modelling tools can transform evolutionary studies, and are providing increasingly new insights into the ecological causes of evolutionary patterns (Kozak et al., 2008; Alvarado-Serrano et al., 2014). In particular, landscape genetics aims to inform on the interactions between landscape features and evolutionary processes, mainly gene flow and selection (Manel et al., 2003; Manel & Holderegger, 2013), being thus especially suitable to use in conservation genetics studies (Holderegger & Wagner, 2006).

1.6 Setting of the present work

In spite of an increasing body of molecular tools available for species diagnosis, there is still a huge knowledge gap regarding the genetic identification of North African ungulates. Most of these species are threatened with extinction, and thus molecular tools based on non-invasive sampling are needed to obtain ecological and genetic information essential to guide conservation actions. Simple, reliable, and low cost tools are required to improve the implementation of efficient management and conservation plans for these threatened ungulates.

Given the remoteness of the Sahara-Sahel and the sampling difficulties associated with megafauna, there are still knowledge gaps in the phylogenetic relationships of threatened gazelles in North Africa. For instance, *Gazella cuvieri* and *G. leptoceros loderi* share morphological and physiological characters, but the former is darker and found in mountain areas, while the latter is lighter and associated to sand dunes, and available mitochondrial DNA data show no genetic divergence between these two sister-species. Given the limitation of mtDNA markers alone in species delineations, the inclusion of nuclear markers to clarify the evolutionary histories and systematics, is needed. These data will help in optimizing local conservation planning.

Although many African ungulates have suffered significant declines in population size and range, there is still a poor understanding of relationships between estimates of genetic diversity and intrinsic and extrinsic factors. Distinct biological and geographic traits, environmental factors and human pressure variables may be distinctly related to genetic diversities in African ungulates. We need to better understand the possible

effects of range regression, latitudinal distribution, biological traits, and environmental variables and human factors that can operate at the genetic diversity level, and how range regression may impact over the realized ecological niche according to environmental variation and human pressure variables.

Despite *Gazella subgutturosa* is known to be mostly distributed in protected areas of the Central Arid Plateaux of Iran, it is still unknown if gene flow occurs between the putatively isolated populations, nor the potential effects of landscape factors on the genetic structure of these gazelle populations. Most likely, geographic distances should have a great influence on gene flow among populations. However, the recent population fragmentation and likely isolation may be associated with the current landscape resistance distances to dispersal and these may have affected the genetic structure of the populations. The understanding of the role of landscape features on the genetic structure and gene flow of populations will be key to disclose the potential dispersal among protected areas, and contribute to a better management of subpopulations.

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CHAPTER 2

Objectives and thesis structure



2.1 General objectives

The general aim of this thesis was to contribute for the conservation planning of threatened ungulates, particularly of North African gazelles. This aim was achieved by delineating four main objectives: i) increasing the available molecular methods for genetic identification of endangered North African ungulates without the need of invasive sampling; ii) clarifying the phylogenetic relationships between threatened North African gazelles and identifying potential occurrence areas; iii) understading relationships between the genetic diversities of 12 African ungulates and their observed range regression patterns and intrinsic and extrinsic factors; and iv) evaluating the genetic structure of an Asian gazelle and the effects of landscape features on gene flow patterns.

2.2 Detailed objectives and thesis structure

This thesis is organized in seven chapters. In the Chapter 1, I provide a general introduction to the thesis, which explores the main biodiversity patterns in deserts and arid regions, presents the Sahara-Sahel and the Central Arid Plateaux of Iran as study areas, with particular traits that turn them interesting to the develop the current thesis, the threatened character of the current conservation status of ungulates in these two study areas, and a brief summary of the major tools and techniques currently available to address conservation genetics studies.

In the current Chapter 2, I provide the objectives and the structure of the thesis.

In the Chapter 3, my colleagues and I developed a molecular method based on polymorphisms in small fragments of the mitochondrial cytochrome b and the nuclear kappa casein genes for identifying endangered North African ungulates. We found that these fragments reveal polymorphisms, including species-specific variation, which allows species identification of the nine ungulate species that co-occur in North Africa. The method was validated across more than 400 samples, including different types of noninvasive samples collected in the field. These findings are published in the following manuscript:

Silva, T.L., Godinho, R., Castro, D., Abáigar, T., Brito, J.C., & Alves, P.C. (2015). Genetic identification of endangered North African ungulates using noninvasive sampling. *Molecular Ecology Resources*, 15: 652–661.

In the Chapter 4, we explored the genetic distinctiveness of *Gazella cuvieri* and *Gazella leptoceros loderi* to characterize their ecological niches and to identify potential occurrence areas across North-West Africa. We found that: i) both taxa comprise a single monophyletic group; ii) the populations of these taxa occupy distinct geographic areas and specific environments; iii) the predicted areas of sympatry were restricted, as a consequence of local sharp transitions in climatic traits. These findings are expressed in the following manuscript:

Silva, T.L., Vale, C.G., Godinho, R., Fellous, A., Hingrat, Y., Alves, P.C., Abáigar, T., Brito, J.C. (2017). Ecotypes and evolutionary significant units in endangered North African gazelles. *Biological Journal of the Linnean Society*, 8: 119–129.

In the Chapter 5, we related the genetic diversities of 12 African ungulates with their geographic and biological traits, and environmental conditions in historical and present distributions, in order to test hypotheses of relationships between genetic diversity levels and range regression and intrinsic/extrinsic factors. We found that: i) genetic diversities are the lowest in the species that have suffered the largest range regressions; ii) genetic diversities were mostly related with environmental variation and human pressure variables and iii) the measured realized ecological niche overlap of historical and present distributions based in environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time. These findings are compiled in a manuscript currently under preparation:

Silva, T.L., Vale, C.G., Abáigar, T., & Brito, J.C. Range regression in threatened African ungulates: implications in genetic diversity and ecological niche. In prep.

In the Chapter 6, we used a landscape genetics approach to evaluate the genetic structure of *Gazella subgutturosa* in Central Iran and the effects of landscape features on gene flow. We found that: i) there are three genetically homogeneous groups and there is restricted dispersal in analysed six populations; ii) there is decreasing relatedness with increasing distance; iii) genetic structure is affected by landscape composition; and iv) there are low effects of anthropogenic barriers on observed genetic structure. These findings are presented in the following manuscript:

Khosravi, R., Hemami, M.-R., Malekian, M., Silva, T.L., Rezaei, H.-R., & Brito, J.C. (2018). Effect of landscape features on genetic structure of the goitered gazelle (*Gazella subgutturosa*) in Central Iran. *Conservation Genetics*, 19: 323-336.

Finally, in Chapter 7, I provide a general discussion, focusing on the key findings of this thesis and their impact on the understanding of the evolutionary history, biogeography and conservation of threatened gazelles.



CHAPTER 3

DNA barcoding and noninvasive sampling

Paper I Genetic identification of endangered North African ungulates using noninvasive sampling

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Genetic identification of endangered North African ungulates using noninvasive sampling

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Abstract

North African ungulates include several threatened and emblematic species, yet are poorly studied mainly due to their remoteness and elusiveness. Noninvasive sampling provides a useful approach to obtain ecological and genetic information essential to guide conservation actions. The very first and most important step in conservation planning is to accurately identify species, and molecular genetics has been proved to be a useful tool. Several molecular genetics protocols are available for species identification, even for samples with poor quality DNA, such as faeces, hairs or bones. Most of these protocols use mitochondrial DNA for barcoding despite this marker being especially prone to problems, including mtDNA introgression, nuclear insert copies, high intraspecific diversity or heteroplasmy. In this work, we developed a molecular method based on polymorphisms in small fragments of the mitochondrial cytochrome b (cytb, mtDNA) and the nuclear kappa casein genes (KCAS, nDNA) for identifying endangered North African ungulates. These fragments revealed polymorphisms, including species-specific variation, which allowed species identification of nine ungulate species that co-occur in North Africa. The method was validated across more than 400 samples, including different types of noninvasive samples collected in the field. The simplicity, high reliability and relative low cost of the described method make it a promising tool to improve ecological studies of the North African ungulates and consequently, the implementation of more efficient management and conservation plans for these endangered ungulates.

Keywords: conservation genetics, cytochrome b, deserts, gazelles, kappa casein, molecular method, species ID

Introduction

Accurate species identification is an essential tool in many disciplines, including conservation biology, ecology and forensics sciences (Long *et al.* 2008; Steele & Pires 2011). For elusive, rare and/or endangered species the use of noninvasive genetic sampling is particularly relevant, as it allows important data collection from the simple use of hairs, faeces, urine, skulls, egg shells, feathers, among others, avoiding the capture, handling, or even the observation of individuals (Beja-Pereira *et al.* 2009; Lampa *et al.* 2013). The molecular identification of species through the use of noninvasive sampling is relatively recent (Waits & Paetkau 2005) and has contributed in the last decade to the study of elusive or rare species (Van Vliet *et al.* 2008; Beja-Pereira *et al.* 2009; Oliveira *et al.* 2009; Chaves *et al.* 2012; Godinho *et al.* 2012; Monterroso *et al.* 2013; Barbosa *et al.* 2013), mainly due to the powerful technical advances in areas such as DNA extraction, sequencing, microsatellite analysis and SNPs development, which expanded the use of molecular methods in conservation (DeSalle & Amato 2004; Beja-Pereira *et al.* 2009).

In the last decade, the concept of DNA barcoding has profoundly increased the molecular identification of species (Floyd *et al.* 2002; Hebert *et al.* 2004) and barcoding initiatives highly accelerated biodiversity assessments (Smith *et al.* 2005; Hajibabaei *et al.* 2007). The idea behind the barcoding concept was to select a universally recognized gene for identification of most, if not all, organisms on Earth. This gene, or few genes, would show high interspecific but low intraspecific levels of variation thus establishing a barcoding gap (Hebert *et al.* 2003). The sequences of such gene(s) could then become the equivalent of species-specific barcodes, providing a dataset representative of Earth's biodiversity. The mitochondrial gene cytochrome *c* oxidase I (COI) is the most popular marker for a global identification system for animals, as it has proven to be useful for identifying many different organisms, from insects to fishes and birds (e.g. Hebert *et al.* 2004; Vila & Björklund 2004). However, this gene is still not the most used for several taxa and in these cases few or no data is available in public databases, as for the North African ungulates. On the other hand, cytochrome *b* (cytb) is a widely used mtDNA gene for phylogenetic studies and species assignment (e.g. Hassanin *et al.* 1998; Fernandes *et al.* 2007; Chaves *et al.* 2012; Barbosa *et al.* 2013; D'Amato *et al.* 2013; Fadakar *et al.* 2013). Despite its usefulness, mtDNA is especially prone to problems due to the occurrence of nuclear inserted copies, high intraspecific diversity, introgression or heteroplasmy (Alves *et al.* 2008; Song *et al.* 2008; White *et al.* 2008; Galtier *et al.* 2009), and thus species identification should be enhanced by the complementary use of a nuclear gene. Additionally, the inclusion of nuclear markers can complement mitochondrial data by allowing the detection of incomplete lineage

sorting, mitochondrial introgression and hybridization (Heckman *et al.* 2007; Godinho *et al.* 2011; Melo-Ferreira *et al.* 2012).

North African ungulates, and in general highly mobile mammals in this region, are poorly studied mainly because of the remoteness and political instability of the region, added to the difficulty of observing or/and capturing these species (Brito *et al.* 2014). Eight wild North African ungulate species are categorized as threatened on IUCN Red Lists (www.iucnredlist.org): *Gazella dorcas*, *Ammotragus lervia* and *Eudorcas rufifrons* as “Vulnerable”; *G. cuvieri* and *G. leptoceros* as “Endangered”; *Nanger dama*, and *Addax nasomaculatus* as “Critically Endangered”; and *Oryx dammah* is now considered “Extinct in the Wild”. In order to preserve North African ungulates, both *in situ* and *ex situ* conservation programs (including reintroduction efforts), have been carried out in several research programs and zoological institutions (for details see the Sahelo-Saharan Interest Group annual reports: www.scf.org). In addition, semi-captive wild populations are kept to reinforce remnant wild populations in several countries, including Morocco, Algeria, Tunisia, and Senegal (Abáigar *et al.* 1997; Aulagnier *et al.* 2001; De Smet & Smith 2001; Smith *et al.* 2001).

Knowledge of the geographical distribution and population abundance of wild ungulates in North Africa is crucial to improve their management and long-term conservation programs, especially given the growing hunting pressure observed in this region (Brito *et al.* 2014; Durant *et al.* 2014). However, the identification of wild ungulates species has been made primarily on the basis of field identifications of footprints, latrines, tracks and faeces (Attum *et al.* 2006; Wronski & Plath 2010; Attum & Mahmoud 2012; Abáigar *et al.* 2013), which may lead to identification errors when species occur in sympatry due to high similarity in both diet and body size (mainly when considering juveniles). For example, doubts may arrive for distinguishing faeces from *G. dorcas* and *G. leptoceros*, or from *G. cuvieri* and *A. lervia*, particularly when including juveniles of the latter species-pair. Additionally, domestic ungulates (namely goat and sheep) are often present in sympatry in many areas, which hampers species identification, and consequently increases errors. Early studies demonstrated the usefulness of using the cytochrome *b* gene for distinguishing the lineages or subspecies within *Gazella* species (Rebholz & Harley 1999; Wronski *et al.* 2010; Wachter *et al.* 2010; Lerp *et al.* 2011; Godinho *et al.* 2012; Fadakar *et al.* 2013), suggesting therefore that this gene is a good candidate molecular marker for barcoding these species. On the other hand, the k-casein (KCAS) gene was successfully used in phylogenetic studies of Antilopini (Bovidae) species (Matthee & Davis 2001; Bärmann *et al.* 2013a), highlighting the potential of this gene for the molecular identification of antelopes.

Here we developed a simple molecular method to identify the endangered ungulate species present in North Africa and to discriminate these species from their domestic forms that inhabit the same areas. By improving the knowledge on these species, we believe that the proposed molecular method can enhance the general understanding of North Africa biodiversity (Brito *et al.* 2014). We selected a mitochondrial (cytb) and a nuclear (KCAS) gene to find diagnostic positions within each gene to enable species identification. Because DNA recovered from noninvasive samples is highly fragmented we selected only physically close variant positions, in order to maximize its successful application in noninvasive samples. Finally we evaluated the existence of a barcoding gap and identify potential genetic variants within the species.

Materials and Methods

Study area and sample collection

A total of 456 noninvasive samples, comprising 415 scats (both fresh and old), 15 hairs (collected directly from carcasses) and 26 bones (collected in the field, undetermined age) of ten species and known geographic origin encompassing seven North African countries (Figure 3.1 and Table 3.1) were subjected to total DNA extraction. All scat samples were of at least four pellets per pile; each pile consisted of pellets produced presumably by a unique individual at once. Scats scattered by the wind were discarded. Scats were putatively classified as “fresh” if mucus was observable or as “old” if not. However, the heterogeneous climatic conditions of the study area, ranging from deserts to Mediterranean forests, may confound such classification. Scats and hairs were preserved in ethanol (96%) until DNA extraction. For scat DNA extraction we used two pellets and followed a protocol adapted by Maudet *et al.* (2004), but using a commercial Kit (E.Z.N.A.® Tissue DNA Kit). Hairs (3-4 with follicle) and bones (100 - 200 mg) samples were extracted using the QIAamp DNA Micro Kit (Qiagen®). Total DNA from 78 tissue samples belonging to nine ungulate species was extracted using the Genomic DNA Minipreps Tissue Kit (EasySpin). To detect potential genetic variants within the species analysed, sampling was distributed throughout different geographical locations of North Africa and from captive populations, including European and North African ones (Table 3.1 and 3S.1). Domestic goat (n=4) and sheep (n=4) samples were used to test the possibility of discriminating domestic ungulates with faeces possibly confusable with the wild counterparts. All noninvasive samples were processed in a dedicated facility with positive air pressure and UV decontamination.

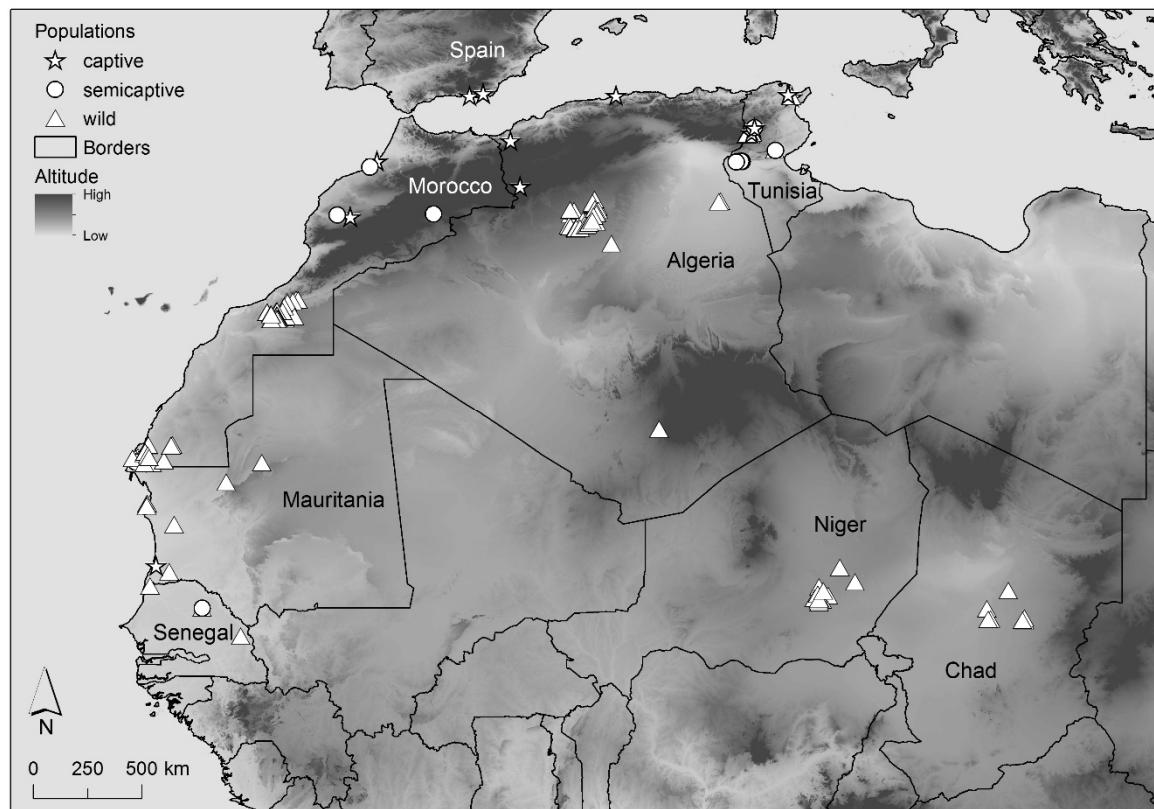


Figure 3.1. Geographic origin and type (wild, captive and semicaptive) of samples used in this work. Captive populations from Hannover and Berlin Zoos are not represented in the map. For additional information see table 3.1 and 3S.1

Molecular analysis

Two genes were selected for species identification: the mitochondrial cytochrome *b* (*cytb*), and the nuclear kappa casein (*KCAS*). A small fragment (450 bp) of the *cytb* gene was amplified using universal primers L14724 (5'-TGACTAATGATAGAAAAACCATCGTTG; Irwin *et al.* 1991, modified by Lerp *et al.* 2011) and H15149 (5'-TAACTGTTGCTCCTCAAAAAGATATTTGTCCTCA; Kocher *et al.* 1989). A fragment comprising part of the exon 4 of the *KCAS* gene (367 bp) was amplified using primers targeting conserved regions among Bovidae species (Matthee *et al.* 2001): *KCAS_E* (5'-GTGGAAGGAAGATGTACAAATC), and *KCAS_D* (5'-CTAACTGCAACTGGCTTTGCATA). Amplifications were performed in a final volume of 10 µl using 5 µl of QIAGEN PCR MasterMix [consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM MgCl₂, dNTP mix, Q solution and HotStart Taq DNA polymerase (QIAGEN, Valencia, CA)], 0.2 µM of each primer and 2 µl of DNA extraction (approximately 10 ng of genomic DNA). The thermocycling for both PCR reactions was performed in a MyCycler (BIO-RAD) and carried out with a first denaturation step at 95°C

for 15 min, followed by 45 cycles at 95°C for 30 s, 56°C for 30 s, 72°C for 30 s and then a final extension step at 60°C for 10 min. PCR amplifications of both fragments were obtained for all target ungulate species. The highest annealing temperature for both fragments, without compromising amplification yield, was selected to reduce unwanted PCR products and maximize specificity (56°C), although lower temperatures may be used to facilitate amplification of poorer quality samples (55 - 51°C; e.g., bones).

Pre- and post-PCR manipulations were conducted in physically separated rooms, always including negative controls, that allowed confirming absence of contaminations. PCR products were purified using ExoSAP-IT® PCR clean-up Kit (GE Healthcare, Piscataway, NJ, USA). Both strands sequences were generated using the amplification primers, as it recommended for example, by Tiedemann *et al.* (2012), allowing the confirmation of sequence consistency and quality. Cycle sequencing reactions were carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). Sequencing products were subsequently separated on a 3130xl Genetic Analyser (Applied Biosystems). Sequence alignment was performed using Clustal W (Thompson *et al.* 1994) implemented in BioEdit software (Hall, 1999) and was manually checked and reassessed for any discrepancy. Additionally, published sequences of the ten species were included in the alignment (see Table 3S.2). All new sequences were submitted to NCBI GenBank (accession numbers from KM582053 to KM582129).

Consensus haplotypes for each species were aligned for both *cytb* and KCAS fragments, using new and available GenBank sequences. Species-specific polymorphisms were identified manually.

Bayesian inference was used for both *cytb* and KCAS to build phylogenetic trees and determine species boundaries. The best-fit model of sequence evolution for each locus alignment was selected based on the Akaike information criterion and using the software jModelTest version 1.0 (Posada 2008). Haplotype trees were generated by the software MrBayes 3.1 (Huelsenbeck & Ronquist 2001) at the Bioportal web-based portal (www.bioportal.uio.no), using the mouse deer (*Tragulius napu*) as outgroup for the *cytb* tree and klipspringer (*Oreotragus oreotragus*) for the concatenated tree. Bayesian posterior probabilities were estimated from two runs with four chains of 10 million generations, with a sampling frequency that provided a total of 10 000 samples for each run, excluding 25% burn-in. Tree visualization was conducted using the software FigTree 1.3.1 (Rambaut 2009).

Intraspecific and interspecific mean pairwise genetic divergences were calculated for both *loci* employing Tamura 3 parameter model -T92 (Tamura 1992) distances using MEGA 5 (Tamura *et al.* 2011). On the basis of these divergence estimates, histograms were built for each species independently, to test for the existence of a barcoding gap between intra

and interspecific genetic divergence (Hebert *et al.* 2003). The extent of divergence was further evaluated using a second graphical analysis of pairwise divergence, which compares the mean and maximum intraspecific divergence of each taxon with the mean and the minimum interspecific divergence of that taxon with the remaining species. We used the Poisson Tree Processes (PTP) model to delimit species on a rooted phylogenetic tree (Zhang *et al.* 2013). In PTP, speciation or branching events are modelled in terms of number of substitutions (represented by branch lengths), estimating species clusters using a phylogenetic tree as input. PTP program assumes that each nucleotide substitution has a fixed probability of being the basis for a speciation event. We used PTP test to assess, in parallel to histograms for each species independently, the existence of a barcoding gap between intra- and interspecific genetic divergence. Analyses were conducted on the web server for PTP (Zhang 2013-2014) using phylogenetic trees obtained with MrBayes 3.1 (Huelsenbeck & Ronquist 2001).

Results

All tissue samples and 412 (90.4%) out of 456 noninvasive samples were successfully amplified for the mitochondrial fragment (cytb). High success rates were achieved for the three types of noninvasive samples tested: bones (88.5%), hairs (93.3%), and scats (90.4%). Ultimately, a fragment of 450 base pairs of the cytb gene was analysed in 571 sequences from eight wild and two domestic ungulates species, including 81 sequences from GenBank. The first position of the alignment corresponds to position 14 515 in *Bos taurus* complete mitochondrial genome; accession number NC_006853 (Table 3S.3). These sequences resulted in 131 different haplotypes with 161 variable sites, of which 28.51% are parsimony informative.

The Bayesian tree inference for cytb was performed using the GTR+I+G model: the general time-reversible substitution model with a proportion of invariable sites and a gamma-distributed rate variation across sites. The tree recovered nine species of ungulates (Figure 3.2) with posterior probabilities ≥ 0.85 , with the exception of *Gazella cuvieri* and *G. leptoceros*, which formed a monophyletic group (posterior probability of 1). Therefore, in the following analyses these two species were treated as a single taxon (GC_GL: *Gazella cuvieri* and *G. leptoceros*, respectively).

Intraspecific variability did not overlap with the interspecific variability (Figure 3.3A), proving the existence of a barcoding gap. The intraspecific values varied from zero (*Addax nasomaculatus* and *Oryx dammah* do not present intraspecific divergence) to 4.8% (*Eudorcas rufifrons*). The maximum interspecific divergence was observed in *Capra sp* (21.9%) and the minimum in *Gazella dorcas* (5.2%). Even with the small divergence

difference of 0.4% between the highest intraspecific divergence and the lowest interspecific, the barcoding gap is observed in all taxa (Figure 3.3B).

Table 3.1. Number of invasive (tissue) and noninvasive (bones, hair, faeces) samples genotyped from each ungulate species. Number of localities and countries of sample origin are specified. The number of captive populations (c) semi-captive groups (sc) and the wild samples (w) are indicated in the Country column. For more details, consult table 3S.1

	Taxa	Total	Tissue	Bone	Hair	Faeces	Localities	Country
Wild	<i>Gazella dorcas</i>	221	47	14	4	156	9	DZ (c2/sc1/w), TD (w), DE (c1), MR (w), MA (c1/sc4/w), NE(w), SN (sc1), ES (c1), TN (c1/sc3)
	<i>Gazella cuvieri</i>	108	9	5	-	94	4	DZ (sc1), MA (w), ES (c1), TN (w)
	<i>Gazella leptoceros</i>	46	4	2	9	31	2	DZ (c1, w), BE (c1)
	<i>Eudorcas rufifrons</i>	3	-	-	1	2	2	MR (w), SN (w)
	<i>Nanger dama</i>	18	2	-	-	16	3	MA (sc1), SN (sc1), ES (c1)
	<i>Oryx dammah</i>	14	4	-	-	10	3	MR (c1), SN (sc1), ES (c1)
	<i>Addax nasomaculatus</i>	18	3	2	-	13	3	DE (c1), MA (sc1), TN (sc1)
Domestic	<i>Ammotragus lervia</i>	49	1	-	-	48	5	DZ (sc1), MA (w), NE(w), ES (c1), TN (c3/w)
	<i>Capra hircus</i>	6	4	-	-	2	2	PT (c1), MA (w)
	<i>Ovis aries</i>	7	4	-	-	3	2	PT (c1), TN (w)
Total		490	78	23	14	375		

Country codes: DZ - Algeria; BE – Belgium; TD - Chad; DE - Germany; MR - Mauritania; MA - Morocco; NE - Niger; PT – Portugal; SN - Senegal; ES – Spain; TN – Tunis

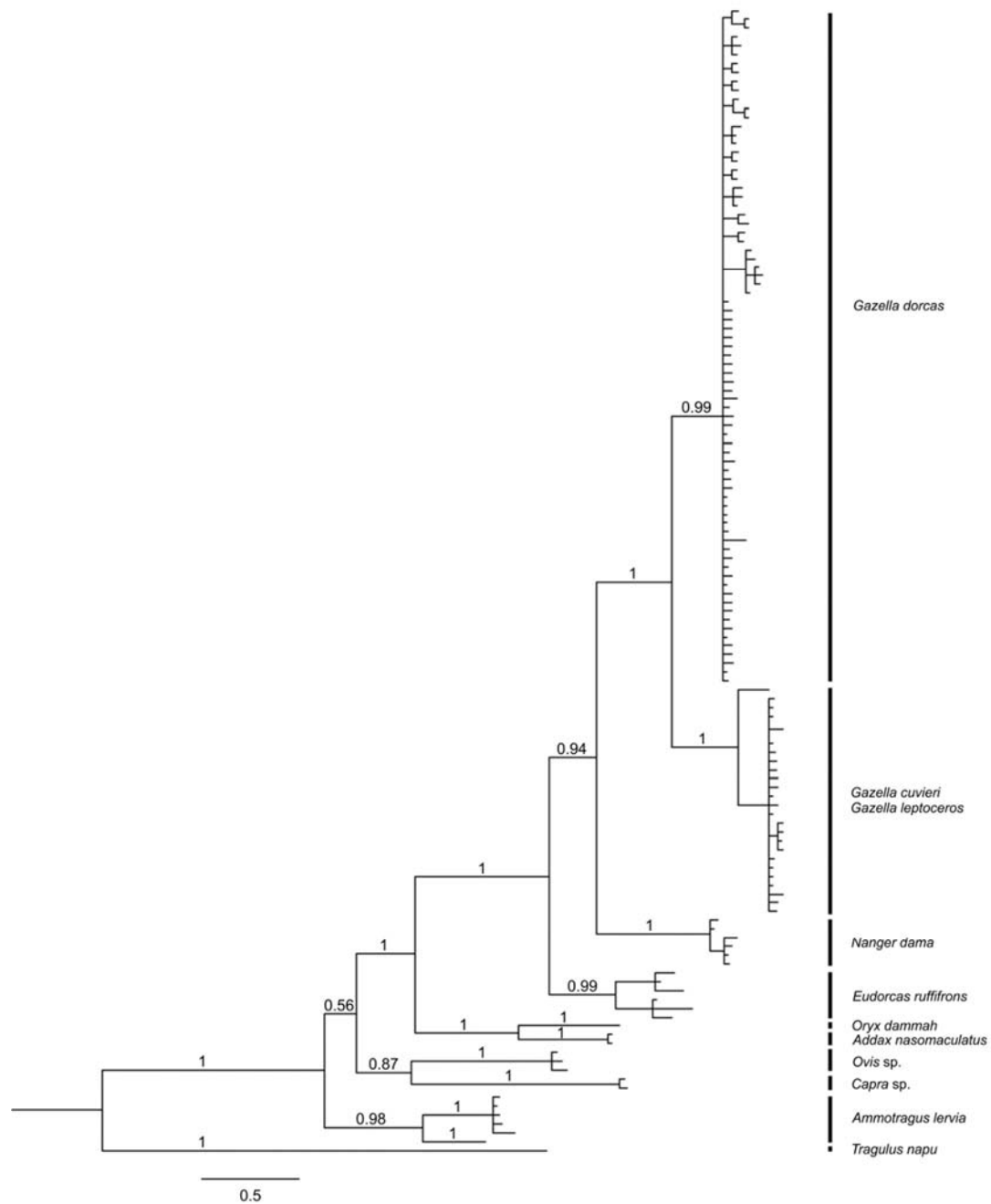


Figure 3.2. Bayesian inference tree for the cytb fragment showing the phylogenetic relationship of all endangered North African ungulates and the domestic species. Posterior probabilities of major nodes are indicated. *Tragulus napu* was used as outgroup.

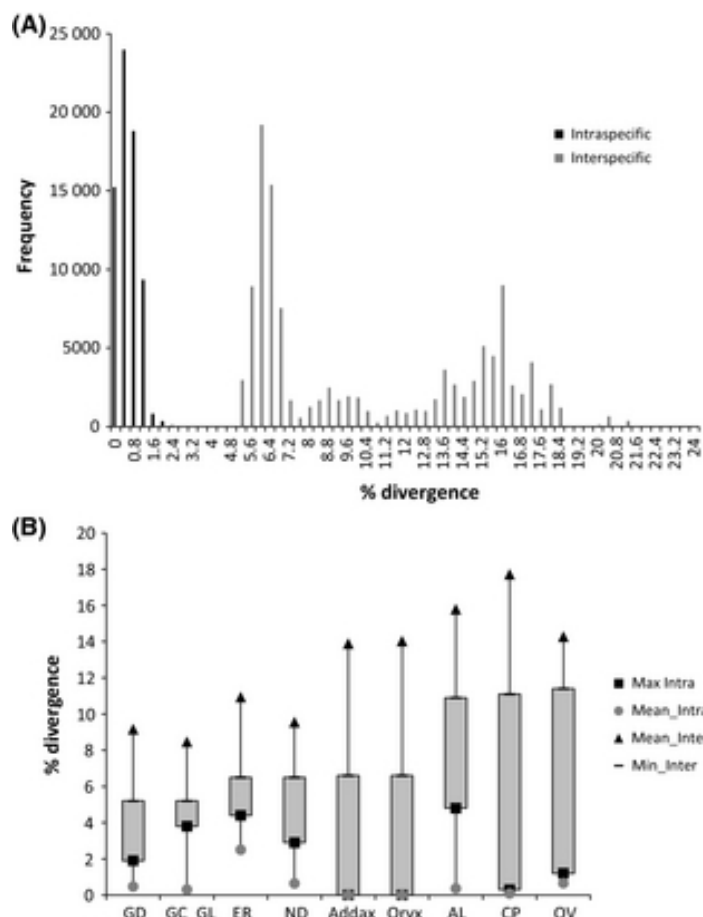


Figure 3.3. (A) Histogram of T92 (Tamura 3-parameter model) cytb divergence values (intraspecific and interspecific), for eight wild and two domestic North African ungulates. (B) Summary of pairwise divergences involving sequences of each species showing mean (circle) and maximum (square) intraspecific divergences and mean (triangle) and minimum (dash) interspecific divergences (comparing sequence from the named species with other species). Grey bars characterize the extent of the barcoding gap. GD: *Gazella dorcas*; GC_GL: *Gazella cuvieri* and *Gazella leptoceros* group ER: *Eudorcas rufifrons*; ND: *Nanger dama*; Addax: *Addax nasomaculatus*; Oryx: *Oryx dammah*; AL: *Ammotragus lervia*; CP: *Capra sp*; OV: *Ovis sp*. Given the unresolved separation between *Gazella cuvieri* and *Gazella leptoceros* these taxa were combined and are referred to as GC_GL.

For the nuclear fragment, all tissue samples and 359 (78.7%) out of 456 noninvasive samples were successfully amplified. High success rates were achieved for the three types of noninvasive samples used: bones (60.0%), hairs (69.2%), and scats (80.0%). A fragment of 367 bp of the KCAS gene was analysed on 444 samples across eight wild and two domestic ungulates present in North Africa (the first position of our sequences correspond to position 12 366 in *Bos taurus* kappa casein (CSN3) gene, CSN3-A allele,

complete coding sequence; accession number AY380228). Sequence assemblage of our data together with seven sequences from GenBank showed a total of 14 out of 23 polymorphic sites exhibiting potential interspecific diagnostic polymorphisms (Table 3.2) and representing a total of nine different haplotypes. This diversity corresponds to 7.33% parsimony informative sites. *Gazella cuvieri* and *Gazella leptoceros* (GC and GL, respectively) did not show any distinctive position for the KCAS region analysed. No overlap between intraspecific and interspecific KCAS divergences was observed (Figure 3.4A), nor intraspecific divergence for any species. Moreover, a barcoding gap was exhibited in all taxon (Figure 3.4B). The values of interspecific divergences between species pairs ranged from 0.3% (*Addax nasomaculatus* and *Oryx dammah*) to 4.3% (*Ammotragus lervia* and *Eudorcas rufifrons*). *Ammotragus lervia* showed the highest mean interspecific divergence.

Both mitochondrial and nuclear markers were useful for identifying and differentiating the studied species. The levels of genetic divergence for the cytb gene were generally higher than those observed for KCAS (Figures 3 and 4), and a barcoding gap was observed between the intraspecific and the interspecific divergences in both molecular markers. Cytb and KCAS sequences produced in the current study were combined in order to improve the resolution of the phylogenetic trees and therefore to better detect intra- and interspecific variations (Figure 3S.1). Together with histograms for each species independently, the PTP method was able to discriminate species regardless of the amount of sequence similarity between the species under comparison. The trees submitted to the species delimitation server confirmed the nine species identified, for both cytb and combined cytb/KCAS.

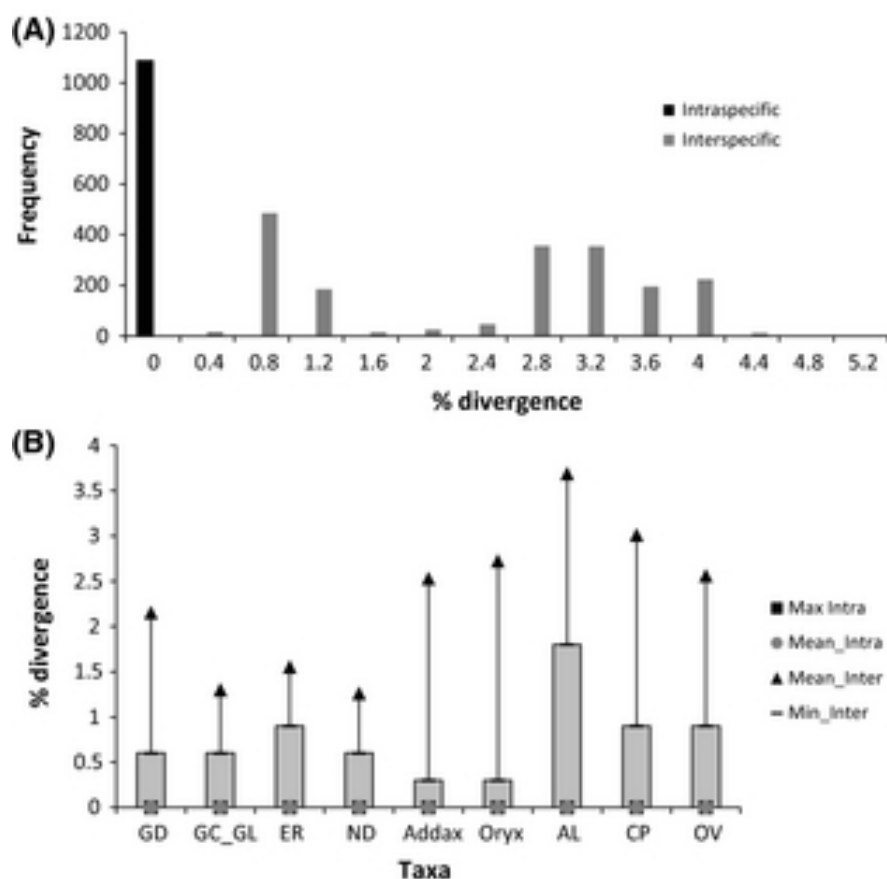


Figure 3.4. (A) Histogram of T92 (Tamura 3-parameter model) KCAS divergence values (intraspecific and interspecific), for eight wild and two domestic North African ungulates. (B) Summary of pairwise divergences involving sequences of each species showing mean (circle) and maximum (square) intraspecific divergences and mean (triangle) and minimum (dash) interspecific divergences. Grey bars characterize the extent of the barcoding gap. GD: *Gazella dorcas*; GC_GL: *Gazella cuvieri* and *Gazella leptoceros* group; ER: *Eudorcas rufifrons*; ND: *Nanger dama*; Addax: *Addax nasomaculatus*; Oryx: *Oryx dammah*; AL: *Ammotragus lervia*; CP: *Capra sp*; OV: *Ovis sp* Given the unresolved separation between *Gazella cuvieri* and *Gazella leptoceros* these taxa were combined and are referred to as GC_GL.

Table 3.2. Interspecific polymorphic positions of a 367bp fragment of the KCAS gene in endangered ungulates species of North Africa. First position corresponds to position 12 366 of *Bos taurus* kappa casein (CSN3) gene, CSN3-A allele, complete cds (accession number AY380228). Boxes represent species-specific nucleotide variations. Points represent similar positions and dashes represent indels.

Taxa / Position	54	79	90	105	108	128	134	137	140	144	161	181	200	204	221	235	243	252	258	259	332	345	362
<i>Gazella dorcas</i>	G	C	G	G	T	A	A	A	A	T	T	T	T	G	C	G	G	T	A	A	C	G	T
<i>Gazella cuvieri</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	.	.	.
<i>Gazella leptoceros</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	.	.	.
<i>Eudorcas rufifrons</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	T	.
<i>Nanger dama</i>	.	.	.	.	.	.	.	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
<i>Oryx dammah</i>	A	A	.	A	C	.	G	.	G	C	.	.	.	A	.	T	.	G	.	.	.	.	.
<i>Addax nasomaculatus</i>	.	A	.	A	C	.	G	.	G	C	.	.	.	A	Y	T	.	G	.	.	.	.	.
<i>Ammotragus lervia</i>	.	A	A	A	C	G	G	.	G	C	C	C	.	A	.	.	.	.	R	.	.	.	C
<i>Capra sp</i>	.	A	.	A	C	.	G	.	G	C	.	.	C	A	.	.	.	.	G	.	T	.	.
<i>Ovis sp</i>	.	A	.	A	C	.	G	.	G	C	.	.	-	A	.	.	.	.	G	.	.	.	.

Discussion

In the last decade, DNA techniques have proved common, inexpensive, rapid, and accurate means for identifying species and assessing biodiversity (Darling & Blum 2007). These techniques have allowed or improved many studies concerning wildlife conservation, ecology and animal forensics. Despite the threatened status of North African ungulates, no DNA-based protocol allowing the unequivocal identification of these species was available to date, especially concerning the application to noninvasive sampling. Our relatively inexpensive, easy and quick molecular method fills this gap, being useful for distinguishing several ungulate species, both wild and domestic, cohabiting in North Africa. Concomitantly, this method has several potential advantages: (i) samples (invasive or noninvasive) can be analysed without any prior assumptions based on morphologic identifications; (ii) it overcomes problems associated with the single use of mitochondrial DNA to species identification because it relies on a co-amplification of a nuclear fragment; (iii) it can be directly applied to noninvasive samples. The selected fragments exhibited high amplification success rates (around 90% and 80% for mtDNA and nDNA, respectively) in agreement with what is generally described for noninvasive samples, in particular to herbivores (Maudet *et al.* 2004; Luikart *et al.* 2008). The main factor that limits such success is the age of the sample, as DNA is easier to amplify in fresher samples (DeMay *et al.* 2013), although relationships between diet and amplification success in herbivores has also been observed (Wehausen *et al.* 2004). In our study, diet was not monitored but the fresh scats collected in captivity had a 100% successful extraction rate, while scats collected in the field had lower rates, which could be related to the freshness at the moment of sample collection. Thus, to maximize amplification success across samples, irrespective of freshness, we used 45 PCR cycles. The few field samples that did not amplify most likely were old or contained inhibitors that affected PCR reactions (DeMay *et al.* 2013).

Both selected fragments show high variability among species allowing the identification of all North African endangered ungulate species. Despite not being commonly used for barcoding purposes, the chosen molecular markers are widely used for species assignment, especially the mitochondrial cytb. Although our fragments were longer than the recommended size for noninvasive genetic samples (300bp; Waits & Paetkau 2005), the amplified fragments are not excessively long, indeed having a size similar to the barcoding fragments (Maudet *et al.* 2004; Harris *et al.* 2010; Adams *et al.* 2011; Kekkonen & Hebert 2014). Additionally, a longer size fragment allows a better resolution of the phylogenies and increases the ability to distinguish intra- and interspecific variations. In our study, the proposed methodology was able to highlight some

uncertainties on the systematics within *Gazella* genus, for instance, no diagnostic positions between *G. cuvieri* and *G. leptoceros* were found, further supporting these species as a monophyletic group (Rebholz & Harley 1999; Wacher *et al.* 2010; Wronski *et al.* 2010; Lerp *et al.* 2011). The lack of genetic differentiation between these two species emphasizes the need for additional studies based on additional nuclear markers to clarify their phylogeny and systematics.

As expected, the intraspecific variability found in this study was higher for the mitochondrial *cytb* than for the nuclear KCAS locus, for which no intraspecific variability was found (Figure 3.3 and 3.4, Table 3.2 and 3S.1). The haplotype diversity for each species, as well as the interspecific divergence, was always lower for the KCAS locus. However, the genetic information retrieved from this locus was useful to distinguish the eight endangered ungulate species, including congeneric species of the *Gazella* genus, which validates its ability as a barcoding marker for species delimitation (Havermans *et al.* 2011). A barcoding gap was detected for all species in both genes, although more pronounced for *cytb* than for KCAS (Figs 3B and 4B). Both histograms for each independent species and the Poisson Tree Processes model were able to distinguish intra- and interspecific differences. However, the short fragment of the KCAS locus revealed higher interspecific diagnostic polymorphism. The threshold for species delimitation using the *cytb* was about 3%, which is consistent with the maximum limit of intraspecific variation detected for the mitochondrial COI in mammals, the gene commonly used for DNA barcoding studies (Luo *et al.* 2011).

The use of single primer pairs for each gene covering all ungulate species is a major advantage for species identification, both concerning laboratory costs and time-consuming processes. Other methods could be used for species identification, based on next-generation sequencing technology (NGS) or in the development of specific primers for each species under analysis (Adams *et al.* 2011). However, despite the ability of these methods to provide huge amounts of data and specificity for species identification, NGS requires the processing of a large number of samples to be cost-efficient and the use of specific primers would have increased costs. Therefore, single primer pair sequencing is a balanced choice, providing additional information for phylogenetic and population studies and allowing for prompt conservation measures.

The number of North African ungulate samples used in the present study provides the largest set of sequences (nuclear and mitochondrial) currently available. Large sequence databases are essential to assure accurate genetic species identification (Darling & Blum, 2007). The described methodology will assist for ecological and population genetic studies using noninvasive samples of endangered ungulates in this region, where ascertaining the species identification is crucial. In addition, the fragment size of both

genes tested in this study can be directly applicable in the high throughput sequencing technologies, which allows other possibilities of analysis, for instance, the simultaneous determination of species and diet using scats (Shehzad *et al.* 2012). Our work reveals, foremost, that we have now a new set of tools amenable for ungulate identification, a pertinent issue given the conservation concern of several species in North Africa. The reported method is therefore useful for biodiversity conservation in North Africa and the management of ungulates in the region and elsewhere.

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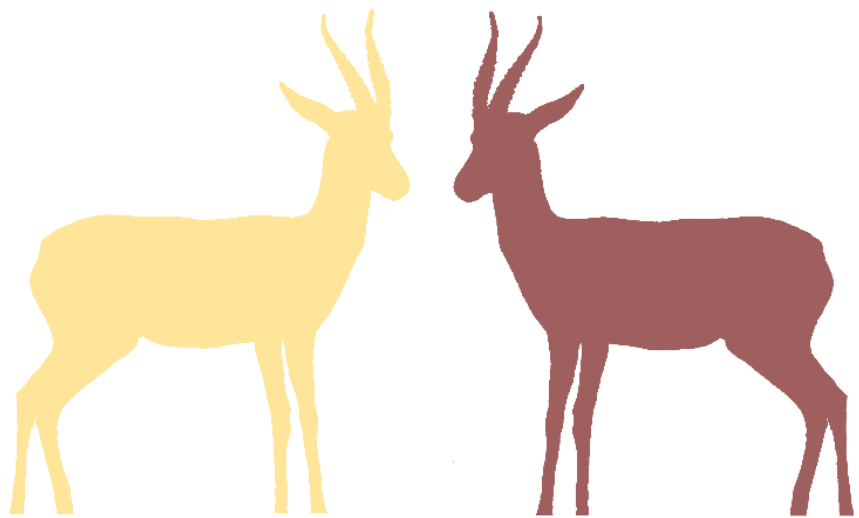
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Data Accessibility

Refer to Table 3S.1 for the DNA sequence data accessibility and sample locations; DNA sequences: GenBank accession numbers from KM582053 to KM582129; DNA haplotype alignments: DRYAD entry doi: 10.5061/dryad.ns7gp

T.L.S., R.G., T.A., P.C.A. and J.C.B. conceived and designed the study; T.L.S., T.A. and J.C.B. collected samples in the field and from collections; T.L.S. and D.C. performed laboratory work, T.L.S. analysed the data and wrote the first draft of the manuscript; T. L. S., R.G., T.A., P.C.A. and J.C.B. discussed the results and contributed to the writing of the final version of the manuscript.



CHAPTER 4

Phylogenetic methods and ecological niche-based modelling approaches

Paper II **Ecotypes and evolutionary significant units in endangered North African gazelles**

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Ecotypes and evolutionary significant units in endangered North African gazelles

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Abstract

Conservation planning of threatened taxa relies upon accurate data on systematics, ecological traits and suitable habitats. The genus *Gazella* includes taxa with distinct morphologies and ecological traits, but close phylogenetic relationships. The North African *Gazella cuvieri* and *Gazella leptoceros loderi* share morphological and physiological characters but the former is darker and found in mountain areas, while the latter is lighter and associated with sand dunes. Here we aim to assess the genetic distinctiveness of these taxa, to characterize their ecological niches and to identify potential occurrence areas, by analysing 327 samples across North-West Africa. Phylogenetic analyses based on mitochondrial (*CYTB*) and five nuclear gene fragments (*KCAS*, *LAC*, *SPTBN1*, *PRKCI* and *THYR*) show that both taxa comprise a single monophyletic group. However, ecological niche-based modelling suggests that populations of these taxa occupy distinct geographic areas and specific environments. Predicted areas of sympatry were restricted, as a consequence of local sharp transitions in climatic traits. The lack of genetic differentiation between these taxa suggests they should be lumped into *G. cuvieri*, while ecological and morphological differences indicate they correspond to distinct ecotypes. Conservation planning of *G. cuvieri* should consider the preservation of both mountain and lowland ecotypes to maintain the overall adaptive potential of the species. This integrative approach provides valuable insights in identifying evolutionary units and should be extended to other gazelles.

ADDITIONAL KEYWORDS: Bayesian dating analysis – ecological niche-based modelling – *Gazella cuvieri* – *Gazella leptoceros* – geographic information systems – phylogeny – Sahara.

Introduction

Effectiveness of species-specific conservation strategies depends on the level of understanding of ecological and evolutionary mechanisms behind current patterns of species diversity (Richardson & Whittaker, 2010; Ishida et al. 2011). Non-random partitioning of diversity might result in the origin of different ecotypes, groups of distinct populations that differ in several traits over space, including genetic diversity (Lowry 2012). As conspecific groups, ecotypes are able to interbreed (Cronin 2006). Yet, they usually exhibit phenotypic differences associated with environmental heterogeneity across the species' range, thus being important evolutionary conservation targets (Courtois et al. 2003; Cunha et al. 2005; Lemay Donnelly & Russello 2013). Ecotype identification may be a simple task when distribution of different forms is clearly bi- or multimodal (e.g. Lin et al. 2008; Gowell, Quinn & Taylor 2012). However, when phenotypic variation has no sharp transition zones between trait states, ecotype designation may be unclear (e.g. Stankowski & Johnson 2014, Carvalho et al. 2016).

The unique diversity of bovids and the absence of taxonomic significance for the “ungulate” term have intensified the debate surrounding the taxonomy of African ungulates (e.g. Simpson, 1945; Gentry, 1965; Fernández & Vrba 2005; Wilson & Mittermeier, 2011; Heller et al. 2013; Cotterill et al. 2014). Most African ungulates, including the genus *Gazella*, were first identified based on morphological (e.g. coat colour, size, craniometry), behavioural and ecological traits, which has occasionally caused taxonomic inflation (e.g. Christy, 1924; Lange, 1972; Kumamoto and Bogart, 1984; Devillers et al. 2005; Wilson & Mittermeier, 2011; Groves & Grubb, 2011). In fact, molecular data has also been able to accurately clarify the position of some species within the groups (Hassanin, Delsuc & Ropiquet 2012). Molecular data along with different species concepts have originated two polarized species delimitation approaches (Frankham et al. 2012): splitting and lumping (Miralles & Vences 2013; Senn et al. 2014). The genus *Gazella* has been successively revised based on the biological and phylogenetics species concept originating its split in several different genera and the elevation of some subspecies to full species level (Groves & Grubb, 2011). For a close genus, *Nanger*, genetic data suggest the best conservation approach is to lump dama gazelle into a single species without subspecific differentiation (Senn et al. 2014).

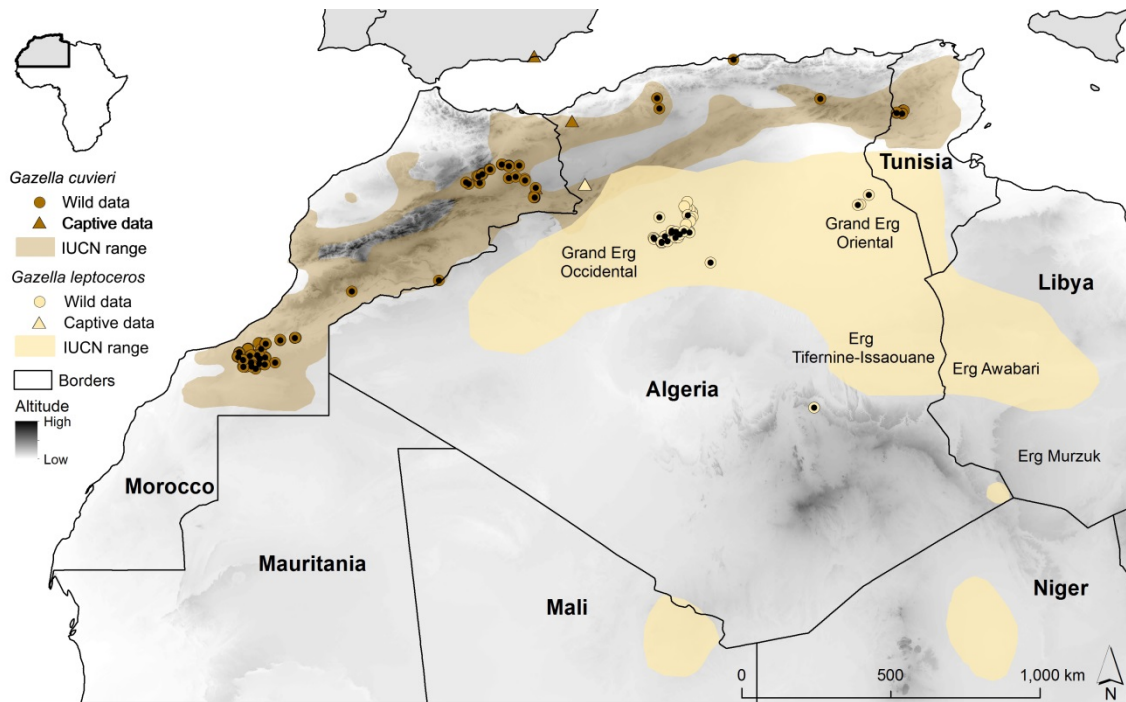


Figure 4.1 Distribution of analysed samples of *Gazella cuvieri* (n= 255) and *Gazella leptoceros loderi* (n= 53) across the study area, distinguishing between wild (dots) and captive or semi-captive populations (triangles). Samples marked with black dots were used for modelling proposes. Polygons depict the IUCN range of each species. Location of the study area in the African context (inset). Major toponymies are indicated in the map.

The Cuvier's gazelle (*Gazella cuvieri*, Ogilby, 1841; Terra typica: Morocco) and the Slender-horned gazelle (*Gazella leptoceros*, Cuvier, 1842; Terra typica: Egypt) dwell in North Africa. While *G. cuvieri*, endemic from North Africa (Escalante 2016), occurs in woodland mountain slopes, *G. leptoceros* is present in lowland sand dunes (Figure 4.1). Both forms show a phenotypic plasticity by matching their habitat background colour; *G. cuvieri* is much darker than *G. leptoceros* (Wilson & Mittermeier, 2011). Ecological and range differences within *G. leptoceros* populations have led to its split into two subspecies based also on the horn size: *G. l. loderi* (Thomas, 1894; Terra typica: Central Sahara), designated by Groves (1969) as the shorter-horned, distributed throughout the west Sahara; *G. l. leptoceros* (designated by Groves (1969) as the longer-horned), which occurs only in Egypt (Table 4.1). Despite such distinctiveness, *G. cuvieri* and *G. leptoceros* share numerous similarities, including body sizes and horn-shapes (Wilson & Mittermeier, 2011), chromosome number (N=33; Robinson et al. 2011) and reproductive traits (twins at birth; Devillers et al. 2005, Wilson & Mittermeier, 2011) (Table 4.1). Available mitochondrial DNA (mtDNA) data show no genetic divergence between these two sister-species (Rebholz & Harley 1999; Wronski et al. 2010; Wachter et al. 2010; Lerp et al. 2011; Hassanin et al. 2012; Bärmann et al. 2013, Bärmann, Rössner & Wörheide 2013) and support their monophyletic status (Hassanin et al. 2012; Silva et al.

2015). However, mtDNA markers alone have limitations in species delineations, especially when the processes of mitochondrial introgression and natural hybridization occur (Alves et al. 2006; Galtier et al. 2009). The inclusion of more nuclear markers is needed to clarify the evolutionary history and systematics of the genus *Gazella* (e.g. Godinho, Crespo & Ferrand 2008; Melo-Ferreira et al. 2012; Vaz Pinto et al. 2016), the only one studied so far (KCAS) did not show any differentiation between the two species (Silva et al. 2015). *G. cuvieri* and *G. leptoceros* are categorized as Vulnerable and Endangered (EN), respectively, on the IUCN red list, mainly due to low population size, overhunting and habitat change (Mallon et al. 2008; Mallon and Cuzin, 2008; Durant et al. 2014). To preserve these endangered North African ungulates and optimize local conservation efforts, an accurate phylogeny and a good habitat/niche characterization is needed.

In the present work, we investigated the genetic diversity and habitat partitioning of *Gazella cuvieri* and *Gazella leptoceros*. Given the known lack of mtDNA variation between the two groups, we hypothesize that these taxa comprise a monophyletic group, probably comprising two ecotypes that range over distinct environmental conditions and exhibit narrow contact zones. This hypothesis was tested in the North-West Africa contact zone, where *Gazella cuvieri* and *Gazella leptoceros loderi* are found with parapatric distributions (Mallon et al. 2008; Mallon & Cuzin, 2008). Given our hypothesis we expect to observe: 1) overall monophyly corresponding to very recent differentiation; 2) ecotype distribution associated to environmental conditions; 3) suitable areas of occurrence with few areas of sympatry between the ecotypes. This multidisciplinary approach provides an updated taxonomy for North African gazelles in a context of environmental partitioning, thus contributing to local conservation planning.

Table 4.1. Summary of trait similarities and differences between *Gazella cuvieri*, *Gazella leptoceros loderi* and *Gazella leptoceros leptoceros*.

	<i>Gazella cuvieri</i>	<i>Gazella leptoceros</i>		References
		<i>Gazella leptoceros loderi</i>	<i>Gazella leptoceros leptoceros</i>	
Caryotype		2n=33		Robinson <i>et al.</i> 2011
Body length	100 cm		105	Wilson & Mittermeier, 2011
Breeding	Gestation length 160 days	Gestation length 156-169 days		Wilson & Mittermeier, 2011
		Twins are common		Devillers <i>et al.</i> 2005
Habitat	Woodland mountain slopes		Lowland dunes	Wilson & Mittermeier, 2011
Coat colour	Greyish-brown		Sandy coloured	Wilson & Mittermeier, 2011
Distribution	Atlas mountains	Ergs of eastern Algeria and the Tunisian border area	Western desert of Egypt	Groves (1969)
Horns (male)	30.97 cm	29.75 cm	33.73 cm	Groves (1969)

Materials and Methods

SAMPLE COLLECTION

A total of 327 samples were used, including wild and semi-captive animals sampled across North-West Africa (N=284) (Figure 4.1) and individuals sampled in captive breeding centres in Europe (N=43), namely Estación Experimental de Zonas Áridas (Almería, Spain), the Antwerp Zoo (Belgium) and the Hannover Zoo (Germany) (Table 4S.1). Samples include tissue from captive and semi-captive breeding specimens (N=34), and scats, bones, skins and hairs from wild (N=270) and captive and semi-captive specimens (N=23) (Table 4S.1). Samples were preserved in ethanol (96%) until DNA extraction. Sample collection was done under permits from the Haut-Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification (HCEFLCD, Morocco) and the Direction Générale des Forêts (Algeria and Tunisia). Analyses were performed at the CITES registered laboratory: 13PT0065/S. IACUC approval was not requested as no animal was sacrificed and there was no animal husbandry, experimentation or care/welfare concerns. All samples from live animals were collected during routine veterinary interventions (See table 4S.1 and acknowledgements section for details).

DNA ISOLATION, AMPLIFICATION AND SEQUENCING

Genomic DNA was extracted from scats, hair and bone following Silva *et al.* (2015). Briefly, DNA from scats was extracted for each sample using 2-3 pellets collected together (Maudet *et al.* (2004), using a commercial Kit (E.Z.N.A.® Tissue DNA Kit). Hair (3-4 with follicle per sample) and bone (100 - 200 mg) samples were extracted using the QIAamp DNA Micro Kit (Qiagen®). All noninvasive samples were processed in a dedicated low-quality DNA facility equipped with positive air pressure and UV lights. Total DNA from tissue samples was extracted using the Genomic DNA Minipreps Tissue Kit (EasySpin).

One mitochondrial gene (cytochrome b [CYTB]) and five nuclear genes (Kappa casein exon 4 [KCAS], α -lactalbumin intron 2 [LAC], b-spectrin nonerythrocytic 1 [SPTBN1], protein kinase C iota [PRKCI], thyroptin beta chain [THYR]) were used in the analysis (for primers, references and PCR conditions see online Table 4S.2). PCR amplifications were performed in a final volume of 10 μ l using 5 μ l of QIAGEN PCR MasterMix (consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM MgCl₂, dNTP mix, Q solution and HotStart Taq DNA polymerase), 0.2 μ M of each primer and 2 μ l of DNA extraction (approximately 10 ng of genomic DNA). The cycling conditions were carried out with a first denaturation step at 95°C for 15 min, followed by 45 cycles at 95°C

for 30 s, Ta (presented in Table 4S.2) for 40 s, 72°C for 30 s and then a final extension step at 60°C for 10 min. PCR's were carried out in a MyCycler thermocycler (BIO-RAD). Pre- and post-PCR manipulations were conducted in physically separated rooms, always including negative controls to monitor for contamination. PCR products were purified using ExoSAP-IT® PCR clean-up Kit (GE Healthcare, Piscataway, NJ, USA). Sequences were generated for both strands following the BigDye Terminator v3.1 Cycle sequencing protocol. Sequencing products were analysed in a 3130 XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). To check sequence consistency and quality, 20% of noninvasive samples were replicated for PCR and sequencing following recommendations by Tiedemann *et al.* (2012).

PHYLOGENETIC ANALYSES

Sequences generated in this study together with a sequence from Lerp *et al.* (2011) [Genbank JN410347] were aligned using Clustal W (Thompson *et al.* 1994) implemented in BioEdit software (Hall, 1999) and were manually checked and reassessed for any discrepancy. For nuclear fragments, nucleotide ambiguities with similar peak heights were considered heterozygous positions and recoded according to IUPAC. DnaSP v.5 (Librado & Rozas 2009) was used to infer haplotypes for nuclear DNA (nDNA) sequences using PHASE 2.1 algorithm (Stephens, Smith & Donnelly 2001). The analysis was conducted for 1.0×10^4 iterations with a thinning interval of 5 and a burn-in value of 1000. Consistency was checked across runs by analysing haplotype frequency estimates and fit measures. The best-fitting model of sequence evolution for each gene was determined using jModeltest v.2.1.3 (Darriba *et al.* 2012) under the Akaike Information Criterion (AIC).

Alignments (online Table 4S.1) were analysed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Bayesian inference of phylogeny was conducted in BEAST 1.8.0 (Drummond *et al.* 2012) using an HKY85 substitution model with gamma model for rate variation, with mitochondrial and nuclear alignments (concatenated) imported separately. Sequence evolution was assumed to follow a strict molecular clock because only intraspecific data were used in this analysis (Drummond *et al.* 2006). A Yule process speciation prior was implemented in each analysis. Five separate MCMC analyses were run for 50 million generations. After a burnin phase of 5 million generations, trees were sampled every 5,000 generations. Chain stationarity and run parameter convergence were checked using Tracer 1.6 (Rambaut *et al.* 2014). *A priori* information on the mean substitution rate per year was available for CYTB (0.015 substitutions per million years; Lerp *et al.* 2011). Independent runs were combined using LogCombiner v.1.8.0 (Drummond *et al.* 2012). Tree topologies were assessed using

TreeAnnotator v.1.8.0 (Drummond *et al.* 2012) and FigTree v.1.4.0 (Rambaut, 2012). A majority consensus tree was computed from the sampled trees. Haplotype networks were reconstructed using the median-joining algorithm (Bandelt, Forster & Röhl 1999) in Network 4.6.1.1 (Fluxus Technology Ltd.; www.fluxus-engineering.com) for each marker separately. Mitochondrial and nuclear diversity and divergence for the whole set of sequences (Table 4S.1) and for each species separately was calculated using DnaSP v.5 (Librado & Rozas, 2009).

Additionally, we performed two phylogenetic reconstructions based on CYTB sequences available in GenBank from *G. cuvieri* and *G. leptoceros* (Table 4S.3), one using 39 sequences of 282bp, and the second using a subset of 10 sequences covering 1013 bp. MrBayes (Huelsenbeck & Ronquist 2001) plugin of GENEIOUS® v6.7.1 was run with HKY85 substitution model with gamma model for rate variation. Two parallel runs were performed using four chains (one cold and three hot) for 1.1×10^6 generations and sampling every 200 generations from the Markov Chain Monte Carlo (MCMC). We determined stationarity by plotting the log likelihood scores of sample points against generation time; when the values reached a stable equilibrium and split frequencies fell below 0.01, stationarity was assumed. Burn-in length was 100,000. The plugin generates a maximum clade credibility (MCC) tree. Bayesian posterior probabilities (BPP) of >0.95 were considered strongly supported (Huelsenbeck & Ronquist 2001). Maximum-likelihood (ML) searches in PHYML (Guindon & Gascuel 2003) were performed with 1000 replicates for bootstrap analyses; nodal support for bootstrap values ≥ 70 were considered significantly supported (Hillis and Bull, 1993).

DISTRIBUTION DATA AND ENVIRONMENTAL VARIABLES

The ecological niche occupied by *Gazella cuvieri* and *Gazella leptoceros loderi* was characterized in an area comprising Morocco, Algeria and Tunisia, northern Mauritania, Mali and Niger, and western Libya (Figure 4.1). It was defined between 17.5°N and 37.5°N and west of 15.5°E, in order to include the global range of *Gazella cuvieri* and the western distribution of *Gazella leptoceros* (75% of total range), according to extent of occurrence polygons for both species (Mallon and Cuzin, 2008: *G. cuvieri*; Mallon *et al.* 2008: *G. leptoceros*). The Eastern part of *G. leptoceros* distribution was excluded from the analyses due to lack of sequenced data available from that region.

A total of 42 and 19 geo-referenced presence points of *Gazella cuvieri* and *Gazella leptoceros loderi*, respectively, were used for characterizing the ecological niche occupied by both taxa. Other presence points were available in the literature but we decided to only include points for which the genetic assignment to a specific unit was confirmed by both mtDNA and nDNA. This was mostly done, to avoid problems of

misidentification related with morphology and all taxonomic problems. Although the sample size for the ecological analyses might be small, by using only genetically validated data, we assure a dataset free of ambiguities related to morphological misidentification and taxonomy.

Thirteen environmental variables were selected for ecological analyses (Table 4S.4), taking into account their representativeness in the study area and their meaningfulness to the distribution of gazelles (e.g. Nazeri *et al.* 2015). Two sets of variables (WGS84 datum) were selected: i) Topoclimatic variables including Slope, derived from a topographical grid (USGS, 2006) using the “Slope” function of ArcGIS, five climate grids from WorldClim (Hijmans *et al.* 2005), and potential evapo-transpiration (Tabucco and Zomer, 2009); and ii) Habitat variables including six distances to habitats grids derived from a land-cover grid for the years 2004 to 2006 (Bicheron *et al.* 2008). To create a gradient of land cover types, categorical land cover variables were converted into continuous variables. For this purpose, one binary grid was created for each habitat type. The Euclidean distance of each grid cell to the closest habitat type cell was calculated (Brito *et al.* 2009). The pixel size of all environmental variables was set taking into account the double of distance covered in one day by *Gazella subgutturosa* (~10km; Bagherirad *et al.* 2014), and projected from the original square pixel size of 30' (~1x1km) to 12' (~20x20km). Correlation between variables was lower than 0.75 in all cases, with the exception of maximum temperature of the warmest month and minimum temperature of the coldest month both with annual mean temperature (0.77 and 0.81, respectively).

ENVIRONMENTAL VARIABILITY

Two Spatial Principal Components Analyses (SPCA) were independently performed to summarize the spatial topoclimatic (PCAtc) and habitat variability (PCAha) of the study area. All environmental variables were previously centered and scaled due to different measurement units. The SPCAs were performed with the “Principal Components Analysis” extension of the GIS ArcMap 10.0 (ESRI, 2011). The first two orthogonal components retained in both PCAtc and PCAha were also used to summarize the spatial topoclimatic and habitat range of each taxon. The values of each retained component of each SPCA were extracted for each taxon observation. The spatial topoclimatic and habitat variability of each taxon was then visually compared with the retained components of both PCAtc and PCAha of the study area.

ECOLOGICAL NICHE-BASED MODELLING

The Maximum Entropy approach implemented in Maxent 3.0.4 beta software (Phillips, Anderson & Schapire 2006) was used to identify environmental variables related to the distribution of each taxon and to derive ecological models for each taxon. This technique requires only presence data as input, and consistently performed well in comparison to other presence-only techniques (such as Bioclim, Domain, GARP), particularly with low sample sizes (Elith *et al.* 2006; Hernandez *et al.* 2006; Aliabadian *et al.* 2016). Two models were built in Maxent for each taxon: a Topoclimatic model using the first three retained orthogonal components of the PCA_{tc}, and a Habitat model using the first three components of the PCA_{ha}, as model inputs. Both models were developed with a total of 10 replicates with 10% of test data chosen by bootstrap with random seed, auto-features, and logistic output (Phillips *et al.* 2006). Area under the curve (AUC) of the receiver-operating characteristics (ROC) plot was taken as a measure of the model's fitness (Fielding & Bell 1997). Finally, the 10 replicates were averaged to generate a forecast of taxa presence probability (Table 4S.5), which is a robust procedure to derive consensus predictions of likelihood of presence (Marmion *et al.* 2009).

The importance of environmental variables for explaining the distribution of each taxon was determined from their average percentage of contribution and permutation importance to each training model type for each morphotype. The relationship between taxa occurrence and variables was determined by visual examination of response curve profiles from univariate models.

The probability models of each taxon were reclassified to display grid cells of probable absence and presence. Given that less restrictive thresholds could be applied for conservation proposes (Liu *et al.* 2005), the minimum training presence threshold (MTP) was used, since it forces all training observations to be considered as predicted. To calculate MTP for each taxon in each model, training observations were intersected with the average probability of occurrence models and the minimum probability value was taken as the MTP (Vale, Tarroso & Brito 2014). The MTP was then used to classify average continuous probability models into binary maps. To identify sympatric areas between both taxa, the binary maps of each taxon were combined. Areas where the predicted suitable areas of each taxon overlapped in space were considered areas of sympatry. Additionally, for visual inspection of the probability of each taxon occurrence and variables in the area of the sympatry, a 500 km transect was designed crossing longitudinally both suitable areas. The location and orientation of the transect was set in order to cross the maximum extent of the sympatric area (~120km) and suitable areas of each taxon (~190km for each taxon suitable area). The values of probability of

occurrence of each taxon and the values of the PC1 of the PCA_{tc} and PCA_{ha} were extracted at intervals of 10km.

Results

PHYLOGENETIC ANALYSES

PCR amplifications of fragments were obtained for all invasive samples. Of the 237 noninvasive samples analysed, 68% showed amplifiable DNA for CYTB, whereas 86% provided positive results for KCAS. Amplification success was lower for the other nuclear markers with larger fragments (Figure 4S.1, Table 4S.2). All sequences where replicates were performed recovered matches across the entire sequence length.

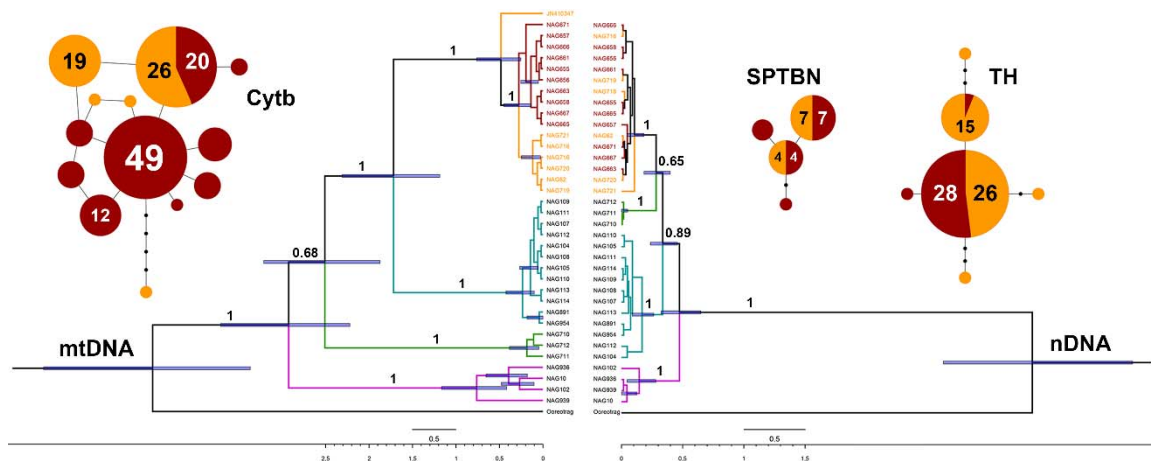


Figure 4.2 Phylogenetic trees based on Bayesian inference showing the relationships among North African gazelles for the mtDNA cytochrome b gene (left tree) and for five nDNA concatenated genes, KCAS, LAC, PRKC, SPTBN, and TH (right tree). Values on branches indicate posterior probability support. Clades are coloured according to the species name: brown for *Gazella cuvieri*, yellow for *G. leptoceros loderi*, blue for *G. dorcas*, green for *N. dama*, purple for *Eudorcas rufifrons*, and black for *Oreotragus oreotragus* (outgroup). The sequences used are indicated in Table 4S.1. Median-joining networks for the CYTB (top left) and for two nuclear genes, TH and SPTBN, (top right) in *Gazella cuvieri* and *Gazella leptoceros loderi*. The number of sequences for each locus are 154 (CYTB), 74 (TH), and 26 (SPTBN). Each circle represents one haplotype and circle area is proportional to the frequency of each haplotype. Circles are coloured according to the species name: brown for *Gazella cuvieri*, yellow for *G. leptoceros*. Total frequency is indicated for more common haplotypes. Branches are proportional to the number of nucleotide differences between haplotypes and dots on branches indicate mutational steps.

The CYTB alignment for phylogenetic reconstruction yielded 37 distinct sequences (500 bp long) from 10 *G. cuvieri* and seven *G. l. loderi*, and 20 outgroup sequences from five other ungulate species (Table 4S.1, Figure 4.2). The final dataset for the reconstruction of the concatenated nuclear phylogeny consisted of 36 sequences (2485 bp long), missing only one sequence of *G. l. loderi* (Table 4S.1). Sequences used for the

phylogenetic analyses among North African gazelles (Table 4S.1) resulted in 84 variable sites for CYTB and 48 variable sites for nuclear genes, of which 14.2 % (71/500) and 1.2 %, (31/2495) respectively, were parsimony informative. Phylogenetic trees recovered four species of ungulates (Figure 4.2, Table 4S.6) with posterior probabilities ≥ 0.95 , with the exception of a monophyletic group containing *G. cuvieri* and *G. l. loderi*. The same monophyly was strongly supported when analysis was performed using available mitochondrial sequences (Figure 4S.3).

Only CYTB and two nuclear markers (SPTBN and TH) exhibited polymorphism across the *G. cuvieri* and *G. l. loderi* sequenced individuals, in which 13, four and six haplotypes, respectively, were found (Table 4S.1, Figure 4.2). CYTB exhibited the highest values of haplotype ($H_d = 0.790$) and nucleotide diversity ($\Pi = 0.003$). KCAS, LAC and PRKC presented only one haplotype for the *G. cuvieri* and *G. l. loderi* dataset (Table 4.2). The CYTB network exhibited 13 haplotypes of which 12 are closely related (corresponding to a divergence of 0.3%) while the last haplotype, corresponding to *G. l. leptoceros* from Egypt, exhibited a genetic divergence five times higher (1.4%) than all other North-West African samples (Figure 4.2, Table 4S.1). One of the two most common CYTB haplotypes was shared between *G. cuvieri* and *G. l. loderi*. The same was observed for TH and SPTBN networks in which the most common haplotypes are shared between *G. cuvieri* and *G. l. loderi*. Given these results, we will hereafter avoid the use of current species nomenclature, referring to *Gazella cuvieri* and *Gazella leptoceros loderi* as ecotypes: *G. cuvieri* corresponding to the mountain type and *G. l. loderi* to the lowland type.

Table 4.2. Genetic diversity observed in *Gazella cuvieri* and *Gazella leptoceros loderi* for the six gene regions included in this study.

Fragments	n	S	h	Hd	k	Π
CYTB	154	13	13	0.790	1.294	0.003
KCAS	233	0	1	0.000	0.000	0.000
LAC	53	0	1	0.000	0.000	0.000
PRKC	54	0	1	0.000	0.000	0.000
SPTBN	26	4	4	0.625	0.883	0.001
TH	74	10	6	0.426	0.602	0.001

n number of samples sequenced; s number of segregating sites; h number of haplotypes; Hd haplotype diversity; K average number of differences between pairs of sequences; Π nucleotide diversity

ECOLOGICAL NICHE-BASED MODELLING

The first two axes of Spatial Principal Components Analyses (SPCA) explained 76% and 72% of the topoclimatic (PCAtc) and habitat variability (PCAha) of the study area, respectively (Table 4S.7). The first principal component (PC1) of the PCAtc accumulated most of the temperature variation of the study area, while the second component (PC2) retained the variability in potential evapo-transpiration. The PC1 of the PCAha

accumulated most of the variation in vegetated areas, while the PC2 retained the variability in bare areas and rocky deserts. Mountain and lowland ecotypes occupied distinct topoclimatic and habitat environmental spaces according to the first two axes of the PCA_{tc} and PCA_{ha} (Figure 4.3a).

The ROC plots of Maxent models exhibited high average AUCs (>0.98) in both training and test data sets and in both ecotypes (Table 4S.5). The distributions of mountain and lowland ecotypes were mostly related with the PC1 of both PCA_{tc} and PCA_{ha}, and the lowland type was also related with the PC2 of the PCA_{tc} (Table 4.3). Presence probability of both ecotypes was unimodal along the gradient of variation of PC1_{ha} (Figure 4.3b), which depicts variation in distances to mosaics of vegetation/cropland (Table 4S.7). The lowland ecotype tended to exhibit narrower unimodal response patterns along PC1_{tc} (temperature variation) and PC2_{tc} (potential evapo-transpiration variation) in comparison to the mountain ecotype. Presence probability of mountain ecotype increased along PC2_{ha} (variation in distances to bare and rocky areas) and decreased in lowland ecotype.

Suitable areas predicted by Maxent models for the mountain ecotype were restricted to the Atlas Mountains, while for the lowland ecotype they cover mostly the sandy areas of the Grand Erg Occidental and Oriental, and the Ergs Tifernine-Issaouane, Awabari, and Murzuk (Figure 4.4). Areas predicted as suitable for both ecotypes were small (1.4% of the total area) and restricted to the transition zones between the southern-faced slopes of the Atlas Mountains and the flat and sandy Grand Ergs of Algeria (Figure 4.4). From mountain to lowland areas, following the established 500 km transect, probability of occurrence of *G. cuvieri* decreased and increased for *G. leptoceros*, and values of variation in PC1_{tc} (temperature) and PC1_{ha} (mosaics vegetation/cropland) decreased (Figures 4.3c and 4S.2).

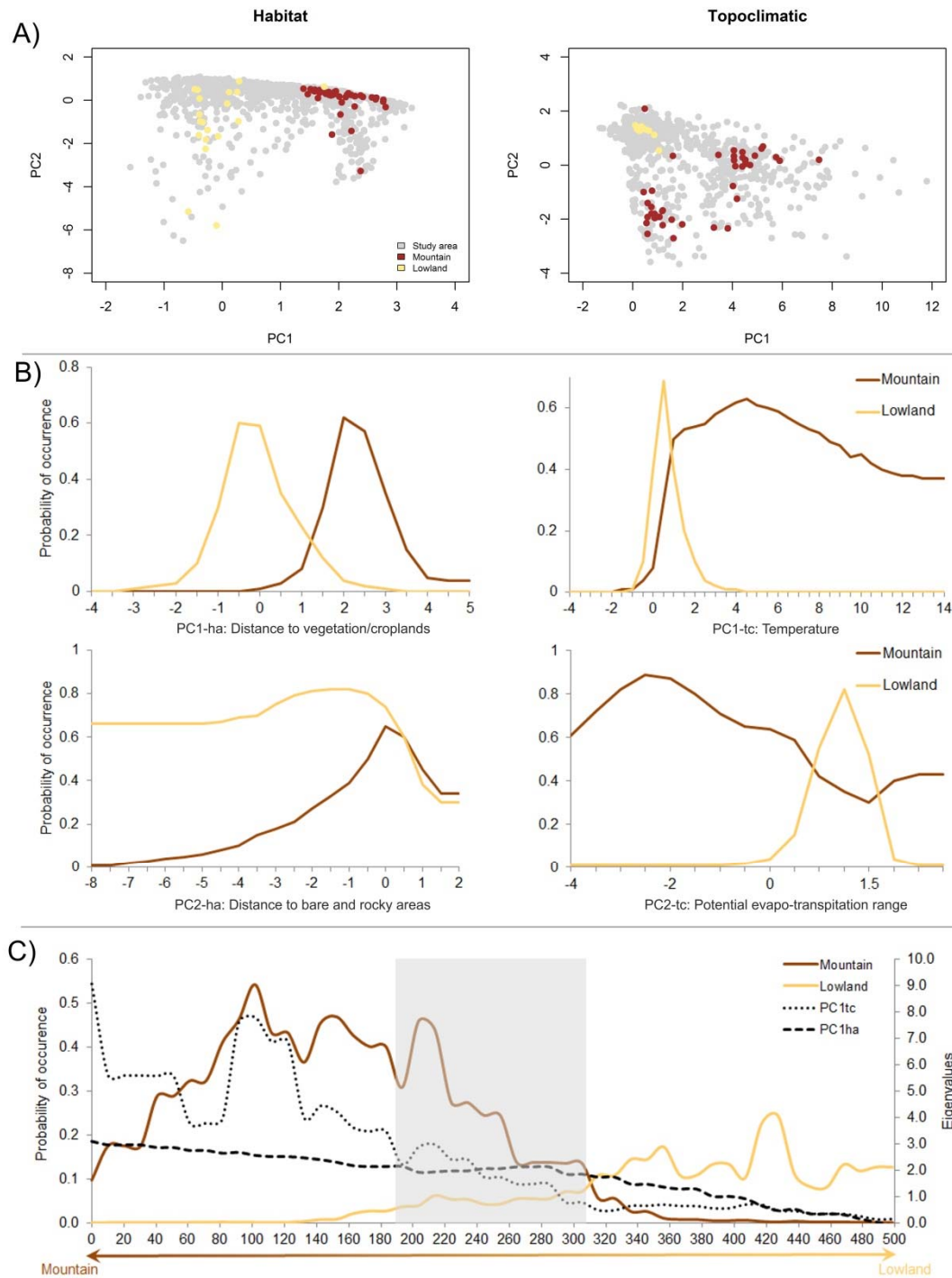


Figure 4.3 A) Habitat and topoclimatic variability of the study area derived by a Spatial Principal Components Analysis and location of each ecotype (mountain and lowland) along the variability. The PC1-habitat represents variation in distances to mosaics vegetation/cropland, the PC2-habitat represents variation in distances to bare and to rocky areas, the PC1-topoclimatic represents temperature variation, and the PC2-topoclimatic represents variation in range of potential evapo-transpiration. B) Response curves for the habitat and topoclimatic factors most related to the distribution of mountain and lowland ecotypes. C) Probability of occurrence of mountain and lowland ecotypes along a 500 km Euclidian transect (see Figure 4.4 for transect location).

Table 4.3. Measures of the contribution of environmental variables to the 10 replicate ecological models for each ecotype (mountain and lowland): percentage of contribution (%) and permutation importance (PI).

		Mountain		Lowland	
		%	PI	%	PI
Topoclimatic	PC1	37.6	34	28.3	15.9
	PC2	9.6	7.0	31.3	60.9
	PC3	1.9	0.2	1.1	1.2
Habitat	PC1	25.9	38.6	21.5	19.6
	PC2	11.5	8.5	16.6	1.5
	PC3	13.5	11.6	1.2	0.9

Models were derived with the first three principal components of both topoclimatic and habitat PCAs.

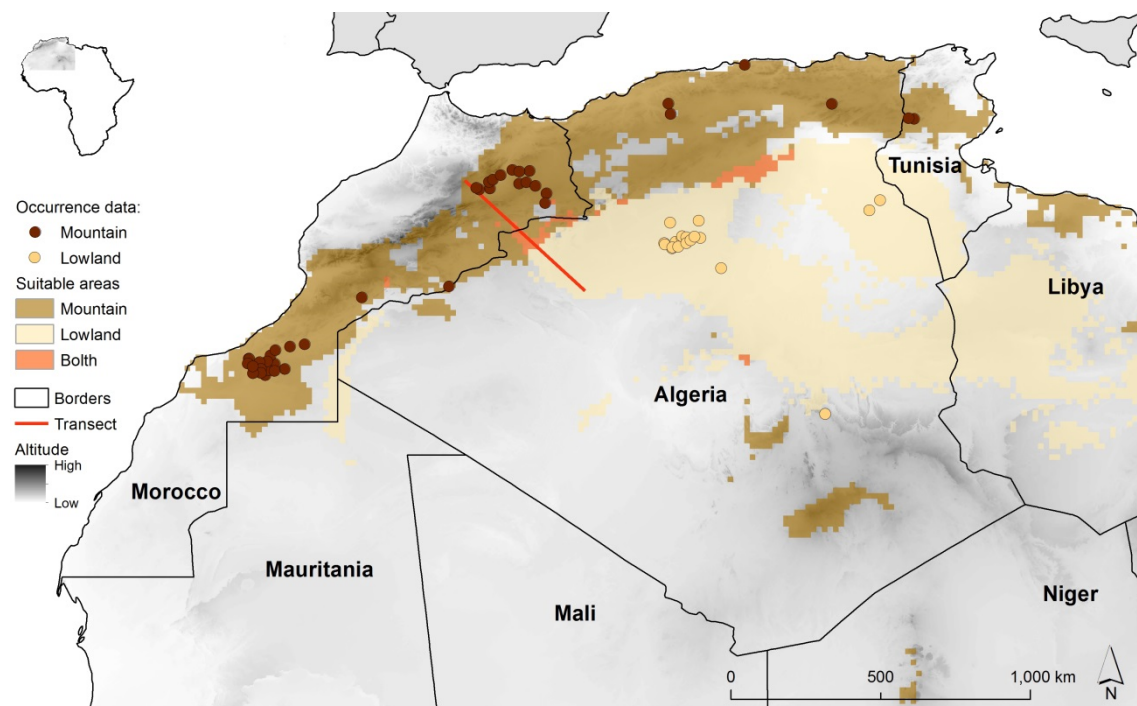


Figure 4.4 Probable suitable areas for each ecotype (mountain and lowland) and areas predicted as suitable for both ecotypes according to 10 replicates of ecological models. Occurrence data of both ecotypes used for modelling purposes. Transect is marked in red.

Discussion

This work clearly shows that *Gazella cuvieri* and *Gazella leptoceros loderi* are genetically similar entities that occupy distinct geographic and environmental spaces. They correspond to two discrete populations lacking genetic differentiation, however exhibiting different ecological (niches) and morphological (coat colour) adaptations (Kingdon *et al.* 2013). Thus, we suggest that *G. cuvieri* and *G. leptoceros loderi* constitute two ecotypes of the same species, corresponding to a mountain and a lowland form, respectively. The two types still qualify under Evolutionary Species Concept (ESC) (Frankham *et al.* 2012), and based in Frankham (*et al.* 2011) the “risk of outbreeding depression tree” is low to

moderate (following an example of a situation of a previous classification as two subspecies, but subsequently revised on the basis of molecular studies). Here we propose that the two taxa should be lumped into one single species, *Gazella cuvieri*, which was described by William Ogilby in 1841, one year before Frédéric Cuvier's description of *G. leptoceros*. We discuss the results found supporting the stated hypothesis and the conservation implications of the proposed systematics rearrangement.

The combination of mitochondrial and nuclear data clearly suggest that *G. cuvieri* and *G. l. loderi* are genetically closely related taxa (Figure 4.2). Both species share the most common haplotypes in all analysed nuclear *loci*, exhibiting only a few apparent ecotype specific variants in very low frequency. We found very low genetic divergence between the two taxa despite the great discriminatory power of the molecular markers used to identify the monophyly of all other *Gazella* species, including *G. l. leptoceros*. Previous studies based in mtDNA alone have also shown the lack of differentiation between *G. cuvieri* and *G. l. loderi* (Rebholz & Harley 1999; Wronski *et al.* 2010; Wachter *et al.* 2010; Lerp *et al.* 2011; Hassanin *et al.* 2012; Bärmann *et al.* 2013; Silva *et al.* 2015). A taxonomic lumping of the two taxa was already suggested by Hassanin *et al.* (2012), although the authors clearly acknowledged the need for additional evidence before definitive conclusions. The analysis of a large number of individuals from several different regions, and the use of five nDNA fragments in the present study excludes confounding effects in species delimitation caused by sample bias or mitochondrial introgression (e.g. Galtier *et al.* 2009; Melo-Ferreira *et al.* 2012; Tarroso *et al.* 2014). In this study, we provide for the first time a clarification of the genus' systematics.

Gazelles constitute an heterogeneous group with several species described based on morphological traits (e.g. Bagherirad *et al.* 2014; Bärmann *et al.* 2013; Gentry 1965; Lerp 2013; Lerp *et al.* 2011; Wachter *et al.* 2010), which has certainly contributed to taxonomic inflation (Senn *et al.* 2014). Interpretations of the genetic data obtained in this work are unequivocal and suggest that these taxa should be synonymised. *Gazella cuvieri* clearly comprises both *G. cuvieri* and *G. l. loderi* (restricted to the northwest of the Sahara), for which mitochondrial genetic divergence is only 0.3%. Specimens of *G. l. leptoceros* from Egypt are probably the only remaining candidate of the species *G. leptoceros*, with a mitochondrial genetic divergence from the other taxa of about 1.4% (see Figure 4.2; Lerp *et al.* 2011; Silva *et al.* 2015). Nevertheless, this mitochondrial differentiation is of the same order of magnitude of the one observed among North African *Gazella dorcas* (Godinho *et al.* 2012). Further analysis, including nuclear data, should be used to assess the species status of the populations assigned to *G. l. leptoceros* in Egypt.

The present study shows that populations assigned to *G. cuvieri* and to *G. l. loderi* occupy distinct eco-geographic spaces. Whereas populations of *G. cuvieri* are mostly restricted to mountain areas characterized by low temperatures, high rainfall, mosaics of vegetation/cropland, populations of *G. l. loderi* occupy plains characterized by high temperatures, low rainfall, and bare and rocky areas. The observed differences in the environmental spaces occupied by each ecotype are consistent with the mountain rocky areas occupied by *G. cuvieri* and the sandy areas (known as Ergs) occupied by *G. l. loderi* (Beudels-Jamar *et al.* 2005; Devillers *et al.* 2005). Remarkably, the predicted suitable areas for each ecotype are in concordance with the currently known distributions of *G. cuvieri* and *G. l. loderi* (Mallon and Cuzin 2008; Mallon *et al.* 2008). Potentially suitable areas for the occurrence of these populations are mostly allopatric, as previously suggested by Beudels-Jamar *et al.* (2005). However, small and fragmented areas of sympatry were predicted between the two forms in the transition zones between the southern-faced slopes of the Atlas Mountains and the flat and sandy Grand Ergs of Algeria. They are located in sharp transition areas of environmental variability, particularly in climatic variation (Figure 4.3). As the distributions of both populations are mostly related to climatic variables, in comparison to habitat factors, and tend to exhibit unimodal responses to distinct values of environmental variation, a bimodal distribution of different forms is expected (Anderson, Alexandersson & Johnson 2010), as well as potentially restricted sympatry areas. Patterns of probability of occurrence along environmental gradients and the predicted suitable areas suggest that populations assigned to *G. cuvieri* and to *G. l. loderi* constitute two different ecological units, involving ecotype formation in parapatric populations.

Taken together, the genetic, ecological and morphological evidence indicate that both taxa should correspond to two ecotypes of *G. cuvieri* (*sensu* Lowry, 2012): the current *G. cuvieri* corresponding to a mountain ecotype and the current *G. l. loderi* corresponding to a lowland ecotype. Given that these taxa range over distinct environmental conditions, the observed morphological differences may result of multiple trait adaptations to the different environments across their range as suggested by Lowry (2012). Interestingly, other ecotypes were recently suggested for the same geographic region for two sister species of fox, *V. rueppellii* as an arid ecotype of North African *V. vulpes*, also showing incomplete genetic differentiation (Leite *et al.* 2015). The contact zone between the two fox ecotypes remarkably coincides with the predicted suitable area for the mountain and lowland *Gazella* ecotypes, the border areas between Morocco and Algeria. The observed distribution patterns associated with the low levels of predicted sympatry also reinforce the ecological differences between *G. cuvieri* and *G. l. loderi*. Given that habitat variation in the predicted contact zone is less pronounced (in comparison to climatic variation),

gene flow events could occur between the two *Gazella* ecotypes, as similarly suggested for North African *Vulpes* ecotypes (Leite *et al.* 2015). Future studies using fast-evolving markers should focus on assessing potential gene flow in the predicted contact zone between the two *Gazella* ecotypes.

The proposed lumping of *G. l. loderi* with *G. cuvieri*, and the possible restriction of *G. leptoceros* to Egyptian populations, justify a reassessment of the current conservation status of these taxa. The reassessment of *G. cuvieri* status needs to consider mountain and lowland ecotypes, which should be listed as distinct evolutionary significant units (ESU's) for conservation purposes (Fraser & Bernatchez 2001). Conserving ecotypes allows the preservation of species adaptive potential under climatic and habitat change and their maintenance assures the preservation of diversity that may promote the species' long term persistence (Morrison 2012). To this point, poaching and habitat degradation have been the main drivers of the low genetic diversity, range contraction and continuous demographic decline experienced by *G. cuvieri* (*sensu* this paper) (Beudels-Jamar *et al.* 2005; Devillers *et al.* 2005; Mallon and Cuzin 2008; Mallon *et al.* 2008; Pauls *et al.* 2013; Durant *et al.* 2014; Payne and Bro-Jørgensen 2015). These threats may likely increase in the near future given the increasing accessibility and exploitation of natural resources within the Sahara-Sahel (Brito *et al.* 2014; Brito *et al.* 2016). Therefore, although these ecotypes constitute a single species, they should be treated as distinct conservation units to preserve their adaptive potential to different environments. This may be particularly important in the Sahara Desert, as predictions of human-induced climate change suggest high warming rates (Loarie *et al.* 2009). It is important to highlight also that we can be in the presence of an epigenetic effect and/or positive phenotypic selection (e.g. Baker *et al.* 2015). Only functional genomics would help us to elucidate this in detail. The integrative approach used in the present work should be applied to all members of the genus *Gazella* to assess cryptic diversity and evolutionary significant units, to evaluate adaptation to local environmental conditions across populations, and to clarify their systematics and taxonomic status.

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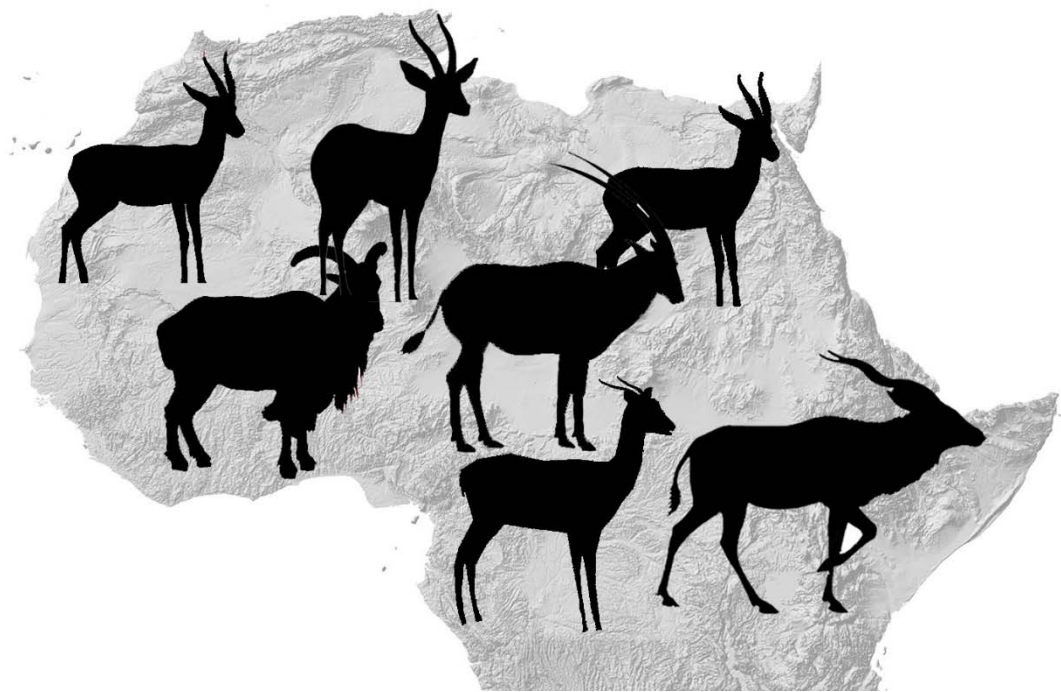
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CHAPTER 5

Relationships between genetic diversity levels and range regression and intrinsic/extrinsic factors

Paper III Range regression in threatened African ungulates: implications in genetic diversity and ecological niche

Silva TL, Vale CG, Abáigar T, Brito JC.

In prep.

Range regression in threatened African ungulates: implications in genetic diversity and ecological niche

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Abstract

The current rates of biodiversity decline associated with increasing human pressures are expected to cause a global decay in genetic diversity. North African ungulates have suffered distinct levels of range regression and thus are suitable cases to test hypotheses of relationships between genetic diversity levels and range regression and intrinsic/extrinsic factors. Genetic diversities of 12 African ungulates were related with geographic and biological traits, and environmental conditions in historical and present distributions. We found that: i) genetic diversities are the lowest in the species that have suffered the largest range regressions, such as *Addax nasomaculatus*, *Gazella leptoceros*, *Nanger dama*, and *Oryx dammah*; ii) genetic diversities were mostly related with environmental variation (high productivity and precipitation variability) and human pressure variables (high remoteness and distance to roads), in comparison to biological or geographic traits; and iii) the measured realized ecological niche overlap of historical and present distributions based in environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time. The present study supports the hypothesis that extant populations are likely occupying the last strongholds of suitable habitats, which are retaining the highest observed genetic diversities among all taxa. Extant populations occur under harsher environmental conditions, and thus may be vulnerable to the frequent drought periods that characterise North Africa. The overall reduced genetic diversities found in this study suggest that these populations may have lost already a portion of the adaptation ability to further environmental change. Relating genetic diversity patterns and changes in environmental and niche conditions offers useful insights for conservation planning of imperilled desert fauna.

Introduction

Genetic diversity is often considered the most fundamental dimension of biodiversity (May and Godfrey, 1994). The amount of genetic variation among individuals within a species, i.e. the intraspecific genetic diversity, provides the critical basis for evolutionary change, such as adaptation potential to increasingly rapid environmental change (Sarre and Georges, 2009).

Ecological and evolutionary processes underlying genetic diversity distribution patterns involve interacting intrinsic factors, such as body size, metabolic rate, mutation rate, and reproductive output, as well as extrinsic factors, such as climate and habitat productivity (Avice, 2004, Hartl and Clark, 2007). For instance, nucleotide substitution rates have been positively correlated to diversification rates and contemporary species richness (Eo and DeWoody, 2010), and there is latitudinal gradient in the distribution of genetic diversity, tropical regions are richer than the poles (Brown, 2014; Miraldo et al., 2016).

The current rates of biodiversity decline associated with increasing human pressures (Butchart et al., 2010) are expected to cause a global decay in genetic diversity (Provan and Maggs, 2012, Bálint et al., 2014). Genetic diversity levels are related to non-equilibrium range expansions and contractions (Segelbacher et al., 2010). Range regression usually leads to decline in population size, and consequently to a decrease in genetic diversity, reproductive fitness, and a limited ability to adapt to environmental change, which increase extinction risks (Furlan et al., 2012) and negatively impact in specie's viability (Frankham et al., 2002, Milot et al., 2007, Pauls et al., 2013). Mammals in particular have been reported to have less genetic diversity than do birds or fishes, and genetic diversity in threatened/endangered mammals to be positively correlated with range size (Doyle et al., 2015). The major losses in the world's large mammal biodiversity are found in ungulates (Cardillo et al., 2014; Ripple et al., 2016). Thus, it is expected that the ungulates that suffered the most extreme range regression should depict presently the lowest genetic diversity levels and the narrowest ecological niches in comparison to other species less affected by range regression.

The African ungulates have suffered distinct levels of range regression and thus are suitable cases to test hypotheses of relationships between genetic diversity levels and range regression and intrinsic/extrinsic factors. In North Africa, from the eight taxa presently known (Figure 5.1), all suffered range regression above 75% and one is extinct in the wild (Durant et al., 2014). Increasing human activities in the region have prompted illegal killing and natural habitat loss (Brito et al., 2014, 2018; Newby et al., 2016), with conservation status ranging from Vulnerable, Endangered, Critically

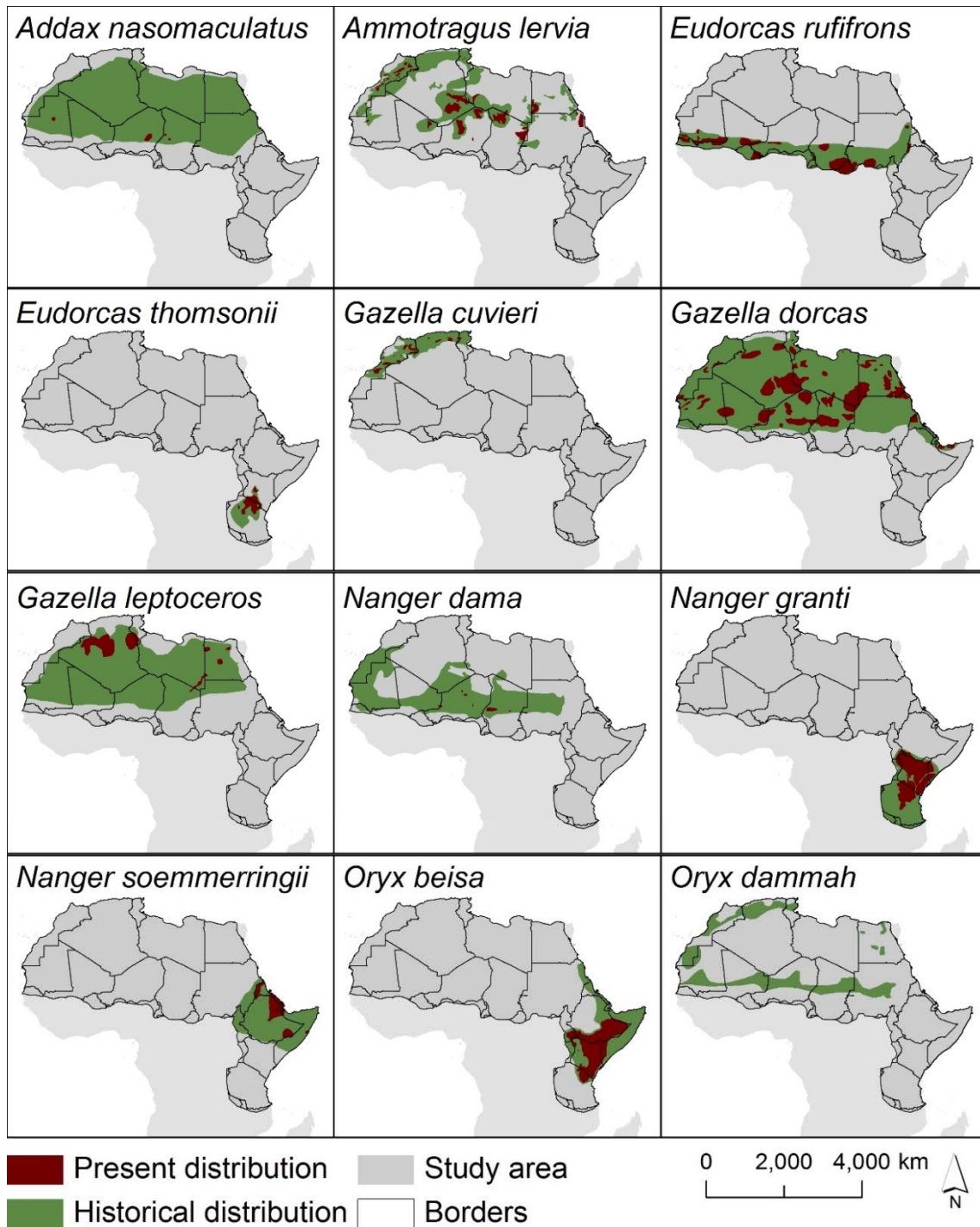


Figure 5.1 Historical and present distributions of the 12 African ungulates studied.

Endangered to Extinct in the Wild (IUCN, 2017). In East Africa, four ungulates are known from similar arid conditions but where range regression has been less pronounced (East, 1988, 1999; Mallon and Kingswood, 2001) and the conservation status ranges from Least Concern to Vulnerable (IUCN, 2017).

The aim of this study is to evaluate the significance of relationships between estimates of genetic diversity and multiple intrinsic/extrinsic predictors, including biological and

geographic traits, environmental factors and human pressure variables. Distinct historical and present distributions of 12 African ungulates will be used to answer the following questions: 1) Is genetic diversity related with range regression and latitudinal distribution? 2) Is genetic diversity related with intrinsic biological traits? 3) Which geographic and environmental predictors are most related with genetic diversity in historical and present distributions? 4) How similar is the realized ecological niche between historical and present distributions according to environmental and human pressure variations? In this work, we examine the different possible effects of range regression, latitudinal distribution, biological traits, and environmental variables and human factors that can operate at the genetic diversity level, and how range regression may impact over the realized ecological niche according to environmental variation and human pressure variables.

Methods

Study area and species

The study area covers the combined distribution of 12 studied ungulates and extends from North Africa to East Africa (Figure 5.1; supplementary material, Figure 5S.1). These include the eight taxa present in North Africa: 1) Addax, *Addax nasomaculatus* (de Blainville, 1816) (Conservation status: CR-Critically Endangered); 2) Barbary sheep, *Ammotragus lervia* (Pallas, 1777) (VU-Vulnerable); 3) Red-fronted gazelle, *Eudorcas rufifrons* (Gray, 1846) (VU); 4) Cuvier's gazelle, *Gazella cuvieri* (Ogilby, 1841) (EN-Endangered); 5) Dorcas gazelle, *Gazella dorcas* (Linnaeus, 1758) (VU); 6) Slender-horned gazelle, *Gazella leptoceros* (Cuvier, 1842) (EN); 7) Dama gazelle, *Nanger dama* (Pallas, 1766) (CR); and 8) Scimitar-horned oryx, *Oryx dammah* (Cretzschmar, 1826) (EW-Extinct in the Wild). In addition, four ungulates ranging in East Africa were included for comparisons, to represent the genus diversity and the variability in conservation statuses: 9) Thomson's gazelle, *Eudorcas thomsonii* (Günther, 1884) (NT-Near Threatened); 10) Grant's gazelle, *Nanger granti* (Brooke, 1872) (LC-Least Concern); 11) Soemmerring's gazelle, *Nanger soemmerringii* (Cretzschmar, 1826) (VU); and 12) Beisa oryx, *Oryx beisa* (Rüppell, 1835) (NT).

The distribution polygons of the 12 species were retrieved from IUCN (2017) and historical and present ranges were built based on reference works (East, 1988, 1999; Mallon and Kingswood, 2001; Durant et al., 2014; Mallon and Jama, 2015; Newby et al., 2016; Brito et al., 2018). The historical range was taken as a representation of the

species distribution in the beginning of the 20th century, while the present range represents the current species distribution observed in the field. The distribution limits of the mountain and lowland ecotypes recently described for *G. cuvieri* (Silva et al., 2017) were not used in this study as historical distributions were unavailable (via IUCN). As such, for the purposes of this study, *Gazella leptoceros* includes also the lowland ecotype of *G. cuvieri*, while *G. cuvieri* includes only the described mountain ecotype (i.e. it was followed the previous taxonomic arrangement).

Genetic data and genetic diversity

All data available on GenBank for the selected taxa was recovered (supplementary material, Table S1) including information on the cytb, the most commonly studied fragment. In order to choose a common fragment, to allowing comparisons, collected sequences were aligned and a common fragment of 926 bp was found. Given that a significant amount of information would be lost due to the size of the available fragment to estimate genetic diversity parameters, two additional small fragments were included: 199 bp and 309 bp. Haplotype (HDI) and nuclear (NDI) diversities were calculated in the three datasets with DnaSP v.5 (Librado and Rozas, 2009). HDI measures relative haplotype frequency of each haplotype in the sample, while NDI measures number of nucleotide differences per nucleotide site between sequences. Therefore, HDI represents the uniqueness of a particular haplotype in a given population while NDI represents the polymorphism within a population. In this study, “populations” are the studied taxa. The analysis involved 161, 378 and 286 nucleotide sequences from each of the datasets with 926, 199 and 309 bp fragments, respectively (supplementary material, Tables 5S.1 and 5S.2). The genetic diversity measures used in following analyses were obtained by averaging the results from each of the datasets (supplementary material, Table 5S.2). Pearson’s correlation coefficient between HDI and NDI was non-significant ($r=0.43$; $p=0.17$).

Predictors of genetic diversity

A total of 34 predictors of genetic diversity were analysed, grouped in four categories: geographic traits, biological traits, environmental factors, and human pressure (Table 5.1). Geographic traits included range size, range regression, and latitudinal distribution. The distribution polygons were projected in a Geographical Information System (GIS; ESRI, 2010) to quantify the range size in historical and present periods. The percentage of area contraction between the historical and the present ranges was taken as an estimation of specie’s range regression. The latitudes of the centroids of the historical

distribution polygons were taken as estimates of the mean latitudinal ranges across Africa.

Table 5.1. List of predictor variables tested against Haplotype and Nucleotide diversities in 12 African ungulates.

Code	Variable	Units
Geographic traits		
LAT	Latitudinal distribution	Degrees
RAS	Range size	Degrees
RRE	Range regression	Percentage
Biological traits		
FEC	Average fecundity	Number of offspring/birth
SMA	Average sexual maturity	Years
LON	Average longevity	Years
BLE	Average body length	cm
BWE	Average body weight	Kg
HOR	Average relation of male horn length/body length	%
GRS	Average social group size	Number of individuals
Environmental factors		
SLO	Slope	Degrees
SST	Slope standardized	Adimensional
SSD	Slope standard deviation	Adimensional
PET	Potential evapo-transpiration	
PEST	Potential evapo-transpiration standardized	Adimensional
PESD	Potential evapo-transpiration standard deviation	Adimensional
ATE	Annual temperature	°C
AST	Annual temperature standardized	Adimensional
ASD	Annual temperature standard deviation	Adimensional
NDVI	Normalized difference vegetation index	Adimensional
NDST	NDVI standardized	Adimensional
NDSD	NDVI standard deviation	Adimensional
PRE	Precipitation	mm
PRST	Precipitation standardized	Adimensional
PRSD	Precipitation standard deviation	Adimensional
Human pressure		
HPD	Human population density	N of persons/km ²
HPSD	Human population density standard deviation	Adimensional
RDS	Distance to roads	Degrees
RSD	Distance to roads standard deviation	Adimensional
ANT	Amount of the range inside wild anthromes	%
REMT	Remoteness	Adimensional
RESD	Remoteness standard deviation	Adimensional
HFP	Human footprint	Adimensional
HSD	Human footprint standard deviation	Adimensional

Biological traits included average fecundity, mean age at sexual maturity, mean longevity, average body length, average body weight, average relation of male horn length/body length, and average social group size. These variables were quantified from literature (List 5S.1, appendix 5S).

Environmental factors included slope, annual average temperature, annual total precipitation, potential evapo-transpiration and productivity. Slope was derived from a topographical grid at about 1km spatial resolution (USGS, 2006), with the “Slope” function of the GIS. Temperature, precipitation and evapo-transpiration data were retrieved from climatic databases also at about 1 km spatial resolution (Hijmans et al., 2005; Trabucco and Zomer, 2009). Productivity was taken from the Global Inventory Modeling and Mapping Studies (GIMMS) Satellite Drift Corrected and NOAA-16 incorporated Normalized Difference Vegetation Index (NDVI), with the temporal resolution of monthly data between 1981 and 2006 and about 1km spatial resolution (Tucker et al., 2005). All environmental variables were centred and scaled due to different measurement units.

Human pressure variables included human population density, human footprint index, roads and linear-infrastructures, wild anthromes, and remoteness. Human population density and human footprint index were available as grids at 1km spatial resolution (WCS-CIESIN-CU, 2005; CIESIN-FAO-CIAT, 2005). Roads and track network were available as a polyline shapefile (CIESIN-ITOS, 2013) and the Euclidian distance to these features was calculated at 1km spatial resolution. Global anthromes were retrieved from a grid dataset at about 10km spatial resolution corresponding to the historical (1900s) and present (2000s) time periods (Ellis et al., 2013) and the wild anthromes (remote croplands, remote rangelands, remote woodlands, inhabited treeless, wild woodlands and wild treeless) were combined to derive a grid representing the distribution of wild anthromes. Remoteness was taken as the inverse of an accessibility grid at 1 km spatial resolution (Nelson, 2008).

With the exception of wild anthromes (available at 10 km resolution), all environmental and human—related variables were upscaled to ~5 km spatial resolution. Then, in the GIS, the distribution polygons of the historical and current time periods of each species were used to extract average and standard deviation statistics from each variable.

Regression analyses

Initially, normally tests were performed on predictor data and genetic diversity values, using both Shapiro-Wilk test (Royston, 1982) and qqnorm plots (Wilk and Gnanadesikan, 1968), and normality distribution was not rejected (data not shown). Pearson's correlation analyses were performed to test for collinearity among predictors.

Correlations were generally low ($r < 0.75$) with the exception of human pressure variables that were correlated to each other and with some environmental factors (especially NDVI and rainfall; supplementary material, Table 5S.3). Then, correlation analyses were performed between all predictors and genetic diversity values. All analyses were conducted in R, using package “Hmisc” package (Frank et al., 2016; R Core Team, 2017).

To identify the model that best describes the relationships between genetic diversity (haplotype diversity or nucleotide diversity) and predictors, Multiple regression-based models (GLZ) were used. Models were ranked according to their Akaike’s information criterion value. Each model’s support was estimated through the difference in AICc with respect to the top-ranked model ($\Delta AICc$) (Burnham and Anderson, 2004). The best model was used to determine the importance of predictors and their significance for explaining genetic diversity. All analyses were conducted in R, using the “MuMIn” package v. 3.0.2 (Kamil, 2016; R Core Team, 2017).

Ecological niche-based analyses

Ecological niche-based analyses were used to compare niche traits between models based in environmental factors (slope, precipitation and temperature) and human pressure variables (wild anthromes). Comparisons were also made between models based in historical and present species distributions. Analyses were conducted in the PCA-ENV approach developed by Broennimann et al. (2012) and updated with functions from ECOSPAT R package (Broennimann et al., 2016; Randin et al., 2016). This method used a Principal Component Analysis (PCA) to create a two-dimensional representation of climatic space, on which it performed comparisons. Niche overlap between distributions was measured using Schoener’s D metric, ranging from 0 (no overlap) to 1 (complete overlap) (Warren et al., 2008). Differences in niche overlap were tested with: i) niche equivalency test, which determined whether the niches of historical and present distributions were equivalent. It tested whether the niche overlap was constant when randomly reallocating 500-times the species occurrences among the two distributions. If observed differences fall within the density of 95% of the simulated values, the null hypothesis of niche equivalency cannot be rejected; and ii) niche similarity test, which determined whether the niche occupied in one distribution is more similar to the one occupied in the other distribution than would be expected by chance. If the observed differences were greater than 95% of 500 simulated values, the species occupied environments in both of its distribution that were more similar to each other than expected by chance (for details see Warren et al., 2008; Broennimann et al., 2012). The Scimitar-

horned Oryx is extinct in the wild and was not considered in ecological analyses, despite the recent re-introduction in Chad (Mallon et al., 2015).

Results

Correlations among predictors and genetic diversity

No significant correlations were observed between genetic diversity, range regression or latitudinal distribution with geographic and biological traits, with the exception of social group size and latitude (Pearson's correlation coefficient = -0.73) (supplementary material, Table 5S.4). Regression analysis identified negative relationships between haplotype and nucleotide diversities and range regression and latitude, but it was only significant between nucleotide diversity and range regression ($r^2 = 0.44$; $p = 0.019$) (supplementary material, Figure 5S.2).

Environmental factors and human pressure variables were in general the ones most frequently related to genetic diversity in both historical and present scenarios (Table 5.2 and supplementary material, Table 5S.4). Significant Pearson's correlation coefficients with haplotype diversity were only observed in the present period and with variables NDVI, distance to roads, and remoteness (Table 5.2). No significant correlations were observed between nuclear diversity and tested variables. In both historical and present periods, latitudinal distribution was negatively correlated with NDVI, precipitation, and human footprint, while range regression was negatively correlated with slope in the historical distribution.

Multiple regression-based models of genetic diversity and predictors

Significant relationships between genetic diversities and the most explanatory geographic and environmental predictors selected by multiple regression-based models (GLZ) models, in both present and historical distribution, were observed between: i) haplotype diversity in the present distributions with NDVI standard deviation (SD) and precipitation SD; and ii) nucleotide diversity in the historical distributions with potential evapo-transpiration SD and NDVI SD (Table 5.3). These relationships were positive with the exception of nucleotide diversity with potential evapo-transpiration (Figure 5.2).

Table 5.2. Pearson’s correlation coefficients between predictors (geographic traits, environmental factors and human pressure) of genetic diversity (HDI: haplotype diversity; NDI: nucleotide diversity), range regression (RRE) and latitudinal distribution (LAT) in 12 African ungulates. * significant differences (p<0.05). See Table 5.1 for variable codes.

	Historical distribution				Present distribution			
	HDI	NDI	RRE	LAT	HDI	NDI	RRE	LAT
Geographic traits								
RAS	- 0.33	- 0.38	0.26	0.41	0.42	0.05	0.16	-0.10
Environmental factors								
SLO	0.18	0.26	-0.65*	-0.20	0.26	0.32	-0.52	-0.07
SST	0.23	0.41	-0.51	-0.25	0.68	0.38	-0.27	-0.19
SSD	0.26	0.30	-0.64*	-0.24	0.35	0.38	-0.48	-0.25
PET	- 0.24	- 0.39	-0.24	-0.01	-0.11	-0.01	-0.25	-0.27
PEST	- 0.19	- 0.44	-0.18	0.06	-0.75	-0.29	0.08	0.14
PESD	0.01	- 0.18	0.13	0.53	0.41	0.25	-0.29	-0.20
ATE	0.07	- 0.16	0.27	-0.20	-0.09	-0.04	-0.27	-0.32
AST	0.09	- 0.23	0.32	-0.12	-0.54	-0.34	0.04	0.02
ASD	0.10	- 0.07	-0.08	0.29	0.35	0.30	-0.27	-0.19
NDVI	0.30	0.49	-0.15	-0.86*	0.26	0.53	-0.04	-0.88*
NDST	0.25	0.46	-0.13	-0.61*	0.81*	0.37	-0.08	-0.47
NDSD	- 0.05	- 0.05	0.09	-0.62*	0.06	-0.16	-0.07	-0.66*
PRE	0.22	0.33	-0.07	-0.87*	0.25	0.48	-0.06	-0.86*
PRST	0.46	0.41	-0.14	-0.69*	0.79	0.37	-0.09	-0.45
PRSD	0.34	0.19	-0.41	-0.68*	0.36	0.28	-0.12	-0.66*
Human pressure								
HPD	0.17	0.25	-0.21	-0.34	0.13	0.47	-0.13	-0.67*
HPSD	- 0.15	0.03	0.24	0.10	0.07	0.50	0.15	-0.72*
RDS	- 0.30	- 0.37	0.37	0.57	- 0.77*	-0.29	0.11	0.34
RSD	- 0.24	- 0.35	0.41	0.57	-0.29	-0.26	0.18	0.40
ANT	- 0.33	- 0.31	0.25	0.69*	-0.29	-0.36	-0.05	0.55
REMT	- 0.30	- 0.33	0.25	0.50	- 0.70*	-0.23	0.04	0.25
RESD	- 0.26	- 0.37	0.35	0.56	-0.29	-0.23	0.09	0.30
HFP	0.31	0.31	-0.25	-0.62*	0.30	0.33	-0.37	-0.69*
HSD	0.37	0.04	-0.08	0.09	0.39	0.23	-0.26	-0.52

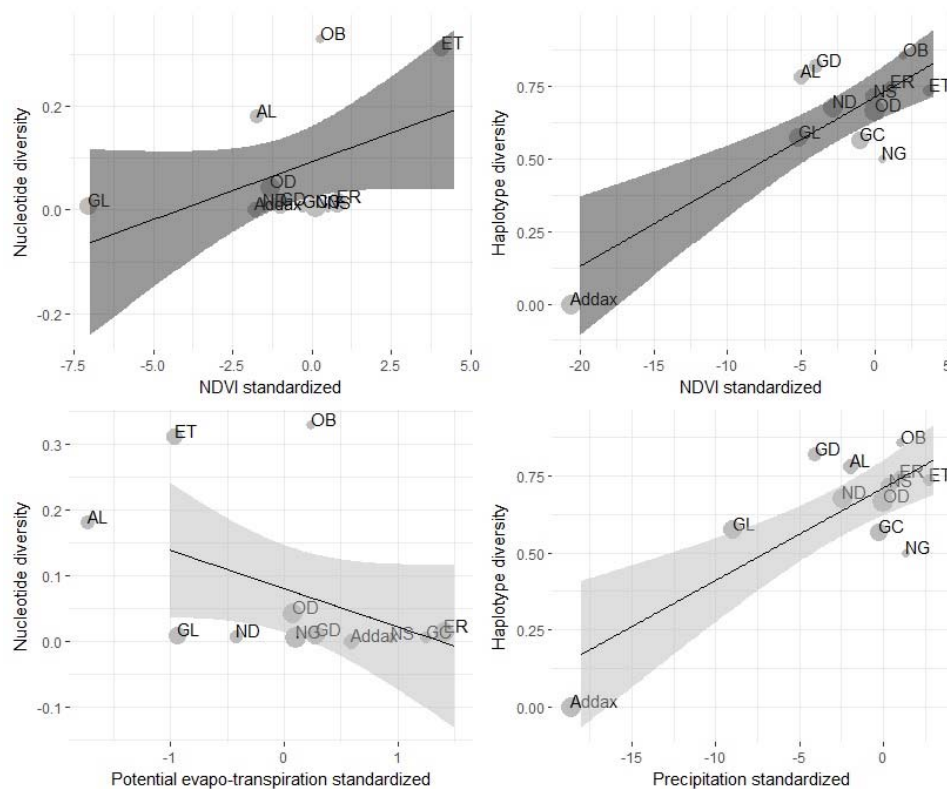


Figure 5.2 Relationships between significant environmental predictors of genetic diversities in 12 African ungulates identified by multiple regression models (see Table 5.3 for detailed results). The size of circles are proportional to the level of range regression experienced by each species, where larger size indicates larger regression. AL: *Ammotragus lervia*, AN: *Addax nasomaculatus*, ER: *Eudorcas rufifrons*, ET: *Eudorcas thomsonii*, GC: *Gazella cuvieri*, GD: *Gazella dorcas*, GL: *Gazella leptoceros*, ND: *Nanger dama*, NG: *Nanger granti*, NS: *Nanger soemmerringii*, OB: *Oryx beisa*.

Significant negative relationships between genetic diversities and the most explanatory geographic and biological predictors selected by GLZ models were observed between nucleotide diversity with latitudinal distribution ($p = 0.017$) and body weight ($P = 0.028$) (supplementary material, Table 5S.5). No significant relationships were found between genetic diversities and human pressure variables (supplementary material, Table 5S.6).

Niche overlap between historical and present distributions

The measured realized ecological niche overlap of historical and present distributions based in environmental variables was very low (average $D=0.002$) for all 11 African ungulates tested, suggesting very distinct niches in both distributions (Table 5.4; Figure 5S.3). Equivalency and similarity tests were non-significant, except in *Nanger granti* in equivalency, and the null hypotheses of niche equivalency and niche similarity between distributions was rejected. Concerning human pressure variables, niche overlap between

historical and present distributions was slightly higher in comparison to environmental variables (average $D=0.122$). In *Gazella cuvieri* and *G. leptoceros* there was no niche overlap. Equivalency and similarity tests in most specie's distributions were non-significant (rejecting the null hypotheses of niche equivalency and niche similarity between distributions), except in *Ammotragus lervia* and *Gazella dorcas* in both tests, and *Nanger dama* in similarity tests (Table 5.4).

For most of the 11 African ungulates tested, there was a trend for decreasing average values in NDVI, precipitation, human footprint, but mostly in human population density, between historical and present distributions (Figure 5.3; Table 5S.7). Increasing average values were observed in distance to roads, percentage of the range inside wild anthromes, and remoteness. The most striking differences, in comparison to historical distributions, were observed in *Addax nasomaculatus*, *A. lervia*, and *G. dorcas*, where the present distribution is located in the areas with reduced human population density and human footprint, and higher remoteness, and in *G. dorcas* that now occurs in areas with reduced precipitation (Figure 5.3).

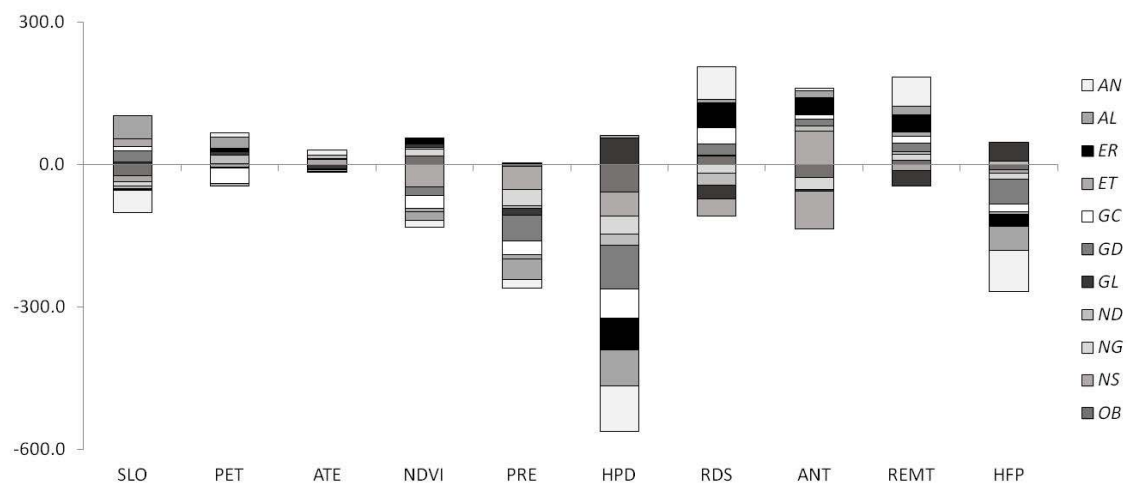


Figure 5.3 Cumulative percent change in environmental factors and human pressure variables in the historical and present distributions for each of the 11 African ungulates studied. Species are AL: *Ammotragus lervia*, AN: *Addax nasomaculatus*, ER: *Eudorcas rufifrons*, ET: *Eudorcas thomsonii*, GC: *Gazella cuvieri*, GD: *Gazella dorcas*, GL: *Gazella leptoceros*, ND: *Nanger dama*, NG: *Nanger granti*, NS: *Nanger soemmerringii*, and OB: *Oryx beisa*. See Table 5.1 for variable codes.

Table 5.3. Multiple regression-based models (GLZ) between Haplotype and Nucleotide diversities and geographic traits and environmental factors in Historical and Present distributions in 12 African ungulates. Most explanatory variables of genetic diversities selected by GLZ, Akaike information criterion corrected (AICc), delta, coefficient (coef), t-value (t), and probability of t ($\Pr(>|t|)$). * significant differences ($p < 0.05$). See Table 5.1 for variable codes.

	Historical distribution					Present distribution				
	AICc	delta	coef	t	$\Pr(> t)$	AICc	delta	coef	t	$\Pr(> t)$
Haplotype diversity										
Geographic traits										
RAS	5.01	2.30	-0.0003	0.966	0.334	-	-	-	-	-
Environmental factors										
SST	5.69	2.99	0.1143	0.672	0.502	-	-	-	-	-
PEST	5.93	3.23	-0.0455	0.537	0.591	-3.46	3.06	-0.0590	1.819	0.069
AST	6.27	3.56	0.0157	0.262	0.793	-	-	-	-	-
NDST	5.57	2.86	-0.0129	0.204	0.838	-6.52	0.00	0.0291	4.392	0.001*
PRST	3.55	0.84	0.0452	1.630	0.134	-5.41	1.11	0.0300	3.087	0.002*
Nucleotide diversity										
Geographic traits										
RAS	-9.94	2.85	-0.0002	1.145	0.252	-8.10	3.63	0.0001	0.152	0.879
Environmental factors										
SST	-10.21	2.57	0.1080	1.233	0.218	-9.97	1.76	0.0531	1.154	0.249
PEST	-10.66	2.12	-0.0786	-2.568	0.030*	-9.14	2.58	-0.0065	0.854	0.393
AST	-	-	-	-	-	-9.53	2.20	-0.0153	1.018	0.309
NDST	-10.90	1.88	0.0296	2.628	0.027*	-9.82	1.91	0.0072	1.106	0.269
PRST	-10.26	2.53	0.0268	1.595	0.111	-9.82	1.90	0.0077	1.107	0.268

Discussion

The comparison between genetic diversity measures and range regression, latitudinal distribution, biological traits, and environmental variables and human factors of 12 African ungulates, allowed retrieving three main patterns: i) genetic diversities are lower in species that have suffered larger range regressions; ii) genetic diversities are mostly related with environmental variation and human pressure variables, in comparison to biological or geographic traits; and iii) the realized niches, according to environmental variation and human pressures, in the present distributions are distinct from the ones of historical distributions. These findings are discussed in the following sections.

Table 5.4. Ecological niche comparisons between historical (A) and present (B) distributions in 11 African ungulates. Comparisons based in environmental factors and human pressure variables (wild anthromes). Measured niche overlap (D), equivalence tests, similarity B->A and A->B tests. * significant differences (p<0.05).

Species	D	Equivalency	Similarity B->A	Similarity A->B
Environmental factors				
<i>Addax nasomaculatus</i>	0.000	1.000	0.998	0.998
<i>Ammotragus lervia</i>	0.000	1.000	0.517	0.475
<i>Eudorcas rufifrons</i>	0.000	1.000	0.996	0.994
<i>Eudorcas thomsonii</i>	0.000	1.000	0.250	0.257
<i>Gazella cuvieri</i>	0.024	0.277	0.108	0.100
<i>Gazella dorcas</i>	0.000	1.000	1.000	1.000
<i>Gazella leptoceros</i>	0.000	0.116	0.156	0.140
<i>Nanger dama</i>	0.000	0.998	1.000	0.998
<i>Nanger granti</i>	0.001	0.002*	0.317	0.305
<i>Nanger soemmerringii</i>	0.000	1.000	0.976	0.980
<i>Oryx beisa</i>	0.000	1.000	0.553	0.535
Human pressure				
<i>Addax nasomaculatus</i>	0.070	0.999	0.182	0.183
<i>Ammotragus lervia</i>	0.157	0.001*	0.002*	0.004*
<i>Eudorcas rufifrons</i>	0.041	1.000	0.172	0.185
<i>Eudorcas thomsonii</i>	0.049	1.000	0.192	0.171
<i>Gazella cuvieri</i>	0.000	1.000	1.000	1.000
<i>Gazella dorcas</i>	0.167	0.001*	0.003*	0.001*
<i>Gazella leptoceros</i>	0.007	1.000	0.191	0.187
<i>Nanger dama</i>	0.171	0.992	0.041*	0.047*
<i>Nanger granti</i>	0.266	0.998	0.069	0.067
<i>Nanger soemmerringii</i>	0.154	1.000	0.085	0.079
<i>Oryx beisa</i>	0.262	1.000	0.231	0.210

Range regression decreases genetic diversity

Negative relationships were found between both haplotype and nucleotide diversities and range regression in the African ungulates studied. The species that suffered the strongest reductions in range size (above 90%), such as *Addax nasomaculatus*, *Gazella leptoceros*, *Nanger dama*, and *Oryx dammah*, were the ones consistently exhibiting the lowest diversities in both molecular markers. These results support the general finding that in mammals, genetic diversity is lower in species with small range sizes compared to those with large range sizes (Doyle et al., 2015). Range regression has been

associated to reductions in population size, which is the main factor affecting genetic diversity (Furlan et al., 2012). Populations of *A. nasomaculatus*, *N. dama*, and *G. leptoceros* have reached critical low numbers, while *O. dammah* was recently re-introduced in Chad (Newby et al., 2016; Brito et al., 2018). Although herbivores generally exhibit high genetic diversities in comparison to other mammals, they are also highly affected by range regression, which contributes greatly to reduce genetic diversities (Doyle et al., 2015). Our findings further support the need for the adequate management of captive and wild populations in order to revert current declining trends.

Environmental factors and human pressure affect genetic diversity

Environmental factors and human pressure variables were mostly related with observed genetic diversities in comparison to both geographic and biological traits. In mammals, lack of relationships between genetic diversity and biological traits has been reported (Doyle et al., 2015). In the present distribution areas, haplotype diversity was larger in areas likely exhibiting the best conditions for population persistence: high productivity, high precipitation variability, high remoteness, and distant to roads. Reductions in genetic diversity typically result from declines in population size, as a consequence of genetic drift and inbreeding (Doyle et al., 2015), while population decline is usually associated to habitat loss (Brito et al., 2014; Newby et al., 2016). As such, results from the present study support the hypothesis that extant populations are likely occupying the last strongholds of suitable habitats, which are retaining the highest observed genetic diversities among all taxa.

Nucleotide diversity was negatively related with latitudinal distribution. This finding mimics the observed global latitudinal gradient in the distribution of genetic diversity, which increases from the poles to the tropics (Brown, 2014; Miraldo et al., 2016). and highlighted the differences between species that occur in lower latitudes (*Eudorcas thomsonii*, *Nanger granti*, *Nanger soemmerringii* and *Oryx beisa*) in relation to the other studied ungulates.

Realized niches are distinct in present and historical distributions

Overall, the measured realized ecological niche overlap of historical and present distributions based in environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time. In most species, present distributions represent a minor portion of the historical distributions and are the result of extensive regressions experienced by most species across their original range (Durant et al., 2014; Newby et al., 2016). The range reductions above 90% observed in

A. nasomaculatus, *G. leptoceros* and *N. dama* are likely reflected in the reduced niche overlap between historical and present distributions.

Range regression was negatively related with slope, suggesting that the population extinction in most species occurred more often in lowland areas in comparison to mountains. Global accessibility to lowland areas is in general facilitated (Nelson, 2008), which may explain such pattern. Population remains of *A. lervia* and *G. dorcas*, *G. cuvieri*, and *N. dama* are presently associated to mountain areas (Silva et al., 2017; Brito et al., 2018). The role of mountains in the Sahara-Sahel as refugia for biodiversity is again emphasised here (Brito et al., 2014, 2016).

The present distributions of several species, but marked in *A. nasomaculatus*, *Ammotragus lervia* and *Gazella dorcas*, are located in areas with reduced human population density and human footprint, and higher remoteness in comparison to the historical distributions. The general over-hunting, disturbance, and modifications of natural habitats associated to the areas with high human population density (Brito et al., 2014, 2016, 2018; Durant et al., 2014; Newby et al., 2016) appear to have restricted extant populations to areas distant from human activities.

The present distribution of most species, but highly marked in *G. dorcas*, tends to occur in areas less productive and with less precipitation in comparison to the historical distributions. Extant populations occur under harsher environmental conditions, and thus may be vulnerable to the frequent climatic oscillations, with frequent drought periods, that characterise the region (Brooks, 2004). Furthermore, the overall reduced genetic diversities found in this study suggest that these populations may have lost already a portion of the adaptation ability to further environmental change (Sarre and Georges, 2009; Pauls et al., 2013).

Methodological limitations and future developments

The use of small gene fragments referring only to the maternal heritage only can be a limiting factor when quantifying genetic diversity (Galtier et al. 2009). However, given that cytochrome b (cytb) is a widely used mtDNA gene (Kekkonen & Hebert 2014), it allows comparisons to be made across wide arrays of taxa, thus providing relevant insights into the main patterns of genetic diversity. Fast evolving markers provide current gene flow measures, which should be useful to accurately define the present genetic structure of populations (Avice 2010), and in the future they could be used to better understand the management implications for these threatened species.

The development of ecological models based in range polygons likely includes non-occurrence areas in modelling exercises. This bias may cause overestimation of parameters, affecting comparisons of ecological niches (Warren et al., 2008). In future

studies, point data should be used in order to increase the accuracy of niche overlap estimations. Still, the results found here are likely reflecting major patterns in ecological niche traits.

In populations with low genetic diversity and small ranges, the risks of extinction likely increase as a consequence of the potential limited ability of species to respond to changes in environmental and habitat conditions (Furlan et al., 2012). As such, relating the genetic diversity patterns and changes in environmental and niche conditions is useful for planning the conservation of these species and for studying species' evolutionary change.

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CHAPTER 6

Landscape genetics approach

Paper IV Effect of landscape features on genetic structure of the goitered gazelle (*Gazella subgutturosa*) in Central Iran

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Effect of landscape features on genetic structure of the goitered gazelle (*Gazella subgutturosa*) in Central Iran

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Abstract

The populations of goitered gazelle suffered significant decline due to natural and anthropogenic factors over the last century. Investigating the effects of barriers on gene flow among the remaining populations is vital for conservation planning. Here we adopted a landscape genetics approach to evaluate the genetic structure of the goitered gazelle in Central Iran and the effects of landscape features on gene flow using 15 polymorphic microsatellite *loci*. Spatial autocorrelation, isolation by distance (IBD) and isolation by resistance (IBR) models were used to elucidate the effects of landscape features on the genetic structure. Ecological modeling was used to construct landscape permeability and resistance map using 12 ecogeographical variables. Bayesian algorithms revealed three genetically homogeneous groups and restricted dispersal pattern in the six populations. The IBD and spatial autocorrelation revealed a pattern of decreasing relatedness with increasing distance. The distribution of potential habitats was strongly correlated with bioclimatic factors, vegetation type, and elevation. Resistance distances and graph theory were significantly related with variation in genetic structure, suggesting that gazelles are affected by landscape composition. The IBD showed greater impact on genetic structure than IBR. The Mantel and partial Mantel tests indicated low but non-significant effects of anthropogenic barriers on observed genetic structure. We concluded that a combination of geographic distance, landscape resistance, and anthropogenic factors are affecting the genetic structure and gene flow of populations. Future road construction might impede connectivity and gene exchange

of populations. Conservation measures on this vulnerable species should consider some isolated population as separate management units.

Keywords: Gene flow, Graph theory, Isolation by distance, Landscape genetics, Resistance distance, Spatial autocorrelation

Introduction

Over the last century, large mammal populations have been declining across the globe mainly due to human-induced habitat fragmentation (HIHF) and landscape changes (Wilkie et al. 2011). Rapid development of connectivity among human populations and the associated HIHF has the potential to disrupt migration routes and exchange of individuals among wildlife populations (Frankham et al. 2010). Lack of individual exchange and gene flow increases extinction risks due to reductions in evolutionary potential and genetic diversity (Coltman et al. 1999). In addition to human development, landscape features have a major role in shaping genetic structure of populations (Pfenninger et al. 2011). These features can act as gene flow barriers and affect the ability of individuals to move across landscapes (Adams and Burg 2015). Migration barriers can also result from historical processes (e.g. mountain ranges), climatic factors (e.g. large arid regions), or microgeographic factors (e.g. natural and human-induced habitat fragmentation; Pérez-Espona et al. 2008).

In a fragmented landscape, species with extensive area requirements (e.g., large terrestrial migratory species) often persist in a metapopulation pattern in which dispersal among subpopulations is essential for regional viability (Wiens 2001). Hence, investigating the effects of landscape barriers on disruption of migration routes, gene flow and consequently the isolation of the populations provides information on how species interact with their environment and is important for conservation purposes (Shuter et al. 2011; Sommer et al. 2013). Several recent studies have used landscape genetics to identify landscape elements influencing gene flow between genetic demes (e.g., invertebrates: Geiser et al. 2013; amphibians: Murphy et al. 2010; reptiles: Klug et al. 2011; and mammals: Galarza et al. 2015). In the conducted researches, topographical features (Smitsen et al. 2013), unsuitable habitat (Zhu et al. 2010) and anthropogenic disturbances (Epps et al. 2005; Yang and Jiang 2011) have all been identified as factors strongly influencing genetic structure.

The goitered gazelle (*Gazella subgutturosa*) is the most widespread species among Asian antelope species (Sorokin et al. 2011) living mainly in semiarid steppes of western

Asia to Mongolia and northern China. Goitered gazelles occur in flat plains, adjacent foothills and mountain valleys dominated by plant species such as *Artemisia siberi*, *Anabasis aphylla*, *Salsola* spp. and *Stipa* spp. (Karami et al. 2002). Water availability in summer was identified as an important habitat constraint (Farahmand 2002). Historically, goitered gazelles covered large distances during winter migrations, to avoid deep snow, and summer migrations, in search of water (Heptner et al. 1961), but current sedentary populations are limited to 2 to 8 km² of home range size (Martin 2000; Durmuş 2010). Gazelle populations in Iran have experienced two contemporaneous decline periods in the region. The first one coincided with the emergence and rapid increase of semi-automatic rifles and offroad vehicles in late 1940s/early 1950s. In 1967, the Game and Fish Department of Iran declared gazelle species and a number of gazelle habitats as protected by law, which resulted in restoration of the retained gazelle populations (Iranian Department of Environment 2012; DOE unpublished data). The second period of decline happened during and shortly after the Islamic revolution in Iran in 1979, when the law enforcement agencies including the DOE collapsed. The following restructuring of the DOE during the 1980s allowed controlling illegal hunting and the recovery of some gazelle populations, though others with small sizes were extirpated within the protected areas (PAs; Hemami and Groves 2001). Other factors such as drought, livestock competition and habitat degradation have also contributed to the reduction of gazelle populations (Zachos et al. 2010). Currently, almost all existing gazelle populations are confined to PAs surrounded by areas of dense human settlement and road network. It is unknown if gene flow occurs between putatively isolated populations, nor the potential effects of landscape factors on genetic structure of gazelles. The nomadic behavior of goitered gazelles is currently restricted by human land uses. Hence, geographic distance may have great influence on gene flow among populations. In addition, resistance distances resulted from disruption of the previously continuous distribution of goitered gazelle across Central Iranian plateau may have affected genetic structure of the populations. Isolation by distance (IBD; Wright 1943) has widely been used for modeling genetic differentiation in natural populations (McRae 2006). The predictive ability of IBD is often hampered by homogeneous and unbounded nature of real populations (two main assumptions of IBD analysis; Slatkin 1985) and spatial variations in their densities and migration rates. To address these shortfalls, isolation by resistance (IBR) was designed to predict the influence of landscape structure on equilibrium genetic structuring of natural populations (McRae 2006). The IBR predicts a relationship between genetic differentiation and the resistance distance, summarizing information on the probability of an individual dispersing from one population to another across all potential paths weighted by the 'friction' to movement (McRae 2006). Long-lived and high dispersal

ability mammals may be less disturbed by geographic distances than landscape matrix (Mullins et al. 2014). Therefore, IBR models may be better related with the genetic structure of populations in comparison to IBD model, because they account for the heterogeneity in species' distributions and migration rates (McRae 2006). IBR models have been successfully used to evaluate effects of landscape features on the genetic structure of multiple vertebrates (e.g. Coulon et al. 2006; Klug et al. 2011; Geiser et al. 2013; Mullins et al. 2014).

Mapping of resistance surface is based on features that influence process of habitat selection and species movements. We used habitat distribution models to map resistance surface. Goitered gazelles occupy heterogeneous habitats and have complex and stochastic attributes of movement. For species exhibiting such traits, multiple interpopulation least cost paths as an 'average' index of the landscape permeability (e.g. CIRCUITSCAPE) have been chosen to show effects of landscape features on genetic composition (Klug et al. 2011; McRae et al. 2008). Hence, we used graph theory to define a measure of landscape resistance for inferring the effects of landscape structure on gazelle's gene flow in Iran. Given the current fragmented distribution of gazelles, we hypothesized that a combination of anthropogenic and natural landscape features influences population genetic structure and connectivity. Our objective was to describe how landscape features have shaped the genetic structure of goitered gazelle in Iran and to identify potential dispersal barriers. The specific objectives were to (1) evaluate genetic diversity of remaining gazelle populations; (2) identify population structure; (3) assess the effects of geographic and resistance distance on gene flow; and (4) evaluate landscape permeability to gazelle migration. The results provide useful framework data for the conservation and management of goitered gazelle in Central Iran.

Materials and Methods

Study Area and Sampling Locations

We sampled six biological populations of goitered gazelle in an area of Central Iran encompassing 363,000 km² (Figure 6.1). The elevation varies from 117 to 4,429 m. Mean annual temperature ranges from 11 to 27 °C. Alborz mountain range from the north and Zagros range from the northwest to southwest prevent moisture bearing systems to pass through to the south and the east respectively. Precipitation ranges from 51 to 329 mm/year. Sampling was performed in all known populations (Figure 6.1) including: BID-Bidui- yeh (E56° 20' N29° 53'), KAL-Kalmand (E54° 20' N31° 05'), KGH-Kolah-Qazi

(E51° 45' N32° 30'), KAH-Kahyaz (E52° 23' N33° 08'), GHAM-Ghamishloo (E49° 95' N36° 14'), and MOT-Mooteh (E50° 10' N33° 20'). A total of 217 fresh fecal samples were collected from observed gazelles congregating at water sources. In addition, 12 tissue samples were obtained from the tissue bank of the DOE and from carcasses of goitered gazelle that died from poaching, hunting, natural enemy predation and disease during sampling sessions (2014–2015). The geographic coordinates of all samples were collected with a Global Positioning System and samples were preserved in 96% ethanol prior to DNA extraction.

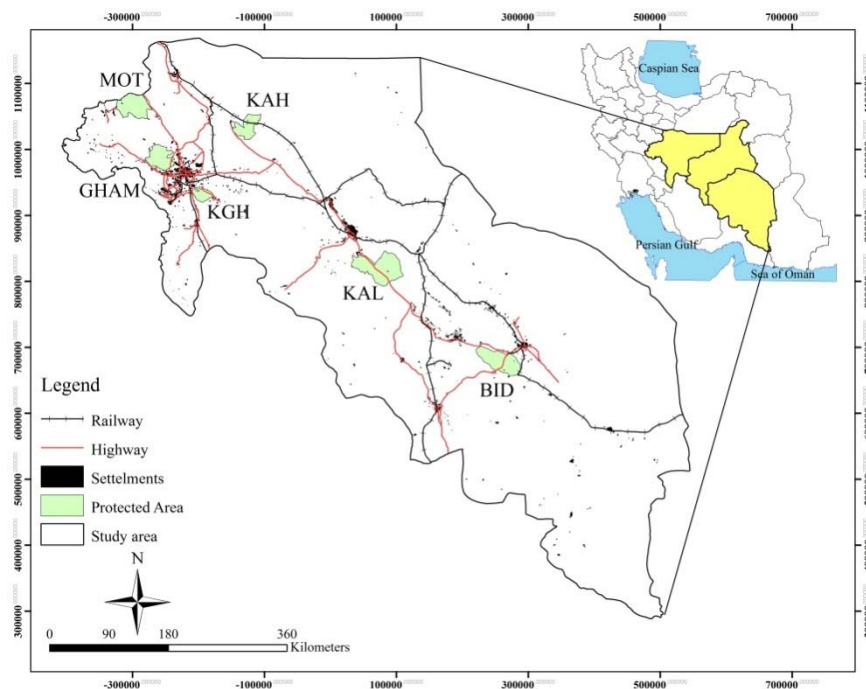


Figure 6.1. Study area in Central Iran and location of the six populations of *G. subgutturosa* remaining in the region. BID-Biduiyeh; KAL-Kalmand; KGH-Kolah-Qazi; KAH-Kahyaz; GHAM-Ghamishloo; MOT-Mooteh

DNA extraction and microsatellite amplification

The 229 samples were subjected to total DNA extraction. Genomic DNA from fecal samples was isolated using DNA Stool Mini-Kit (Yekta-Tajhiz Azma, Iran). Extraction blanks were used as negative controls. All genetic identifications were performed according to Silva et al. (2015) methodology, using as toll a mitochondrial fragment (450 bp of the cyt b gene) and a nuclear one (367 bp of KCAS gene). Both strand sequences were generated using the amplification primers, as it is recommended for example, by Tiedemann et al. (2012), allowing the confirmation of sequence consistency and quality. Cycle sequencing reactions were carried out using BIGDYE TERMINATOR v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). Sequencing products were subsequently separated on a 3130xl Genetic Analyser (Applied Biosystems). Sequence

alignment was performed using Clustal W (Thompson et al. 1994) implemented in BIOEDIT software (Hall 1999) and was manually checked and reassessed for any discrepancy. We excluded the samples generating weak amplifications from further analyses.

We amplified 15 dinucleotide species transferred microsatellite *loci*: HSC, Maf65, McM527 (Di Stasio 2001); HPN79, HPN111 HPN91, HpN5, HPN12, HPN2, (Pinto et al. 2015); BM302, BM415, INRA40 (Kappes et al. 1997); INRA5 (Beja-Pereira et al. 2004); ILST008 (Kumar et al. 2009) and INRA6 (Vaiman et al. 1994). Forward primers of the 15 markers were fluorescently labeled with VIC, FAM, PET and NED. The primer pairs were arranged into five separate multiplex reactions. The PCR conditions for the five separate multiplex reactions were done using QIAGEN PCR MasterMix in optimized PCR condition (Table 6S.3, Appendix 6S). We conducted a minimum of four replicates polymerase chain reactions per fecal sample per locus to minimize genotyping errors (Text 6S.3, Online Appendix 6S) resulting from potential degraded DNA from fecal. Fragment analysis was carried out using a 3130xl Genetic Analyser (Applied Biosystems) under standard run conditions with LIZ500 as the internal size standard. Alleles were scored and binned using GENEMARKER 1.7. We used the program MICRO-CHECKER (Van Oosterhout et al. 2004) to check scoring error, large allele dropout, stutter or null alleles.

Population Genetic Diversity

The departure of *loci* from Hardy–Weinberg equilibrium (HWE) and the linkage disequilibrium across all *loci* were performed using the program GENEPOP (Raymond and Rousset 1995). We calculated the levels of genetic diversity (the number of alleles (A), observed heterozygosity (HO), and expected heterozygosity (HE)), using default Markov chain parameters. The resultant P values were adjusted for multiple tests via the sequential Bonferroni method (Rice 1989) Allelic richness for each population was calculated using FSTAT 2.9.3 (Goudet 2001). Since no locus showed deviations from Hardy–Weinberg equilibrium in some populations randomly, hence all *loci* were used in the analysis. A two phased mutation model (TPM) with 90% SMM (stepwise mutation model) and 10,000 iterations was fitted to detect the genetic imprint of recent bottlenecks in program BOTTLENECK (Piry et al. 1999). We implemented Wilcoxon signedrank test along with a mode shift to test significant heterozygote excess in populations. We also calculated M-Ratio (mean number of alleles in a population divided by the allelic size range; Garza and Williamson 2001) in ARLEQUIN 3.11 to test for bottleneck events that may have occurred over longer time periods (Wall et al. 2014). The M-ratio test is a more sensitive index of population bottleneck than the heterozygosity excess tests (Piry et al.

1999). When alleles are lost from a population, the number of alleles decreases at a faster rate than the allelic size range. So, M-ratio lower than critical value (0.68) indicates population bottleneck (Garza and Williamson 2001).

Population Genetic Structure and Identification of Dispersal Events

We used FreeNP with ENA correction for null alleles (Chapuis and Estoup 2007) to calculate pairwise F_{ST} -values between populations with 95% confidence intervals based on 100,000 bootstrap replicates (Text 6S.2, Appendix 6S). Genetically homogeneous structure was assessed using two Bayesian clustering approaches to estimate the number of genetic clusters in the whole dataset. First, we used non-spatial prior distribution clustering implemented in STRUCTURE (Pritchard et al. 2000). Each simulation was performed using admixture model with correlated allele frequencies without prior population information. We run ten separate iterations for $1 \leq K \leq 8$ with 1,000,000 MCMC iterations, discarding the first 100,000 as burn-in. Both delta K (DK; Evanno et al. 2005) using STRUCTURE Harvester (Earl and Von Holdt 2012) and LnPr (X|K; Pritchard et al. 2000) were used to determine K. We used the 'recessive alleles' settings implemented in STRUCTURE to estimate null allele frequencies for each locus in the clusters (Senn and Pemberton 2009). R package GENELAND (Guillot et al. 2005) was used to corroborate the STRUCTURE results and to obtain estimates of population structure in a geographic context. We ran the MCMC ten times, allowing K to vary, with the following parameters: 106 MCMC iterations with a thinning of 100, maximum rate of the Poisson process fixed to 200, minimum K = 1, maximum K = 8, maximum number of nuclei in the Poisson–Voronoi tessellation fixed to 300. The Dirichlet model was used as a prior for all allele frequencies and we let the algorithm account for the presence of null alleles (Guillot 2008). The cluster membership for each individual for all runs was selected and plotted on a map. We used first-generation migrant detection in GeneClass v. 2 (Piry et al. 2004) to identify dispersal events between sampled populations. Tests were performed according to the Bayesian method of Rannala and Mountain (1997) using the Monte Carlo resampling of Paetkau et al. (2004) with 10,000 simulated individuals and a significance level of 0.05. We implemented two different likelihood-based test statistics as the appropriate model for migrant detection ($L = L_{\text{home}}$ and $L = L_{\text{home}}/L_{\text{max}}$).

Landscape Genetic Analysis

Spatial Autocorrelation

Spatial autocorrelation analysis was performed in GENALEX 6.5 (Peakall and Smouse 2012) to assess the fine-scale patterns of genetic structure across the entire landscape (Klug et al. 2011; Mullins et al. 2014). A correlation coefficient (r) between pairwise

genetic and geographic distance for 26 distance classes was calculated. Distance classes were set so that each class had approximately equal sample size. A null distribution of r values for each distance class was calculated by permutation ($N = 9999$) and the confidence intervals estimated by bootstrapping ($N = 9999$). The results were plotted in a correlogram. The random permutations generated an estimate of r around the null hypothesis. We confirmed significance if r exceeded the null distribution for a given distance class and if the 95% CI around r did not overlap zero (bootstrapping).

Isolation by distance (IBD)

The hypothesis of IBD was tested using Mantel test in GENALEX 6.5. Genetic distances among populations were calculated using corrected genetic differentiation ($F_{ST}/[1-F_{ST}]$). Linear Euclidian distances were measured based on each population's average geographic coordinates of sample locations within the ArcGIS v.9.3 (ESRI, Redlands, CA, USA). Isolation by distance analysis (IBD) was performed by coding all cells equal resistance and calculation Euclidean distance between populations. Therefore, we included a null model in our analysis. Also, we used a partial Mantel test, comparing the genetic and geographic distances when controlling for resistance distance to test for IBR vs IBD. Significance was determined using 9999 permutations.

Isolation by resistance (IBR)

Isolation by resistance (IBR) was used to investigate permeability of landscape features to gene flow. We used CIRCUITSCAPE 3.5.2 (McRae et al. 2008) to assess landscape connectivity and calculate resistance distances and gene flow between all pairs of populations, analyzing all possible inter-populations paths through a raster resistance surface (McRae 2006). We used MaxEnt model (Phillips et al. 2006) to map resistance surface and define the functional landscape connectivity. We took 260 presence records from gazelles and run MaxEnt using 12 uncorrelated variables in four classes (climatic, topographic, vegetation and anthropogenic; Table 6S.4, Appendix 6S) at spatial accuracy of 250 m. Spatial downscaling method (Flint and Flint 2012) was used to transfer the original 1-km resolution of WorldClim data obtained from 1970 to 2000 representing current climate to the target resolution of 250 m with FORTRAN using Microsoft Visual Studio. This model combines a spatial gradient and inverse-distance-squared (GIDS) weighting to WorldClim data with multiple regression (See Khosravi et al. 2016 for details and Text 6S.3, Appendix 6S). We used this fine resolution to capture relevant landscape elements also the computer processing intensity of the connectivity analyses. Environmental variables were applied to run the MaxEnt program using 10 replicates and the cross-validate run type (See Khosravi et al. 2016 for details). We used

logistic output in MaxEnt model which gives an estimate of habitat suitability value between 0 and 1 with higher values corresponding to higher habitat quality. To assess how gazelles might respond to landscape heterogeneity, we constructed resistance values for each grid using habitat suitability values obtained from MaxEnt by coding each pixel as 1 = most permeable to dispersal (i.e. the most habitat suitability value), 100 = least permeable (fewest habitat suitability value), or barrier = not permeable, with intermediate values scaled linearly in between. Mantel tests (Mantel 1967) were used to compare correlations between matrixes of pairwise genetic and average resistance distances to test for the impact of landscape heterogeneity on gene flow (Smouse et al. 1986). The Mantel tests were performed in library VEGAN version 1.6-7 (Dixon 2003; Oksanen 2005) of R (R Development Core Team 2005). We also ran a partial Mantel test, comparing the genetic and resistance distances when controlling for Euclidean distance. Due to the different permeability of landscapes for gazelles, if the resistance distance was a better estimator than Euclidean distance, we could infer that landscape composition affects gene flow.

Effect of anthropogenic features on population structure

We generated a categorical matrix for three main types of anthropogenic landscape features, human settlements, highways and railways, describing whether certain types of anthropogenic features were present (=1) or not (=0) between six sampled populations. As geographical distance and landscape features are dependent, partial Mantel tests (Smouse et al. 1986) were used to assess the relative effect of each significant factor inferred in the Mantel tests. All tests were executed using IBD (Bohonak 2002) with 10,000 permutations to determine statistical significance.

Results

Population Genetic Diversity

All the microsatellite *loci* were polymorphic and the number of alleles per locus varied from 4 at Maf65, ILST008 and HPN91 to 19 at HPN5 (Table 6S.5, Appendix 6S). The average number of alleles across all *loci* detected per each population ranged from 2.33 (in KAH) to 4.80 (in KAL; Table 6.1). For the entire samples, five *loci* (HSC, BM302, INRA40, HPN111, HpN5) deviated significantly (with $p < 0.01$) from Hardy Weinberg equilibrium. However, at the population level the number of significant deviations from HWE varied from none in KAH and MOT to five *loci* in KAL (Table 6S.6, Appendix 6S). A significant departure from linkage disequilibrium was observed, even after Bonferroni

correction, for 3 out of the 15 *loci* combinations (2.8%). No evidence of linkage disequilibrium was found within each population after correcting for multiple comparisons. Among the 15 markers, evidence of null alleles ($r > 0.2$) across some clusters in STRUCTURE analysis was detected in BM302, BM415, INRA40, HPN79, HPN91 and HPN2 (Table 6S.7, Appendix 6S). Taking in account the r values close to 0.2 and the non-significant results of MICRO-CHECKER for all populations (data not shown), we did not discard any *loci* from further analyses. Observed heterozygosity (H_O) ranged from 0.28 in KAH, to 0.58, in MOT (Table 6.1), and mean number of alleles (A) varied from 2.33 (KAH) to 4.80 (KAL). Inbreeding coefficient (F_{IS}) was highest in the KAH population (0.474) and smallest in MOT population (0.007). F_{IS} values were not significant for all populations ($p > 0.05$).

Evidences of heterozygosity excess under mutation–drift equilibrium were not detected in the six analyzed populations, except in KAH. In the latter population, the allele frequency distribution exhibited a shifted mode indicating a recent bottleneck event. Also, two-tailed Wilcoxon signedrank test for GHAM was significant (p value = 0.001). Contrary to Wilcoxon signed-rank test in BOTTLENECK, the average M -ratio across all populations averaged 0.33 (± 0.19 , SD) and were lower than the critical threshold value of 0.68 (Garza and Williamson 2001) indicating a severe bottleneck event in gazelle populations over longer time periods.

Population Genetic Structure and Dispersal Events

Pairwise F_{ST} between sampling populations ranged from 0.035 (GHAM and MOT; Table 6.2) to 0.264 (KAH and BID). Values for the number of migrants successfully entering a population per generation (N_m) ranged from 0.697 (KAH and BID) to 6.966 (GHAM and MOT). These results indicated significant genetic differentiation among some population pairs.

Table 6.1. Diversity indices for Goitered gazelle populations in Central Iran.

ID	Approximate Population size	Distance to next population (km)	N extracted samples	N analyzed sample	A	Ar	PA	H_E	H_O	F_{IS}
BID	150	228	50	22	4.26	1.59	11	0.50	0.40	0.303
KAL	100-150	228	53	29	4.80	1.60	16	0.57	0.41	0.348
KGH	500-1000	85	38	19	3.73	1.60	11	0.57	0.45	0.268
KAH	100-120	128	10	7	2.33	1.40	3	0.47	0.28	0.474
GHAM	1000-1500	85	47	21	4.47	1.88	9	0.58	0.47	0.341
MOT	2000-2500	88	31	11	3.20	1.86	4	0.58	0.58	0.007
Total			229	109	3.80	1.65		0.54	0.43	0.290

ID - Population abbreviation; N - sample size; A - mean number of alleles across all *loci* detected per each population; Ar- mean allelic richness across all *loci*; PA- number of private alleles; H_O - observed heterozygosity; H_E - expected heterozygosity; F_{IS} - inbreeding coefficient.

Structure analysis showed that the most likely number of clusters in the dataset was three (G1–G3, Figure 6.2). The maximum value of $\ln P(D)$ was obtained at $K = 4$ and STRUCTURE HARVESTER showed that the most likely partitioning of the genetic diversity based on delta K was obtained with $K = 3$ (Figure 6.2b). The sequential procedure under the seven levels of organization highlighted a subdivision in three clusters (Figure 6.2 and 6S.1, Appendix 6S). Using three inferred clusters, the q_i of each predefined group into three clusters were estimated. For $K = 3$, the majority of the individuals from BID were assigned to cluster G1, and most of the individuals from KGH and KAH belonged to G2. Also, for $K = 3$, more than 95% of the gazelles from MOT, GHAM, and KAL were assigned to a distinct cluster (G3; Figure 6.2). GENELAND analysis clearly confirmed the STRUCTURE results, supporting the genetic subdivision of the goitered gazelles in Central Iran into three genetic clusters (Figure 6S.2, Appendix 6S). Individual assignments performed in GENELAND with K fixed to 3 identified all BID samples and assigned them in the first cluster, all KGH samples to the second one, and the remaining samples to the third cluster (Figure 6S.2, Appendix 6S). With the exception of KAL, all populations grouped into the cluster three are geographically in close proximity.

GeneClass identified seven individuals as migrants ($p < 0.01$), six with the $L_{\text{home}}/L_{\text{max}}$ ratio and one with L_{home} (Table 6.3). The individuals identified with $L_{\text{home}}/L_{\text{max}}$ assignment were in accord with the clustering results from STRUCTURE exception B14 and G7.

Table 6.2. Pairwise estimated values of F_{ST} (below diagonal) and N_m ($F_{ST} = 1/(1 + 4N_m)$; above diagonal) for each Goitered gazelle population.

	BID	KAL	KGH	KAH	GHAM	MOT
BID	-	2.012	1.121	0.697	1.847	1.444
KAL	0.111*	-	1.760	1.232	5.701	2.581
KGH	0.182*	0.124*	-	2.249	2.667	4.013
KAH	0.264*	0.169*	0.100	-	2.316	2.020
GHAM	0.119*	0.042	0.086*	0.097*	-	6.966
MOT	0.148*	0.088	0.059	0.110	0.035	-

*Indicates significance after Bonferroni correction ($P\text{-value} < 0.0033$).

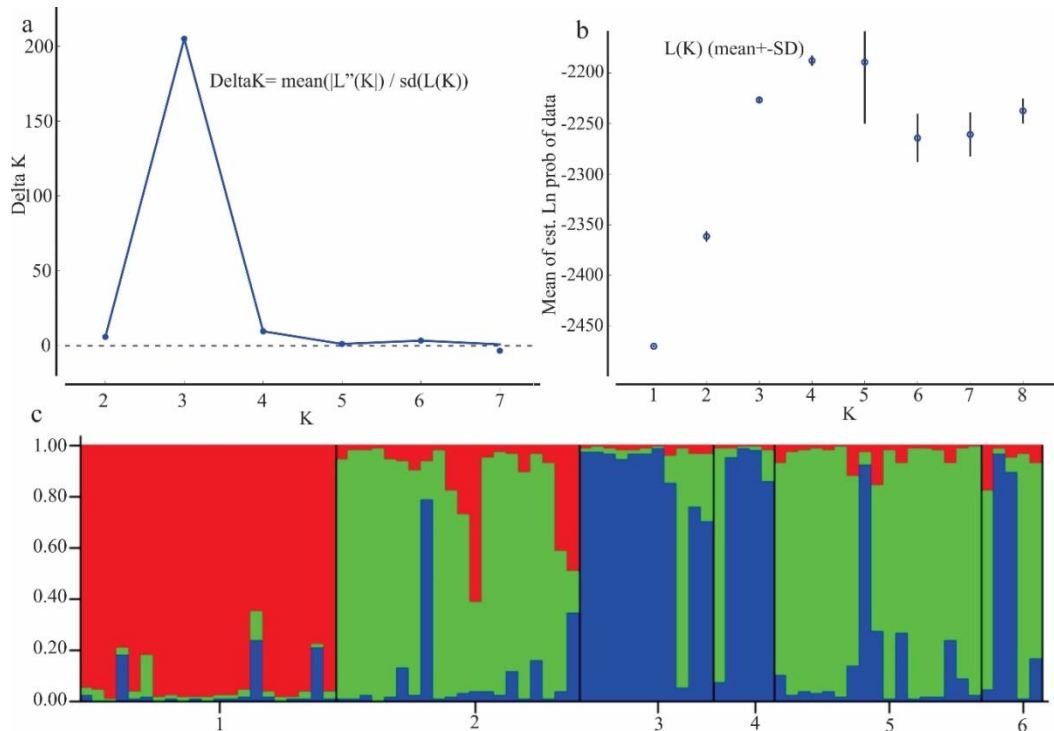


Figure 6.2. Detecting the most likely number of genetically distinct groups within the Goitered gazelles in Central Iran based on (a) Mean $L(K) \pm SD$ over 10 runs per K, (b) DK (Evanno et al. 2005) and (c) percentage of population assignments. The sampling populations for individuals are shown as 1–6 including 1 = BID (G1); 2 = KAL (G3); 3= KGH (G2); 4= KAH (G2); 5= GHAM (G3); 6= MOT (G3)

Table 6.3. Results of migrant detection analyses

Sample	Geographic origin	STRUCTURE cluster (G1/G2/G3). No prior population information, $K = 3$	GeneClass F_0 migrant probability using L_{home} (**) and $L_{\text{home}}/L_{\text{max}}$ (*)	GeneClass locality of highest probability
B14	BID	0.786; 0.181; 0.033	0.0077*	MOT
K19	KAL	0.017; 0.011; 0.972	0.0073*	GHAM
F13	KGH	0.011; 0.969; 0.021	0.0077*	KAH
F39	KGH	0.009; 0.048; 0.943	0.0085*	GHAM
Ka1	KAH	0.010; 0.069; 0.921	0.0047*	GHAM
G7	GHAM	0.064; 0.233; 0.703	0.0012*	KAH
T10	MOT	0.174; 0.043; 0.788	0.0028**	GHAM

Landscape Genetic Analysis

Spatial autocorrelation

The autocorrelation analysis revealed significant coefficients ($p < 0.01$) over distances. The r -values were significantly positive for the first four classes (up to 40 km) and a pattern of decreasing relatedness among all samples with increasing geographic distance was evident in the first four distance classes (5, 10, 20, 40 km; Figure 6S.3, Appendix 6S). The r coefficients remained positive with few 95% CI and r -values dropping below zero until a distance class of 200 km. The relationship between r and distance was not significantly negative until 300 km (Figure 6S.3, Appendix 6S).

Generally, a gradual cline in genetic differentiation was observed over increasing geographic distance.

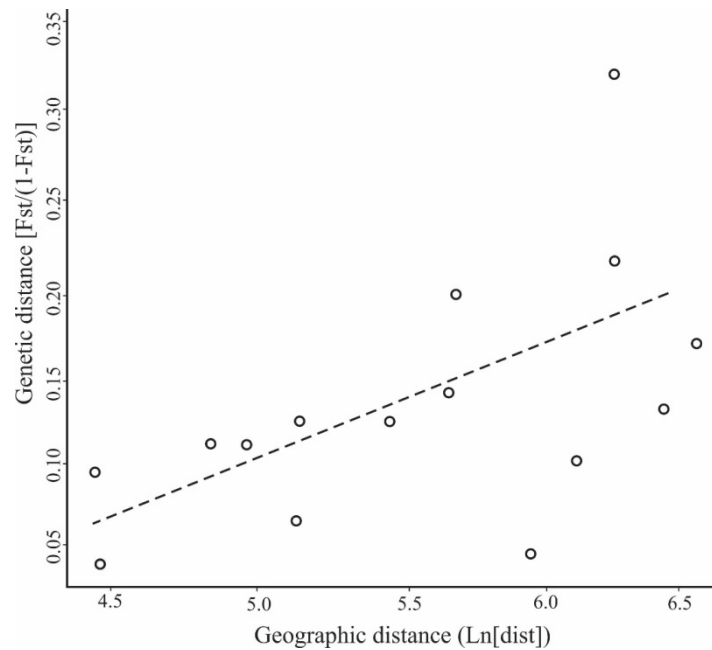


Figure 6.3. Analysis of isolation by distance showing the relationship between inter-population genetic distance as a function of geographical distance for Goitered gazelle in Central Iran. Mantel's $r = 0.55$, $p = 0.026$

Isolation by distance (IBD)

The IBD tests revealed a slightly significant correlation between geographic and genetic distance (Figure 6.3). However, when BID population (the southernmost population with a distance of about 300 km from the nearest gazelle population, KAL) was removed from the dataset, Mantel tests revealed a non-significant relation ($r^2 = 0.28$, $p > 0.05$). These results indicated that populations located closer together were less differentiated than those further apart.

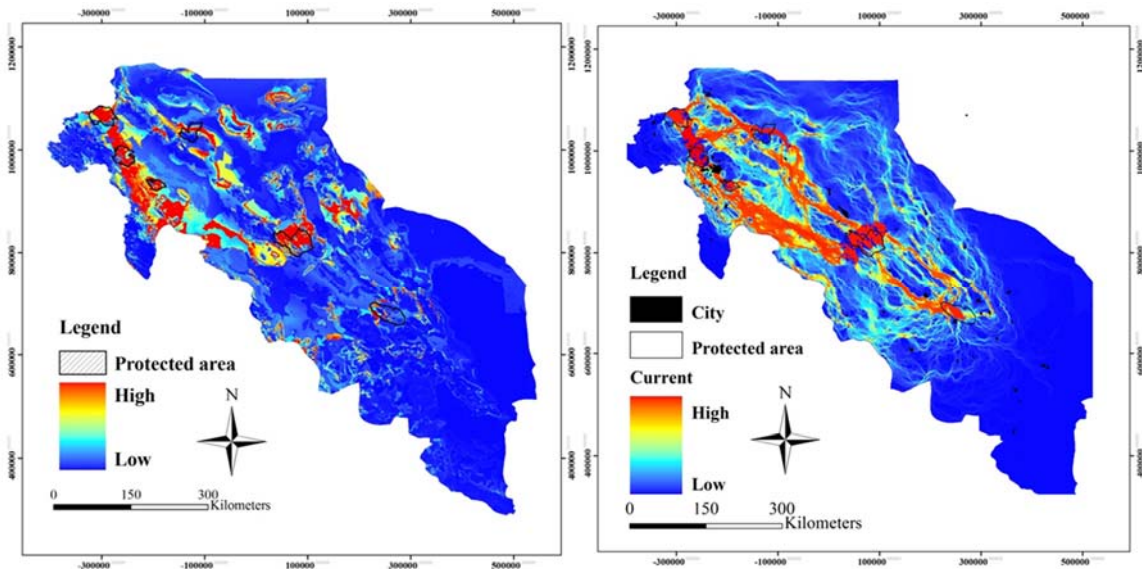


Figure 6.4. Goitered gazelle potential distribution map (left) and the current map output from CIRCUITSCAPE (right). Results depicting cumulative resistance between populations of Goitered gazelle based on the combined effect of environmental variables. High stands for the highest current flow.

Table 6.4. Correlations between genetic distance ($F_{ST}/(1-F_{ST})$) and geographical distance, human settlements, roads, railways as measured by Mantel tests and partial Mantel tests. Significant correlations marked *

Mantel test and partial mantel test	r	P value
Geographical distance *	0.55	0.03
Human settlement *	0.31	0.02
Road	0.34	0.06
Railway	0.23	0.15
Geographical distance (controlling for human settlement) *	0.48	0.04
Geographical distance (controlling for road)	0.47	0.06
Geographical distance (controlling for Railway) *	0.52	0.05
Human settlement (controlling for geographical distance)	0.03	0.45
Human settlement (controlling for road)	-0.11	0.65
Human settlement (controlling for Railway)	-0.24	0.84
Road (controlling for geographical distance)	0.13	0.37
Road (controlling for Railway)	-0.25	0.84
Road (controlling for Human settlement)	0.18	0.22
Railway (controlling for geographical distance)	0.09	0.39
Railway (controlling for roads)	0.00	0.65
Railway (controlling for Human settlement)	0.11	0.34

Isolation by resistance (IBR)

The MaxEnt model showed high performance with AUC equal to 0.957 (Figure 6.4-left). Based on the jackknife analysis and percentage of contribution, the environmental variables bioPCA1 (The first PC axis of a PCA analysis of bioclimatic variables; Appendix 6S), vegetation type, elevation, bioPCA2 (The second PC axis of a PCA analysis of bioclimatic variables) and density of vegetation 1_2 were the ones most strongly related with species distribution (Table 6S.8, Appendix 6S). CIRCUITSCAPE analysis (Figure 6.4-right) showed that genetic distance was partially explained by average resistance distances between populations ($r^2 = 0.42$; $p = 0.02$; Figure 6S.4, Appendix 6S). Resistance maps did not explained further variation in genetic distance after controlling for Euclidean distance ($P = 0.234$).

Effect of anthropogenic features on population structure

The Mantel and partial Mantel tests conducted between categorical matrixes including human settlements, roads, and railways indicated a significant positive association between genetic differentiation and presence of human settlements (Table 6.4), but when controlling for geographic distance, R values were lower. Human activities are apparently related with population structure but isolation by distance is the most relevant factor.

Discussion

This is the first study examining genetic diversity and geographical structure of Goitered gazelle populations in a relatively large area of Iran. Our results showed relatively low to moderate genetic diversity and also shallow genetic structuring in remaining gazelle populations. We found that a combination of geographic distance, landscape permeability and anthropogenic factors have most likely affected recently the genetic structure and gene flow in Goitered gazelle populations.

While Mantel test suffer from some limitations (Legendre and Fortin 2010), this method is widely used to test correlations between the elements in distance matrices. The domain of application of the Mantel test is the set of genetic questions that are originally formulated in terms of distances (such as F_{ST} vs. geographic distance or resistance distance; Legendre and Fortin 2010). Testing the isolation by distance in landscape genetic analysis is one of these applications. Hence, in the present study, we used this

method to test the effects of geographic and resistance distance on genetic differentiation (F_{ST}) among gazelle populations.

Success Rate of Amplification And Sources Of Uncertainty

The selected fragments used for genetic identification exhibited high amplification success rates (94% and 74% for *cytb* and *KCAS*, respectively) in agreement with what is generally described for noninvasive samples from herbivores (Silva et al. 2015). The higher success for mtDNA is explained by the high ratio of mitochondria to nuclei in cells (Kovach et al. 2003). The average success rate of amplifications for 15 microsatellites *loci* (62%) was low but not outside rates found in studies on Przewalski's gazelle (62.5 to 79.63%, Duo et al. 2015; 88%, Yang and Jiang 2011) and Mongolian gazelle (95%, Okada et al. 2015). The lower success rate of microsatellites in the present study may be due to the use of highly degraded DNA from samples collected during the warmest season of the year.

Departures from the Hardy Weinberg equilibrium estimated in some populations were likely caused by the presence of *loci* with null allele frequencies. Null allele frequency in the studied populations was below the threshold 0.20 (Table 6S.7, Appendix 6S; Okello et al. 2005). The observed null alleles can be due to preferential amplification of short alleles because of low quality extracted DNAs or phylogenetic distances between the Goitered gazelle and the taxon in which microsatellites were initially developed (Chapuis and Estoup 2007). As the 'recessive allele option' in STRUCTURE was used and the effects of null alleles were considered while calculating F_{ST} -values, we are confident that our results and conclusions are biologically meaningful.

Genetic Diversity and Population Structure

Our results revealed low to moderate nuclear genetic diversity in Goitered gazelle. Observed heterozygosity in the studied populations was lower than in *G. dorcas*, *Procapra przewalskii*, *Procapra gutturosa* and in a population of *G. subgutturosa* from Sohrein plain, Iran (Table 6.5). Results of mtDNA analysis showed extremely low diversity of haplotype and nucleotides in the studied populations, only five haplotypes in a total of 170 samples (Khosravi et al. in prep). The low genetic variation found in both markers was not surprising as all populations underwent a strong bottleneck in size during the past decades, which probably accounts for erosion and depletion of genetic diversity.

Although the expected mutation-drift heterozygosity was not significant for some populations, there was evidence of past genetic bottleneck based on the M-ratio within the six sampled populations. The M-ratio method is more sensitive than mutation-drift

heterozygosity and shows that bottleneck events may have occurred over longer time periods (Girod et al. 2011)

Statistically non-significant positive values of FIS were obtained within the populations with the minimum and maximum rate of inbreeding, MOT and KAH, respectively. There is a small population of less than 100 Goitered gazelles in KAH, while MOT incorporates over 4000 gazelles. The low coefficient of inbreeding and high heterozygosity observed in MOT and Ghamishloo (GHAM) populations is likely to be explained by the large effective population size and the potential individual exchange between these populations (85 km apart). The present study supports the idea that past bottleneck and genetic drift resulting from a small population size are the main factors accounting for the low genetic variability observed, even in the larger analyzed populations, such as MOT, GHAM and Kolah-Qazi (KGH).

Table 6.5. A summary of the measures of microsatellite diversity in gazelle species

Species	Reference	N	P	n	A	AR	H _E	H _O
<i>Gazella subgutturosa</i>	Present study	109	6	15	3.80	2.9	0.54	0.43
<i>G. subgutturosa</i>	Zachos et al. 2010	57	1	7	10.6	-	0.72	0.46
<i>G. dorcas</i>	Godinho et al. 2012	103	7	13	4.18	3.10	0.56	0.56
<i>N. granti</i>	Arctander et al. 1996	-	-	-	-	-	0.61	-
<i>Procapra przewalskii</i>	Duo et al. 2015	133	6	12	7.83	-	0.82	0.91
<i>P. przewalskii</i>	Yang and Jiang 2011	169	9	13	5.85	0.58	0.55	0.53
<i>P. gutturosa</i>	Okada et al. 2015	138	-	10	15	-	0.85	0.85

N number of genotyped individuals, P number of studied populations, n total number of *loci*, A mean number of alleles per locus, AR allelic richness, H_E expected heterozygosity, H_O observed heterozygosity

Analyses of genetic structuring based on F-statistics and Bayesian clustering revealed significant population structuring resulting in three genetic groups across the study area. Our results also suggest panmixia among gazelle's populations of GHAM, MOT, KAH, and KAL despite long geographic distance between KAL and the other three populations. In fact, graph analyses suggested high flow among KAL, KAH, GHAM and MOT, which formed a genetically homogenous group (cluster G3). On the contrary, effective resistances calculated among BID and KAL and low flow between these two populations supported the high genetic differentiation found between them. Spatial analyses also suggested multiple suitable habitat patches between these populations, which likely reflects a historical panmictic distribution of Goitered gazelle. The weak genetic structuring found can be partly explained by the land-use changes that occurred in Central Iran. The region was undisturbed until the last half century but urbanization and the constructions of roads jeopardized the connection of protected areas and the

persistence of gazelle's populations. The results of population structure support the current local isolation because of recent fragmentation and extirpation of local populations has led to shallow population structure in Goitered gazelles in Iran. While, B14 was identified as first generation migrant in GeneClass analysis, STRUCTURE assigned these samples to same genetic cluster as the same site in which the individuals were captured suggesting that the true source population was not represented in our sampled populations. Also, G7 classified as migrants in GeneClass, but not clearly assigned as residents in STRUCTURE, suggest that these individuals are the products of admixture between localities. Unlike structure, GeneClass does not assume that all source populations have been sampled and so it is more apt to detect migrants from such unsampled populations.

Effects of Geographic and Resistance Distances on Gene Flow

Spatial autocorrelation and isolation by distance (IBD) analyses indicated that gazelles become more genetically different than expected on average from 200 km (Klug et al. 2014). As a limited dispersal pattern was revealed, it seems that geographical distance is an important dispersal barrier. Gazelle dispersal most likely occurs between neighboring populations. The existence of a past continuous distribution was supported by the significant positive relationship found between geographic and genetic distances at the population level within the entire landscape, and isolation by distance could explain most of the genetic differentiation observed in gazelle populations. However, for this migratory species, we did not expect to recognize a significant IBD pattern between closer populations in the northwestern part of the study area (GHAM, MOT and KAH). Although current strict protection of BID has led to a population increase from 300 to about 1000 individuals (Kerman Provincial DoE, unpublished data), still long distance to nearest population (KAL) has led isolation and reduced genetic variation in this population. So, a combination of geographic distance and past decline in population size seems to account for the present low genetic variation and shallow structure in KAL population.

Our landscape genetic analysis showed that anthropogenic factors (roads and human settlements) are moderately related to the genetic structure of gazelle populations. The effects of recent road fragmentation on genetic structure have been observed in desert bighorn sheep (*Ovis canadensis nelsoni*; Epps et al. 2005) and Przewalski's gazelle (*Procapra przewalskii*; Yang et al. 2011). Empirical studies have found that effects of anthropogenic barriers on raising genetic differentiation to a detectable level has a lag time of about 1-200 generations depending on the species characteristic including generation time, dispersal ability and population size. For species with high dispersal

ability, it will take less time for a barrier to show its effect on FST and genetic structure (Landguth et al. 2010). If we considered generation time of about two years (female of the Goitered gazelle becomes sexually mature at one year of age and has a gestation period of 5 to 6 months; Mallon and Kingswood 2001), therefore a time frame of more than 50 years is reasonable for changing genetic patterns in response to recent anthropogenic change. However, given that Goitered gazelle is long-lived and have relatively large population sizes in some protected areas (PA), the likely effects of human activities and of disruption of gene flow might still be undetectable on FST and genetic sub-structuring for the moment.

Studied gazelle's populations exhibited a gradient of genetic differentiation as a function of potential habitat resistance. For instance, gazelles in BID are genetically differentiated from other populations and ecological models highlighted habitat suitability discontinuities between BID and other gazelle populations, while gene flow is likely to occur between northwestern populations (GHAM, MOT, KAH) given the observed low FST values and ecological modeling predicted suitable habitats in the landscape matrix between them. As such, landscape resistance is associated with population differentiation and apparently dispersal between isolated gazelle populations in PAs is mediated by the distribution of unsuitable habitats between regions.

Landscape Permeability to Gazelle Migration

Several previous studies have revealed the effects of anthropogenic and natural landscape factors on the functional connectivity and gene flow among populations (e.g. Yang and Jian 2011). Our landscape genetic analysis also yielded evidence for the effects of roads and human settlements on population structure of Goitered gazelle. As the species is apparently unable to traverse mountain ridges, topography and geographic distance have great influence in landscape permeability and gene flow among populations. For example, in the case of the KGH populations, the high mountains to the west, the long geographical distances to other eastern gazelle populations (i.e. KAH, BID and KAL), and the intense human activities to the north (around Isfahan city) have apparently restricted dispersal in KGH population and disrupted connectivity with other populations (DOE, unpublished data). On the contrary, resistance distances obtained from distribution map support the hypothesis of past continuous distribution across central Iran. Populations in KAL and KAH may exchange individuals via barrier-free desert in western and northwestern parts of the study area. In the southern areas, ecological modeling showed that patch-to-patch functional connectivity is weak and that permeability to dispersal between BID and other populations may be restricted by warm

and dry climatic conditions, poor vegetation cover, and the presence of anthropogenic barriers.

Conservation Implications

Goitered gazelle populations in Central Iran can be considered as a single evolutionarily significant unit (ESU), given the lack of significant genetic and/or morphological differentiation among populations (Hayatgheib et al. 2011). However, the levels of divergence found in the current study suggest that three management units (MUs) could be defined within our study area. The populations of MOT, GHAM, KAH, and KAL could be managed as one conservation unit, while the populations of BID and KGH should be treated as distinct. The identification of distinct MUs for this species is crucial for short-term management, biological conservation of the populations, monitoring of the population trends, and regulating the effects of human activity upon the abundance of populations.

Small isolated populations are generally affected by genetic drift, inbreeding and reduced gene flow that can decrease their long-term survival (Furlan et al. 2012). Low levels of genetic variability have been associated with low fitness in populations to overcome the effects of stochastic events (Keller and Waller 2002; Jamieson et al. 2008; Allentoft and O'Brien 2010). As such, special conservation consideration should be given to the KAH population, which exhibits the smallest census population (presently c.100 individuals). The construction of a recent highway with protected guardrails has likely decreased population connectivity even more, which is a problem that may also affect other populations located close to urban areas (for instance, GHAM and MOT from KAH). Hence, the potential effects of road networks on dispersal of Goitered gazelle should be taken into account when designing local conservation programs. In addition, the increasing road network suggests that conserving dispersal corridors becomes crucial, especially the establishment of passages and mitigation measures (e.g. overpass, noise barrier, and wildlife crosswalk) when projecting the construction of new highways. Further studies with more genetic markers will be also fundamental to allow monitoring population trends.

Conclusions

Goitered gazelle census populations in Iran seem to have lost genetic variation due to bottleneck effects and to small population size. The observed shallow structure and low gene flow among populations likely result from the combined effects of anthropogenic and natural landscape features promoting population isolation. Combined analysis of genetic and spatial data suggests that current genetic variation patterns and spatial

genetic structure of gazelles in the region have been more influenced by geographic distance in comparison to landscape resistance to movement and anthropogenic factors. In the following years human activities may significantly contribute for decreasing gene flow. Therefore, restoration of degraded habitats for re-colonization of extinct populations in the available suitable habitat patches and provision of suitable corridors for genetic exchange between the remnant wild populations seems to be vital for maintaining genetic diversity and assure the long-term persistence of Goitered gazelle populations in Iran.

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CHAPTER 7

General Discussion



CHAPTER 7 – General Discussion

The general aim of this thesis was to contribute for the conservation planning of threatened ungulates, particularly in North African gazelles. Four main objectives were targeted: i) increase the available molecular methods for genetic identification of threatened North African ungulates based on non-invasive sampling; ii) enlighten the phylogenetic relationships between threatened North African gazelles and identify potential occurrence areas; iii) understand relationships between the genetic diversities of 12 African ungulates and their observed range regression patterns and intrinsic and extrinsic factors; and iv) evaluate the genetic structure of an Asian gazelle and the effects of landscape features on gene flow patterns.

The first section of this chapter presents the key findings of each of the four main objectives. The second section presents the future prospects that can be derived based on the results found. The third section presents the major conclusions of this thesis.

7.1 Key findings

7.1.1 Genetic identification tools

A major achievement during this thesis was the development of genetic identification tools for nine co-occurring ungulates from North Africa, based on non-invasive sampling (Chapter 3). Despite their threatened status, there was no available protocol for ungulate identification especially developed for application to non-invasive sampling. The major advantages of the developed method based in two small fragments of a mitochondrial and nuclear marker, are to provide for the very first time a protocol to accurately identify species that accounts for species-specific variation and allows species identification from non-invasive sampling. The simplicity, high reliability and relative low cost of the described method make it a promising tool to improve ecological studies of the North African ungulates. The thesis allowed deriving a tool that contributes to the easiest and fastest identification of species, prior to the delineation of efficient management and conservation plans for these endangered ungulates.

7.1.2 Ecotypes and evolutionary significant units

An important contribution of this thesis to the conservation of ungulates in North Africa was the clarification of phylogenetic relationships in genus *Gazella* and the identification of ecotypes and evolutionary significant units in *Gazella cuvieri* and *Gazella leptoceros* (Chapters 3 and 4). Advances were made in three aspects:

i) the phylogenetic structure and relationships within genus *Gazella* were clearly defined from both mtDNA and nDNA sequences. Deep genetic structure based on mtDNA sequences had been previously reported (Hassanin et al., 1998; Lerp et al., 2011, 2013; Ropiquet and Hassanin, 2005; Wronski et al., 2010), but the taxonomy and systematics remained unclear due to potential biases caused by relationships being derived exclusively based in mtDNA sequences (Chapter 3). The most recent phylogeny available (Lerp et al., 2016) reconstructed two clusters: A) a predominantly Asiatic clade that includes *G. bennettii*, *G. subgutturosa*, *G. marica*, *G. leptoceros*, and *G. cuvieri*; and B) a predominantly African clade that includes *G. dorcas*, *G. spekei*, *G. gazella*, and *G. arabica*. Although the distribution of these two clusters tend to be allopatric, there is broad parapatry and sympatry in North Africa and Arabian Peninsula (Lerp et al., 2016; Chapter 3). The use of both mtDNA and nDNA sequences was able to clear uncertainties on the systematics within *Gazella* genus. For instance, diagnostic positions between former *G. cuvieri* and *G. leptoceros* were not found, further supporting these species as

a monophyletic group (Rebholz & Harley 1999, Wacher et al. 201, Wronski et al. 2010, Lerp et al. 2011; Chapter 3). Additional nuclear markers were missing for clarifying the phylogenetic relationships, identifying evolutionary significant units, and resolving the systematics of the group;

ii) the analyses of both mtDNA and nDNA sequences revealed further genetic substructure within genus *Gazella* that is most likely associated to processes of adaptive species formation in the course of divergent, ecological selection (Lerp et al., 2016; Chapters 3 and 4). Broadly, two groups of species exhibiting distinct life-history strategies may be identified: one group of species adapted to the extreme environmental conditions of deserts, and a second group of species inhabiting mountain areas of higher altitude, humidity and productivity, in comparison to the first group. Ecological and behavioural differences are also observable: the desert species tend to be non-territorial, feeding mainly on grasses, and forming larger herds, while the mountain species tend to be sedentary and territorial, feeding mainly on leaves, and only found in small groups (Lerp et al., 2016);

iii) the phylogenetic relationships found between distinct populations of former *G. cuvieri* and *G. leptoceros* allowed the clarification of the genetic structure, systematics and distribution of these two species (Chapter 4). The Cuvier's gazelle, *G. cuvieri*, comprises two ecotypes: A) a mountain ecotype which broadly corresponds to the populations formerly assigned to *G. cuvieri*, ranging from the Jbel Ouarkiz along the Atlases mountains of Morocco until western Tunisian mountains; and B) a lowland ecotype which broadly corresponds to the western populations formerly assigned to *G. leptoceros*, distributed in the Grand Erg Occidental and Grand Erg Oriental of Algeria and Tunisia (Figure 4.1). The Slender-horned gazelle, *G. leptoceros*, is now presumably restricted to Egypt, south-eastern Libya, and north-eastern Chad (see Figures 3.2 and 4.2, Tables 3S.2, 4S.1 and 4S.3). This assumption is based in the restricted genetic information available regarding *G. leptoceros*, two *cytb* sequences: Genbank JN410346 and JN410347. Still Egypt, exhibited a genetic divergence five times higher (1.4%) than all other North-West African samples (Chapter 4 discussion, Figure 4.2, Table 4S.1). In the future, conservation planning of *G. cuvieri* should consider the preservation of both ecotypes to help maintaining the overall adaptive potential of the species.

7.1.3 Updated distributions

A major finding of this thesis is the continuous range decline in several threatened ungulates in North Africa. Comparing the updated extents of occurrence derived in this

thesis (Chapters 4 and 5) with the extents from the last consulted IUCN assessments (IUCN, 2016), there were local population extinctions across the ranges of *Ammotragus lervia* and *Eudorcas rufifrons*, and most notably of *Gazella dorcas* (Figure 7.1).

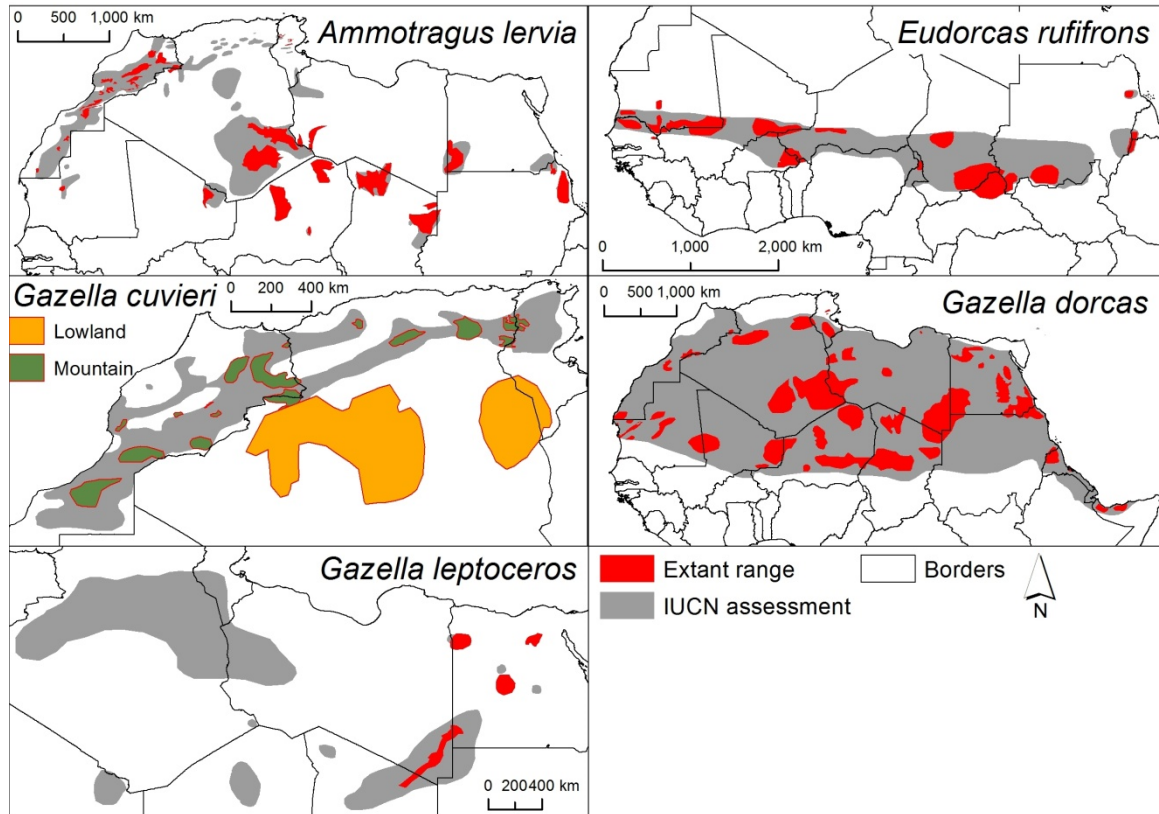


Figure 7.1. - Distribution of threatened ungulates in Africa according to IUCN assessments (IUCN, 2016) and to the present thesis (extant range). In *Gazella cuvieri*, the extant range displays both mountain and lowland ecotypes.

A range regression pattern was observed in the updated ranges of *G. cuvieri* and *G. leptoceros* (Figure 7.1), following the phylogenetic relationships found among these two species and the systematic rearrangements needed (Chapter 4). The updated range of *G. leptoceros* decreased mostly due to the allocation of the previous western populations to the lowland ecotype of *G. cuvieri*, but range contraction was also detected in the south-eastern populations. In *G. cuvieri*, there was an overall range increase due to the allocation of the previous western populations of *G. leptoceros* to the lowland ecotype of *G. cuvieri*, but there were two additional findings: i) range contraction was very strong in the mountain ecotype, particularly in populations located in the Saharan and Tellian Atlases mountains of Algeria and Tunisia, from where *A. lervia* was also almost extinct; ii) in comparison to the consulted IUCN assessments (IUCN, 2016), the updated range of the lowland population is now fragmented into two putative subpopulations, ranging in the Grand Erg Occidental and Grand Erg Oriental, respectively. These two findings

suggest that extant populations of *G. cuvieri* and *A. lervia* surviving in the mountains of Morocco need strong protection, as they represent the current population strongholds of these species: the mountain ecotype of *G. cuvieri* and the northernmost populations of *A. lervia*. Likewise, the same principle applies to the lowland ecotype populations of *G. cuvieri* ranging in Algeria and southern Tunisia.

Another finding is that the consulted IUCN assessments (IUCN, 2016) of the range map of these ungulates need to be updated (Figure 7.1).

7.1.4 Genetic diversity, range regression and ecological niches

The comparison between genetic diversity measures and range regression, latitudinal distribution, biological traits, and environmental variables and human factors of 12 African ungulates made in this thesis allowed retrieving three main patterns (Chapter 5):

- i) genetic diversities are lower in species that have suffered larger range regressions, such as *Addax nasomaculatus*, *G. leptoceros*, *Nanger dama*, and *Oryx dammah* (Durant et al., 2014; Newby et al., 2016; Brito et al., 2018). These findings support the need for the development of adequate management of captive and wild populations in order to revert current declining trends;
- ii) genetic diversities were mostly related with environmental variation and human pressure variables, in comparison to biological or geographic traits. Larger genetic diversity was found in remote areas exhibiting high productivity and precipitation variability, and distant from roads. These results support the hypothesis that extant populations are likely occupying the last strongholds of suitable habitats, which are retaining the highest observed genetic diversities among all taxa;
- iii) the measured realized ecological niche overlap of historical and present distributions based in environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time. It was found that extant populations occur under harsher environmental conditions in comparison to historical distributions. The positive relationships found between genetic diversity and productivity levels, and the fragmented and restricted character of the extant ranges, suggest that present populations should be vulnerable to the frequent drought periods that characterise North Africa. The overall reduced genetic diversities found suggest that most species may have lost already a portion of the adaptation ability to further environmental change.

7.1.5 Landscape features and genetic structure

A key finding of this thesis was that the genetic variation patterns and the spatial genetic structure of *Gazella subgutturosa* populations from the Central Arid Plateaux of Iran have been more influenced by geographic distance in comparison to landscape resistance to movement and anthropogenic factors (Chapter 6). The combined analysis of genetic and spatial data revealed shallow genetic structure and low gene flow levels among populations presently restricted to protected areas, which likely resulted from the combined effects of anthropogenic and natural landscape features promoting population isolation. Iranian populations seem to have lost genetic variation due to bottleneck effects and to small population size. Shallow geographic structure in genetic variation has also been reported in *G. dorcas* (Godinho et al., 2012), with weak spatial isolation and evidence of existing gene flow from western to eastern populations (data not shown). In Iran, human activities may have significantly contributed to the decreasing of gene flow between populations of *G. subgutturosa*. The restoration of degraded habitats for recolonization of extinct populations in the vicinity of available suitable habitat patches and provision of suitable corridors for genetic exchange between the remnant wild populations seem to be vital for maintaining genetic diversity and assure the long-term persistence of gazelle populations in arid regions.

7.1.6 Sampling gap

The analysis of a large number of individuals from several different regions, and the use of five nDNA fragments in the present study excluded the possibility of confounding effects in species delimitation caused by sampling biases or mitochondrial introgression (Chapter 4). Although the sample size for some analyses might be small, by using only genetically validated data, we assured a dataset free of ambiguities related to morphological misidentification and taxonomy (Chapter 5). Ideally, the origin of samples should be always known and for future we highly recommend to the scientific community to always make such information available in order to increase the quality and quantity of shared data.

7.2 Future prospects

Despite the contributions here presented to the conservation planning of threatened ungulates in North Africa and Iran, there are still several knowledge gaps and research subjects that need further development. There is a generalised increase in the understanding of biodiversity distribution patterns and evolutionary processes in North Africa (reviewed in Brito et al., 2014) and regional conservation priorities (Brito et al., 2016), but general remoteness and regional growing insecurity still hamper the development of biodiversity surveys (Brito et al., 2018). Detailed quantifications of species diversity and abundance, and genetic diversity present in the region are needed to provide framework data for the identification of local priority conservation areas (Brito et al., 2016). As such, the DNA barcoding approaches based on non-invasive sampling developed in this thesis (Chapter 3) offer the possibility of uncovering genetic diversity and likely cryptic diversity. The method should now be applied in both local and regional studies for rapid and effective surveying of ungulate populations.

The generalised lack of high spatial-resolution distribution data (collected with a Global Positioning System) forced the ecological analyses in this thesis (Chapters 4 and 5) to be based in distribution polygons depicting the range map of species. These polygons are defined by the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a species, i.e. the minimum convex polygon that contains all sites of occurrence (IUCN, 2016). Therefore, distribution polygons may include large areas of unsuitable habitat, i.e. non-occurrence areas, which may affect ecological modelling exercises. This bias may cause overestimation of parameters, affecting comparisons of ecological niches (Warren et al., 2008). In future studies, point data should be used in order to increase the accuracy of the estimations of niche differentiation and overlap.

The quantifications of genetic diversity developed in this thesis (Chapter 5) were mostly based in the use of a small gene fragment from the mtDNA (cytochrome b) that refers only to the maternal heritage. Such constraint may be a limiting factor when quantifying genetic diversity (Galtier et al., 2009). In future studies, fast evolving markers should be additionally used, as they provide as well detailed information on the spatial structure of genetic variation and gene flow levels between retrieved population clusters (Avise, 2010; e.g. Chapter 6). They may be also used to further explore the evolutionary relationships between the closely related ecotypes found in *G. cuvieri* (Chapter 4), for instance the putative occurrence of gene flow and hybridisation in contact zones. Finally, fast evolving markers may also be used to better understand demographic processes (e.g. Chapter 6) and to estimate effective population sizes in evolutionary significant units

targeted for conservation. Overall, the broad application of additional markers should be developed in the future for defining management strategies to the conservation of threatened ungulates.

The role of landscape features in shaping the spatial genetic structure of ungulate populations and the distribution of genetic diversity is still poorly known, as well as the spatial patterns in genetic diversity that can be associated with opportunities for dispersal events. The methodologies developed in this thesis, combining analysis of genetic and spatial data in *G. subgutturosa* (Chapter 6), should be expanded in the future to other threatened ungulates. Additional molecular markers and landscape genetics approaches are needed to quantify population sub-structuring and effective population size, to measure gene flow among populations, and to understand functional connectivity patterns among populations. Integrative genetic and spatial/ecological analyses identifying both the dispersal patterns and the landscape and environmental constraints to the dispersal of individuals will help in establishing efficient ecological networks (Baguette et al., 2013).

7.3 Concluding remarks

By integrating genetic and ecological/environmental data, and phylogenetic, populational, and ecological modelling tools, this thesis has contributed for the conservation planning of threatened ungulates, particularly of North African gazelles. In summary, the main achievements and conclusions are:

- 1) a molecular method based on polymorphisms in small fragments of the mitochondrial cytochrome b and the nuclear kappa casein genes was developed for laboratory identification of threatened North African ungulates;
- 2) the developed method allows the identification of the nine ungulate species that co-occur in North Africa and was validated across more than 400 samples, including different types of non-invasive samples collected in the field;
- 3) *Gazella cuvieri* and *G. leptoceros loderi* comprise a single monophyletic group and the populations of these taxa occupy distinct geographic areas and specific environments;

- 4) the populations of *Gazella cuvieri* and *G. leptoceros loderi* correspond to mountain and lowland ecotypes of *G. cuvieri*, respectively;
- 5) genetic diversities of 12 African ungulates are the lowest in the species that have suffered the largest range regressions;
- 6) genetic diversities of 12 African ungulates were mostly related with environmental variation and human pressure variables;
- 7) the measured realized ecological niche overlap of historical and present distributions of 12 African ungulates based on environmental variables and human pressure variables was low or completely non-overlapping, indicating strong niche shifts along time;
- 8) *Gazella subgutturosa* in the Central Arid Plateaux of Iran comprises three genetically homogeneous populations and there is restricted dispersal between populations;
- 9) Geographic distance in *G. subgutturosa* is related with genetic diversity and there are low effects of anthropogenic barriers on observed genetic structure.

7.4 References

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CHAPTER 8

Appendices



Appendix 3S – Supplementary material of chapter 3

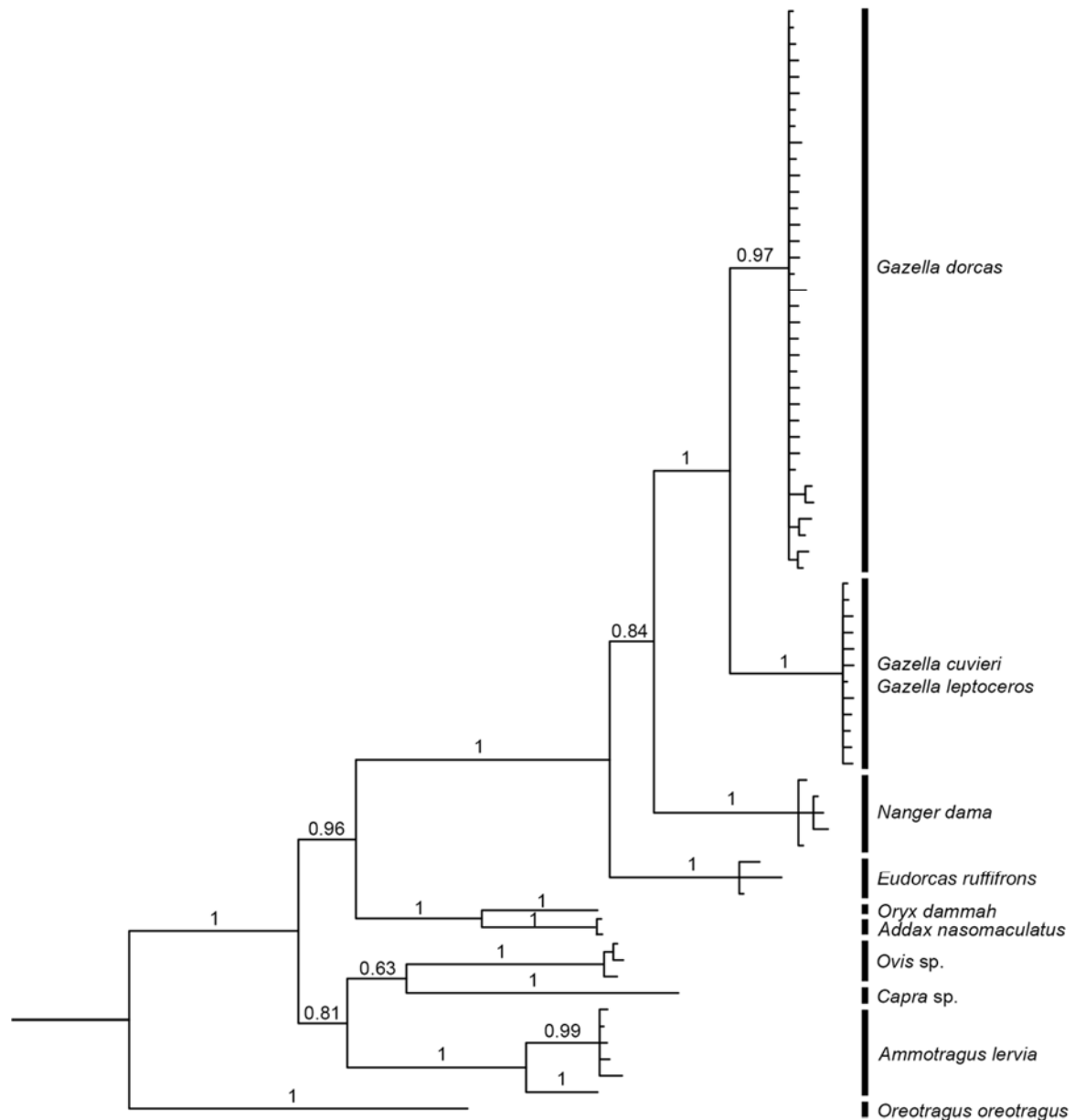


Figure 3S.1 Bayesian inference tree for the cytb and KCAS concatenated fragments showing the phylogenetic relationship of all endangered North African ungulates and the domestic species. Posterior probabilities of major nodes are indicated. *Oreotragus oreotragus* was used as outgroup. The Bayesian tree inference for cytb was performed using the GTR+I+G model

Table 3S.1. Species, type, origin, haplotype codes, GenBank accession numbers and country of the North African ungulate samples used in this study

Taxa	Type of sample				Origin of sample			Haplotype		GenBank Accession number		Country									
	Bone	Hair	Faeces	Tissue	Wild	Captive	Semicaptive	Cytb	KCAS	Cytb	KCAS	DZ	BE	TD	DE	MR	MA	NE	SN	ES	TN
Gazella dorcas	1	1	24	5	24	2	5	hap16_GD		JN410238-JN410240, 44, 48; JN410326				2		21	5		2	1	
	2	1	58	22	43	17	23	hap22_GD		JN410256; JN410338		1			1	32	25	1		10	13
			2	8		8	2	hap46_GD		KM582062									2	8	
			1	3	1	3		hap47_GD		KM582063							1			3	
				1		1		hap48_GD		KM582064										1	
			2	5	1		6	hap49_GD		KM582065							6				1
	1				1			hap50_GD		KM582066							1				
			11		8	3		hap51_GD		KM582067		5				3	3				
			30		11	5	14	hap52_GD		KM582068		1					10				19
			1		1			hap53_GD		KM582069		1									
		1			1			hap54_GD		KM582070		1									
			1		1			hap55_GD		KM582071							1				
			1		1			hap56_GD		KM582072							1				
				3			3	hap57_GD		KM582073										3	
	1	1			2			hap58_GD		KM582074						1		1			
			1		1			hap59_GD		KM582075						1					
			6		6			hap60_GD		KM582076						6					
			1		1			hap61_GD	GD_KCAS	KM582077	KM582053			1							
			1		1			hap62_GD		KM582078					1						
			1		1			hap63_GD		KM582079					1						
	2		3		5			hap64_GD		KM582080					2				3		
	1				1			hap65_GD		KM582081									1		
			2		2			hap66_GD		KM582082									2		
	2				2			hap67_GD		KM582083								1	1		
			1		1			hap68_GD		KM582084									1		
			2				2	hap69_GD		KM582085											2
			2				2	hap70_GD		KM582086											2
			1				1	hap71_GD		KM582087											1
	1						1	hap72_GD		KM582088										1	
	2						2	hap73_GD		KM582089										2	
			1		1			hap74_GD		KM582090								1			
			1		1			hap75_GD		KM582091								1			
			1		1			hap76_GD		KM582092								1			
	1				1			hap77_GD		KM582093		1									
			1				1	hap78_GD		KM582094		1									
Taxa	Type of sample				Origin of sample			Haplotype		GenBank Accession number		Country									
	Bone	Hair	Faeces	Tissue	Wild	Captive	Semcaptiv e	Cytb	KCAS	Cytb	KCAS	DZ	BE	TD	DE	MR	MA	NE	SN	ES	TN

<i>G. cuvieri</i> / <i>G. leptoceros</i>			48	1	48	1		hap84_GCGL		JN632636		48	1		
			5		3	2		hap85_GCGL		KM582095		3	2		
			5		1	4		hap86_GCGL		KM582096		1	4		
			5		5			hap87_GCGL		KM582097		5			
			8		8			hap88_GCGL		KM582098		8			
			2		2			hap89_GCGL		KM582099		2			
	1	9	32	4	34	11	1	hap90_GCGL	GCGL_KCAS	KM582100	KM582054	23	3	17	
	1					1		hap91_GCGL		KM582101				1	
	4			8		12		hap92_GCGL		KM582102				12	
	1		18		19			hap102_GCGL		KM582112		19			
			1		1			hap103_GCGL		KM582113		1			
			1		1			hap104_GCGL		KM582114		1			
	<i>Eudorcas rufifrons</i>		1		1			hap108_ER		KM582115		1			
	1			1			hap109_ER	ER_KCAS	KM582116	KM582055			1		
		1		1			hap110_ER		KM582117				1		
<i>Nanger dama</i>			2			2		hap112_ND		JN632665				2	
			12	1		6	7	hap113_ND		KM582118		4	3	6	
				1		1		hap114_ND	ND_KCAS	KM582119	KM582056			1	
			1				1	hap115_ND		KM582120		1			
			1				1	hap116_ND		KM582121		1			
<i>Oryx dammah</i>		10	4		5	9	hap117_Oryx	Oryx_KCAS	AJ222685; JN632677	KM582057			9	4	1
<i>Addax nasomaculatus</i>	2	13	3		3	15	hap120_Addax	Addax_KCAS	KM582122	KM582058	3	6			9
<i>Ammotragus lervia</i>		13		8	5		hap121_AL		FJ207522		8			5	
		1		1			hap122_AL		NC_009510			1			
		29	1	20	9	1	hap123_AL		KM582123					1	28
		2			2		hap124_AL	AL_KCAS	KM582124	KM582059	1				2
		2				2	hap125_AL		KM582125		2				
		1				1	hap126_AL		KM582126		1				
<i>Capra sp</i>		2		2			hap128_CP	CP_KCAS	KM582127	KM582060		2			
<i>Ovis sp</i>		1		1			hap129_OV		NC_001941						1
		1		1			hap130_OV	OV_KCAS	KM582128	KM582061					1
		1		1			hap131_OV		KM582129						1

Country codes: DZ - Algeria; BE – Belgium; TD - Chad; DE - Germany; MR - Mauritania; MA - Morocco; NE - Niger; SN - Senegal; ES – Spain; TN – Tunisia.

Table 3S.2. GenBank sequences for Cytb and KCAS genes from North African ungulates included in this study

Gene/Taxa	Accession Number	Locality	Reference
<i>Cytb</i>			
<i>Gazella dorcas</i>	AF030616	Almeria (Spain)	(Rebholz & Harley 1999)
	JN410219 - JN410258	North Africa	(Lerp <i>et al.</i> 2011)
	JN410314 - JN410322	North Africa	(Lerp <i>et al.</i> 2011)
	JN410325 - JN410339	North Africa	(Lerp <i>et al.</i> 2011)
	JN632637 - JN632638	North Africa	(Hassanin <i>et al.</i> 2012)
	JQ676941 - JQ676952	Morocco	(Godinho <i>et al.</i> 2012)
	JX274672, JX274673	Mali, Somalia	(Bärmann <i>et al.</i> 2013b)
	JX647822	Qatar	(Bärmann <i>et al.</i> 2013a)
	KC188752	Israel	(Lerp <i>et al.</i> 2012)
<i>Gazella cuvieri</i>	AF030609	Almeria (Spain)	(Rebholz & Harley 1999)
	HQ316154	Saudi Arabia	(Wacher <i>et al.</i> 2010)
	JN410342, JN410343	Almeria (Spain)	(Lerp <i>et al.</i> 2011)
	JN632636	San Diego	(Hassanin <i>et al.</i> 2012)
<i>Gazella leptoceros</i>	AF030610	Algeria?	(Rebholz & Harley 1999)
	AF187699	Saudi Arabia	(Hammond <i>et al.</i> 2001)
	HQ316152, HQ316153	Saudi Arabia	(Wacher <i>et al.</i> 2010)
	JN410259	Algeria	(Lerp <i>et al.</i> 2011)
	JN410344, JN410345	Tunisia	(Lerp <i>et al.</i> 2011)
	JN410346, JN410347	Egypt: Western Desert	(Lerp <i>et al.</i> 2011)
	JN632641	San Diego Zoo (USA)	(Hassanin <i>et al.</i> 2012)
<i>Eudorcas rufifrons</i>	JN632633, JN632634	Sudan	(Hassanin <i>et al.</i> 2012)
	AF030606		(Rebholz & Harley 1999)
<i>Gazella (Nanger) dama</i>	JN632665	San Diego Zoo (USA)	(Hassanin <i>et al.</i> 2012)
	AF030603		(Rebholz & Harley 1999)
<i>Oryx dammah</i>	JN632677	Vincennes Zoo	(Hassanin <i>et al.</i> 2012)
	AJ222685	Paris MNHN	(Hassanin & Douzery 1999)
	NC_016421	Sudan	(Zhang <i>et al.</i> 2012)
<i>Addax nasomaculatus</i>	AF034722	Vincennes Zoo MNHN	(Hassanin <i>et al.</i> 1998)
<i>Ammotragus lervia</i>	NC_009510		(Mereu <i>et al.</i> 2007)
	FJ207522	Paris MNHN	(Hassanin <i>et al.</i> 2009)
<i>Capra hircus</i>	GU295658	Paris MNHN	(Hassanin <i>et al.</i> 2010)
<i>Ovis aries</i>	NC_001941	European	(Hiendleder 1998)
<i>KCAS</i>			
<i>Gazella dorcas</i>	JX647813, JX647814	Qatar	(Bärmann <i>et al.</i> 2013a)
<i>Gazella (Nanger) dama</i>	JN018099		(Matthee & Davis 2001)
<i>Oryx dammah</i>	AF210157		(Matthee & Davis 2001)
<i>Ammotragus lervia</i>	AY670670	Paris MHN	(Ropiquet & Hassanin 2005)
<i>Capra hircus</i>	AF165800		(Matthee <i>et al.</i> 2001)
<i>Ovis aries</i>	AF165800		(Matthee <i>et al.</i> 2001)

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Table 3S.3. Interspecific polymorphic positions of the cyt b 450bp fragment analysed in endangered ungulates species from North Africa. First position corresponds to position 14 515 in *Bos taurus* complete mitochondrial genome (accession number NC_006853). Points represent similar positions and dashes represent indels.

[illegible]

[illegible]

[illegible]

		1 1 1 1 1 1 2 2 2 2 3 3 4 4 5 5 5 6 6 6 6 7 8 8 8 9 9 9 0 0 0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3 4 4 4																																																						
Haplotype / Taxa / Position		4	5	8	0	1	5	7	8	9	0	2	3	7	9	2	8	1	7	3	6	9	2	5	6	6	6	7	8	8	8	9	9	9	0	0	0	0	0	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4		
Hap_100	Gazella cuvieri / Gazella leptoceros	N	N	N	N	N	N	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.				
Hap_101		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.						
Hap_102		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.						
Hap_103		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.						
Hap_104		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.						
Hap_105	Eudorcas rufifrons	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	T	.	C	.	.	T	.				
Hap_106		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	C	.	.	T	.	C	.	.	T	.					
Hap_107		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	C	.	.	T	.	C	.	.	T	.					
Hap_108		.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	A	.	T	.	.	.	.	.	C	.	.	T	.	C	.	.	T	.						
Hap_109		.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	T	T	T	.	C	.	.	A	.	T	T	C	.	.	C	.	.	T	.	C	.	.	T	.				
Hap_110	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	A	.	T	.	.	.	.	.	C	.	.	T	.	C	.	.	T	.							
Hap_111	Nanger dama	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	.	.	.	.	.	.	C	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.	.					
Hap_112		.	T	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.						
Hap_113		.	T	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.						
Hap_114		.	T	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.						
Hap_115		.	T	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.						
Hap_116	.	T	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	C	T	.	.	T	.	G	C	.	.	.	T	.	C	.	.	.							
Hap_117	Oryx dammah	C	.	.	T	T	.	A	.	.	.	.	.	.	.	.	G	G	T	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	A	.	C	.	.	C	.	T	.	G	.	.	T	.	T	.				
Hap_118		C	.	.	T	T	.	A	.	.	.	.	.	.	.	.	.	G	G	T	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	A	.	C	.	.	C	.	T	.	G	.	.	T	.	T	.			
Hap_119	Addax nasomaculatus	C	T	.	T	T	.	A	.	.	.	.	.	.	.	.	G	.	T	.	.	T	C	.	.	.	.	C	T	.	.	.	A	.	.	.	.	A	.	C	.	.	C	.	.	G	.	C	T	.	.	.				
Hap_120		C	T	.	T	T	.	A	.	.	.	.	.	.	.	.	.	G	.	T	.	.	T	C	.	.	.	.	C	T	.	.	.	A	.	.	.	.	A	.	C	.	.	C	.	.	G	.	C	T	.	.	.			
Hap_121	Ammotragus lervia	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	T	.	C	.	.	C	.	T	T	.	C	T	.	.	T	.			
Hap_122		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	.	.	.	C	.	.	T	T	.	C	.	.	.					
Hap_123		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	.	.	T	.	C	.	.	C	.	T	T	.	C	T	.	.	T	.
Hap_124		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	.	.	.	C	.	.	C	.	T	T	.	C	T	.	.	T	.	
Hap_125		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	.	.	.	C	.	.	C	.	T	T	.	C	T	.	.	T	.	
Hap_126	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	A	.	C	.	.	.	A	C	.	.	.	.	.	.	C	.	.	C	.	T	T	.	C	T	.	.	T	.		
Hap_127	Capra sp	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	A	.	C	.	.	.	A	.	A	.	.	.	.	A	.	T	C	.	.	T	.	C	.	G	.	.				
Hap_128		C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	A	.	C	.	.	.	A	.	A	.	.	.	.	A	.	T	C	.	.	T	.	C	.	G	.	.			
Hap_129	Ovis sp	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	T	.	T	.	.	.	A	.	T	.	.	.	.	C	.	T	.	T	T	.	G	.	.	.	.					
Hap_130		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	T	.	T	.	.	.	A	.	T	.	.	.	.	C	.	T	.	T	T	.	G	.	.	.					
Hap_131		.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	T	.	T	.	.	.	A	.	T	.	.	.	.	.	C	.	T	.	T	T	.	G	.	.	.				

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Appendix 4S – Supplementary material of chapter 4

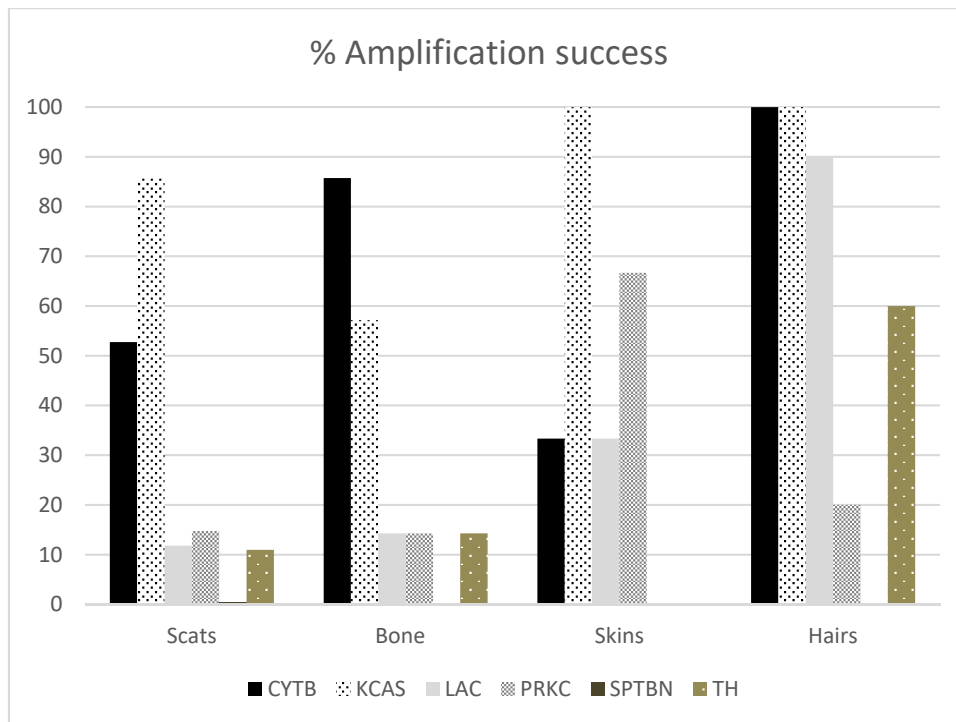


Figure 4S.1 Amplification success (%) for the cytochrome b (CYTB), Kappa casein exon 4 (KCAS), α -lactalbumin intron 2 (LAC), b-spectrin nonerythrocytic 1 (SPTBN), protein kinase C iota (PRKC) and thyroptin beta chain (TH) fragment genes on noninvasive material (scats, bones, skins and hairs).

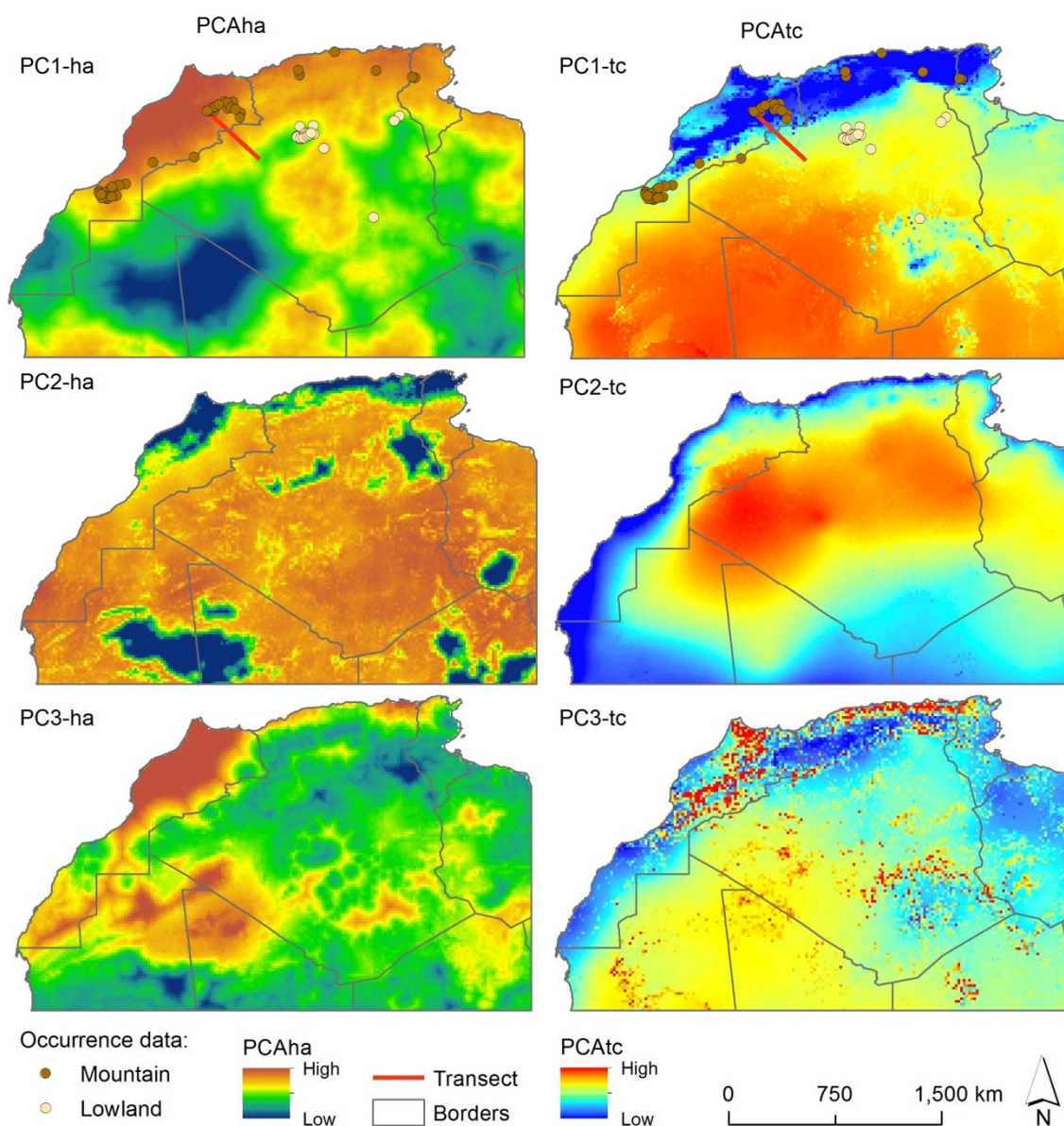


Figure 4S.2. Distribution of mountain and lowland ecotypes in relation to the spatial variation of the first three principal components (PC1, PC2 and PC3) of a Spatial Principal Components Analyses of topoclimatic (tc) and habitat (ha) variation in the study area.

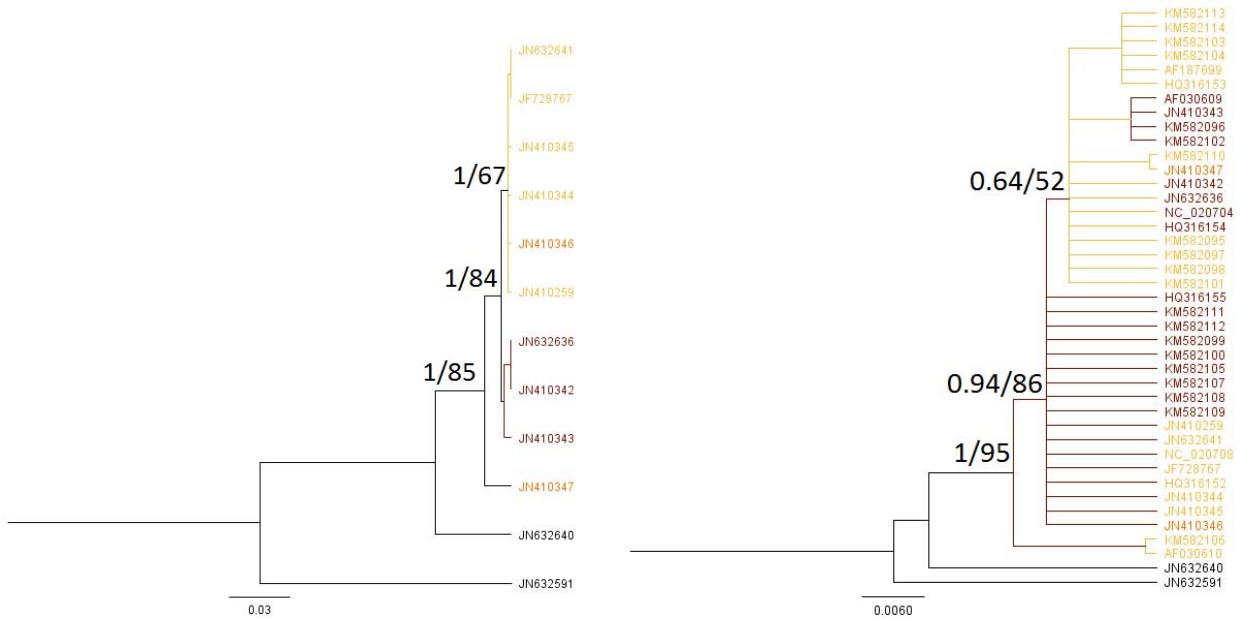


Figure 4S.3. Phylogenetic reconstructions based on Bayesian and Maximum Likelihood inference of CYTB sequences, showing the relationships among *Gazella cuvieri* and *Gazella leptoceros*. Left tree was reconstructed with a 1013 bp common fragment and the right tree with a 282 bp common fragment. Posterior probabilities of major nodes (Bayesian) and bootstrap proportion values (ML) are indicated in respectively order. Colours represent the distinct ecotypes: mountain in brown (corresponding to *G. cuvieri*), lowland in yellow (corresponding to *G. leptoceros loderi*) and the Egypt samples in orange (corresponding to *G. leptoceros leptoceros*). *Gazella gazella* and *Addax nasomaculatus* were used as outgroups (in black).

Table 4S.1. Codes, country and coordinates, type, origin, genetic identification, methods for which samples were used [Mod: ecological models; Net: network (Figure 4.2) and Tree: phylogenetic analysis (Figure 4.2)] and GenBank accession numbers of the North African ungulate samples used in this study.

Lab Code	Country	Latitude	Longitude	Sample	Population	Genetic ID	Methods	GenBank Accession numbers					
								CYTB	KCAS	LAC	PRKC	SPTBN	TH
19-448	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL				
19-449	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			#TH3
13-401	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			#TH3
13-402	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			#TH2
13-403	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			#TH3
13-404	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			
13-405	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			
13-406	Algeria	32.98122	-0.92733	Hair	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			
NAG13	Algeria	31.98053	1.32008	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG14	Algeria	32.22031	2.11493	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG17	Algeria	31.55960	2.21176	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net		#KCAS_GCGL				
NAG19	Algeria	31.51370	2.22723	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG20	Algeria	32.13070	2.25321	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2					
NAG22	Algeria	32.13234	2.30108	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG23	Algeria	32.13490	2.30130	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH3
NAG24	Algeria	32.20696	2.24012	Faeces	Wild	<i>G. leptoceros</i>	Mod	#cytb3					
NAG26	Algeria	31.36169	1.14534	Faeces	Wild	<i>G. leptoceros</i>	Mod						
NAG29	Algeria	32.24639	2.13687	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG32	Algeria	31.20212	1.38191	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL			
NAG33	Algeria	31.40016	1.48506	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG34	Algeria	31.51113	1.67569	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG35	Algeria	31.56794	1.68858	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH2
NAG36	Algeria	31.54081	1.81596	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb11	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH3
NAG37	Algeria	32.44674	2.15871	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH3
NAG38	Algeria	31.31946	1.16323	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH2
NAG39	Algeria	31.32204	1.16507	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH2
NAG40	Algeria	31.32041	1.16377	Bone	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL			
NAG41	Algeria	32.30376	2.07208	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG42	Algeria	32.03967	2.17904	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL				
NAG43	Algeria	32.04001	2.17874	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3					
NAG44	Algeria	32.04187	2.17850	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG45	Algeria	31.99286	2.32182	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		#TH3

NAG46	Algeria	31.94274	2.24602	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH2
NAG48	Algeria	31.85815	2.24565	Hair	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG49	Algeria	31.75784	2.11442	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2				
NAG50	Algeria	31.23978	1.36478	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL		#PRKC_GCGL	
NAG51	Algeria	31.23692	1.38903	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3				
NAG52	Algeria	31.25756	1.56639	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb12				
NAG53	Algeria	31.36122	1.80739	Bone	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL		#PRKC_GCGL	#TH3
NAG54	Algeria	31.37089	1.87969	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL		#PRKC_GCGL	
NAG55	Algeria	31.45467	1.93658	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3				#TH3
NAG56	Algeria	31.55883	2.05986	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb3	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG59	Algeria	32.65053	7.63769	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net		#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	
NAG60	Algeria	32.36825	7.38006	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH1;#TH2
NAG61	Algeria	32.34942	7.30764	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH2
NAG62	Algeria	30.60626	2.85958	Tissue	Wild	<i>G. leptoceros</i>	Mod+Net+Tree	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH2
NAG65	Morocco	28.27639	-10.11867	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG66	Morocco	28.28195	-10.12130	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG68	Morocco	28.26983	-10.11347	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb6	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG69	Morocco	28.28090	-10.11989	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb6				
NAG70	Morocco	28.16313	-10.55953	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	
NAG74	Morocco	28.16691	-10.57499	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL		#TH3
NAG75	Morocco	28.16258	-10.55908	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG76	Morocco	28.16345	-10.55984	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb6	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG77	Morocco	28.16345	-10.55984	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG78	Morocco	28.16345	-10.55984	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3;#TH5
NAG79	Morocco	28.16345	-10.55984	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb7	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG88	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb6				
NAG89	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net		#KCAS_GCGL			
NAG90	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb7				
NAG91	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb7				
NAG92	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb6	#KCAS_GCGL	#LAC_GCGL		
NAG93	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb7	#KCAS_GCGL		#PRKC_GCGL	
NAG94	Spain	36.83806	-2.46083	Faeces	Captive	<i>G. cuvieri</i>	Net	#cytb7	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#TH3
NAG236	Morocco	27.57742	-10.63322	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL			#TH3
NAG241	Morocco	27.55957	-10.67374	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb8	#KCAS_GCGL			
NAG242	Morocco	27.57742	-10.63322	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL			
NAG243	Morocco	27.58072	-10.69046	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL			
NAG245	Morocco	27.76723	-10.58803	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL			

NAG246	Morocco	27.74971	-10.56150	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG247	Morocco	27.74560	-10.57166	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG248	Morocco	27.75596	-11.38266	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG251	Morocco	27.76527	-11.39653	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL	#TH3
NAG252	Morocco	27.76865	-11.40685	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL	
NAG254	Morocco	27.76720	-11.40271	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG255	Morocco	27.76927	-11.39465	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG256	Morocco	27.76618	-11.39655	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG257	Morocco	27.76618	-11.39655	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	#TH3
NAG261	Morocco	27.78557	-11.40236	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG263	Morocco	27.57479	-10.63669	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG265	Morocco	27.57834	-10.63102	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG266	Morocco	27.57472	-10.71456	Faeces	Wild	<i>G. cuvieri</i>	Mod			#TH3
NAG267	Morocco	27.58822	-10.27378	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL	
NAG268	Morocco	27.57872	-10.68934	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG270	Morocco	27.58417	-10.63028	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG272	Morocco	27.56164	-10.68658	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG273	Morocco	27.55500	-10.69000	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG274	Morocco	27.55500	-10.75584	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG275	Morocco	28.00412	-10.69687	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG276	Morocco	27.55973	-10.68753	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG277	Morocco	27.76619	-11.39586	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb9		
NAG278	Morocco	27.76894	-11.39363	Faeces	Wild	<i>G. cuvieri</i>	Mod			
NAG279	Morocco	27.77627	-11.39881	Faeces	Wild	<i>G. cuvieri</i>	Mod			
NAG280	Morocco	27.77638	-11.39581	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1		
NAG283	Morocco	27.56622	-10.64889	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL	
NAG285	Morocco	27.58145	-10.62926	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG306	Morocco	27.47133	-11.23728	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG309	Morocco	27.46781	-11.23091	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG321	Morocco	27.46766	-11.23076	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL	
NAG322	Morocco	27.46787	-11.23114	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG336	Morocco	27.81151	-10.80600	Faeces	Wild	<i>G. cuvieri</i>	Mod			
NAG337	Morocco	27.81809	-10.99126	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL	
NAG338	Morocco	27.79805	-11.03689	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb9	#KCAS_GCGL	
NAG339	Morocco	27.80868	-11.01761	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb8		
NAG341	Morocco	27.81392	-10.99994	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb8	#KCAS_GCGL	
NAG342	Morocco	27.81392	-10.75777	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL	

NAG343	Morocco	27.79708	-11.03101	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb8	#KCAS_GCGL
NAG344	Morocco	27.81246	-10.99637	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb8	#KCAS_GCGL
NAG348	Morocco	27.63724	-10.97878	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG349	Morocco	27.64043	-11.00039	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL
NAG350	Morocco	27.59379	-10.99309	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	
NAG351	Morocco	27.64083	-11.00133	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG352	Morocco	27.63977	-10.97869	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	
NAG354	Morocco	27.63253	-10.98586	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL
NAG356	Morocco	27.46687	-11.16823	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL
NAG357	Morocco	27.46843	-11.16209	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb5	#KCAS_GCGL
NAG358	Morocco	27.46882	-11.16616	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	
NAG359	Morocco	27.49840	-10.94302	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	
NAG360	Morocco	27.50111	-10.97172	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG362	Morocco	27.50727	-10.94961	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	
NAG363	Morocco	27.46932	-11.15394	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG364	Morocco	27.52210	-11.00802	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	
NAG365	Morocco	27.50251	-10.97037	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG366	Morocco	27.47297	-11.23730	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG372	Morocco	27.47428	-10.99126	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG373	Morocco	27.46449	-11.22645	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG374	Morocco	27.46575	-11.22661	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	
NAG376	Morocco	27.68397	-11.24658	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG379	Morocco	27.67531	-11.23440	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL
NAG381	Morocco	27.66804	-11.24177	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL
NAG382	Morocco	27.68087	-11.22558	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL
NAG383	Morocco	27.68091	-11.22565	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb1	#KCAS_GCGL
NAG384	Morocco	27.66805	-11.24172	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL
NAG467	Tunisia	35.19319	8.64069	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	
NAG476	Tunisia	35.20350	8.67692	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL
NAG478	Tunisia	35.20350	8.67692	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	
NAG480	Tunisia	35.20728	8.67061	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL
NAG489	Tunisia	35.18706	8.64761	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	
NAG498	Tunisia	35.18547	8.64992	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	
NAG505	Tunisia	35.19933	8.68556	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	
NAG508	Tunisia	35.19933	8.68556	Faeces	Wild	<i>G. cuvieri</i>	Mod		
NAG512	Tunisia	35.10636	8.63839	Faeces	Wild	<i>G. cuvieri</i>	Mod		
NAG516	Tunisia	35.10325	8.63672	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL

NAG523	Tunisia	35.12058	8.47425	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG524	Tunisia	35.12058	8.47425	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG525	Tunisia	35.12058	8.47425	Faeces	Wild	<i>G. cuvieri</i>	Mod						
NAG526	Tunisia	35.12058	8.47425	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2					
NAG527	Tunisia	35.12058	8.47425	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2					
NAG536	Tunisia	35.14397	8.48053	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2					
NAG537	Tunisia	35.14397	8.48053	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL				
NAG538	Tunisia	35.14397	8.48053	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG539	Tunisia	35.14397	8.48053	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG540	Tunisia	35.14397	8.48053	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL				
NAG620	Spain	36.83806	-2.46083	Bone	Captive	<i>G. cuvieri</i>	Net	#cytb10					
NAG626	Spain	36.83806	-2.46083	Skin	Captive	<i>G. cuvieri</i>	Net		#KCAS_GCGL				
NAG630	Spain	36.83806	-2.46083	Bone	Captive	<i>G. cuvieri</i>	Net	#cytb4	#KCAS_GCGL				
NAG651	Spain	36.83806	-2.46083	Bone	Captive	<i>G. cuvieri</i>	Net	#cytb4	#KCAS_GCGL				
NAG652	Spain	36.83806	-2.46083	Bone	Captive	<i>G. cuvieri</i>	Net	#cytb4	#KCAS_GCGL				
NAG653	Spain	36.83806	-2.46083	Bone	Captive	<i>G. cuvieri</i>	Net	#cytb4					
NAG655	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN1; #SPTBN2	
NAG656	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2; #SPTBN3	
NAG657	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN3	
NAG658	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG661	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2; #SPTBN4	
NAG663	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2; #SPTBN3	
NAG665	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN1	
NAG666	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree		#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG667	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb4	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL		
NAG671	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. cuvieri</i>	Net+Tree	#cytb1	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2	
NAG716	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net+Tree	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2	#TH3
NAG717	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net	#cytb2	#KCAS_GCGL			#SPTBN2	
NAG718	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net+Tree	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2; #SPTBN3	
NAG719	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net+Tree	#cytb2	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN2; #SPTBN3	#TH3; #TH4
NAG720	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net+Tree		#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	#SPTBN3	
NAG721	Belgium	51.21016	4.40490	Tissue	Captive	<i>G. leptoceros</i>	Net+Tree		#KCAS_GCGL		#PRKC_GCGL		#TH3; #TH5
NAG835	Algeria	34.88278	-1.31667	Faeces	SemiCaptive	<i>G. cuvieri</i>	Mod+Net	#cytb2	#KCAS_GCGL		#PRKC_GCGL		#TH3; #TH2
NAG896	Morocco	27.53314	-10.58175	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL				
NAG897	Morocco	27.53314	-10.58175	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL				
NAG898	Morocco	27.53314	-10.58175	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL				
NAG991	Morocco	30.07409	-5.33679	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL				

NAG1002	Morocco	29.74386	-7.95871	Skin	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL	#LAC_GCGL	#PRKC_GCGL	
NAG1003	Morocco	33.00959	-4.10367	Skin	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1004	Morocco	33.53882	-2.91408	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1005	Morocco	32.86633	-2.40822	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1006	Morocco	32.86633	-2.40822	Faeces	Captive	<i>G. cuvieri</i>	Net	#KCAS_GCGL			
NAG1027	Algeria	26.23583	5.98444	Faeces	Wild	<i>G. leptoceros</i>	Mod+Net	#KCAS_GCGL			#SPTBN2
NAG1064	Algeria	35.25424	1.31967	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1065	Morocco	28.34217	-9.64897	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1066	Morocco	28.34312	-9.67500	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1067	Morocco	28.33507	-9.67964	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1068	Morocco	28.34320	-9.64839	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1069	Morocco	28.34734	-9.67129	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL		#PRKC_GCGL	
NAG1075	Morocco	27.83328	-10.84775	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1078	Morocco	27.67192	-10.74754	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1084	Morocco	27.66479	-10.72463	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1085	Morocco	27.70636	-10.73002	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1086	Morocco	27.40702	-10.85953	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1097	Morocco	27.88260	-11.35886	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1104	Morocco	28.00849	-11.09313	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1107	Morocco	28.00974	-11.09223	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1109	Morocco	27.85041	-11.24871	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1110	Morocco	28.18437	-10.74727	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1111	Morocco	27.87819	-11.36055	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1112	Morocco	27.85747	-11.23217	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1113	Morocco	27.86884	-11.36234	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1115	Morocco	27.89735	-11.34903	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1116	Morocco	27.89735	-11.34903	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1117	Morocco	27.86272	-11.30867	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1118	Morocco	27.89643	-11.34805	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1121	Morocco	28.01526	-11.08875	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1123	Morocco	27.85341	-11.24628	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1124	Morocco	27.88811	-11.34873	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1127	Morocco	27.88493	-11.35828	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1128	Morocco	28.01831	-11.08768	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1129	Morocco	28.18392	-10.76222	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL			
NAG1130	Morocco	27.85811	-11.30119	Faeces	Wild	<i>G. cuvieri</i>	Mod				
NAG1131	Morocco	27.88553	-11.35402	Faeces	Wild	<i>G. cuvieri</i>	Mod				

NAG1132	Morocco	27.86552	-11.36930	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1133	Morocco	28.00639	-11.09478	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1134	Morocco	27.84915	-11.25070	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1135	Morocco	28.01325	-11.09941	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1136	Morocco	27.86582	-11.36580	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1137	Morocco	28.18493	-10.76091	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1138	Morocco	28.00639	-11.09478	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1139	Morocco	27.88732	-11.34972	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1141	Morocco	27.88525	-11.35786	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1142	Morocco	27.90787	-11.36084	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1143	Morocco	27.87797	-11.36059	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1145	Morocco	27.85747	-11.23217	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1146	Morocco	27.86295	-11.30908	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1147	Morocco	27.87267	-11.36149	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1148	Morocco	27.90718	-11.35959	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1150	Morocco	28.00888	-11.09276	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1152	Morocco	27.86179	-11.30698	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1153	Morocco	27.88425	-11.35876	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1154	Morocco	27.87679	-11.35939	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1156	Morocco	28.17717	-10.77012	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1172	Algeria	35.56667	1.25000	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1173	Algeria	35.56667	1.25000	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1174	Algeria	35.56667	1.25000	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1175	Algeria	35.56667	1.25000	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1176	Algeria	36.72778	3.55389	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1177	Algeria	35.55000	6.16667	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1178	Algeria	35.55000	6.16667	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1180	Algeria	35.55000	6.16667	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1181	Morocco	27.89651	-11.34807	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1183	Morocco	27.88493	-11.35828	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1184	Morocco	27.87221	-11.36164	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1186	Morocco	28.00483	-11.09560	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1187	Morocco	28.00483	-11.09560	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1188	Morocco	27.89643	-11.34805	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1189	Morocco	27.88425	-11.35876	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1208	Morocco	33.13397	-2.74512	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1209	Morocco	33.13403	-2.74500	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL

NAG1210	Morocco	33.22425	-4.17561	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1211	Morocco	33.30034	-4.01678	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1212	Morocco	33.21046	-4.13708	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1213	Morocco	33.27820	-4.03141	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1214	Morocco	33.21088	-4.13853	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1216	Morocco	33.52081	-3.22739	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1217	Morocco	33.57252	-3.43520	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1218	Morocco	33.52074	-3.22759	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1219	Morocco	33.52300	-3.22862	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1221	Morocco	32.98059	-4.42940	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1222	Morocco	32.97946	-4.43025	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1223	Morocco	33.27820	-4.03141	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1224	Morocco	33.52017	-3.22738	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1226	Morocco	33.52758	-3.23199	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1227	Morocco	33.52808	-3.22915	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1228	Morocco	33.54006	-3.22560	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1229	Morocco	33.54051	-3.22650	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1230	Morocco	33.54122	-3.23062	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1231	Morocco	33.54134	-3.23018	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1232	Morocco	33.54035	-3.23023	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1233	Morocco	32.97946	-4.43025	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1234	Morocco	32.97951	-4.43026	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1235	Morocco	33.15688	-3.22409	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1236	Morocco	33.15607	-3.22420	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1237	Morocco	33.27745	-4.02551	Faeces	Wild	<i>G. cuvieri</i>	Mod	
NAG1238	Morocco	33.27820	-4.03141	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1240	Morocco	33.29820	-4.01578	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1241	Morocco	33.29758	-4.01475	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1242	Morocco	33.21572	-4.14008	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1243	Morocco	33.52283	-3.22275	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1244	Morocco	33.52269	-3.22268	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1245	Morocco	33.51974	-3.22610	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1246	Morocco	33.27585	-4.02442	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1247	Morocco	33.27853	-4.02649	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1248	Morocco	33.27678	-4.02787	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1249	Morocco	33.27973	-4.03059	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net	#KCAS_GCGL
NAG1250	Morocco	33.57543	-3.43100	Faeces	Wild	<i>G. cuvieri</i>	Mod	

NAG1251	Morocco	33.57662	-3.42919	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1253	Morocco	33.52808	-3.22555	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1254	Morocco	33.52416	-3.22364	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1255	Morocco	33.52197	-3.22375	Faeces	Wild	<i>G. cuvieri</i>	Mod							
NAG1265	Morocco	33.42072	-3.80274	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1266	Morocco	33.42193	-3.80324	Faeces	Wild	<i>G. cuvieri</i>	Mod							
NAG1270	Morocco	33.42758	-3.79826	Faeces	Wild	<i>G. cuvieri</i>	Mod							
NAG1271	Morocco	33.41796	-3.78892	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1273	Morocco	33.19852	-3.01847	Faeces	Wild	<i>G. cuvieri</i>	Mod							
NAG1277	Morocco	33.02846	-4.51344	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1278	Morocco	32.57148	-2.44614	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1279	Morocco	32.57514	-2.45389	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1280	Morocco	32.57507	-2.45968	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1281	Morocco	32.57579	-2.46205	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1282	Morocco	32.57609	-2.46303	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1283	Morocco	32.58018	-2.46929	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
NAG1287	Morocco	33.09225	-2.73625	Faeces	Wild	<i>G. cuvieri</i>	Mod+Net		#KCAS_GCGL					
JN410347	Egypt	xxx	xxx	xxx	xxx	<i>G. leptoceros</i>	Net+Tree	#cytb13	xxx	xxx	xxx	xxx	xxx	xxx
NAG104	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD					
NAG105	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD		#PRKC_GD1			#TH_GD1
NAG107	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD	#LAC_GD1				
NAG108	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD2	#KCAS_GD	#LAC_GD1		#SPTBN_GD1		
NAG109	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD2	#KCAS_GD	#LAC_GD1	#PRKC_GD1	#SPTBN_GD2		
NAG110	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD		#PRKC_GD1			
NAG111	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD	#LAC_GD1	#PRKC_GD1			#TH_GD2
NAG112	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD	#LAC_GD2	#PRKC_GD1			#TH_GD2
NAG113	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD	#LAC_GD1	#PRKC_GD1	#SPTBN_GD3		#TH_GD2
NAG114	Spain	36.83806	-2.46083	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD1	#KCAS_GD	#LAC_GD1	#PRKC_GD1			#TH_GD2
NAG891	Germany	52.37104	9.73201	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD3	#KCAS_GD		#PRKC_GD2			#TH_GD2
NAG954	Algeria	36.74722	-3.00944	Tissue	Captive	<i>G. dorcas</i>	Tree	#cytb_GD3	#KCAS_GD	#LAC_GD3	#PRKC_GD3			#TH_GD2
NAG10	Senegal	14.33333	-12.50000	Hair	Wild	<i>E. rufifrons</i>	Tree	#cytb_ER1	#KCAS_ER	#LAC_ER	#LAC_ER_ND			#TH_ER1
NAG102	Senegal	16.39993	-16.24652	Faeces	Wild	<i>E. rufifrons</i>	Tree	#cytb_ER2	#KCAS_ER		#LAC_ER_ND			
NAG936	Senegal	15.48556	-14.09472	Tissue	SemiCaptive	<i>E. rufifrons</i>	Tree	#cytb_ER3	#KCAS_ER	#LAC_ER	#LAC_ER_ND			#TH_ER2
NAG939	Senegal	15.48556	-14.09472	Tissue	SemiCaptive	<i>E. rufifrons</i>	Tree	#cytb_ER3	#KCAS_ER	#LAC_ER	#LAC_ER_ND			#TH_ER2
NAG710	Spain	36.83806	-2.46083	Tissue	Captive	<i>N. dama</i>	Tree	#cytb_ND1	#KCAS_ND	#LAC_ND	#LAC_ER_ND	#SPTBN_ND		#TH_ND1
NAG711	Spain	36.83806	-2.46083	Tissue	Captive	<i>N. dama</i>	Tree	#cytb_ND2	#KCAS_ND	#LAC_ND	#LAC_ER_ND	#SPTBN_ND		#TH_ND2
NAG712	Spain	36.83806	-2.46083	Tissue	Captive	<i>N. dama</i>	Tree	#cytb_ND1	#KCAS_ND	#LAC_ND	#LAC_ER_ND	#SPTBN_ND		

Table 4S.2. List of PCR primer pairs used in this study and approximate length of PCR products (PCR). Ta: optimal annealing temperature; bp: base pairs.

Marker	Primer	Primer sequence	Ta	PCR	Reference
CYTB	L14724	TGACTAATGATAGAAAAACCATCGTTG	56	450 bp	[1]
	H15149	TAACTGTTGCTCCTCAAAAAGATATTTGTCCTCA			[2]
KCAS	Kcasein_D	CTAACTGCAACTGGCTTTGCATA	56	367 bp	[3]
	Kcasein_E	GTGGAAGGAAGATGTACAAATC			
LAC	LacA	ATCTGTAACATCTCCTGTGA	59-51	423 bp	[4]
	LabB	TCAGTAAGRTCATCATCCAG			
Th	ThNAG_Fw	GCATGTGGGCARRCAATGTC	61-53	590 bp	[3]
	ThNAG_Rw	ATGGCYTCATGTATRCAGTCA			
SPTBN	SPTBN1_C	GAAGACCTGTTACAGAAGCA	61-50	785 bp	[3]
	SPTBN1_D	TCTGCTGCCAACTGGCAAAGC			
PRKC	PRKC1_C	AGTTATGCTAAAGTACTGTTG	60-52	464 bp	[3]
	PRKC1_D	GGACGCCTGTTCAAAGACATG			

The highest annealing temperature for all fragments, without compromising amplification yield, was selected to reduce unwanted PCR products and maximize specificity, although lower temperatures may be used to facilitate amplification of poorer quality samples (3 °C decrease in Ta indicated above; e.g. bones). [1]: Irwin *et al.* (1991) modified by Lerp *et al.* (2011); [2]: Kocher *et al.* (1989); [3]: Matthee *et al.* (2001); [4]: Hassanin & Douzery (2003).

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Table 4S.3. GenBank sequences for CYTB genes from *G. cuvieri* and *G. leptoceros* included in phylogenetic reconstruction (Figure 4S.3).

Accession Number	Taxa	Sequence length (bp)	Geographic Source	Reference
AF030609	<i>Gazella cuvieri</i>	300		Rebholz & Harley, 1999
HQ316154	<i>Gazella cuvieri</i>	333	Saudi Arabia	Wacher <i>et al.</i> 2010
HQ316155	<i>Gazella cuvieri</i>	333	Saudi Arabia	Wacher <i>et al.</i> 2010
JN410342	<i>Gazella cuvieri</i>	1084	Almeria (Spain)	Lerp <i>et al.</i> 2011
JN410343	<i>Gazella cuvieri</i>	1095	Almeria (Spain)	Lerp <i>et al.</i> 2011
JN632636	<i>Gazella cuvieri</i>	1132		Hassanin <i>et al.</i> 2012
NC_020704	<i>Gazella cuvieri</i>	1140		Hassanin <i>et al.</i> 2012
KM582095	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582096	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582110	<i>Gazella cuvieri</i> x <i>leptoceros</i>	435	North West Africa	Silva <i>et al.</i> 2015
KM582111	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582112	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582113	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582114	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582097	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582098	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582099	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582100	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582101	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582102	<i>Gazella cuvieri</i> x <i>leptoceros</i>	450	North West Africa	Silva <i>et al.</i> 2015
KM582103	<i>Gazella cuvieri</i> x <i>leptoceros</i>	374	North West Africa	Silva <i>et al.</i> 2015
KM582104	<i>Gazella cuvieri</i> x <i>leptoceros</i>	333	North West Africa	Silva <i>et al.</i> 2015
KM582105	<i>Gazella cuvieri</i> x <i>leptoceros</i>	333	North West Africa	Silva <i>et al.</i> 2015
KM582106	<i>Gazella cuvieri</i> x <i>leptoceros</i>	300	North West Africa	Silva <i>et al.</i> 2015
KM582107	<i>Gazella cuvieri</i> x <i>leptoceros</i>	385	North West Africa	Silva <i>et al.</i> 2015
KM582108	<i>Gazella cuvieri</i> x <i>leptoceros</i>	389	North West Africa	Silva <i>et al.</i> 2015
KM582109	<i>Gazella cuvieri</i> x <i>leptoceros</i>	432	North West Africa	Silva <i>et al.</i> 2015
AF030610	<i>Gazella leptoceros</i>	300		Rebholz & Harley, 1999
AF187699	<i>Gazella leptoceros</i>	375		Hammond <i>et al.</i> 2001
HQ316152	<i>Gazella leptoceros</i>	333	Saudi Arabia	Wacher <i>et al.</i> 2010
HQ316153	<i>Gazella leptoceros</i>	333	Saudi Arabia	Wacher <i>et al.</i> 2010
JF728767	<i>Gazella leptoceros</i>	1140	Hannover Zoo (Germany)	Schikora <i>et al.</i> in prep
JN410259	<i>Gazella leptoceros</i>	1152	Algeria	Lerp <i>et al.</i> 2011
JN410344	<i>Gazella leptoceros</i>	1013	Tunisia	Lerp <i>et al.</i> 2011
JN410345	<i>Gazella leptoceros</i>	1025	Tunisia	Lerp <i>et al.</i> 2011
JN410346	<i>Gazella leptoceros</i>	1104	Egypt	Lerp <i>et al.</i> 2011
JN410347	<i>Gazella leptoceros</i>	1118	Egypt	Lerp <i>et al.</i> 2011
JN632641	<i>Gazella leptoceros</i>	1200	San Diego Zoo (USA)	Hassanin <i>et al.</i> 2012
NC_020708	<i>Gazella leptoceros</i>	1140		Hassanin <i>et al.</i> 2012

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Table 4S.4. Description, range (minimum and maximum), and units of the topoclimatic and habitat variables used to characterize the environmental variability of the study area and for modelling the distribution of each ecotype (mountain and lowland).

Topoclimatic	Units	Range
Annual average temperature	° C	6.2 - 30.7
Maximum temperature of warmest month	° C	23.7 - 48.9
Minimum temperature of coldest month	° C	-9.7 - 16.4
Annual average total precipitation	mm	1 - 1376
Annual average total precipitation of driest month	mm	0 - 18
Potential evapo-transpiration annual range	mm	50 - 217
Slope	%	0 - 45
Habitat (distance to)		
Mosaic cropland (50-70%) /vegetation (20-50%)	°	0 - 6.67
Mosaic vegetation (50-70%) / cropland (20-50%)	°	0 - 7.02
Sparse (<15%) vegetation or grassland	°	0 - 4.09
Bare areas	°	0 - 1.30
Consolidated bare areas (rocky desert)	°	0 - 2.09
Non-consolidated bare areas (sandy desert)	°	0 - 4.76

Table 4S.5. Number of observations of each ecotype (mountain and lowland) that were used to train and test (N train - test) the 10 replicate ecological models, and the respective average and standard deviation (SD) of training and test AUC of replicates.

	N train - test	Train AUC (SD)	Test AUC (SD)
Mountain	38 – 4	0.98 (0.01)	0.98 (0.02)
Lowland	18 – 1	0.99 (0.01)	0.99 (0.02)

Table 4S.6. Variable positions found for the mtDNA cytochrome b gene and for five concatenated nDNA genes, SPTBN, KCAS, LAC, PRKC, and TH. Informative positions are coloured according to the species name and with Figure2: brown for *Gazella cuvieri*, yellow for *G. leptoceros loderi* (orange for shared positions between this taxa), blue for *G. dorcas*, green for *N. dama* and purple for *Eudorcas rufifrons*. The sequences used are indicated in Table 4S.1. NAG113 (*G. Dorcas*) is presented for reference.

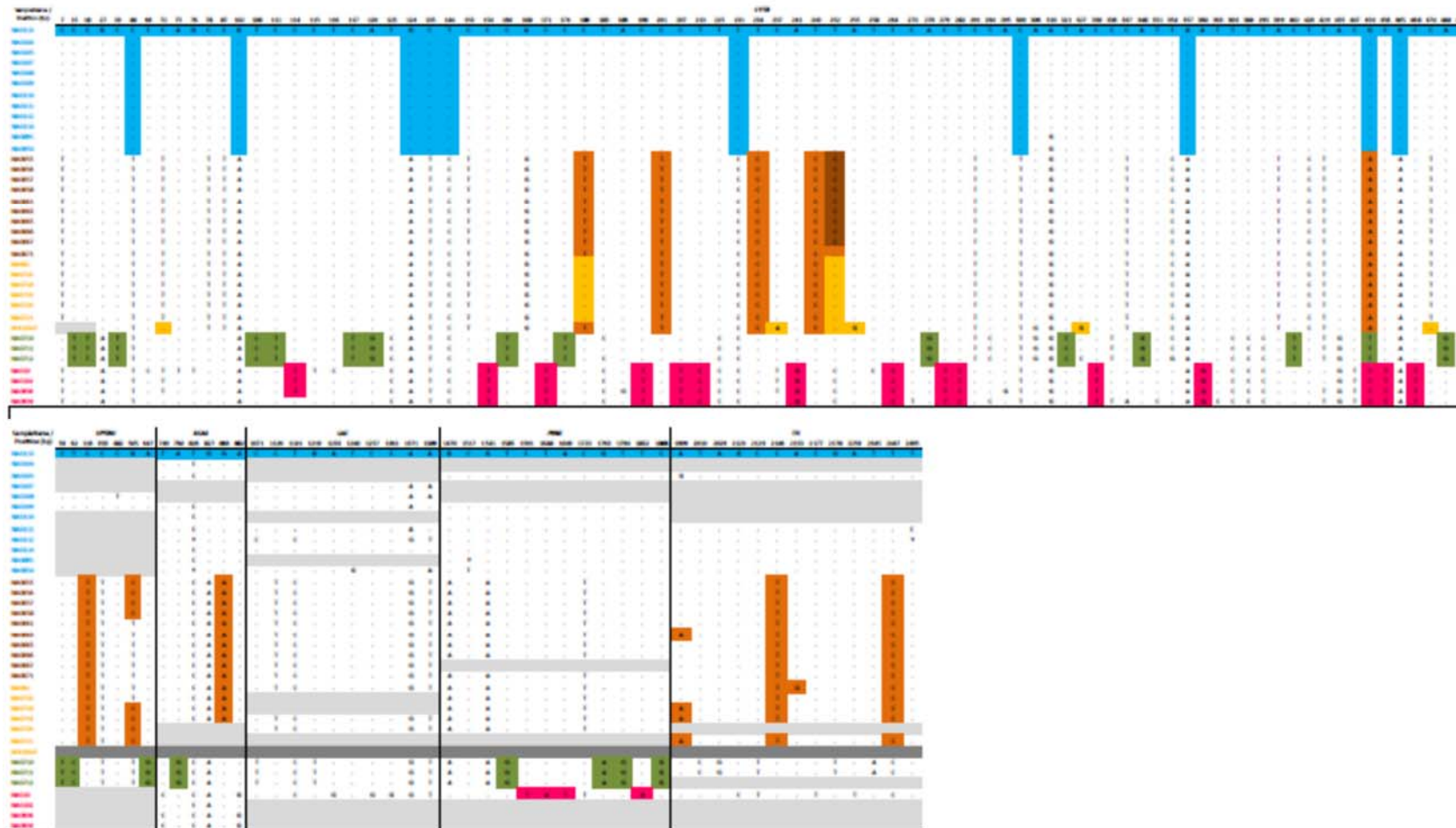


Table 4S.7. Eigenvalues (percentage of explanation) of each principal component and loading scores of the environmental variable derived from a Spatial Principal Components Analyses built with topoclimatic (PCAtc) and habitat (PCAha) variables, independently.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
PCAtc	2.9 (52.6)	1.3 (23.1)	0.6 (10.4)	0.4 (7.8)	0.2 (4.4)	0.1 (1.2)	0.0 (0.06)
Precipitation	0.4	-0.2	0.2	0.4	0.7	-0.2	0.0
Temperature	-0.5	-0.1	0.3	0.2	-0.1	-0.3	0.7
Potential evapo-transpiration	0.1	0.8	0.1	0.1	0.2	0.5	0.3
Precipitation of driest month	0.4	0.0	-0.0	0.7	-0.6	0.0	0.1
Slope	0.3	-0.1	0.9	-0.4	-0.2	0.1	0.0
Maximum temperature	-0.4	0.4	0.4	0.3	-0.0	-0.3	-0.6
Minimum temperature	-0.4	-0.5	0.2	0.3	0.1	0.7	-0.1
PCAha	2.0 (41.8)	1.4 (30.2)	0.6 (12.1)	0.4 (7.8)	0.2 (4.5)	0.2 (3.7)	
Mosaic cropland/vegetation	-0.6	0.1	0.2	0.2	-0.3	0.7	
Mosaic vegetation/cropland	-0.5	0.2	0.3	-0.8	0.2	-0.2	
Sparse vegetation or grassland	-0.5	-0.2	0.2	0.6	0.1	-0.6	
Bare areas	-0.1	-0.7	-0.2	-0.3	-0.7	-0.2	
Rocky desert	-0.0	-0.7	-0.1	-0.0	0.7	0.3	
Sandy desert	0.4	-0.2	0.9	-0.0	-0.1	-0.0	

Appendix 5S – Supplementary material of chapter 5

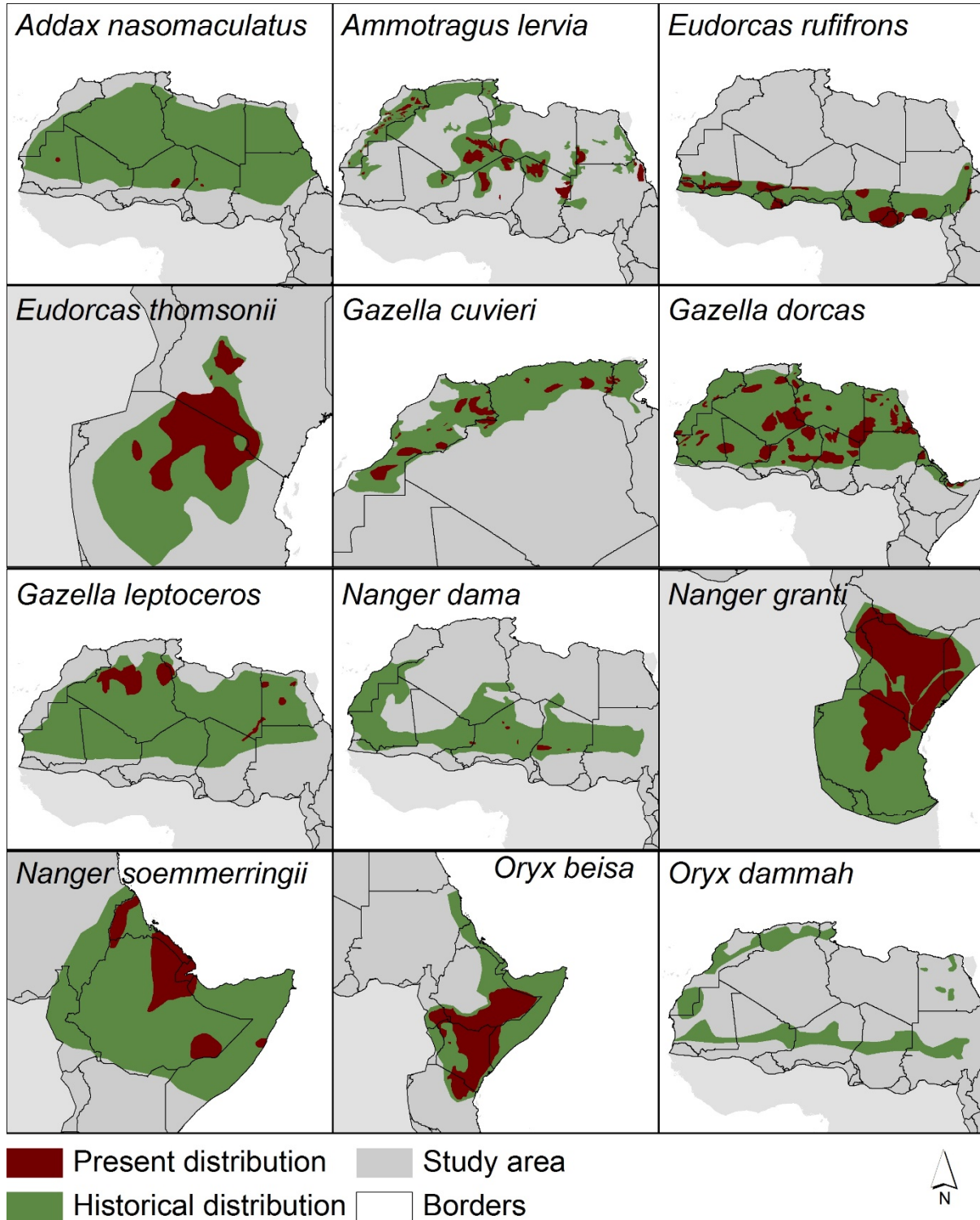


Figure 5S.1. Zooms on the historical and present distributions of the 12 African ungulates studied.

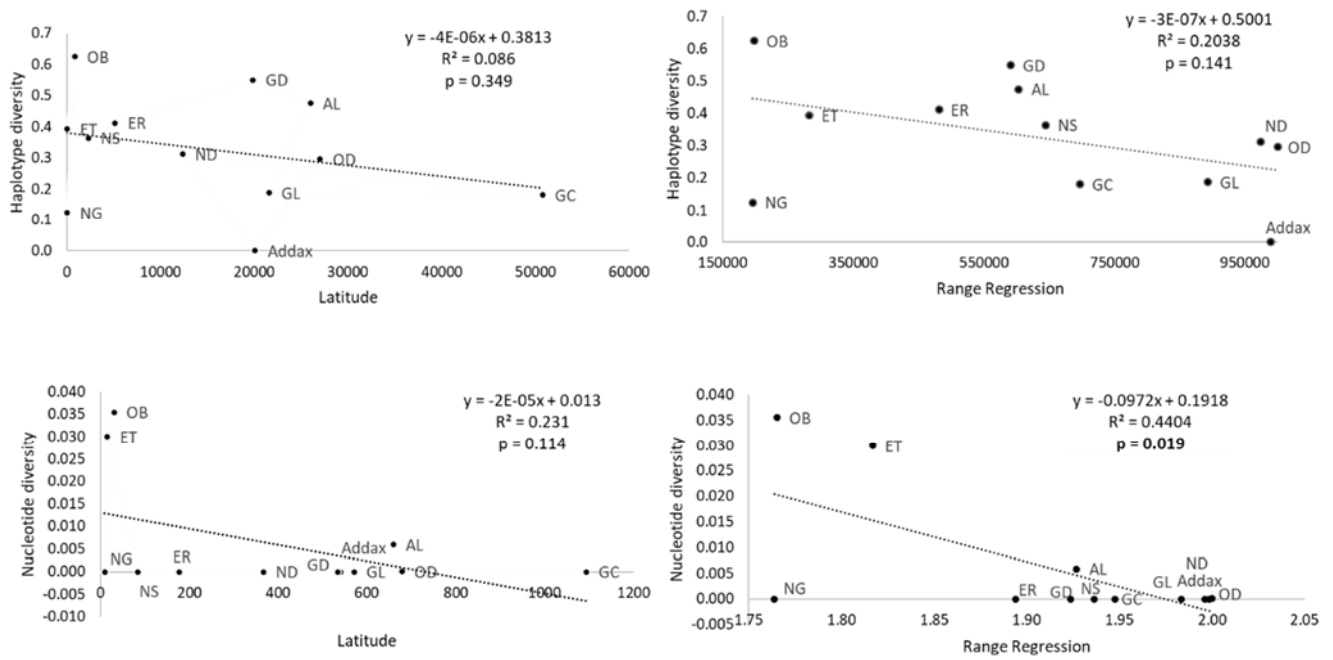
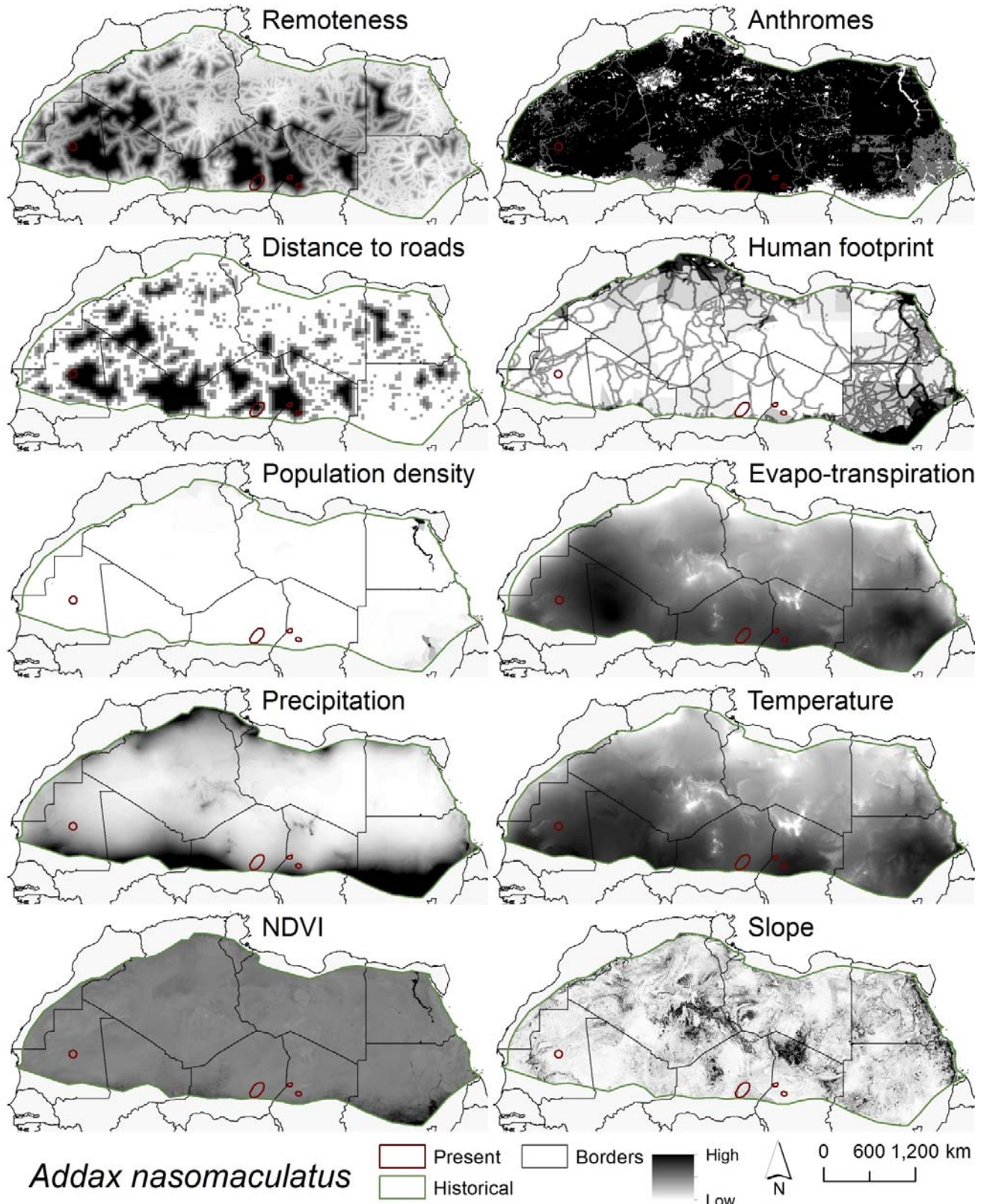
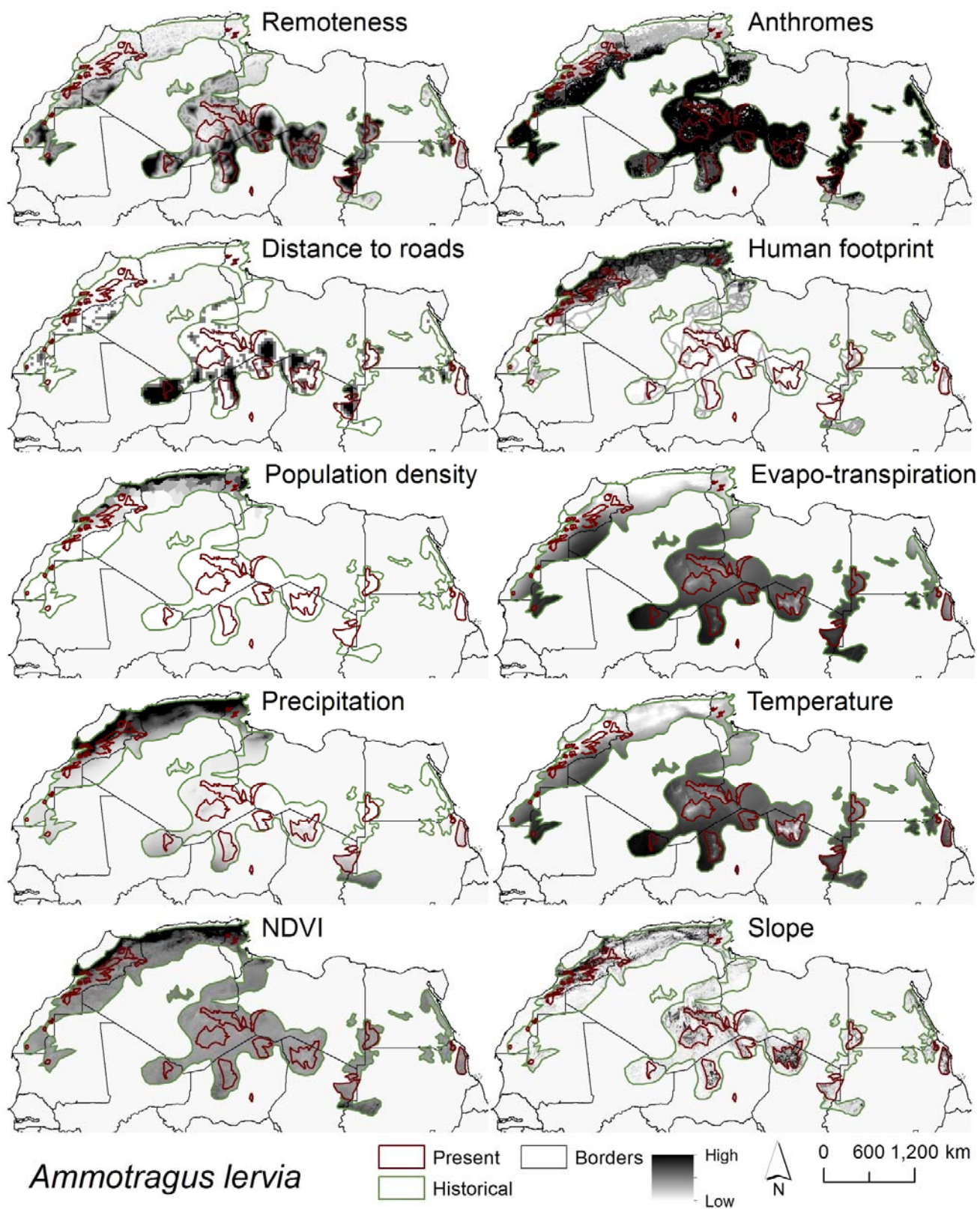
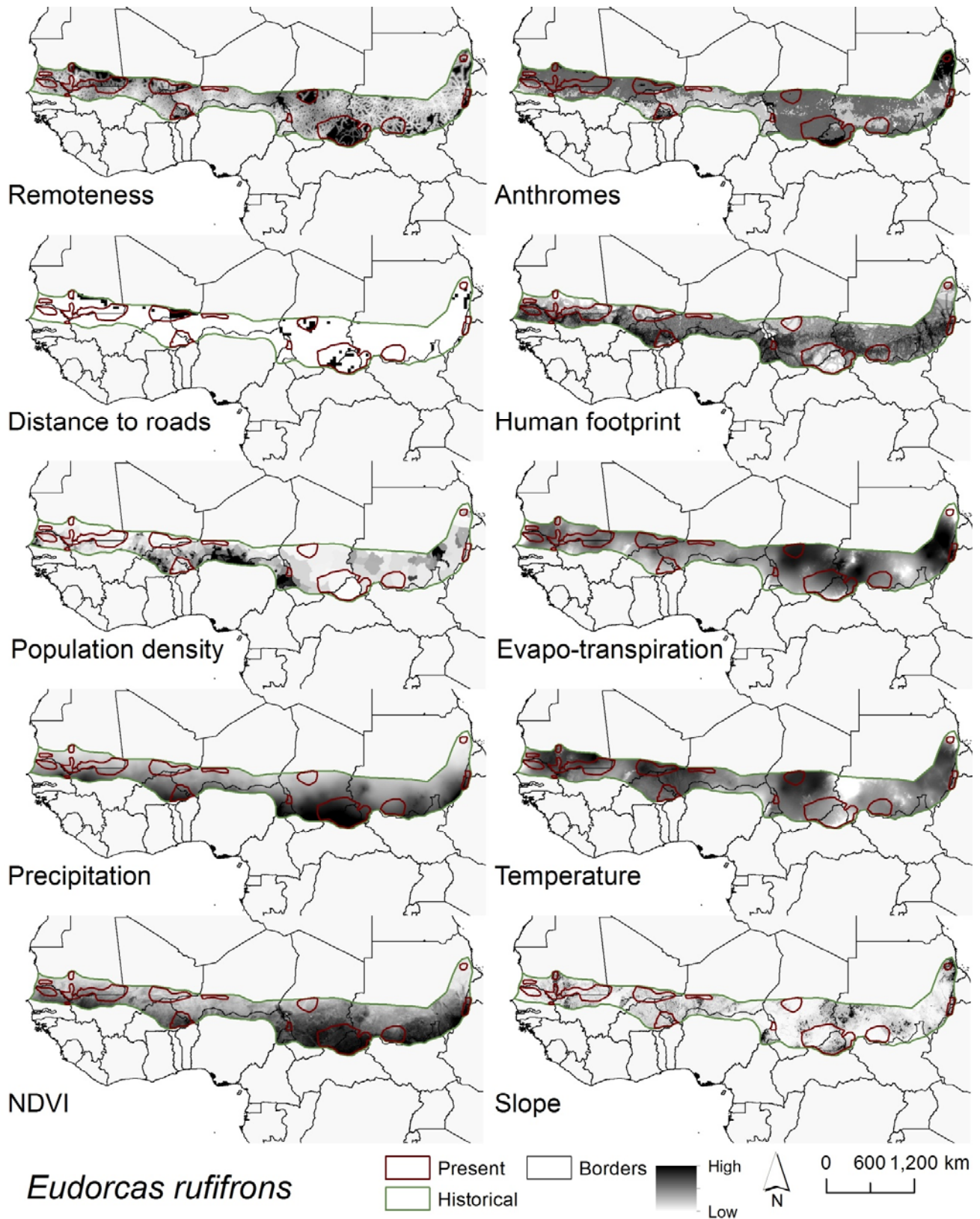


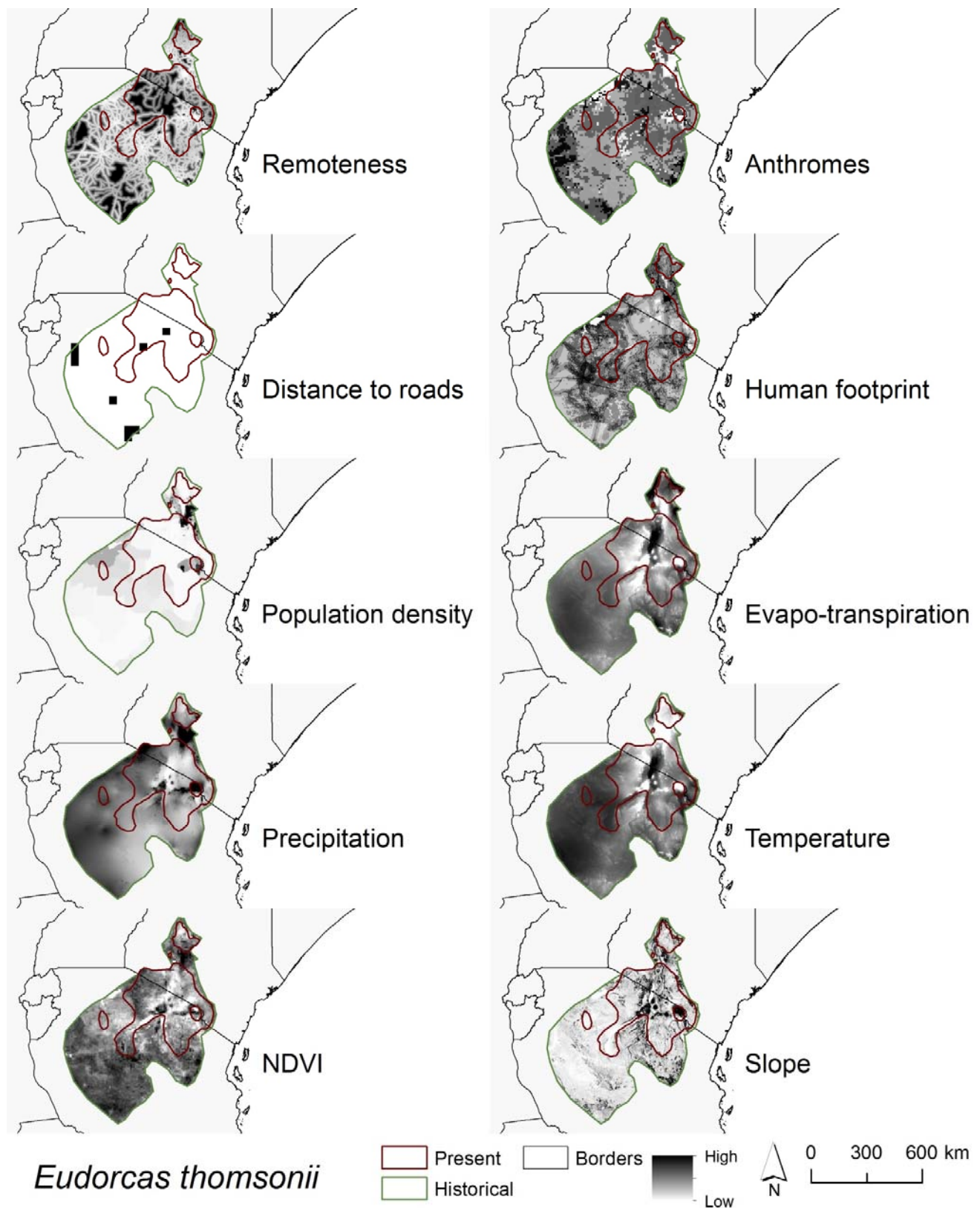
Figure 5S.2. Correlations between haplotype diversity (top row) and nucleotide diversity (bottom row) against latitude (left column) and range regression (right column) in 12 African ungulates. Significant correlations are represented in bold. Addax: *Addax nasomaculatus*, AL: *Ammotragus lervia*, ER: *Eudorcas rufifrons*, ET: *Eudorcas thomsonii*, GC: *Gazella cuvieri*, GD: *Gazella dorcas*, GL: *Gazella leptoceros*, ND: *Nanger dama*, NG: *Nanger granti*, NS: *Nanger soemmerringii*, OB: *Oryx beisa*.

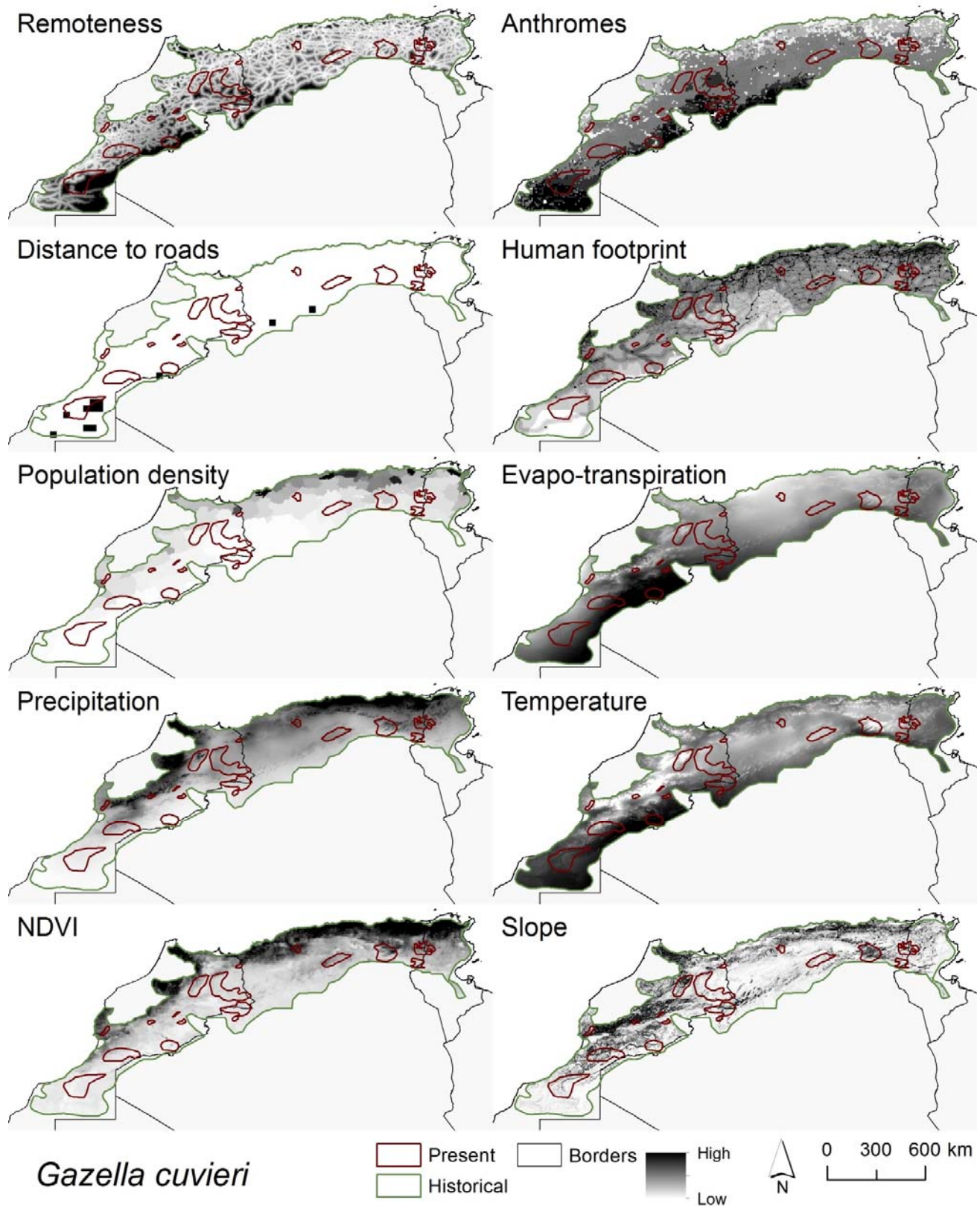
Figure 5S.3. Geographic variation of human pressure variables (remoteness, anthromes, distance to roads, human footprint, and population density) and environmental factors (evapo-transpiration, precipitation, temperature, NDVI, slope) within the historical and present distribution of 12 African ungulates: *Addax nasomaculatus*, *Ammotragus lervia*, *Gazella cuvieri*, *Gazella dorcas*, *Gazella leptoceros*, *Eudorcas rufifrons*, *Eudorcas thomsonii*, *Nanger dama*, *Nanger granti*, *Nanger soemmerringii*, *Oryx beisa*, and *Oryx dammah*.

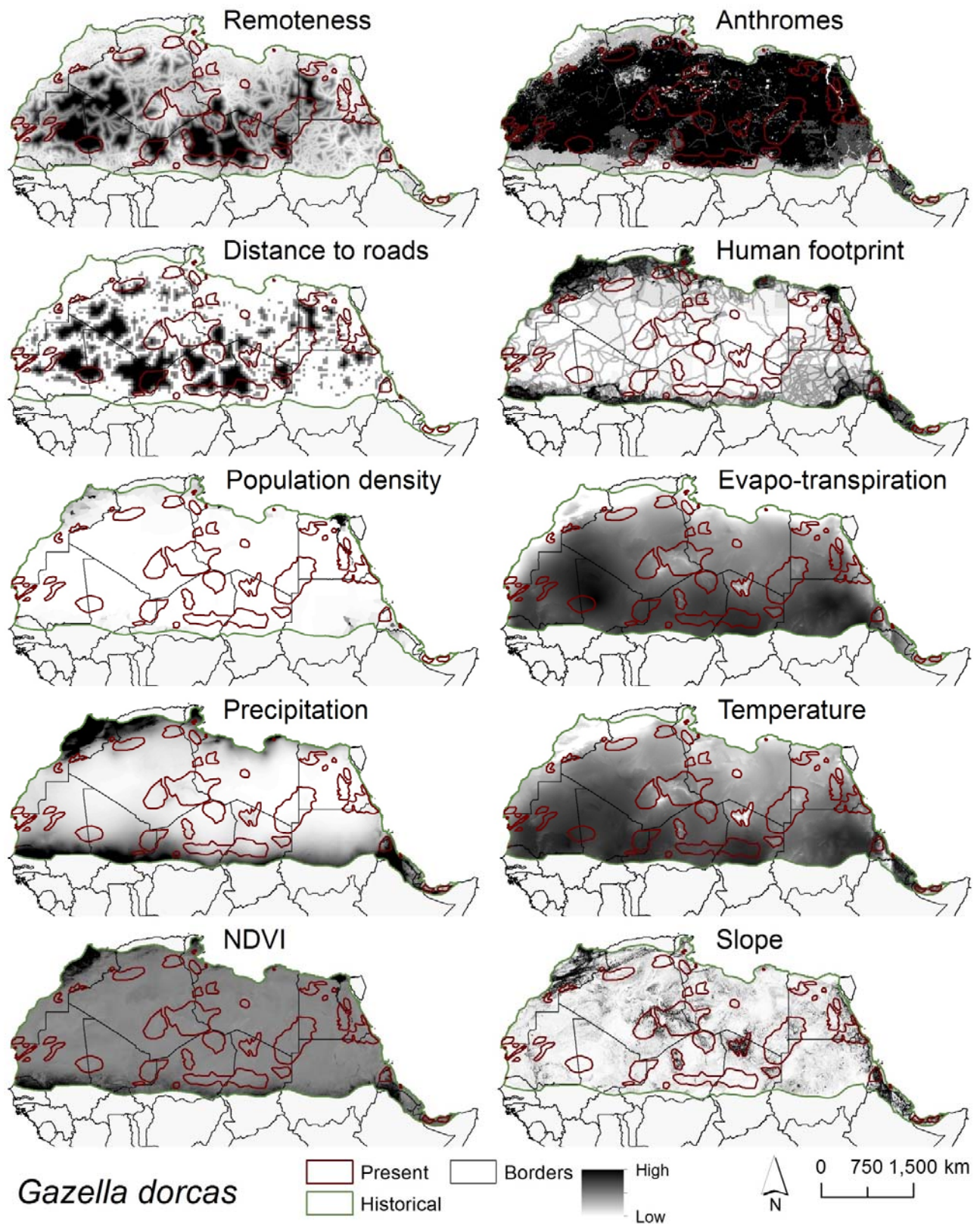


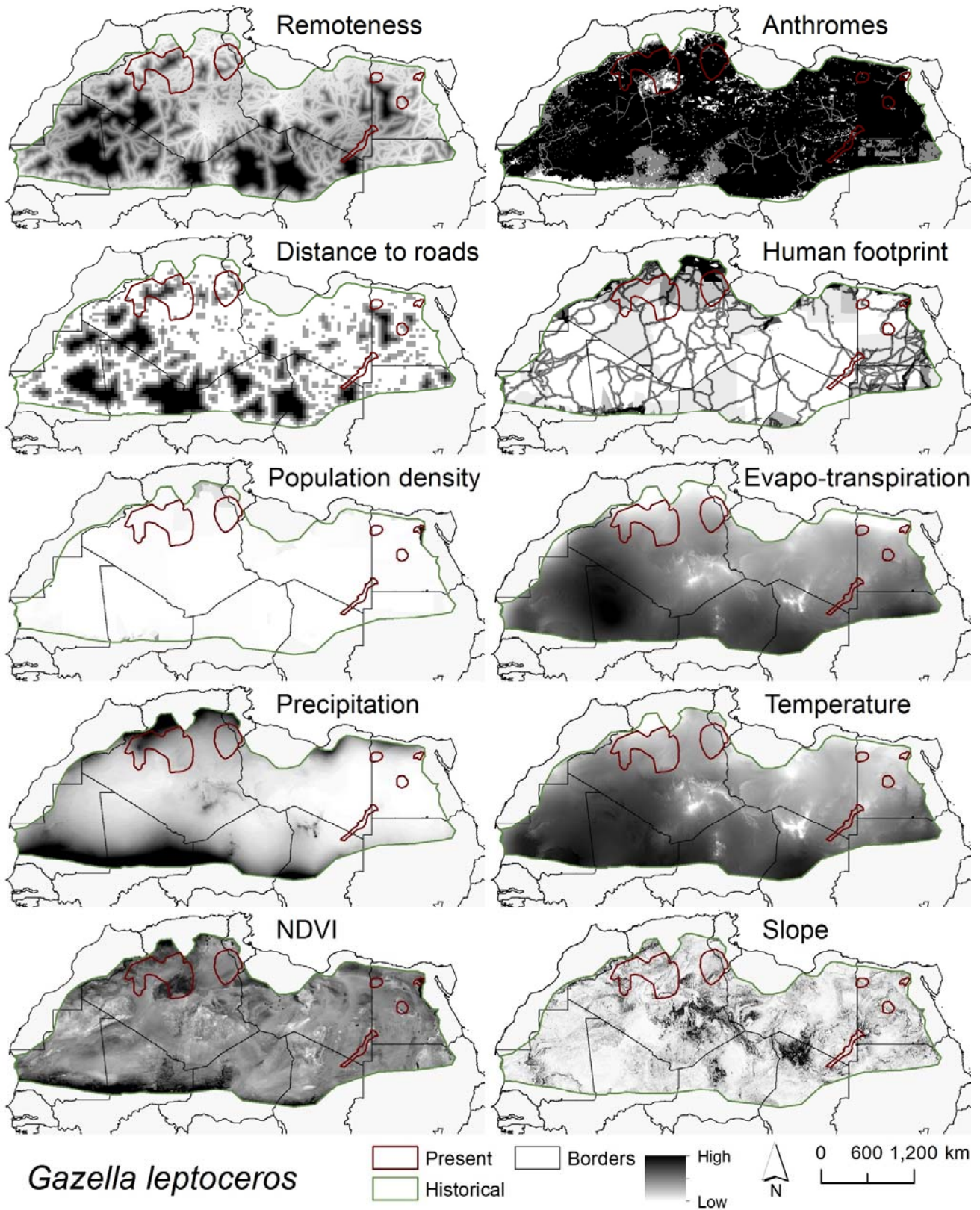


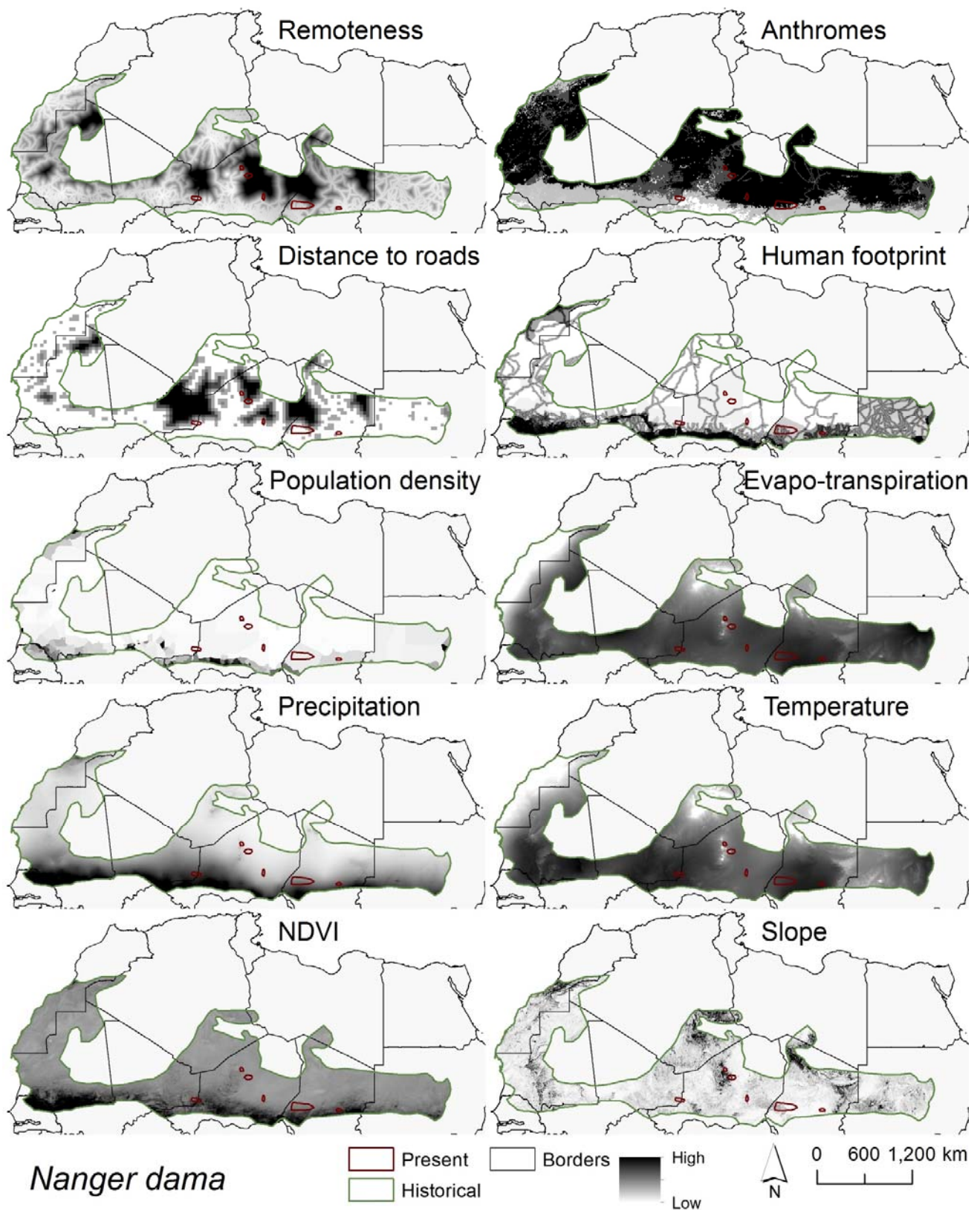


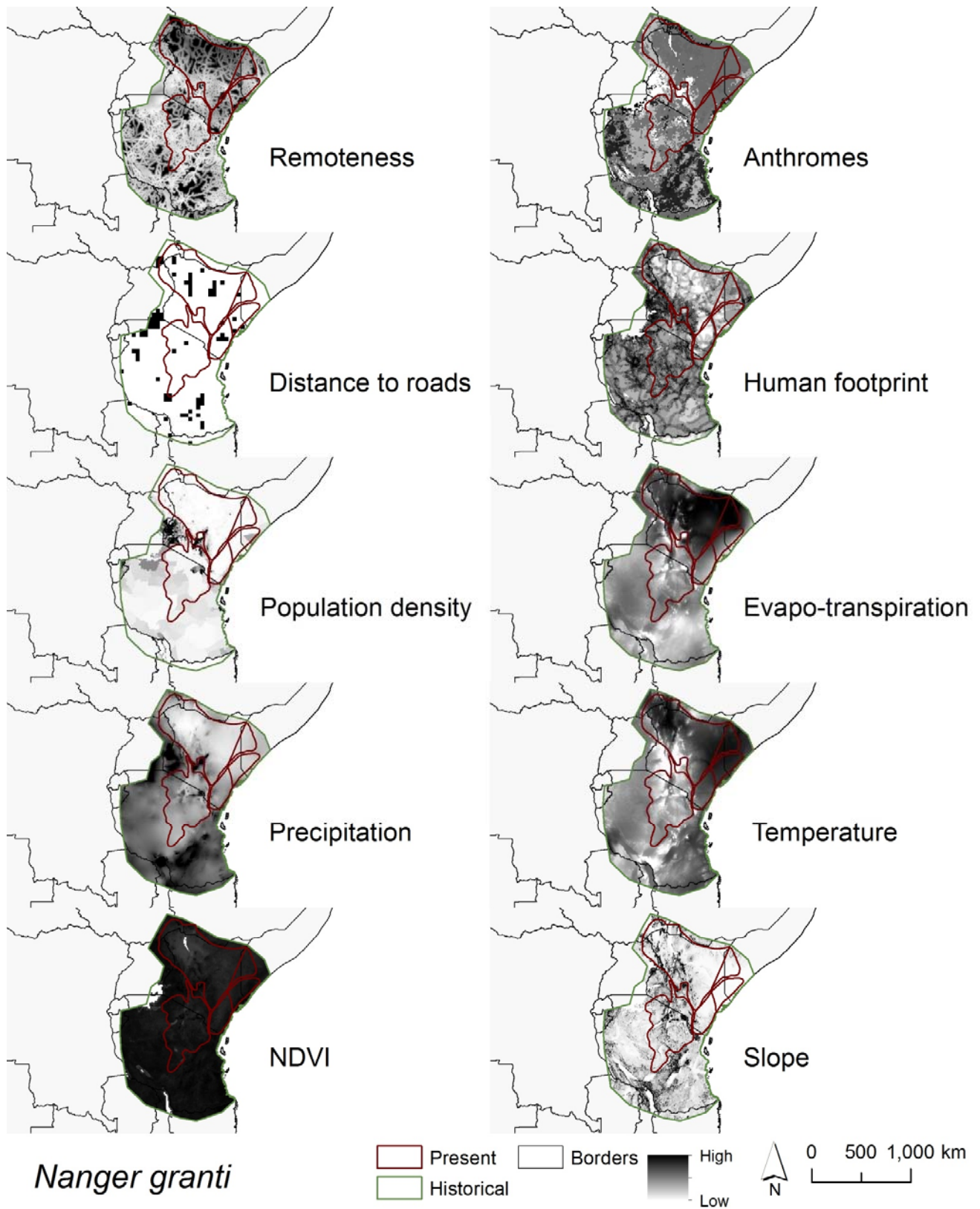


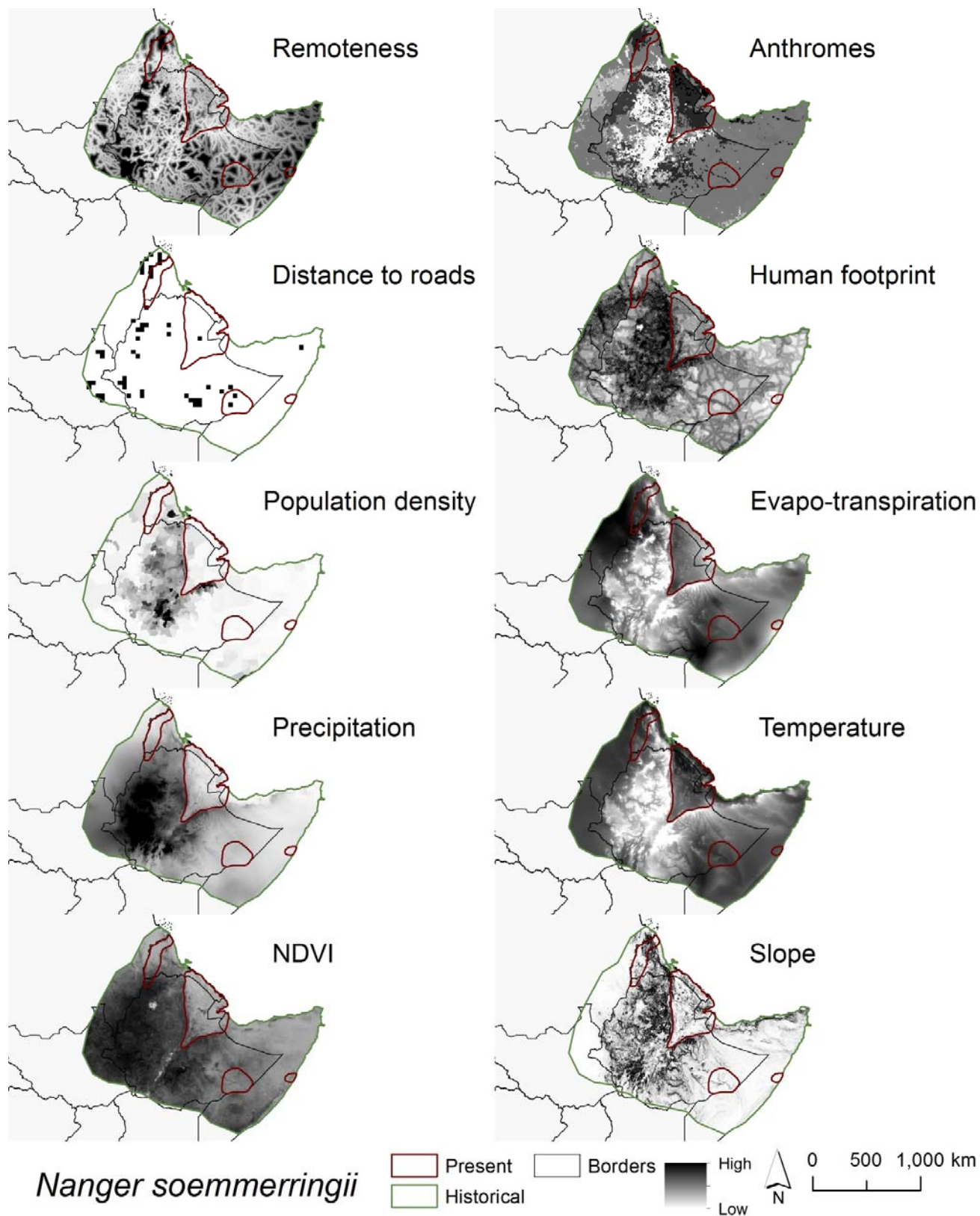


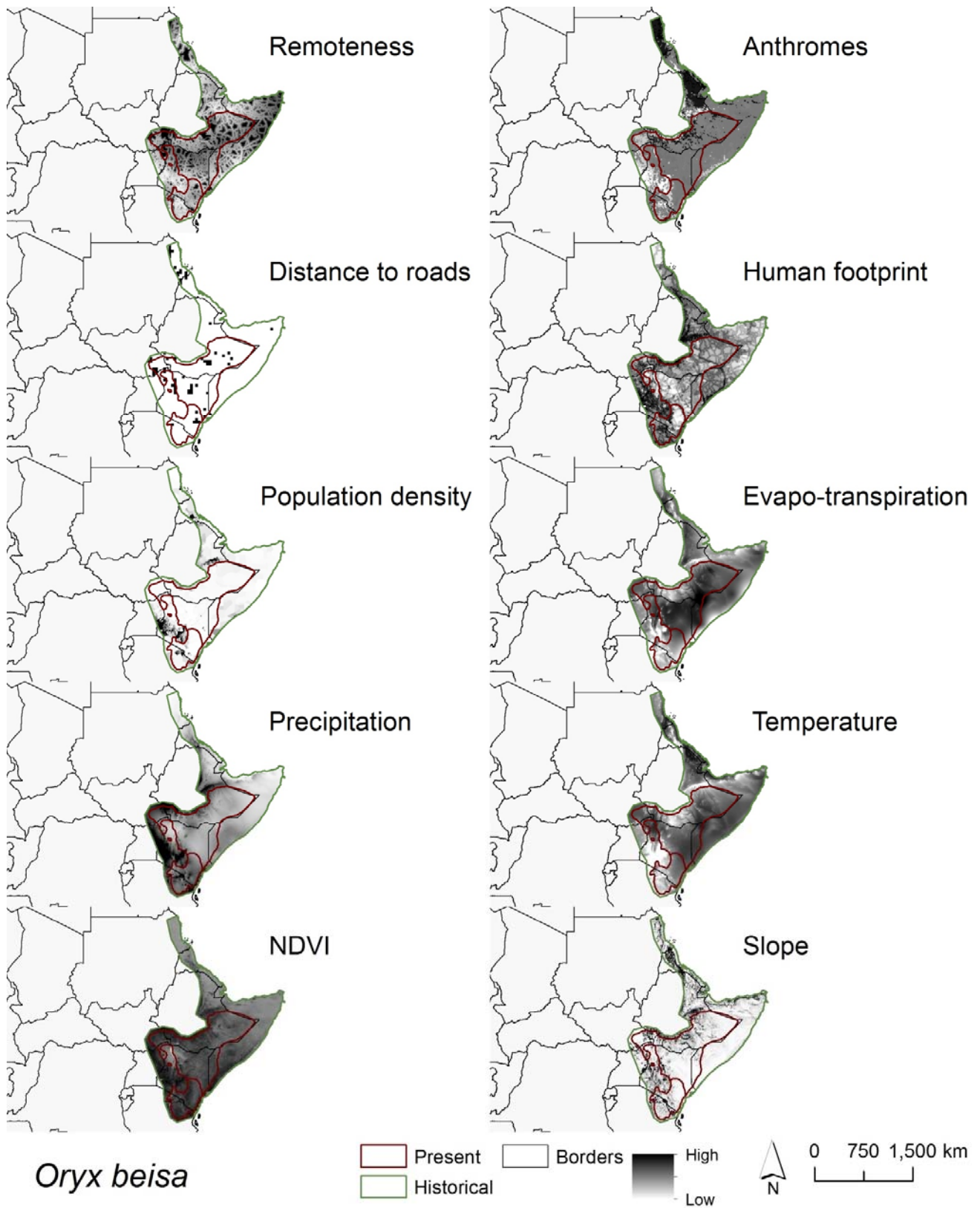












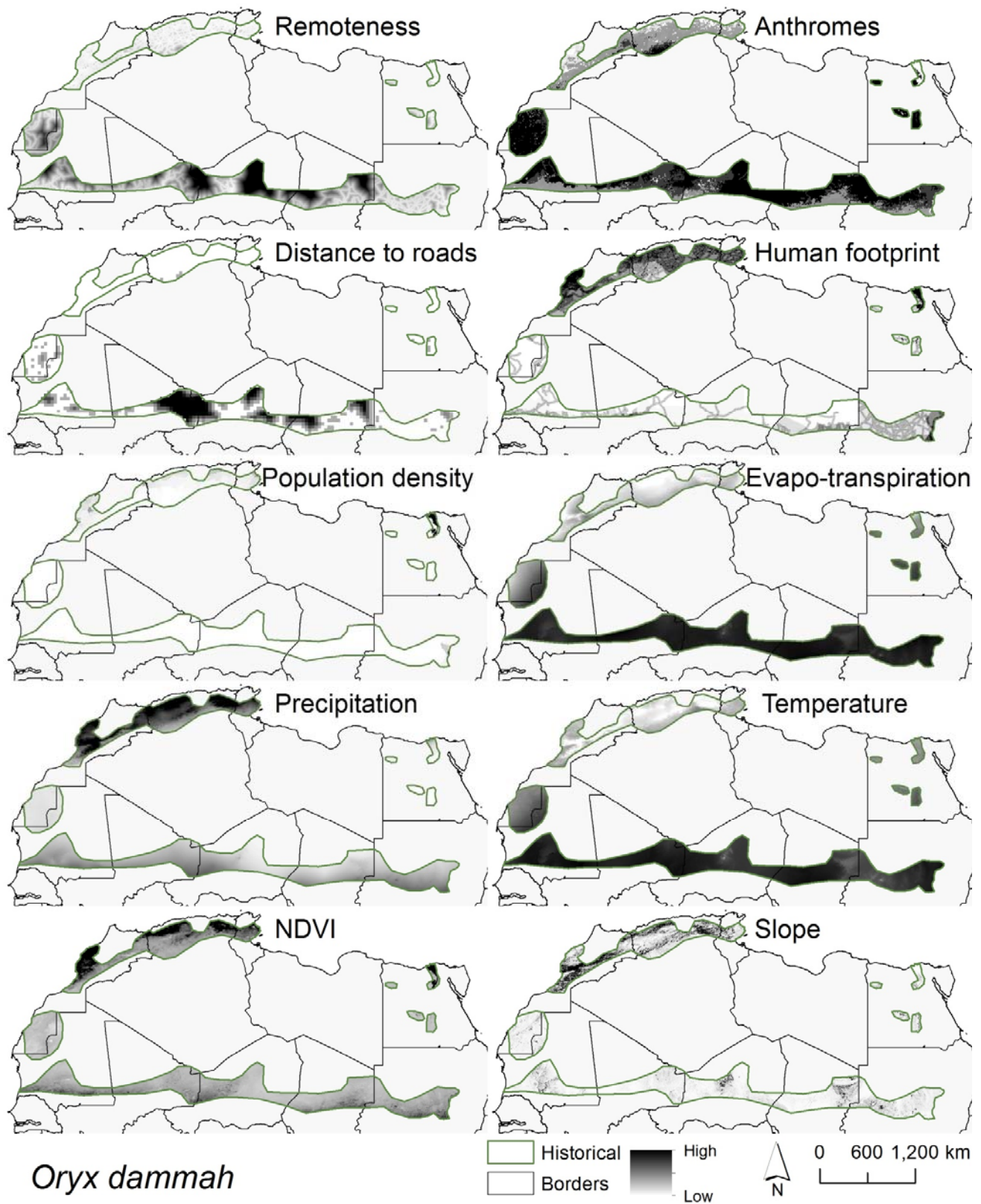


Table 5S.1. List of sequences retrieved from Genbank from 12 African ungulates and used in genetic analysis, including accession number, sequence length (base pairs), subset used to calculate genetic diversity (A: 926 bp, N=161; B: 309 bp, N=286; and C: 199 bp, N=378; see also Table 5S.2), and reference.

Accession	Species	Sequence length	Subset	Reference
JN632591	<i>Addax nasomaculatus</i>	16751	A, B, C	Hassanin et al., 2012
KM582122		450	B, C	Silva et al., 2015
NC_020674		16751	A, B, C	Hassanin et al., 2012
AF034731	<i>Ammotragus lervia</i>	1143	C	Hassanin et al., 1998
EF466060		16530	A, C	Mereu et al., 2007
EU878385		1073	C	Chaichoune et al., 2008
EU878386		1085	C	Chaichoune et al., 2008
FJ207522		16540	A, C	Hassanin et al., 2009
FJ556568		1083	C	Tungsudjai et al., 2008
FJ556577		562	C	Tungsudjai et al., 2008
KM582123		450	C	Silva et al., 2015
KM582124		450	C	Silva et al., 2015
KM582125		450	C	Silva et al., 2015
KM582126		450	C	Silva et al., 2015
NC_009510		16530	A, C	Mereu et al., 2007
AF030606		300	C	Rebholz and Harley 1999
JF728764		1140	A, B, C	Schikora et al., 2011
JN632633		342	C	Hassanin et al., 2012
JN632634	<i>Eudorcas rufifrons</i>	16418	A, B, C	Hassanin et al., 2012
JN632634		343	C	Hassanin et al., 2012
KM582115		450	C	Silva et al., 2015
KM582116		450	C	Silva et al., 2015
KM582117		450	C	Silva et al., 2015
AY534346		441	C	Kimwele et al., 2004
DQ470793		440	C	Kimwele et al., 2004
DQ470794		440	C	Kimwele et al., 2004
DQ470795		440	C	Kimwele et al., 2004
FJ556559		1138	C	Tungsudjai et al., 2008
FJ785352		321	C	Kimwele et al., 2009
FJ785353		321	C	Kimwele et al., 2009
FJ785387		363	C	Kimwele et al., 2009
FJ785388		363	C	Kimwele et al., 2009
JF728765		1140	C	Schikora et al., 2011
AF030609	<i>Eudorcas thomsonii</i>	300	C	Rebholz and Harley 1999
HQ316154		333	C	Wacher et al., 2010
HQ316155		333	C	Wacher et al., 2010
JN410342		1084	A, B	Lerp et al., 2011
JN410343		1095	A, B	Lerp et al., 2011
JN632636		1132	A, B	Hassanin et al., 2012
NC_020704		16427	A, B	Hassanin et al., 2012
KM582095		450	C	Silva et al., 2015
	<i>Gazella cuvieri</i>			
	<i>Gazella cuvieri x leptoceros</i>			

KM582096	<i>Gazella cuvieri x leptoceros</i>	450	C	Silva et al., 2015
KM582097		450	C	Silva et al., 2015
KM582098		450	C	Silva et al., 2015
KM582099		450	C	Silva et al., 2015
KM582100		450	C	Silva et al., 2015
KM582101		450	C	Silva et al., 2015
KM582102		450	C	Silva et al., 2015
KM582103		374	C	Silva et al., 2015
KM582104		333	C	Silva et al., 2015
KM582105		333	C	Silva et al., 2015
KM582106		300	C	Silva et al., 2015
KM582107		385	C	Silva et al., 2015
KM582108		389	C	Silva et al., 2015
KM582109		432	C	Silva et al., 2015
KM582110		435	C	Silva et al., 2015
KM582111		450	C	Silva et al., 2015
KM582112		450	C	Silva et al., 2015
KM582113		450	C	Silva et al., 2015
KM582114		450	C	Silva et al., 2015
AF187694		375	C	Hammond et al., 2001
AF187695		375	C	Hammond et al., 2001
AF187704		375	C	Hammond et al., 2001
AF187705		375	C	Hammond et al., 2001
AF187708		375	C	Hammond et al., 2001
AF187709		375	C	Hammond et al., 2001
AF187719		375	C	Hammond et al., 2001
EU723704		375	C	Korrida et al., 2008
EU723705		375	C	Korrida et al., 2008
EU723706		375	C	Korrida et al., 2008
JF728768		1140	A, B, C	Schikora et al., 2011
JN410219		1152	A, B, C	Lerp et al., 2011
JN410220		1152	A, B, C	Lerp et al., 2011
JN410221		1152	A, B, C	Lerp et al., 2011
JN410222		1152	A, B, C	Lerp et al., 2011
JN410223		1152	A, B, C	Lerp et al., 2011
JN410224		1152	A, B, C	Lerp et al., 2011
JN410225		1152	A, B, C	Lerp et al., 2011
JN410226		1152	A, B, C	Lerp et al., 2011
JN410227		1151	A, B, C	Lerp et al., 2011
JN410228		1152	A, B, C	Lerp et al., 2011
JN410229		1152	A, B, C	Lerp et al., 2011
JN410230		1152	A, B, C	Lerp et al., 2011
JN410231		1152	A, B, C	Lerp et al., 2011
JN410232		1152	A, B, C	Lerp et al., 2011
JN410233		1152	A, B, C	Lerp et al., 2011
JN410234		1152	A, B, C	Lerp et al., 2011

JN410235	<i>Gazella dorcas</i>	1152	A, B, C	Lerp et al., 2011
JN410236		1152	A, B, C	Lerp et al., 2011
JN410237		1152	A, B, C	Lerp et al., 2011
JN410238		1152	A, B, C	Lerp et al., 2011
JN410239		1152	A, B, C	Lerp et al., 2011
JN410240		1152	A, B, C	Lerp et al., 2011
JN410241		1152	A, B, C	Lerp et al., 2011
JN410242		1152	A, B, C	Lerp et al., 2011
JN410243		1152	A, B, C	Lerp et al., 2011
JN410244		1152	A, B, C	Lerp et al., 2011
JN410245		1152	A, B, C	Lerp et al., 2011
JN410246		1152	A, B, C	Lerp et al., 2011
JN410247		1152	A, B, C	Lerp et al., 2011
JN410248		1152	A, B, C	Lerp et al., 2011
JN410249		1152	A, B, C	Lerp et al., 2011
JN410250		1152	A, B, C	Lerp et al., 2011
JN410251		1152	A, B, C	Lerp et al., 2011
JN410252		1152	A, B, C	Lerp et al., 2011
JN410253		1152	A, B, C	Lerp et al., 2011
JN410254		1152	A, B, C	Lerp et al., 2011
JN410255		1152	A, B, C	Lerp et al., 2011
JN410256		1152	A, B, C	Lerp et al., 2011
JN410257		1152	A, B, C	Lerp et al., 2011
JN410258		1152	A, B, C	Lerp et al., 2011
JN410314		810	B	Lerp et al., 2011
JN410315		1106	A, B, C	Lerp et al., 2011
JN410316		1095	A, B, C	Lerp et al., 2011
JN410317		314	C	Lerp et al., 2011
JN410318		1130	A, B, C	Lerp et al., 2011
JN410319		1130	A, B, C	Lerp et al., 2011
JN410320		403	C	Lerp et al., 2011
JN410321		403	C	Lerp et al., 2011
JN410322		403	C	Lerp et al., 2011
JN410325		822	B	Lerp et al., 2011
JN410326		821	B	Lerp et al., 2011
JN410327		401	C	Lerp et al., 2011
JN410328		408	C	Lerp et al., 2011
JN410329		403	C	Lerp et al., 2011
JN410330		406	C	Lerp et al., 2011
JN410331		357	C	Lerp et al., 2011
JN410332		1092	A, B, C	Lerp et al., 2011
JN410333		1126	A, B, C	Lerp et al., 2011
JN410334		807	B	Lerp et al., 2011
JN410335		1132	A, B, C	Lerp et al., 2011
JN410336		983	A, B, C	Lerp et al., 2011
JN410337		1040	A, B, C	Lerp et al., 2011

JN410338	<i>Gazella dorcas</i>	1122	A, B, C	Lerp et al., 2011
JN410339		1058	A, B, C	Lerp et al., 2011
JN632637		1172	A, B, C	Hassanin et al., 2012
JN632638		1163	A, B, C	Hassanin et al., 2012
JQ676941		716	B	Godinho et al., 2012
JQ676942		716	B	Godinho et al., 2012
JQ676943		716	B	Godinho et al., 2012
JQ676944		716	B	Godinho et al., 2012
JQ676945		716	B	Godinho et al., 2012
JQ676946		716	B	Godinho et al., 2012
JQ676947		716	B	Godinho et al., 2012
JQ676948		716	B	Godinho et al., 2012
JQ676949		716	B	Godinho et al., 2012
JQ676950		716	B	Godinho et al., 2012
JQ676951		716	B	Godinho et al., 2012
JQ676952		716	B	Godinho et al., 2012
JX274672		336	C	Bärmann et al., 2013
JX274673		336	C	Bärmann et al., 2013
JX647822		336	C	Bärmann et al., 2013
KC188752		1132	A, B, C	Lerp et al., 2013
KM523351		790	B	Hadas et al., 2015
KM523353		790	B	Hadas et al., 2015
KM523354		790	B	Hadas et al., 2015
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KM523356		790	B	Hadas et al., 2015
KM523357		760	B	Hadas et al., 2015
KM523358		789	B	Hadas et al., 2015
KM523360		790	B	Hadas et al., 2015
KM523361		790	B	Hadas et al., 2015
KM523362		790	B	Hadas et al., 2015
KM523363		790	B	Hadas et al., 2015
KM523364		790	B	Hadas et al., 2015
KM523365		790	B	Hadas et al., 2015
KM523366		790	B	Hadas et al., 2015
KM523367		790	B	Hadas et al., 2015
KM523368		790	B	Hadas et al., 2015
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KM523371		790	B	Hadas et al., 2015
KM523372		790	B	Hadas et al., 2015
KM523373		790	B	Hadas et al., 2015
KM523374		760	B	Hadas et al., 2015
KM523375		790	B	Hadas et al., 2015
KM523376		783	B	Hadas et al., 2015
KM523377		790	B	Hadas et al., 2015
KM523378		790	B	Hadas et al., 2015

KM523379	<i>Gazella dorcas</i>	790	B	Hadas et al., 2015
KM523448		776	B	Hadas et al., 2015
KM582062		450	C	Silva et al., 2015
KM582063		450	C	Silva et al., 2015
KM582064		450	C	Silva et al., 2015
KM582065		450	C	Silva et al., 2015
KM582066		450	C	Silva et al., 2015
KM582067		450	C	Silva et al., 2015
KM582068		450	C	Silva et al., 2015
KM582069		450	C	Silva et al., 2015
KM582070		450	C	Silva et al., 2015
KM582071		450	C	Silva et al., 2015
KM582072		450	C	Silva et al., 2015
KM582073		450	C	Silva et al., 2015
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KM582076		450	C	Silva et al., 2015
KM582077		450	C	Silva et al., 2015
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KM582079		450	C	Silva et al., 2015
KM582080		450	C	Silva et al., 2015
KM582081		450	C	Silva et al., 2015
KM582082		450	C	Silva et al., 2015
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KM582084		450	C	Silva et al., 2015
KM582085		450	C	Silva et al., 2015
KM582086		450	C	Silva et al., 2015
KM582087		450	C	Silva et al., 2015
KM582088		450	C	Silva et al., 2015
KM582089		450	C	Silva et al., 2015
KM582090		450	C	Silva et al., 2015
KM582091		450	C	Silva et al., 2015
KM582092		450	C	Silva et al., 2015
KM582093		450	C	Silva et al., 2015
KM582094		450	C	Silva et al., 2015
NC_020705		16432	A, B, C	Hassanin et al., 2012
AF030610	<i>Gazella leptoceros</i>	300	C	Rebholz and Harley 1999
AF187699		375	C	Hammond et al., 2001
HQ316152		333	C	Wacher et al., 2010
HQ316153		333	C	Wacher et al., 2010
JF728767		1140	A, B, C	Schikora et al., 2011
JN410259		1152	A, B, C	Lerp et al., 2011
JN410344		1013	A, B, C	Lerp et al., 2011
JN410345		1025	A, B, C	Lerp et al., 2011
JN410346		1104	A, B, C	Lerp et al., 2011
JN410347		1118	A, B, C	Lerp et al., 2011

JN632641	<i>Gazella leptoceros</i>	1200	A, B, C	Hassanin et al., 2012
NC_020708		16439	A, B, C	Hassanin et al., 2012
AF025954	<i>Nanger dama</i>	1140	A, B, C	Matthee et al., 1999
AF030603		300	C	Rebholz and Harley 1999
AF030604		300	C	Rebholz and Harley 1999
HQ122592		517	B	Herrero et al., 2010
JF728775		1140	A, B, C	Schikora et al., 2011
JN632665		1221	A, B, C	Hassanin et al., 2012
KM582118		450	C	Silva et al., 2015
KM582119		450	C	Silva et al., 2015
KM582120		450	C	Silva et al., 2015
KM582121		450	C	Silva et al., 2015
NC_020724		1140	A, B, C	Hassanin et al., 2012
AY534343	<i>Nanger granti</i>	404	C	Kimwele et al., 2004
FJ785380		363	C	Kimwele et al., 2009
FJ785381		363	C	Kimwele et al., 2009
JN632666		16381	C	Hassanin et al., 2012
JF728776	<i>Nanger soemmerringii</i>	1140	A, B, C	Schikora et al., 2011
JN632667		16379	A, B, C	Hassanin et al., 2012
KC188777		1140	A, B, C	Lerp et al., 2013
NC_020726		16379	A, B, C	Hassanin et al., 2012
DQ138195	<i>Oryx beisa</i>	1136	A, C	Masembe et al., 2006
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DQ138199		1136	A, C	Masembe et al., 2006
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DQ138201		666	C	Masembe et al., 2006
DQ138202		666	C	Masembe et al., 2006
DQ138203		666	C	Masembe et al., 2006
DQ138204		666	C	Masembe et al., 2006
DQ138205		666	C	Masembe et al., 2006
DQ138206		666	C	Masembe et al., 2006
DQ138207		666	C	Masembe et al., 2006
DQ138208		666	C	Masembe et al., 2006
DQ138209		666	C	Masembe et al., 2006
DQ138210		666	C	Masembe et al., 2006
HM209249		666	C	Kimwele et al., 2009
HQ122598		517	C	Herrero et al., 2010
JN632676		16518	A, C	Hassanin et al., 2012
NC_020793		16518	A, C	Hassanin et al., 2012
JF728778	<i>Oryx dammah</i>	1140	A, B, C	Schikora et al., 2011
JN869311		16756	A, B, C	Zhang et al., 2012
NC_016421		16756	A, B, C	Zhang et al., 2012

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Table 5S.2. Measures of genetic diversity of 12 African ungulates: haplotype diversity (HDI) and nucleotide diversity (NDI). Measures calculated in three datasets: A) 926 bp, N=161; B) 309 bp, N=286; and C) 199 bp, N=378. Final values are the average of the three subsets.

	Subset A		Subset B		Subset C		Final values	
	HDI	NDI	HDI	NDI	HDI	NDI	HDI	NDI
<i>Addax nasomaculatus</i>	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
<i>Ammotragus lervia</i>	0.66700	0.03528	-	-	0.89400	0.32568	0.78050	0.18048
<i>Eudorcas rufifrons</i>	0.66670	0.00936	0.66670	0.00647	0.90000	0.02529	0.74447	0.01371
<i>Eudorcas thomsonii</i>	-	-	-	-	0.73300	0.31076	0.73300	0.31076
<i>Gazella cuvieri</i>	0.42860	0.00231	0.42860	0.00416	0.61540	0.00353	0.56558	0.00524
<i>Gazella dorcas</i>	0.92860	0.01057	0.88410	0.01238	0.64590	0.00466	0.81953	0.00920
<i>Gazella leptoceros</i>	0.73330	0.00684	0.23330	0.00604	0.53620	0.01143	0.57313	0.00882
<i>Nanger dama</i>	0.71430	0.00818	0.71110	0.00575	0.61050	0.00719	0.67863	0.00704
<i>Nanger granti</i>	-	-	-	-	0.50000	0.00503	0.50000	0.00503
<i>Nanger soemmerringii</i>	0.71430	0.00231	0.71430	0.00555	-	-	0.71430	0.00393
<i>Oryx beisa</i>	0.95200	0.01225	-	-	0.73300	0.31076	0.85500	0.32863
<i>Oryx dammah</i>	0.66670	0.04824	0.66670	0.04531	0.66670	0.03685	0.66670	0.04347

Table 5S.3. Pearson's correlation coefficients (below diagonal) and p values (above diagonal) between predictors of genetic diversity. Significant correlations ($p < 0.05$) are represented in bold. See Table 5.1 for variable codes.

	RRE	LAT	FEC	SM A	LON	BLE	BW E	HOR	GRS	RAS	SLO	SST	SSD	PET	PES T	PES D	ATE	AST	ASD	NDV I	NDS T	NDS D	PRE	PRS T	PRS D	HPD	HPS D	RDS	RSD	ANT	REM T	RES D	HFP	HSD
RRE	-	1.0	0.6	0.4	0.7	1.0	0.1	0.4	0.1	0.4	0.0	0.1	0.0	0.5	0.6	0.7	0.4	0.3	0.8	0.6	0.7	0.8	0.8	0.7	0.2	0.5	0.5	0.2	0.2	0.4	0.4	0.3	0.4	0.8
LAT	0.0	-	0.1	0.8	1.0	0.0	0.2	0.4	0.0	0.2	0.5	0.4	0.4	1.0	0.9	0.1	0.5	0.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.8	0.1	0.1	0.0	0.1	0.1	0.0	0.8
FEC	-0.2	0.5	-	0.5	0.5	0.1	0.4	0.5	0.6	0.9	0.5	0.9	0.8	0.7	0.9	0.9	0.1	0.2	0.7	0.4	0.1	0.5	0.4	0.2	0.3	0.7	0.6	0.8	0.9	0.6	0.9	1.0	0.8	0.7
SMA	-0.3	0.1	-0.2	-	0.0	0.3	0.6	0.0	0.6	0.4	0.6	0.4	0.4	0.4	0.5	0.1	1.0	0.8	0.1	0.8	0.8	0.9	0.8	0.9	0.8	0.6	0.0	0.7	0.7	0.9	0.9	0.7	0.9	0.4
LON	0.1	0.0	-0.2	0.8	-	0.1	0.1	0.0	0.7	0.7	1.0	0.7	0.7	0.4	0.5	0.1	0.7	0.9	0.0	0.8	0.9	0.5	1.0	0.9	0.7	0.6	0.0	0.6	0.5	1.0	0.7	0.6	0.9	0.5
BLE	0.0	-0.8	-0.5	0.4	0.5	-	0.0	0.1	0.0	0.5	0.9	0.9	0.7	0.3	0.3	0.2	0.2	0.3	0.7	0.0	0.2	0.0	0.0	0.1	0.0	0.5	0.7	0.2	0.2	0.0	0.3	0.2	0.1	0.9
BWE	0.5	-0.4	-0.3	0.2	0.6	0.6	-	0.0	0.6	0.7	0.8	0.9	0.9	0.4	0.4	0.9	0.6	0.6	0.8	0.5	0.7	0.0	0.2	0.5	0.4	0.5	0.3	0.8	0.8	0.4	0.7	0.8	0.6	0.8
HOR	0.3	-0.3	-0.2	0.6	0.9	0.5	0.8	-	0.4	0.4	0.6	0.3	0.4	0.4	0.6	0.1	0.8	0.6	0.1	0.7	0.7	0.1	0.3	0.5	0.4	0.2	0.0	1.0	0.9	0.6	0.8	1.0	0.6	0.4
GRS	-0.5	-0.7	-0.2	0.2	0.1	0.6	0.2	0.2	-	0.0	0.0	0.0	0.0	0.2	0.3	0.2	0.6	0.6	0.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.3
RAS	0.2	-0.1	-0.2	-0.4	-0.3	0.1	-0.1	-0.2	-0.1	-	1.0	0.6	0.5	0.4	0.4	0.1	0.4	0.6	0.1	0.7	0.7	0.8	1.0	0.8	0.6	0.6	0.7	0.9	0.2	0.6	1.0	0.3	0.9	0.6
SLO	-0.5	-0.1	0.2	-0.2	-0.4	-0.3	-0.3	-0.2	0.4	0.0	-	0.7	0.0	0.7	0.1	0.0	0.8	0.1	0.0	0.5	0.4	0.4	0.4	0.2	0.3	0.1	0.4	0.2	0.5	0.7	0.3	0.5	0.1	0.0
SST	-0.3	-0.2	0.1	0.0	-0.1	-0.2	-0.2	0.2	0.4	0.2	0.0	-	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.5	0.0	0.4	0.4	0.0	0.4	0.1	0.4	0.0	0.1	0.1	0.0	0.1	0.1	0.1
SSD	-0.5	-0.3	0.1	-0.3	-0.4	-0.1	-0.2	-0.1	0.5	0.2	1.0	0.8	-	0.5	0.1	0.0	0.6	0.1	0.0	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.3	0.2	0.5	0.6	0.3	0.5	0.0	0.0
PET	-0.3	-0.3	-0.1	-0.6	-0.7	0.2	-0.2	-0.6	0.1	0.3	0.1	-0.4	0.2	-	0.4	0.3	0.0	0.1	0.3	0.3	0.4	0.5	0.4	0.4	0.3	0.9	1.0	0.2	0.1	0.1	0.1	0.0	0.4	0.2
PEST	0.1	0.1	-0.3	0.1	0.1	0.2	0.2	-0.1	-0.3	-0.3	-0.5	-0.9	-0.6	0.2	-	0.1	0.2	0.0	0.1	0.5	0.0	0.5	0.5	0.0	0.4	0.2	0.3	0.0	0.1	0.2	0.0	0.0	0.2	0.1
PESD	-0.3	-0.2	0.1	-0.4	-0.4	-0.1	-0.2	-0.2	0.3	0.6	0.8	0.7	0.9	0.3	-0.6	-	0.4	0.1	0.0	0.4	0.3	0.2	0.4	0.2	0.2	0.3	0.6	0.3	0.9	1.0	0.4	1.0	0.2	0.0
ATE	-0.3	-0.3	-0.2	-0.5	-0.6	0.2	-0.1	-0.6	0.1	0.3	0.1	-0.4	0.2	1.0	0.4	0.3	-	0.5	0.4	0.3	0.5	0.3	0.4	0.5	0.2	1.0	1.0	0.3	0.1	0.2	0.1	0.0	0.4	0.2
AST	0.0	0.0	-0.4	0.1	0.1	0.3	0.2	-0.1	-0.2	-0.2	-0.6	-0.9	-0.6	0.5	0.9	-0.6	0.1	-	0.1	0.8	0.0	1.0	0.7	0.0	0.9	0.2	0.2	0.0	0.2	0.3	0.0	0.1	0.4	0.3
ASD	-0.3	-0.2	0.1	-0.4	-0.5	-0.1	-0.2	-0.2	0.3	0.5	0.8	0.6	0.9	0.3	-0.5	1.0	0.3	-0.5	-	0.3	0.4	0.3	0.4	0.2	0.2	0.2	0.5	0.3	0.9	1.0	0.5	0.9	0.2	0.0
NDVI	0.0	-0.9	-0.3	-0.2	-0.2	0.7	0.2	0.0	0.8	0.1	0.2	0.2	0.4	0.3	-0.2	0.3	0.3	-0.1	0.3	-	0.6	0.1	0.0	0.1	0.0	0.0	0.0	0.2	0.1	0.0	0.3	0.2	0.0	0.0
NDST	-0.1	-0.5	0.0	0.0	-0.1	0.1	-0.1	0.1	0.6	0.1	0.3	0.7	0.4	-0.3	-0.8	0.3	-0.2	-0.6	0.3	0.1	-	0.2	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NDSD	-0.1	-0.7	-0.2	-0.1	0.1	0.5	0.6	0.4	0.7	0.1	0.2	0.3	0.4	0.2	-0.2	0.4	0.3	0.0	0.3	0.6	0.4	-	0.0	0.2	0.0	0.2	0.4	0.2	0.2	0.2	0.3	0.2	0.0	0.0
PRE	-0.1	-0.9	-0.2	-0.2	-0.2	0.6	0.2	0.1	0.8	0.0	0.3	0.3	0.4	0.3	-0.2	0.3	0.3	-0.1	0.3	1.0	0.6	0.6	-	0.6	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.0
PRST	-0.1	-0.5	-0.2	0.0	-0.1	0.1	0.0	0.1	0.6	0.1	0.4	0.8	0.5	-0.3	-0.8	0.4	-0.2	-0.6	0.4	0.5	1.0	0.4	0.0	-	0.0	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PRSD	-0.1	-0.7	-0.2	-0.2	-0.2	0.6	0.2	-0.1	0.7	0.2	0.3	0.3	0.4	0.4	-0.3	0.4	0.4	-0.1	0.4	0.9	0.6	0.7	0.9	0.6	-	0.1	0.3	0.1	0.1	0.0	0.2	0.2	0.0	0.0
HPD	-0.1	-0.7	0.1	-0.2	-0.2	0.2	0.1	0.1	0.7	-0.2	0.5	0.5	0.6	0.0	-0.4	0.3	0.0	-0.4	0.4	0.7	0.5	0.4	0.8	0.5	0.5	-	0.0	0.2	0.1	0.1	0.2	0.1	0.0	0.0
HPSD	0.2	-0.7	-0.1	-0.3	-0.2	0.3	0.2	0.2	0.5	-0.1	0.3	0.3	0.3	0.0	-0.3	0.2	0.0	-0.4	0.2	0.7	0.4	0.3	0.7	0.3	0.3	0.9	-	0.4	0.2	0.1	0.4	0.3	0.0	0.2
RDS	0.1	0.3	-0.1	0.0	0.0	-0.1	0.1	-0.1	-0.5	0.0	-0.4	-0.8	-0.4	0.4	0.9	-0.4	0.3	0.7	-0.3	-0.4	-1.0	-0.4	-0.5	-1.0	-0.5	-0.4	-0.3	-	0.0	0.0	0.0	0.0	0.0	0.0
RSD	0.2	0.4	-0.1	-0.4	-0.4	-0.4	-0.3	-0.5	-0.7	0.4	-0.2	-0.5	-0.2	0.5	0.5	0.0	0.5	0.4	0.0	-0.5	-0.7	-0.4	-0.6	-0.7	-0.5	-0.5	-0.4	0.8	-	0.0	0.0	0.0	0.0	0.2
ANT	-0.1	0.6	0.2	-0.4	-0.4	-0.6	-0.4	-0.5	-0.7	0.2	-0.1	-0.5	-0.2	0.5	0.4	0.0	0.4	0.4	0.0	-0.7	-0.7	-0.4	-0.7	-0.7	-0.6	-0.6	-0.5	0.7	0.9	-	0.0	0.0	0.0	0.2
REMT	0.0	0.3	-0.2	-0.1	-0.1	0.0	0.0	-0.2	-0.5	0.0	-0.3	-0.8	-0.3	0.5	0.9	-0.3	0.5	0.8	-0.2	-0.3	-0.9	-0.3	-0.4	-0.9	-0.4	-0.4	-0.3	1.0	0.8	0.7	-	0.0	0.1	0.1
RESD	0.1	0.3	-0.1	-0.4	-0.5	-0.3	-0.3	-0.5	-0.6	0.4	-0.2	-0.6	-0.2	0.6	0.6	0.0	0.6	0.5	0.0	-0.4	-0.7	-0.4	-0.5	-0.7	-0.4	-0.5	-0.3	0.8	1.0	0.9	0.9	-	0.1	0.3
HFP	-0.4	-0.7	0.0	-0.2	-0.3	0.4	0.1	0.0	0.9	-0.1	0.5	0.4	0.6	0.2	-0.4	0.4	0.3	-0.3	0.4	0.8	0.7	0.7	0.9	0.6	0.8	0.8	0.6	-0.6	-0.6	-0.6	-0.5	-0.6	-	0.0
HSD	-0.3	-0.5	0.2	-0.5	-0.5	0.2	0.0	-0.3	0.6	0.2	0.6	0.5	0.7	0.4	-0.5	0.7	0.4	-0.3	0.6	0.8	0.6	0.6	0.8	0.6	0.9	0.6	0.4	-0.6	-0.4	-0.4	-0.5	-0.3	0.9	-

Table 5S.4. Pearson's correlation coefficients between predictors (geographic and biological traits) of genetic diversity (HDI: haplotype diversity; NDI: nucleotide diversity), range regression (RRE) and latitudinal distribution (LAT) in 12 African ungulates. * significant correlations ($p < 0.05$). See Table 5.1 for variable codes

	HDI	NDI	RRE	LAT
Geographic traits				
RRE	-0.14	-0.29	-	-
LAT	-0.19	-0.46	-	-
Biological traits				
FEC	-0.14	-0.26	-0.18	0.48
SMA	-0.18	0.17	-0.27	0.08
LON	-0.29	-0.07	0.13	0.01
BLE	-0.12	0.30	0.01	-0.75
BWE	-0.48	-0.35	0.51	-0.40
HOR	-0.30	-0.12	0.27	-0.25
GRS	0.24	0.45	-0.47	-0.73*

Table 5S.5. Multiple regression-based models (GLZ) between Haplotype and Nucleotide diversities and geographic and biological traits of 12 African ungulates. Most explanatory variables of genetic diversities selected by GLZ, Akaike information criterion corrected (AICc), delta, coefficient, t-value (t), and probability of t (Pr(>|t|)). * significant variables (p<0.05). See Table 5.1 for variable codes.

	AICc	delta	coefficient	t	Pr(> t)
Haplotype diversity					
Geographic traits					
RRE	6.14	3.43	-0.0021	0.389	0.698
LAT	5.95	3.24	-0.0066	0.969	0.333
Biological traits					
FEC	6.15	3.44	-0.0799	0.385	0.700
SMA	5.99	3.28	-0.0868	0.503	0.615
LON	5.32	2.62	-0.0155	0.841	0.400
BWE	3.24	0.53	-0.0023	-1.729	0.114
HORs	5.22	2.51	-0.0047	0.884	0.377
GRS	5.65	2.95	0.0035	0.692	0.488
Nucleotide diversity					
Geographic traits					
LAT	-13.00	0.00	-0.0074	-2.941	0.017*
Biological traits					
BWE	-13.00	0.00	-0.0017	-2.608	0.028*
GRS	-10.84	2.16	0.0038	1.503	0.133

Table 5S.6. Multiple regression-based models (GLZ) between Haplotype and Nucleotide diversities and human pressure variables of 11 African ungulates. Estimates of coefficients (estimate), standard deviation (Std.), standard error (error), and probability of t (Pr(>|t|)). See Table 5.1 for variable codes.

	Haplotype diversity				Nucleotide diversity			
Historical	estimate	Std.	error	Pr(> t)	Estimate	Std.	Error	Pr(> t)
ANT	-0.248	0.361	-0.686	0.617	-0.036	0.038	-0.962	0.512
HFP	-0.758	1.141	-0.664	0.627	-0.130	0.135	-0.961	0.513
HSD	-3.979	5.833	-0.682	0.619	-0.742	0.612	-1.213	0.439
LAT	0.451	0.662	0.681	0.619	0.071	0.064	1.113	0.466
HPD	0.379	0.542	0.699	0.612	0.054	0.049	1.114	0.466
HPSD	-0.009	0.011	-0.796	0.572	0.000	0.001	-0.182	0.885
REMT	-	-	-	-	-0.001	0.002	-0.413	0.751
RES	0.026	0.036	0.723	0.602	0.003	0.005	0.660	0.628
RDS	-360.000	513.200	-0.702	0.611	-62.570	49.390	-1.267	0.425
RSD	196.500	280.500	0.700	0.611	37.030	28.120	1.317	0.413
RRE	0.042	0.065	0.638	0.639	-	-	-	-
Present	estimate	Std.	error	Pr(> t)	Estimate	Std.	Error	Pr(> t)
ANT	-0.005	0.002	-2.687	0.227	-0.005	0.001	-3.898	0.160
HFP	0.048	0.023	2.060	0.288	-	-	-	-
HSD	-0.074	0.028	-2.609	0.233	-0.017	0.014	-1.200	0.442
LAT	0.015	0.008	1.832	0.318	0.010	0.004	2.704	0.226
HPD	-0.029	0.016	-1.775	0.327	-0.014	0.008	-1.844	0.316
HPSD	0.003	0.002	1.790	0.324	0.003	0.001	3.196	0.193
REMT	0.000	0.000	1.072	0.478	0.001	0.000	2.671	0.228
RES	-	-	-	-	-0.001	0.001	-1.848	0.316
RDS	-3.071	1.144	-2.685	0.227	-3.323	1.182	-2.812	0.218
RSD	3.096	0.707	4.379	0.143	3.126	1.142	2.736	0.223
RRE	-0.006	0.003	-1.847	0.316	-0.009	0.002	-4.029	0.155

Table 5S.7. Average values of environmental factors (top) and human pressure variables (bottom) for each of the 12 African ungulates studied in the historical (Hist) and present (Pres) distribution. Species are Addax: *Addax nasomaculatus*, AL: *Ammotragus lervia*, GC: *Gazella cuvieri*, GD: *Gazella dorcas*, GL: *Gazella leptoceros*, ER: *Eudorcas rufifrons*, ET: *Eudorcas thomsonii*, ND: *Nanger dama*, NG: *Nanger granti*, NS: *Nanger soemmerringii*, OB: *Oryx beisa*, and OD: *Oryx dammah*. See Table 5.1 for variable codes.

Environmental	RR	Slope		PET		ATE		NDVI		PRE	
		Hist	Pres	Hist	Pres	Hist	Pres	Hist	Pres	Hist	Pres
Addax	100	0.8	0.4	1965.9	2149.5	246.5	274.6	107.2	93.2	57.4	46.8
AL	84	1.9	3.7	1379.3	1805.3	221.4	219.6	134.2	108.4	118.0	66.1
ER	78	0.6	0.5	1968.0	2122.8	277.8	279.2	439.5	500.9	537.8	555.4
ET	66	2.3	2.8	1742.5	1644.2	212.6	203.9	677.9	631.4	814.5	753.1
GC	89	3.7	4.1	2114.6	1423.3	170.5	167.2	245.2	178.6	331.3	234.8
GD	83	1.0	1.4	1921.8	1918.7	244.2	244.0	131.0	106.2	103.8	46.8
GL	94	0.8	0.8	1680.5	1795.2	246.0	222.8	102.8	111.4	46.3	39.3
ND	99	0.7	0.7	1774.5	2157.2	265.2	282.1	136.7	141.8	135.5	128.8
NG	58	2.3	2.0	1874.0	1847.0	234.2	241.7	458.9	531.6	856.7	563.9
NS	86	3.3	2.8	2036.4	1927.0	245.4	272.1	473.4	253.4	627.8	326.3
OB	58	2.4	1.9	1894.7	1933.9	251.8	252.3	421.0	512.4	497.8	475.3
OD	100	1.3	0.0	1877.6	0.0	243.1	0.0	158.4	0.0	181.6	0.0
Average (SD)	82.9	1.8 (1.1)	1.8 (1.3)	1852.5	1727.0	238.2	221.6	290.5	264.1	359.0	269.7
Human	RR	HPD		RDS		ANT		REMT		HFP	
		Hist	Pres	Hist	Pres	Hist	Pres	Hist	Pres	Hist	Pres
Addax	100	7.4	0.4	0.2	0.8	94.0	100.0	1251.5	3168.3	4.3	0.6
AL	84	12.9	3.1	0.1	0.2	80.0	92.0	881.5	1079.4	7.5	3.8
ER	78	22.0	7.2	0.0	0.0	10.0	16.0	421.3	665.5	22.5	16.9
ET	66	51.6	54.8	0.0	0.0	15.0	3.0	349.1	386.7	24.7	23.2
GC	89	58.6	22.2	0.0	0.0	31.0	34.0	253.4	294.2	21.1	17.5
GD	83	14.9	1.4	0.2	0.3	83.0	96.0	1083.3	1312.9	7.1	3.4
GL	94	2.9	6.5	0.3	0.2	97.0	94.0	1394.6	937.4	3.1	5.0
ND	99	3.0	2.3	0.3	0.2	81.0	92.0	1229.2	1305.6	5.7	6.2
NG	58	41.3	25.7	0.0	0.0	12.0	9.0	436.7	499.6	20.5	18.0
NS	86	37.0	18.3	0.0	0.0	14.0	46.0	574.9	503.9	22.3	20.8
OB	58	28.7	11.8	0.0	0.0	18.0	13.0	567.7	620.7	18.4	16.2
OD	100	34.2	0.0	0.3	0.0	71.0	0.0	1068.7	0.0	9.6	0.0
Average (SD)	82.9	26.2	12.8	0.1 (0.1)	0.1 (0.2)	50.5	49.6	792.6	897.8	13.9 (8.3)	11.0 (8.5)

List 5S.1 – List of bibliographic references from where data on biological traits (Table 5.1) of 12 African ungulates were collected.

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Appendix 6S – Supplementary material of chapter 6

Text 6S.1. Reliability of genotyping results

All tissue samples (n=12) and 204 fecal samples (94%) were successfully amplified for cytb fragment. The KCAS fragment was successfully amplified in all tissue samples and in 161 fecal samples (74%). Both mitochondrial and nuclear markers allowed identifying and recovering 190 samples (87%) as *G. subgutturosa*, to proceed to genotyping. After removing low quality DNA samples (high missing data) and duplicated genotypes, we obtained a dataset of 109 individuals. In the final microsatellite dataset, we did not observe any case of genotyping error (allelic dropout and stuttering) among the consensus genotypes of the samples that we identified as duplicates. The results of MICRO-CHECKER for all populations were not significant (data not shown). Also, the test of null alleles within our dataset based on “RECESSIVEALLELES=1” option in STRUCTURE indicated low to moderate frequencies (0.00 - 0.28) of null alleles for some *loci* in some clusters and this was not consistent for any *loci*. Increasing the number of clusters (K) in the STRUCTURE analyses did not result in lower estimates of null alleles, indicating that undetected population substructure was not primarily responsible for the high null allele frequencies (Table 6S.5).

Text 6S.2. Null allele's frequency

The frequency of null alleles at all *loci* was estimated simultaneously using the “RECESSIVEALLELES=1” option, in STRUCTURE. This function enables suspected null alleles in the data to be nominated and estimates null allele frequency for each *loci*. The presence of null alleles has the potential to affect the estimation of population differentiation, by reducing the genetic diversity within populations (Paetkau and Strobeck 1995). So, we used FreeNP with ENA correction for null alleles (Chapuis and Estoup 2007) to calculate pairwise F_{ST} -values between population pairs. Chapuis & Estoup (2007) suggested that null allele frequency increase with increasing phylogenetic distance between target species and species in which the microsatellite was developed first. As the microsatellite markers were originally developed for sheep, therefore a possible reason for null allele frequency might be cross-species amplification. As we used the ‘recessive allele option’ in STRUCTURE and accounted for null alleles in calculating F_{ST} -values, so we are confident that our results and conclusions are biologically meaningful and provide useful insights for the conservation of Goitered gazelles.

References

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Text 6S.3. Environmental variables used to model species distribution

Twelve uncorrelated environmental variables were used to model the distribution of Goitered gazelle (Table 6S.1). NDVI values were calculated for 12 months in 2012 separately using MODIS images. Spatial downscaling method (Flint and Flint 2012) was used to transfer the original 1-km resolution of WorldClim data to the target resolution of 250 m (Khosravi et al. 2016). We obtained information on climatic conditions within the study area from the WorldClim database (<http://www.worldclim.org>; developed by Hijmans et al., 2005). We used a spatial downscaling method to transfer the original 1-km resolution of WorldClim data to the target resolution of 250 m (Flint and Flint, 2012). This model combines a spatial gradient and inverse-distance-squared (GIDS) weighting to WorldClim data with multiple regression. The location and elevation of the new fine-resolution grid cell relative to a coarse-resolution grid cell is used to weight the parameters based on the following equation:

$$Z = \left[\sum_{i=1}^N \frac{Z_i + (X - X_i) * C_x + (Y - Y_i) * C_y + (E - E_i) * C_e}{d_i^2} \right] / \left[\sum_{i=1}^N \frac{1}{d_i^2} \right]$$

where Z is the estimated climatic variable at the specific location defined by easting (X) and northing (Y) coordinates and elevation (E); Zi is the climatic variable from the 1-km grid cell i; Xi, Yi, and Ei are easting and northing coordinates and elevation of the 1-km grid cell i, respectively; N is the number of 1-km grid cells in a specified search radius; Cx, Cy, and Ce are regression coefficients for easting, northing, and elevation, respectively; and di is the distance from the 250-m site to 1-km grid cell i (Flint and Flint, 2012). We used a 30-km search radius to calculate bioclimatic data at the 250-m resolution.

As inclusion of all 19 bioclimatic variables and also all NDVI values calculated for 12 months in model may cause overfitting and uncertainties due to the high degree of correlation among variables, so we conducted principal component analysis PCA to reduce the correlative NDVI indices and bioclimatic variables. The first and second axes of the PCA analysis on bioclimatic variables accounted for 66% and 25% of the total variance, respectively (Table A). In addition, the results of a PCA analysis on 12 NDVI indices showed that the first two axes of the PCA analysis accounted for 87% of the total variance (Table 6S.2). PC1 was mainly correlated with the NDVI of autumn and winter months and PC2 was correlated with the NDVI index of spring months ($r > 0.8$). We used the two first PCs in the Maxent model.

Table 6S.1. Summary of the principal components analysis of the 19 bioclimatic variables extracted from the occurrence points of Goitered gazelle in Central Iran.

Component	PCA1	PCA2
Eigenvalue	9.55	1.06
Percent	79.10	7.50
Cumulative percent	79.10	86.60
Contribution of variables		
NDVI_1, March	0.86	0.02
NDVI_2, April	0.79	0.10
NDVI_3, May	0.86	0.04
NDVI_4, June	0.90	0.03
NDVI_5, July	0.47	0.23
NDVI_6, August	0.59	0.26
NDVI_7, September	0.79	0.09
NDVI_8, October	0.89	0.05
NDVI_9, November	0.75	0.03
NDVI_10, December	0.89	0.03
NDVI_11, January	0.89	0.00
NDVI_12, February	0.87	0.03

Table 6S.2. Summary of the principal components analysis of the 12 NDVI indices extracted from the occurrence points of Goitered gazelle in Central Iran.

Component	PCA1	PCA2
Eigenvalue	11.92	4.44
Percent	66.22	24.66
Cumulative percent	66.22	90.87
Contribution of variables		
BIO1, Annual mean temperature	0.95	0.25
BIO2, Mean diurnal range	−0.32	0.82
BIO3, Isothermality	−0.19	0.97
BIO4, Temperature seasonality	0.61	−0.77
BIO5, Max temperature of warmest month	0.97	0.00
BIO6, Min temperature of coldest month	0.91	0.34
BIO7, Temperature annual range	0.15	−0.89
BIO8, Mean temperature of wettest quarter	0.85	0.48
BIO9, Mean temperature of driest quarter	0.98	−0.06
BIO10, Mean temperature of warmest quarter	0.97	0.11
BIO11, Mean temperature of coldest quarter	0.87	0.44
BIO12, Annual precipitation	−0.98	−0.09
BIO13, Precipitation of wettest month	−0.95	0.05
BIO14, Precipitation of driest month	0.00	0.00
BIO15, Precipitation seasonality	0.13	0.86
BIO16, Precipitation of wettest quarter	−0.95	0.15
BIO17, Precipitation of driest quarter	0.88	−0.20
BIO18, Precipitation of warmest quarter	−0.89	0.11
BIO19, Precipitation of coldest quarter	−0.97	0.06

References

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Table 6S.3. Primer sequences dye and multiplex PCR conditions of 15 microsatellite *loci* used in the study.

Multiplex	Locus	Dye	AT or TD	Amplicon fragment (range)
1	HSC	VIC	56°	288 – 322
	Inra5	FAM		133 – 153
	Maf65	VIC		132 – 140
2	ILST008	VIC	56°	194 – 202
	Inra6	PET		125 – 159
	McM527	NED		170 – 196
3	BM302	NED	53°/48°	134 – 180
	BM415	FAM		127 – 162
	INRA40	VIC		178 – 242
4	HPN111	FAM	60°/55°	149 – 169
	HPN79	VIC		111 – 133
	HPN91	NED		275 – 281
5	HPN12	VIC	56°/54°	192 – 210
	HPN2	PET		199 – 229
	HpN5	FAM		177 – 231

AT annealing temperatures

TD touchdown temperatures

Table 6S.4. Environmental predictor variables used to derive a habitat suitability index for Goitered gazelle in Central Iran.

Abbreviation	Category	Description	Source
Bio-PCA1	Climatic	The first PC axis of a PCA analysis of 19 bioclimatic variables	WorldClim 2004
Bio-PCA2	Climatic	The second PC axis of a PCA analysis of 19 bioclimatic variables	WorldClim 2004
Elevation	Topographic	Elevation	USGS 2012
Roughness	Topographic	Roughness	USGS 2012
SP	Topographic	Slope position	USGS 2012
RT	Vegetation	Vegetation type	Iranian Department of Environment 2012
NDVI-PCA1	Vegetation	The first PCs of PCA analysis of 12 NDVI layers	The Iranian Space Agency 2012
NDVI-PCA2	Vegetation	The first PCs of PCA analysis of 12 NDVI layers	The Iranian Space Agency 2012
Rng1_2	Vegetation	Density of vegetation types 1 and 2 (dense and semi-dense rangeland with more than 25% canopy cover)	Iranian Department of Environment 2012
Rng3	Vegetation	Density of vegetation type 3 (scarce rangeland with 5% to 25% canopy cover)	Iranian Department of Environment 2012
SD	Anthropogenic	Settlement density	Iranian Department of Environment 2012
FD	Anthropogenic	Farmland density	Iranian Department of Environment 2012

Table 6S.5. Characteristics of the final panel of microsatellite *loci* used in this study for all samples. na = number of alleles, H_E = expected heterozygosity; H_O = observed heterozygosity.

Locus	na	H_E	H_O
HSC	12	0.55	0.11
INRA5	8	0.74	0.60
Maf65	4	0.44	0.40
ILST008	4	0.16	0.09
INRA6	9	0.75	0.66
McM527	6	0.58	0.47
BM302	12	0.75	0.33
BM415	8	0.77	0.29
INRA40	9	0.59	0.08
HPN111	6	0.72	0.23
HPN79	9	0.77	0.23
HPN91	4	0.34	0.04
HpN12	9	0.79	0.41
HPN2	13	0.89	0.61
HPN5	19	0.69	0.18

Table 6S.6. Probability of deviation from Hardy–Weinberg equilibrium for each population.

Locus	Population						Global test
	Biduiyeh	Kalmand	Kolah-Qazi	Kahyaz	Ghamishloo	Mooteh	
HSC	1.00	0.00	1.00	1.00	0.01	1.00	0.00
Maf65	0.81	0.03	0.16	0.62	0.65	0.87	0.14
INRA5	0.84	1.00	0.94	0.11	1.00	0.20	0.66
ILST008	0.02	1.00	1.00	0.87	1.00	1.00	0.10
INRA6	0.93	0.03	0.01	0.14	0.97	0.34	0.06
McM527	1.00	0.79	1.00	0.07	0.03	1.00	0.46
BM302	0.00	0.00	0.01	0.14	0.09	0.43	0.00
BM415	0.00	0.07	0.02	0.20	0.01	0.60	0.10
INRA40	0.10	0.00	0.11	1.00	0.14	1.00	0.00
HPN111	0.04	0.04	1.00	1.00	0.09	0.34	0.00
HPN79	0.00	0.00	0.00	1.00	0.00	0.60	0.10
HPN91	0.33	1.00	1.00	0.61	1.00	1.00	0.38
HpN5	0.12	0.02	0.04	0.42	0.61	1.00	0.01
HPN12	0.42	0.29	0.09	1.00	0.16	0.39	0.04
HPN2	0.00	0.00	0.01	1.00	0.33	1.00	0.10

Table 6S.7. Estimated null allele frequencies for the 15 microsatellite *loci* using “RECESSIVEALLELES” option in STRUCTURE.

	Cluster																			
	K = 2		K = 3			K = 4				K = 5					K = 6					
	1	2	1	2	3	1	2	3	4	1	2	3	4	5	1	2	3	4	5	6
HSC	0.19	0.16	0.14	0.18	0.14	0.19	0.14	0.15	0.20	0.17	0.17	0.10	0.18	0.16	0.17	0.12	0.15	0.09	0.17	0.17
Maf65	0.02	0.07	0.02	0.08	0.04	0.06	0.01	0.03	0.05	0.05	0.04	0.01	0.04	0.03	0.04	0.03	0.02	0.01	0.03	0.04
INRA5	0.02	0.06	0.01	0.05	0.04	0.02	0.01	0.03	0.05	0.02	0.06	0.01	0.06	0.03	0.01	0.02	0.02	0.01	0.04	0.04
ILST008	0.06	0.10	0.08	0.09	0.06	0.09	0.08	0.08	0.11	0.09	0.10	0.07	0.10	0.09	0.07	0.09	0.07	0.06	0.10	0.10
INRA6	0.02	0.08	0.02	0.08	0.05	0.06	0.01	0.03	0.04	0.06	0.05	0.01	0.05	0.04	0.04	0.02	0.03	0.01	0.04	0.04
McM527	0.00	0.05	0.01	0.04	0.03	0.03	0.01	0.03	0.04	0.03	0.05	0.01	0.05	0.03	0.02	0.02	0.02	0.00	0.03	0.03
BM302	0.12	0.11	0.11	0.21	0.18	0.16	0.09	0.15	0.17	0.16	0.17	0.07	0.17	0.12	0.27	0.09	0.11	0.06	0.16	0.15
BM415	0.20	0.17	0.21	0.17	0.16	0.12	0.21	0.15	0.16	0.12	0.16	0.22	0.16	0.15	0.11	0.10	0.18	0.22	0.16	0.17
INRA40	0.19	0.12	0.10	0.12	0.19	0.15	0.17	0.20	0.24	0.15	0.25	0.16	0.15	0.16	0.37	0.24	0.21	0.18	0.13	0.12
HPN111	0.14	0.15	0.12	0.14	0.14	0.11	0.13	0.14	0.14	0.11	0.13	0.11	0.13	0.15	0.11	0.11	0.17	0.11	0.14	0.15
HPN79	0.12	0.12	0.21	0.16	0.12	0.13	0.19	0.18	0.20	0.34	0.19	0.18	0.19	0.19	0.17	0.21	0.16	0.17	0.19	0.10
HPN91	0.10	0.13	0.23	0.14	0.13	0.12	0.23	0.12	0.13	0.11	0.13	0.23	0.13	0.12	0.20	0.18	0.22	0.29	0.21	0.22
HpN5	0.07	0.10	0.07	0.11	0.08	0.09	0.07	0.09	0.10	0.06	0.07	0.06	0.07	0.07	0.08	0.07	0.10	0.09	0.10	0.11
HPN12	0.03	0.09	0.02	0.07	0.07	0.14	0.02	0.05	0.06	0.15	0.06	0.02	0.06	0.04	0.13	0.02	0.03	0.01	0.04	0.04
HPN2	0.13	0.12	0.26	0.14	0.24	0.09	0.26	0.22	0.13	0.08	0.14	0.27	0.15	0.22	0.08	0.22	0.19	0.28	0.16	0.15

Table 6S.8. Analysis of variable contribution for the best model fit explaining the distribution of Goitered gazelle in Central Iran.

Variable	Contribution (%)	Variable	Contribution (%)
Bio-PCA1	42.1	SP	4.8
RT	16.5	NDVI-PCA1	1.9
Elevation	10.9	FD	1.8
Bio-PCA2	7.4	Roughness	1.7
Rng1_2	5.4	NDVI-PCA2	1.4
Rng3	5.4	SD	0.3

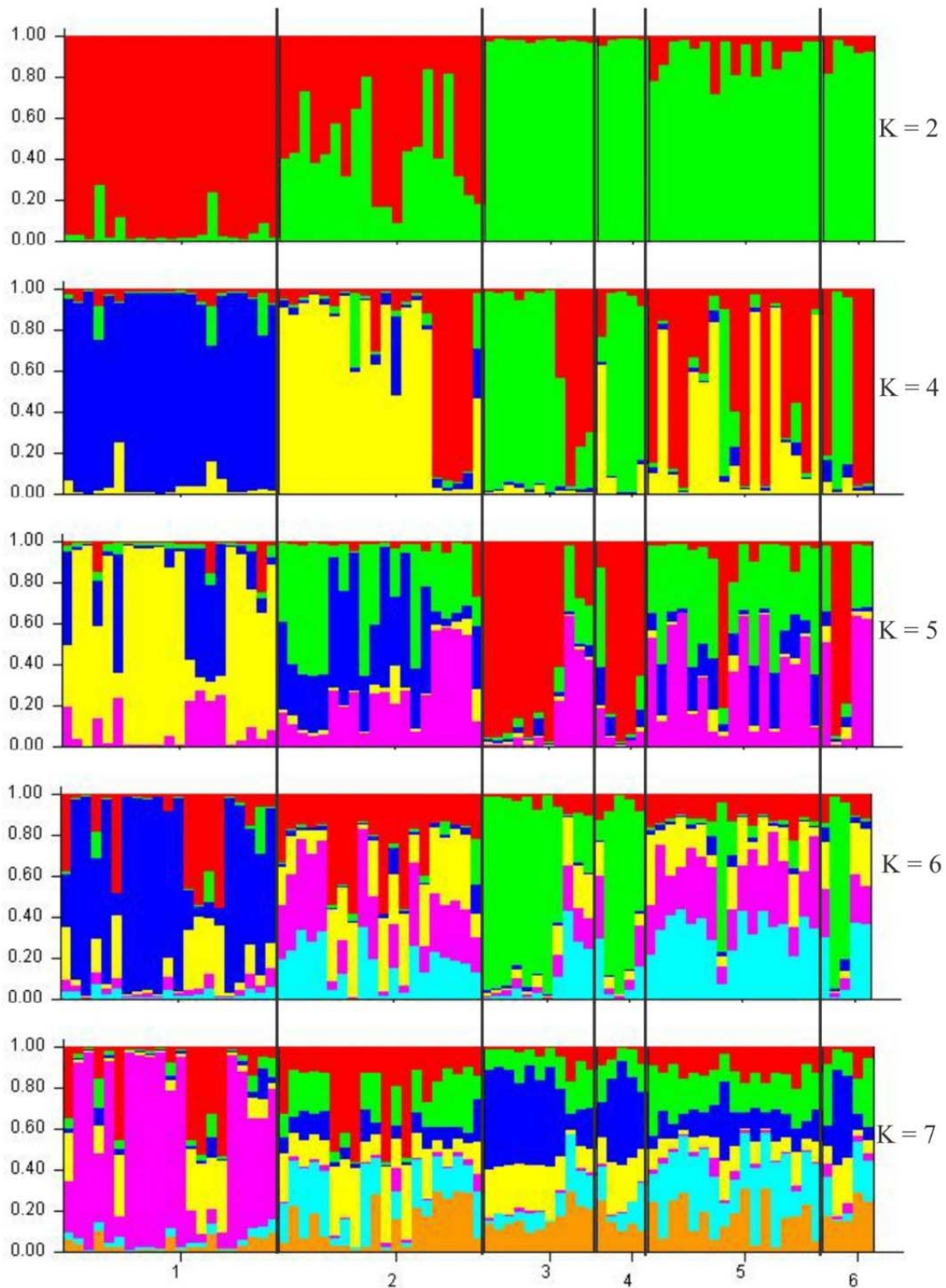


Figure 6S.1. Percentage population assignments to inferred genetic clusters with K ranging from 2 to 7, except k=3 that is the main text (Figure 2). The sampling populations for individuals are shown as 1–6 including 1 = Biduiyeh; 2 = Kalmand; 3= Kolah-Qazi; 4= Kahyaz; 5= Ghamishloo; 6= Mooteh.

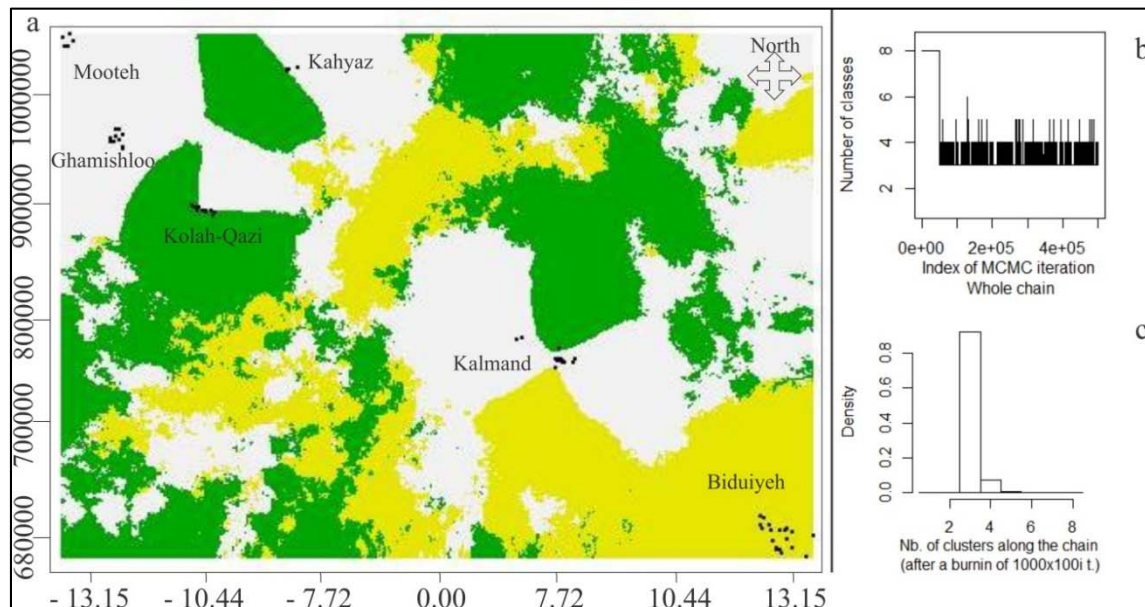


Figure 6S.2. A Voroni tessellation map depicting the sorting of all genotypes into three clusters: white (GHAM, MOT, KAL and KAH), green (KGH) and yellow (BID). The map was produced by GENELAND with the highest mean posterior density. Black dots are GPS points at which samples were collected (a). The trace of number of population along the MCMC runs (b). Relative density of the number of populations along the MCMC chain following burn-in using GENELAND algorithm (c). BID-Biduiyeh; KAL-Kalmand; KGH-Kolah-Qazi; KAH-Kahyaz; GHAM-Ghamishloo; MOT-Mooteh.

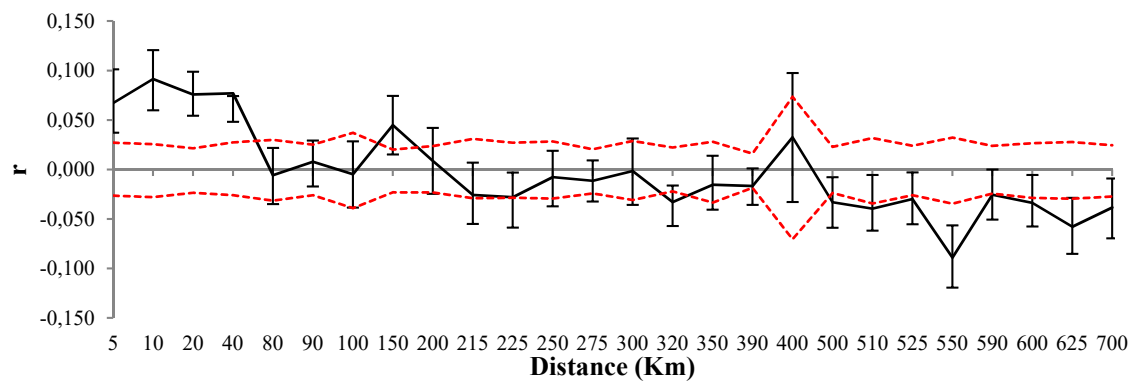


Figure 6S.3. Correlogram of genetic correlation (r) plotted as a function of geographic distance for Goitered gazelle in Central Iran. The permutated 95 % confidence intervals (dashed lines) and bootstrapped 95 % error bars are shown.

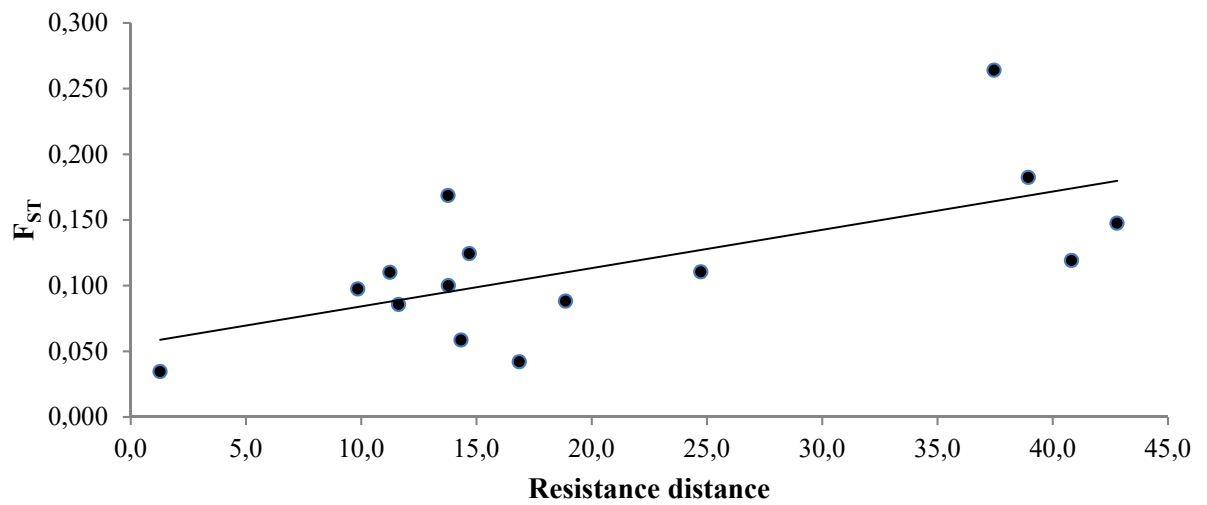


Figure 6S.4. Correlation between pairwise resistance of landscape movement of Goitered gazelles and geographic distances among focal patches based on a circuit theory analysis. Mantel's $r^2 = 0.42$, p -value = 0.02.

Appendix 8S – Other publications

Other papers published during the PhD project

Brito JC, Durant S., Pettorelli N, Newby J, Canney S, Algadafi W, Rabeil T, Crochet P-A, Pleguezuelos JM, Wachter T, de Smet K, Gonçalves DV, da Silva MJF, Martínez-Freiría F, Abáigar T, Campos JC, Comizzoli P, Fahd S, Fellous A, Garba HHM, Hamidou D, Harouna A, Hacha MH, Nagy A, **Silva TL**, Sow AS, Vale CG, Boratyński Z, Rebelo H, Carvalho SB (2018). Armed conflicts and wildlife decline: Challenges and recommendations for effective conservation policy in the Sahara-Sahel. *Conservation Letters*, doi:10.1111/conl.12446

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POLICY PERSPECTIVE

WILEY Conservation Letters
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Armed conflicts and wildlife decline: Challenges and recommendations for effective conservation policy in the Sahara-Sahel

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Diversity and Distributions, (Diversity Distrib.) (2016) 22, 371–384



Conservation Biogeography of the Sahara-Sahel: additional protected areas are needed to secure unique biodiversity

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ABSTRACT

Aim Identification of priority conservation areas and evaluation of coverage of the current protected areas are urgently needed to halt the biodiversity loss. Identifying regions combining similar environmental traits (climate regions) and species assemblages (biogroups) is needed for conserving the biodiversity patterns and processes. We identify climate regions and biogroups and map species diversity across the Sahara-Sahel, a large geographical area that exhibits wide environmental heterogeneity and multiple species groups with distinct biogeographical affinities, and evaluate the coverage level of current network of protected areas for biodiversity conservation.

Location Sahara-Sahel, Africa.

Methods We use spatially explicit climate data with the principal component analysis and model-based clustering techniques to identify climate regions. We use distributions of 1147 terrestrial vertebrates (and of 125 Sahara-Sahel endemics) and apply distance clustering methods to identify biogroups for both species groups. We apply reserve selection algorithms targeting 17% of species distribution, climate regions and biogroups to identify priority areas and gap analysis to assess their representation within the current protected areas.

Results Seven climate regions were identified, mostly arranged as latitudinal belts. Concentrations of high species richness were found in the Sahel, but the central Sahara gathers most endemic and threatened species. Ten biogroups (five for endemics) were identified. A wide range of biogroups tend to overlap in specific climate regions. Identified priority areas are inadequately represented in protected areas, and six new top conservation areas are needed to achieve conservation targets.

Main conclusions Biodiversity distribution in Sahara-Sahel is spatially structured and apparently related to environmental variation. Although the majority of priority conservation areas are located outside the areas of intense human activities, many cross multiple political borders and require internationally coordinated efforts for implementation and management. Optimized biodiversity conservation solutions at regional scale are needed. Our work contradicts the general idea that deserts are uniform areas and provide options for the conservation of endangered species.

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Vale CG, Campos JC, **Silva TL**, Gonçalves DV, Sow AS, Martínez-Freiría F, Boratyński Z, Brito JC (2016). Biogeography and conservation of mammals from the West Sahara-Sahel: an application of ecological niche-based models and GIS. *Hystrix-Italian Journal of Mammology*, 27(1). doi:10.4404/hystrix-27.1-11659



Research Article

Blogeography and conservation of mammals from the West Sahara-Sahel: an application of ecological niche-based models and GIS

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Abstract

The West Sahara-Sahel is a remote region where knowledge gaps on mammal distribution have hampered accurate local biodiversity assessments and the development of optimised conservation planning in the region. Using a geographical information system and ecological niche-based modelling, this study combines high-resolution presence data from 22 mammals and environmental factors from the West Sahara-Sahel to identify suitable areas for mammal occurrence, biogeographic affinities among taxa, and local hotspots of species richness. The maximum entropy approach was used to relate environmental factors with mammal distributions and to predict suitable areas of species' occurrence. Biogeographic groups were defined based on the spatial similarities of predicted distributions. Ecological niches of analysed taxa were summarized through Principal Components Analyses based on topoclimatic and habitat variables. Distributions of most mammals were related with climate and/or habitat features, and some were associated with topography. Suitable areas were predicted mostly within known distributions of mammalian taxa. Low values of niche breadth were estimated for all taxa, indicating a tendency for specialization in the study area. Shared distributional ranges among taxa allowed the identification of five groups with different biogeographic affinities. Most groups occurred in the ecoregions North Saharan steppe and woodlands and Sahelian *Acacia* savanna, reflecting their availability and stressing the importance of these ecoregions for local mammal conservation. Predicted suitable areas were poorly represented in the current network of protected areas, especially in Mauritania. The southern M'ritanian mountains contained suitable habitat for most of the studied mammals, but are largely unprotected. The fine scale ecological niche-based model is built with high resolution data can be used to identify key-areas for conservation and management, and could be applied to other remote regions and taxonomic groups worldwide.

Introduction

Global biodiversity loss is a major concern for humanity, given its crucial role for ecosystem services (Butchart et al., 2010; Cardinale et al., 2012). The strategic goals of the Aichi Biodiversity Targets (CBD, 2010) listed key-targets to halt biodiversity loss by 2020. Among targets are the global identification of priority areas for conservation and the evaluation of the effectiveness of current protected areas. To achieve them, accurate knowledge about biodiversity distribution is needed and spatial mapping at high-resolution is particularly crucial to optimize conservation planning (Ferrier et al., 2002).

Deserts and arid regions (aridity index <0.20 ; Ward, 2009) are generally perceived as homogeneous and species-poor (Durant et al., 2014). However, they contain about 25% of terrestrial vertebrate species (Mace et al., 2005), provide local biodiversity hotspots, and harbour many endemic species with unique adaptations to extreme environmental conditions (Murphy et al., 2012; Wilson and Pitts, 2012). The West Sahara-Sahel of Africa covers twelve ecoregions (Olson et al., 2001) from coastal sandy beaches to inland mountain areas (Fig. 1).

The dramatic climatic oscillations affecting the region since the Pliocene (5.3 Mya) have caused multiple dry-wet cycles (Le Houérou, 1997) and are hypothesised to shape present-day biodiversity patterns (Britto et al., 2014). Local biodiversity hotspots have been identified in mountain rock pools (locally known as *guellets*), where isolated refugial populations of Afro-tropical taxa are found (Vale et al., 2015a). Parts of the region have been identified as priority areas for the conservation of Sahara-Sahel endemics, but coverage by the current network of protected areas is low (Britto et al., 2016). In general, knowledge about biodiversity distribution in West Sahara-Sahel is scarce, in comparison with other regions (Britto et al., 2014; Durant et al., 2014). The history of conflicts since mid-1970s combined with general remoteness (Britto et al., 2014) have hampered biodiversity assessments and consequently, optimised conservation planning in the region is still underdeveloped.

Mammals are a diverse group of animals that show particular adaptations to arid environments (Ward, 2009). Although 105 mammals have been described in West Sahara-Sahel (IUCN, 2015), knowledge on their distribution is poor. Some regions have been relatively well sampled, such as the lower Drâa river valley (Cuzin, 2003; LeDauphin, 2005) or coastal Mauritania (Granjon et al., 1997, 2002), but available distribution data from inland and remote areas is scarce and displays

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Brito JC, Godinho R, Martínez-Freiría F, Pleguezuelos JM, Rebelo H, Santos X, Vale CG, Velo-Antón G, Boratyński Z, Carvalho SB, Ferreira S, Gonçalves DV, **Silva TL**, Tarroso P, Campos JC, Leite JV, Nogueira J, Álvares F, Sillero N, Sow AS, Fahd S, Crochet P.-A., Carranza S (2014). Unravelling biodiversity, evolution and threats to conservation in the Sahara-Sahel. *Biological Reviews*, 89: 215–231. doi: 10.1111/brv.12049

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Unravelling biodiversity, evolution and threats to conservation in the Sahara-Sahel

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ABSTRACT

Deserts and arid regions are generally perceived as bare and rather homogeneous areas of low diversity. The Sahara is the largest warm desert in the world and together with the arid Sahel displays high topographical and climatic heterogeneity, and has experienced recent and strong climatic oscillations that have greatly shifted biodiversity distribution and community composition. The large size, remoteness and long-term political instability of the Sahara-Sahel, have limited knowledge on its biodiversity. However, over the last decade, there have been an increasing number of published scientific studies based on modern geomatic and molecular tools, and broad sampling of taxa of these regions. This review tracks trends in knowledge about biodiversity patterns, processes and threats across the Sahara-Sahel, and anticipates needs for biodiversity research and conservation. Recent studies are changing completely the perception of regional biodiversity patterns. Instead of relatively low species diversity with distribution covering most of the region, studies now suggest a high rate of endemism and larger number of species, with much narrower and fragmented ranges, frequently limited to micro-hotspots of biodiversity. Molecular-based studies are also unravelling cryptic diversity associated with mountains, which together with recent distribution atlases, allows identifying integrative biogeographic patterns in biodiversity distribution. Mapping of multivariate environmental variation (at 1 km × 1 km resolution) of the region illustrates main biogeographical features of the Sahara-Sahel and supports recently hypothesised dispersal corridors and refugia. Micro-scale water-features present mostly in mountains have been associated with local biodiversity hotspots. However, the distribution of available data on vertebrates highlights current knowledge gaps that still apply to a large proportion of the Sahara-Sahel. Current research is providing insights into key evolutionary and ecological processes, including causes and timing of radiation and divergence for multiple taxa, and associating the onset of the Sahara with diversification processes for low-mobility vertebrates. Examples of phylogeographic patterns are showing the importance of allopatric speciation in the Sahara-Sahel, and this review presents a synthetic overview of the most commonly hypothesised diversification mechanisms. Studies are also stressing that biodiversity is threatened by increasing human activities in the region, including overhunting and natural resources prospection, and in the future by predicted global warming. A representation of areas of conflict, landmines, and natural resources extraction illustrates how human activities and regional insecurity are hampering biodiversity research and

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Monterroso P, Castro D, **Silva TL**, Ferreras P, Godinho R, Alves PC (2013) Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe. *Journal of Zoology*, 289(4), 243–250, doi: 10.1111/jzo.12000.

Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe

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Keywords

mammalian mesocarnivores; scat identification; accuracy rates; non-invasive genetics; conservation.

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Abstract

Research on terrestrial carnivore ecology frequently relies on scat identification and analysis. However, species assignment is commonly based on scat morphology. Potential errors in scat identification are rarely accounted for and might contribute to substantial bias of the final results. Using molecular methods, we evaluate the accuracy of species identification based on morphological characteristics of mammalian mesocarnivore scats collected in two areas in the Iberian Peninsula. Our results revealed that error rates in species assignment of scats based on morphology were highly variable, ranging from 14%, for putative red fox *Vulpes vulpes* samples, to 88%, for putative wildcats *Felis silvestris*. The developed models revealed that putative species, season, study area and target species abundance are among the factors involved in identification accuracy. However, the low variability explained suggests that unaccounted factors also had significant effects on accuracy rates. The error rates in scat species assignment constitute a potential source of bias in ecological studies, with serious consequences for the management of threatened species, as unrealistic estimates of status and distribution are prone to occur. Our results suggest that scat identification accuracy rates are circumstance-specific and therefore should not be transferred or extrapolated. We suggest that scat-based studies should implement measures (molecular or others) that allow researchers to determine their own circumstance-specific error rates in scat identification, which should be incorporated in subsequent analyses, ensuring reliable ecological inferences.

Introduction

Adequate and adjusted conservation planning relies on the collection, analysis and interpretation of field data. For this reason, the accuracy and reliability of data collected in the field assumes a crucial role in wildlife conservation. Data collection on mammalian carnivores is particularly challenging because they typically occur in low densities, are crepuscular and/or nocturnal, and elusive (Wilson & Delahay, 2001). As a result, knowledge on these species frequently relies on indirect methods, namely on species presence signs rather than on the observation or capture of the animals themselves (Heinemeyer, Ulizio & Harrison, 2008). Among the indirect field methods employed for carnivores, scat searching is one of the most frequently used (Davison *et al.*, 2002). This method has been argued as being one of the most efficient methods for the detection and monitoring of European mammalian mesocarnivores (Sadler *et al.*, 2004; Barea-Azcón *et al.*, 2006; Rosellini *et al.*, 2008). Moreover, scat analysis has the potential to provide information on many other ecological aspects (e.g. Trites & Joy, 2005; Janko *et al.*, 2011; Asa, 2012). However, all

the potential information retrieved from carnivore scats can only be useful upon correct species identification. During recent years, advances in non-invasive molecular methods have allowed the extraction and amplification of fragmented and degraded DNA (Broquet, Ménard & Petit, 2007; Beja-Pereira *et al.*, 2009) and species-specific markers have been developed (Livia *et al.*, 2006; Oliveira *et al.*, 2010). The application of genetic scatology has highlighted the fact that the evaluation of scat morphology alone is prone to misidentifications among sympatric carnivore species, even when evaluated by experienced field technicians (Davison *et al.*, 2002; Janečka *et al.*, 2008; Harrington *et al.*, 2010). Regardless, monitoring programmes and ecological research on carnivore species are still mainly carried out based on morphologically identified scats, without acknowledging potential biases induced by misidentifications. However, morphology-based scat searching methods are often the only available alternative for conducting large-scale surveys on carnivore species because of the reduced costs and labour when compared with other methods (Wilson & Delahay, 2001; Barea-Azcón *et al.*, 2006). Moreover, information on the diet of species as elusive as most carnivores

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Splitting or Lumping? A Conservation Dilemma Exemplified by the Critically Endangered Dama Gazelle (*Nanger dama*)

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Abstract

Managers of threatened species often face the dilemma of whether to keep populations separate to conserve local adaptations and minimize the risk of outbreeding, or whether to manage populations jointly to reduce loss of genetic diversity and minimise inbreeding. In this study we examine genetic relatedness and diversity in three of the five last remaining wild populations of dama gazelle and a number of captive populations, using mtDNA control region and cytochrome b data. Despite the sampled populations belonging to the three putative subspecies, which are delineated according to phenotypes and geographical location, we find limited evidence for phylogeographical structure within the data and no genetic support for the putative subspecies. In the light of these data we discuss the relevance of inbreeding depression, outbreeding depression, adaptive variation, genetic drift, and phenotypic variation to the conservation of the dama gazelle and make some recommendations for its future conservation management. The genetic data suggest that the best conservation approach is to view the dama gazelle as a single species without subspecific divisions.

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Introduction

Fragmented populations of endangered species present a conservation dilemma [1]. If numbers dwindle to the extent that conservation intervention is necessary, should they be managed separately to conserve locally adapted genetic diversity and minimise the risk of outbreeding? Or should they be managed jointly to preserve evolutionary potential and to reduce the risk of inbreeding? This dilemma has been discussed recently both from taxonomic [2–3] and management [4] perspectives and is present in many wild and captive management scenarios [5–10]. The dilemma is typified in regard to the Critically Endangered dama gazelle (*Nanger dama*) [11].

There are around 300 dama gazelles left in the wild [11–12] and their range has shrunk by 99% during the period 1960–90 [13–14]. They now only exist in confirmed isolated populations in Chad, Niger, and Mali (Figure 1) and possibly in Sudan.

Approximately 550 individuals are thought to exist within zoos and breeding centres across Europe, the USA and the Middle East, with further populations in private collections in the Middle East and particularly the USA accounting for an estimated 1000 individuals.

The gazelle's range formerly spanned the entire Sahel and Atlantic Saharan region (Figure 1) from south of the Anti-Atlas Mountains to the west coast of Morocco, south to Senegal and Mauritania, and east to the River Nile in Sudan. It is likely this range was once continuous (Figure 1), although a possible break in southern Mauritania has been debated [15–16]. The current wild populations are situated in the central part of the former range. Captive populations are known to have originated from two distant points of the original geographical distribution and are currently maintained as separate breeding populations (Figure 1, Table 1).

