The phylogeny of the genus *Gazella* and the phylogeography and population genetics of Arabian species

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I Summary

Biodiversity is caused by a fundamental evolutionary process: speciation. When species can spread into new habitats and are allowed to colonize new ecological niches, speciation can become accelerated and is then called radiation. This can happen, *e.g.*, when formerly separated land masses become connected. A prime example of such a scenario is the Arabian Peninsula that connects Africa and Asia since the Oligocene (approx. 30 Ma ago). Since then, the peninsula promoted several faunal exchanges between both continents. The mammalian genus *Gazella* is an excellent candidate for investigating this faunal exchange. Species are distributed on both, the African and Asian continent as well as on the Arabian Peninsula that is located in between. The aim of my thesis was to cast new light on the evolution and speciation of the genus and, furthermore, to evaluate the currently problematic taxonomy to infer suggestions for improved conservation actions for threatened gazelle species. Therefore, I investigated the taxon *Gazella* genetically and identified factors that promoted the speciation of this diverse genus. I assessed intraspecific genetic variability for species that inhabited the Arabian Peninsula to infer the past demography of those species and to estimate the history of species divergence and past population parameters.

In the first part of my thesis I inferred a mitochondrial phylogeny based on cytochrome *b* gene sequences using samples of all nine extant species of *Gazella* and also of closely related taxa (chapter 2). Besides the monophyly of the genus *Gazella* two reciprocally monophyletic clades were detected that evolved in allopatry: one predominantly African and one predominantly Asian clade. Within both clades species pairs could be inferred with species being ecologically adapted to different habitats: one species is a desert-dweller (probably the ancestral character state combination), while the other one is adapted to rather mountainous and humid habitats. These adaptations also correlate with the behavior of the species with the mountainous forms being sedentary, territorial and living in small groups and the desert forms being migratory, non-territorial and living in larger herds.

The second part of my thesis focuses on the Arabian gazelle species. In a study about *G. subgutturosa* I could show that the Arabian form *G. marica* (sand gazelle)—previously recognized as a subspecies of *G. subgutturosa*—is genetically distinct from the nominate form (chapter 3). Moreover, a phylogenetic tree based on cytochrome *b* gene sequences revealed a polyphyly of *G. subgutturosa* and *G. marica* with sand gazelles being more closely related to *G. leptoceros* and *G. cuvieri* of North Africa. Consequently, I suggested the restoration to full species level for *G. marica* corroborating earlier conservation practices of breeding both taxa separately in captivity.

In case of *G. dorcas* such a genetic differentiation could not be detected (chapter 4). Despite the large distribution range from Mali in the west to Saudi Arabia in the east only low genetic variation was detectable in mitochondrial sequence data. Statistically parsimony network analyses revealed pronounced haplotype sharing across regions. Using a coalescence approach I observed a steep population decline that started about 25,000 years ago and which is still ongoing. The decline could be correlated with human hunting activities in the Sahara. Hence, hunting of *G. dorcas* (already in ancient times) had a much larger impact on gazelle populations than previously thought and even led to the extinction of the Arabian form of *G. dorcas*.

In chapter 5 of my thesis I provided a rigorous test to genetically distinguish between the potential species *G. gazella* and *G. arabica*. Previously recognized as a single species mitochondrial sequence analyses provided first hints for the separation of both taxa. But without the investigation of nuclear loci the observed pattern could also be the result of male biased dispersal combined with female philopatry. Therefore, I amplified mitochondrial sequence markers and nuclear microsatellite loci for both taxa and found support for the earlier view of two separate species. No signs of recurrent gene flow could be detected between neighboring populations of *G. arabica* and *G. gazella*. The split of both species could be estimated one million years ago and the recommendation of breeding both taxa separately in captivity for conservation purposes is fully justified.

Several populations of *G. arabica* suffer from a severe decline. In chapter 6 I asked whether the population occurring on the Farasan archipelago—being at stable individual numbers for decades—may serve as potential source for future reintroduction on the Arabian mainland, although the gazelles show a reduced body size. Analyzing the genetic differentiation of Farasan gazelles, a genetic cluster could be inferred being endemic to the archipelago. However, only approx. 70% of Farasan individuals were assigned to this specific cluster, while the others showed at least intermediate or even complete assignment to the mainland cluster. This indicates ongoing introgression that is probably mediated by human translocations of gazelles from and onto the islands. Considering the uniform dwarfism of Farasan gazelles, reasons for the smaller body size might be direct consequences of resource limitations, *i.e.*, phenotypic plasticity. If the population decline on the mainland will hold on Farasan gazelles could serve as stocks for future reintroductions.

II Zusammenfassung

Die Erforschung der Entstehung der Arten bildet einen Schlüssel für das Verständnis von Evolution und Biodiversität. Der Prozess der Artbildung kann beschleunigt werden, wenn Arten neue Habitate oder neue ökologische Nischen besiedeln können. Dieser Prozess wird als Radiation bezeichnet. Bestimmte geologische Prozesse können Radiationen auslösen, wenn Arten sich dadurch ausbreiten können. Solche eine Radiation kann zum Beispiel durch geologische Prozesse ausgelöst werden, die es Organismen überhaupt erst ermöglichen sich weiter auszubreiten. Zum Beispiel kann die Verbindung zweier Kontinente durch eine Landbrücke für Festland bewohnende Arten ein solches geologisches Ereignis darstellen. Die wissenschaftliche Untersuchung solcher Radiationsereignisse kann damit zum Verständnis von Artbildung und Evolution beitragen.

Ein Beispiel für eine solche Verbindung stellt die Arabische Halbinsel dar. Sie repräsentiert eine biogeographische Kontaktzone der Flora und Fauna Afrikas und Asiens. Seit dem Oligozän (vor etwa 30 Millionen Jahren) hat es mehrfach Austausche zwischen den beiden Kontinenten über die Arabische Halbinsel hinweg gegeben. Dabei war der Transfer der Fauna asymmetrisch und änderte sich über geologische Zeiträume (für eine Erläuterung siehe Kapitel 2). Ob eine Art über die Arabische Halbinsel erfolgreich neue Habitate besiedeln konnte, hing dabei von ihren ökologischen Voraussetzungen und den vorherrschenden klimatischen Bedingungen ab.

Eine Tiergruppe, die sich besonders zur Untersuchung einer Radiation in dieser Region eignet, ist die Gattung *Gazella*, da sie sowohl auf den Kontinenten Afrika und Asien als auch auf der Arabischen Halbinsel selbst verbreitet ist. Mit neun rezenten Arten gehört sie zu einer der artenreichsten Gattungen innerhalb der Bovidae. Allerdings gehört die Gattung *Gazella* auch zu einer der am wenigsten verstandenen Gruppen innerhalb der Säugetiere. Viele, zum Teil widersprüchliche, Studien über ihre Entstehung und ihre Taxonomie wurden bereits veröffentlicht. Doch beruhen diese Studien hauptsächlich auf morphologischen Eigenschaften oder der Anzahl und Eigenschaft der Chromosomen der verschiedenen Arten. Genetisch ist die Gruppe der Gazellen bisher unzureichend charakterisiert, vor allem bezogen auf die Feststellung innerartlicher, genetischer Variabilität. Dies wirkst sich zudem negativ auf Bemühungen aus, diese gefährdeten

Gazellenarten zu schützen. So besteht zum Beispiel Unsicherheit darüber, welche Populationen an welchen Orten wieder ausgewildert werden können.

Ziel meiner Dissertation war es daher eine molekulare Phylogenie der Gattung *Gazella* zu erstellen und dabei alle rezenten Arten mit einzubeziehen. Des Weiteren wurde untersucht, welche Faktoren die Diversifizierung der Gattung unter Berücksichtigung der demographischen Entwicklung, Ökologie und des Verhaltens der entsprechenden Arten vorangetrieben haben (erster Teil der Dissertation, der einen bereits veröffentlichten Artikel enthält). Um die innerartliche, genetische Variabilität ausgewählter Arten – also derer, die auf der Arabischen Halbinsel verbreitet sind – einschätzen zu können, wurden kleiner skalierte Studien mit mehr Individuen (möglichst aus dem gesamten Verbreitungsgebiet der Art) durchgeführt (zweiter Teil der Dissertation der drei bereits veröffentlichte und einen eingereichten Artikel enthält). Damit sollte auch das Verständnis historischer Wanderbewegungen und die Besiedlung neuer Habitate durch bestimmte Gazellenarten im Speziellen und somit der Evolution der Gattung *Gazella* im Allgemeinen gesteigert werden.

Kapitel 2 repräsentiert den ersten Teil der Arbeit. Es wurde eine molekulare Phylogenie der Gattung *Gazella* mit Hilfe der Sequenzen des Cytochrom-*b*-Gens erstellt. Innerhalb der Gattung *Gazella* konnte ich zwei Kladen rekonstruieren, die vermutlich allopatrisch entstanden sind: (1) eine vornehmlich asiatische Klade (mit den Arten *G. bennettii*, *G. subgutturosa*, *G. marica*, *G. leptoceros* und *G. cuvieri*) und (2) eine vornehmlich afrikanische Klade (mit den Arten *G. dorcas*, *G. spekei*, *G. gazella* und *G. arabica*). Heute überlappen sich die Verbreitungsgebiete beider Kladen in Nordafrika und besonders auf der Arabischen Halbinsel. Innerhalb der beiden Kladen konnte ich weitere Strukturen aufdecken, die sich besser durch adaptive Artbildung im Zuge divergenter, ökologischer Selektion erklären lassen. So lassen sich drei Artenpaare nachweisen, bei denen eine Art an das Wüstenleben angepasst ist (dies stellt wahrscheinlich den anzestralen Merkmalszustand dar), während die andere Art höher gelegene, feuchtere Bergregionen besiedeln konnte. Diese divergente Lebensweise spiegelt sich auch in der Ökologie und dem Verhalten der entsprechenden Arten wider. Die Bergform ist sedentär und territorial, ernährt sich hauptsächlich von Blättern und ist nur in kleinen Gruppen anzutreffen. Die Wüstenform hingegen wandert, ist nicht-territorial (mit Ausnahme der Brunftzeit), ernährt sich mehrheitlich von Gräsern und kann größere Herden bilden.

Im zweiten Teil meiner Dissertation (Kapitel 3 – 6) wurden verschiedene Gazellenarten, die auf der Arabischen Halbinsel verbreitet sind (oder erst kürzlich dort ausgestorben sind) näher untersucht, um ihre genetische Variabilität und somit ihr historische Demographie zu ermitteln. Hierzu wurde eine größere Zahl an Individuen beprobt, wobei eine große Abdeckung des Verbreitungsgebietes der jeweiligen Art im Fokus stand, um mögliche genetische Strukturierungen innerhalb einer Art aufdecken zu können.

So konnte in Kapitel 3 gezeigt werden, dass die arabische Form von *G. subgutturosa*, vormals als Unterart *G. s. marica* beschrieben, genetisch stark von der Nominalform abweicht. Hierzu wurden Proben von *G. s. marica* von der Arabischen Halbinsel, Jordanien, Syrien und dem Irak gesammelt und mit *G. s. subgutturosa* Proben verglichen, die unter anderem in Aserbaidschan und Chinesisch-Turkestan gesammelt wurden. Es wurde ein Teil des Cytochrom-*b*-Gens sequenziert und phylogenetisch ausgewertet. Dabei konnte ich festgestellen, dass das Taxon *G. subgutturosa*, wie es bisher verstanden wurde, polyphyletisch ist. Eine mögliche Monophylie beider untersuchter Unterarten *G. s. subgutturosa* und *G. s. marica* wurde statistisch signifikant abgelehnt. Stattdessen zeigte sich, dass *G. s. marica* näher mit den nordafrikanischen Gazellenarten *G. leptoceros* und *G. cuvieri* verwandt ist. Daher wurde vorgeschlagen der vormals arabischen Unterart von *G. subgutturosa* einen vollen Artstatus zuzuerkennen: *G. marica*. Die Ergebnisse dieser Studie haben auch Auswirkungen auf Maßnahmen des Naturschutzes, die bereits ins einigen Ländern des Nahen Ostens durchgeführt werden. So wird zum Beispiel die Trennung beider Taxa in Zuchtprogrammen empfohlen.

Kapitel 4 meiner Dissertation beschäftigt sich mit der Dorkasgazelle (*G. dorcas*), einer Art mit einer sahelo-saharischen Verbreitung, deren arabische Form (Erstbeschreibung als *G. saudiya*) heute bereits ausgestorben ist. Wie die meisten Gazellenarten leidet *G. dorcas* unter einer starken Reduktion der Populationszahlen aufgrund von Bejagung und Verdrängung durch Nutztiere. Schutz- und Wiederauswilderungsmaßnahmen werden jedoch durch das schlechte Verständnis der Verwandtschaftsbeziehungen der einzelnen Populationen zu einander erschwert. In dieser Studie wurden daher 73 Dorkasgazellen aus dem gesamten Verbreitungsgebiet von der westlichen Sahara bis nach Saudi-Arabien beprobt (bei letzterem Museumsproben von *G. saudiya*) und mit zwei mitochondrialen Sequenzmarkern ausgewertet. Hierbei konnte nur eine geringe genetische Variabilität festgestellt werden. Des Weiteren ließen sich keine größeren phylogenetischen Aufspaltungen innerhalb der monophyletischen *G. dorcas* nachweisen; vielmehr konnte ein hohes Maß an Genfluss zwischen verschiedenen Verbreitungsregionen beobachtet werden. Mit Hilfe eines Koaleszenz-Ansatzes konnte ich außerdem einen starken Rückgang der Populationszahlen rekonstruieren, der vor etwa 25 000 Jahren begann. Dies fällt mit der Verbesserung menschlicher Jagdtechniken in der Sahara zusammen, da die Jagd auf *G. dorcas* erst mit der Erfindung der Speerschleuder ermöglicht wurde und (in geänderter Form) über das Neolithikum bis heute andauert.

Eine hohe genetische Strukturierung auf Basis mitochondrialer Sequenzmarker konnte bereits durch phylogenetische Analysen zwischen *G. gazella* auf der einen Seite und *G. arabica* auf der anderen Seite festgestellt werden (beide Arten wurden vormals als eine Art *G. gazella* betrachtet). Da die Auswertung mitochondrialer Marker allein aber zu Fehlinterpretationen führen kann (siehe Kapitel 1), habe ich in Kapitel 5 neben diesen auch nukleäre Marker, d.h., Mikrosatelliten angewandt, um die Trennung von *G. gazella* und *G. arabica* in zwei Arten zu verifizieren. Ich konnte keinerlei Hinweise auf bestehenden Genfluss benachbarter Populationen beider Arten zeigen und somit deren phylogenetische Trennung bestätigen. Diese Trennung konnte ich auf einen Zeitpunkt vor etwa einer Million Jahre datieren. Dies bedeutet, dass beide Taxa in zukünftigen Naturschutz- und Zuchtmaßnahmen als zwei separate Arten behandelt werden sollten.

Das letzte Kapitel meiner Dissertation (Kapitel 6) befasst sich genauer mit verschiedenen Populationen von *G. arabica*, die über das gesamte Verbreitungsgebiet der Art vorkommen. Ein besonderer Fokus lag dabei auf einer Population, die auf den Farasan-Inseln beheimatet ist. Da die Populationszahlen auf der Arabischen Halbinsel stark sinken, gleichzeitig auf den Farasan-Inseln aber konstant auf hohem Niveau bleiben, war es für mich von großem Interesse zu untersuchen, ob sich die Inselgazellen für zukünftige Wiederauswilderungen in anderen Gebieten eignen würden. Die Inseltiere zeigten eine im Durchschnitt reduzierte Körpergröße und eine Untersuchung der genetischen Differenzierung zwischen Farasan- und Festlandsgazellen mit Hilfe von Mikrosatelliten ergab eine Zuordnung zu einer genetischen Gruppierung, die einzig auf den Farasan-Inseln gefunden wurde. Etwa 30% der untersuchten Inseltiere konnten jedoch dem Festlandsgenotyp (zumindest teilweise) zugeordnet werden und zeigten damit Zeichen von anhaltender Introgression. Mit Hilfe eines Isolations-Migrations-Modells konnte ich einen bi-direktionalen Austausch von Gazellen zwischen den Inseln und dem Festland rekonstruieren, der vermutlich auf menscheninduzierte Translokation zurückzuführen ist. Der auffallend gleichförmige Zwergwuchs der Inselformen kann daher als phänotypische Plastizität interpretiert werden, d.h. als Reaktion auf die Limitierung der Ressourcen. Sollte der anhaltende Rückgang von *G. arabica* auf der Arabischen Halbinsel nicht gestoppt werden können, könnten Farasangazellen also als mögliche Quelle für Wiederauswilderungen genutzt werden.

III Contents

— Part I Phylogeny of the Genus *Gazella* —

— Part II Phylogeography and Population Genetics of Arabian Gazelle Species —

IV Preface

My PhD thesis comprises scientific work I conducted within the last four years. In order to meet the prerequisites of a cumulative PhD thesis I introduce and critically discuss the research ideas that motivated my thesis and conclude the major lines of discussion of each chapter (chapter 1). The following represents a collection of scientific publications, which were submitted to international, peer-reviewed journals or books. I divided my thesis in two parts with the first one focusing on the phylogeny of the genus *Gazella* (chapter 2). Here, I reviewed the geological history setting the course of gazelle evolution, conducted a phylogeny of *Gazella* and closely related genera and, discuss potential factors that promoted the divergence within the group. The second part of my thesis addresses the phylogeography and population genetics of different Arabian gazelle species. In chapter 3 I could show the polyphyly of goitered gazelles and suggest the full species level for sand gazelles (*G. marica*). In another study that was covered by local media after publication (see my Curriculum Vitae for details) I demonstrated a low genetic differentiation within Dorcas gazelles (*G. dorcas*) and inferred a steep population decline that started already in ancient times (chapter 4). In chapters 5 and 6 I focused on Arabian gazelles (*G. arabica*) and provided a rigorous test for delimitating Arabian from mountain gazelles (chapter 5). Moreover, I inferred recent introgression of mainland Arabian gazelles into an island population, thus, supporting the conclusion that those island gazelles are no distinct conservation management unit (chapter 6).

During the time I worked on my thesis, I was additionally involved in other projects (see my Curriculum Vitae for details). I conducted studies investigating sand gazelles' mate choice behavior and life history traits. I inferred the genetic differentiation and consequences of convergent colonizations of sulfidic springs by different poeciliid species. Therefore, I developed new microsatellite markers out of a 454 sequence run, applied them to sulfidic and non-sulfidic populations of three river drainages and correlated the gene flow patterns with data from translocation and mate choice experiments. I was further involved in a project showing that the key adaptation towards an H_2S-tolerant phenotype in the *P. mexicana*-species complex arose at least three times in parallel from clear water ancestors.

Chapter 1

by Hannes Lerp

An introduction to the phylogeny of the genus *Gazella* and the phylogeography and population genetics of Arabian species with critical discussion

Speciation is a fundamental evolutionary process creating what has been termed species richness or γ diversity (Whittaker 1960; Crist *et al.* 2003). In natural systems three basic modes of speciation are operating within and among populations, namely allopatric, parapatric and sympatric speciation (Bush 1975). Allopatric speciation occurs when extrinsic gene flow barriers emerges, whereas parapatric and sympatric speciation rely on intrinsic gene flow barriers (Bush 1975). Speciation is often accelerated—then called radiation—in cases where species could increase their former distribution range, *i.e.*, colonize new habitats with new ecological niches (*e.g.*, Darwin's finches on Galapagos, Darwin 1839). This can be the case when new land bridges emerge connecting land masses that were previously separated (Vermeij 1991), *e.g.*, the Great American Biotic Interchange after the closure of the Isthmus of Panama (Webb 2006) or the connection of India and South-East Asia (Klaus *et al.* 2010; Li *et al.* 2013). Hence, biological invasions or immigrations of animals can promote speciation (Flynn *et al.* 1991; Vermeij 1991). The investigation of such radiation events, including the paleoclimatic settings in which they occurred, can provide valuable insights into the question of how environmental factors (like the climatological and vegetational setting) affected speciation, *e.g.*, the radiation of bovids that followed the radiation of grasses in expanding savannah and steppe ecosystems in Africa (Matthee & Robinson 1999; Matthee & Davis 2001; Strömberg 2011).

One example for a reconnection of two continents is the land mass bridging Africa and Asia: the Arabian Peninsula. It represents a biogeographic contact zone with floral and faunal elements of both continents coming into contact (Vincent 2008). From the mid-Oligocene (ca. 30 Ma) (Bosworth *et al.* 2005) until now there has been repeated faunal exchange between both continents (Tchernov 1988). As reviewed in chapter 2 (see below) this exchange was asymmetric and changed over geological times. Dispersal abilities of different taxonomic groups were obviously dependent on taxon-specific ecological requirements and physiological capabilities (Sexton *et al.* 2009; Angert *et al.* 2011). Most importantly, ecological and climatic conditions, which naturally change over geological time scales, played a significant role. For terrestrial animals the formation of land bridges is essential for their dispersal (of course, flying animals—especially birds—are less dependent on the existence of land bridges, *e.g.*, Voelker and Light 2011, but see do Amaral *et al.* 2009). Today, the Arabian Peninsula connects Africa and Asia only via the Sinai Peninsula, but there is a lively debate on the former existence of another land bridge located at the Bab al-Mandeb strait between the Red Sea and the Gulf of Aden after the Miocene (*e.g.*, Wildman *et al.* 2004; Winney *et al.* 2004; Fernandes *et al.* 2006; Bailey *et al.* 2009; Fernandes 2009). On the one hand, paleooceaongraphic and paleoecological data militate for the absence of a land bridge after the Miocene and, especially, during the glacial cycles (Fernandes *et al.* 2006), but on the other hand several mammalian and reptilian taxa show a disjunct distribution at the Horn of Africa and southwestern Arabia and a dispersal via a land bridge seems not unlikely (Wildman *et al.* 2004; Winney *et al.* 2004; Portik & Papenfuss 2012).

Amongst mammals, a candidate genus for the investigation of a radiation event in this region is *Gazella*. The genus' present distribution ranges from North Africa (from the Mediterranean coast into the Sahel zone) over the Arabian Peninsula, the Middle East and India to the wide plains of China and Mongolia. The genus *Gazella* belongs to the subfamily Antilopinae, for which the earliest fossil remains were reported from Kenya dating back to the early Miocene (Thomas 1981). However, it remains unclear where the first representatives of gazelle-like antelopes emerged—in Africa (Kingdon 1988), or Asia (Vrba & Schaller 2000). With nine extant species (following the chapters below) the genus *Gazella* is one of the most species-rich genera within the family Bovidae. In Africa, four species can be found, namely Dorcas gazelle (*G. dorcas*; with an extinct subspecies the distribution previously extended onto the Arabian Peninsula; chapter 4), Speke's gazelle (*G. spekei*) being endemic to the Horn of Africa (Heckel *et al.* 2008), slender-horned gazelle (*G. leptoceros*) being distributed in the Sahara (Devillers *et al.* 2006) and Cuvier's gazelle (*G. cuvieri*) being endemic to the Atlas Mountains (Lafontaine *et al.* 1999; Beudels-Jamar, Lafontaine, *et al.* 2006). Today, three species occur on the Arabian Peninsula: Arabian gazelle (*G. arabica*; chapters 5 and 6), Mountain gazelle, representing the nominate form (*G. gazella*; chapter 5), whose distribution extends into Turkey (Kasparek 1986; Kankilic *et al.* 2012), and sand gazelle (*G. marica*; chapter 3), occurring eastwards as far as the Tigris-Euphrates drainage basin. Asian representatives of the genus are chinkara (*G. bennettii*) from Iran, Afghanistan, Pakistan and India (Mallon 2008), and goitered gazelles (*G. subgutturosa*), occurring from east of the Tigris-Euphrates drainage basin onwards to China and Mongolia (chapter 3).

All species of the genus *Gazella* are included in the IUCN Red List of Threatened Species and all except one were categorized "vulnerable" or worse (IUCN 2012). The most important threads for gazelles are intensified hunting due to the use of firearms and motorized vehicles, competition with domestic livestock and habitat destruction leading to an overall decrease of individual numbers in all species (Mallon & Kingswood 2001; Beudels-Jamar, Devillers, *et al.* 2006; IUCN 2012). In order to stop this devastating trend hunting was prohibited by law in most countries and protected areas were established (UNEP/CMC 1998; Mallon & Kingswood 2001). Furthermore, conservation measures were realized in several countries, *e.g.*, captive breeding programs of different gazelle species that aim at the reintroduction of those species into their former ranges (Mallon & Kingswood 2001). But progress of these *ex situ* breeding programs is still hampered by taxonomic uncertainties concerning the assessment of conservation management units (*sensu* Vogler & DeSalle 1994).

The aim of my thesis was to infer a phylogeny of the genus *Gazella* including all extant species and to assess information on possible factors promoting the diversification of the genus in the context of the demographic history, ecology and behavior of the investigated species. The genus *Gazella* was so far phylogenetically and taxonomically poorly defined, and potential intraspecific genetic variation was assessed in only one species (*G. arabica*: Wronski *et al.* 2010). Therefore, phylogeographic and population genetic analyses were conducted for selected species with a focus on the Arabian Peninsula (Fig. 1; *G. marica, G. dorcas*, *G. arabica* and *G. gazella*). I used a large number of individuals to infer knowledge about the spatial and temporal processes causing observable genetic variation within and between closely related species (see Knowles 2009 for a review). To do so, I inferred the past demography of the species by use of coalescence approaches that allow for a direct estimate of the history of divergence and past population parameters, and so, to disentangle complex species' histories (Knowles 2009). Combined with ecological and climatological data coalescent models of population structure can improve the understanding of factors promoting population divergence (Carstens & Richards 2007), and thus, the evolution of the genus *Gazella*. Furthermore, results obtained from those investigations allow for improved conservation decisions (Ashley *et al.* 2003) for threatened gazelle species, especially regarding captive breeding and reintroduction initiatives, and for the assessment of conservation management units (Vogler & DeSalle 1994).

When investigating speciation in a phylogenetic framework, a unifying concept of what is to be considered a species and, more specifically, how species delimitation is accomplished is crucial (Wiens 2007). A variety of species concepts were forwarded, and at least some of them are incompatible (for a review of the most important concepts see, *e.g.*, Coyne & Orr 2004; de Queiroz 2007). In an attempt to reconcile the different movements into one "Integrative Species Concept", de Queiroz (2007) identified an element common in all concepts: that species are separately evolving metapopulation lineages. In this context lineages are defined as ancestor-descendant series and metapopulations as inclusive populations made up of connected subpopulations (de Queiroz 2007 and references therein). Here, the term metapopulation is used to distinguish species from demes and family groups (de Queiroz 2007). Conflicts of different species concepts forwarded to date arose from secondary species criteria that, depending on the concept, arise at different points in time during the speciation process (de Queiroz 2007). According to the Integrative Species Concept no secondary species criterion is necessary, and the properties formerly used to define species become contingent properties, *i.e.*, they are considered as lines of evidence when evaluating the separation of lineages (de Queiroz 2007). Therefore, the Integrative Species Concept separates the theoretical concept of speciation from the operational lines of evidence that are empirically applicable. To delimitate species multiple lines of evidence are strongly recommended, because single operational lines of evidence might be misleading when interpreted inappropriately (de Queiroz 2007). Throughout my thesis this species concept was applied, because it provides an alternative to the debates about different species definitions and encourages the use of multiple lines of evidence, *i.e.*, the application of different methods in delimitating species.

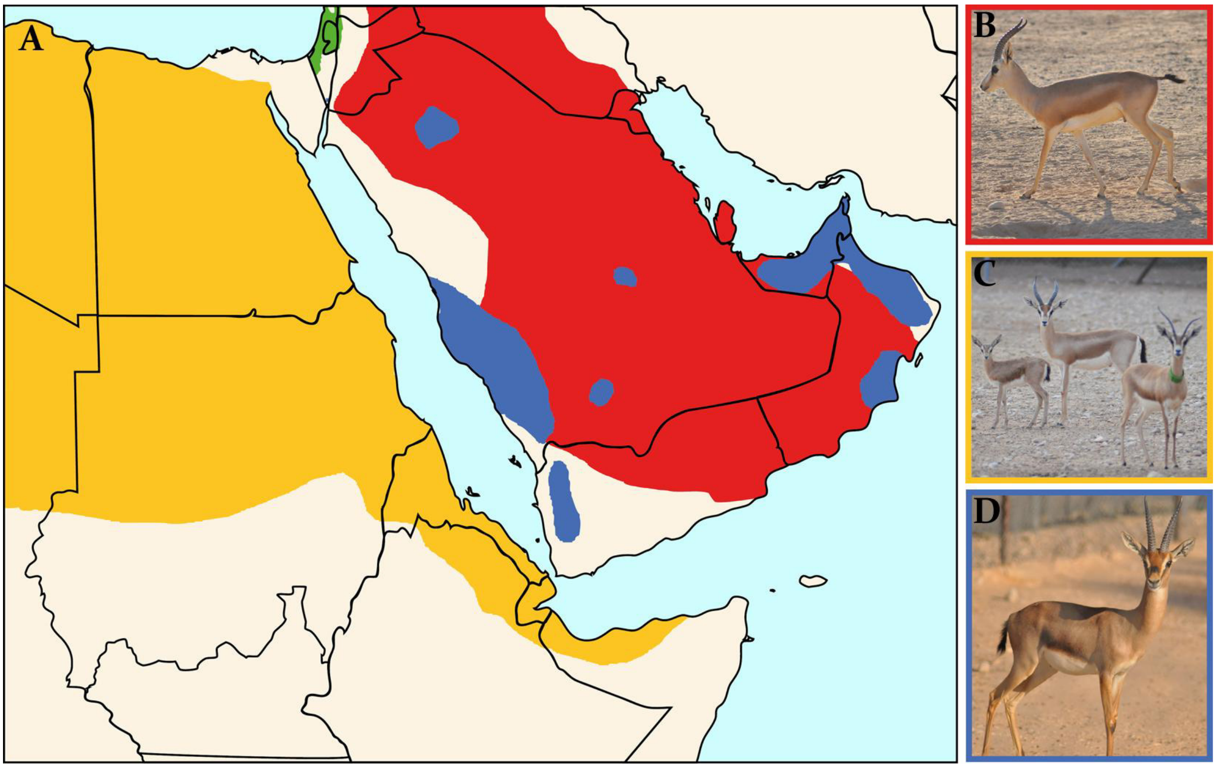

Figure 1 *Arabian gazelle species. (A) Map of the Arabian Peninsula with potential distribution areas of Arabian gazelle species (compiled and modified from IUCN/SSC Antelope Specialist Group 2008a; b; c) with* G. marica *(red shade),* G. dorcas *(yellow shade; Arabian forms have already gone extinct),* G. arabica *(blue shape) and* G. gazella *(green shape). Note: current distribution areas are likely to be smaller. (B – D) Arabian gazelle species held at King Khalid Wildlife Research Centre, Saudi Arabia, for captive breeding and reintroduction purposes; all photos taken by Hannes Lerp at the center. (B)* G. marica. *(C)* G. dorcas. *(D)* G. arabica.

Chapter overview

In this section I will provide a short introduction into the following chapters of my thesis and explain the main methodology, the most important results and summarize major lines of discussion. Each chapter consists of a scientific publication, which was submitted to an international, peer-reviewed journal or book. In total, my thesis comprises five publications: one book chapter (chapter 2), three articles already published in international journals (chapters 3-5) and one submitted article (chapter 6). All of them address either the phylogeny of gazelles (chapter 2) or the phylogeography and population genetics of an Arabian gazelle species (chapters 3-6).

Speciation of Arabian gazelles (chapter 2)

In chapter 2 an overview over the geological and climatic settings under which the genus *Gazella* evolved is given. The first connection of Africa and Asia took place at the Proboscidean Datum Event (ca. 20 Ma; Madden & van Couvering 1976), when a new land bridge (*i.e.*, the *Gomphotherium* Land Bridge; Rögl 1999) emerged separating the Mediterranean Sea and Indian Ocean. Thus, Asian faunal elements were for the first time enabled to disperse into the Arabian Peninsula (Rögl & Steininger 1983) that was previously inhabited by African fauna only (Tchernov 1988; Delany 1989). Due to intense rifting of the Red Sea during the early Miocene the Arabian Peninsula became increasingly separated from Africa (Bosworth *et al.* 2005). Simultaneously, a radiation of several bovid tribes seems to have taken place in Africa (Matthee & Robinson 1999) once several representatives of bovids were able—after their adaptation to arid desert environments—to cross the Saharo-Arabian arid belt (Thomas 1979; Tchernov 1988). At the boundary of Miocene and Pliocene (6 Ma) the Messinian Salinity Crisis took place (Krijgsman *et al.* 1999) corresponding with the regression of water levels in the Mediterranean Sea. This led to an increased faunal exchange between Africa and Eurasia (Hassanin & Douzery 1999; Agusti *et al.* 2006). After the Messinian Salinity Crisis the Arabian Peninsula became gradually more isolated because in the west the water inflow into the Red Sea increased and hampered dispersal from Africa, while in the east the Zagros Mountains rose, separating Arabia from Asia (Tchernov 1988; Bosworth *et al.* 2005). The faunal interchange between the Arabian Peninsula and Africa via the Sinai Peninsula became asymmetric in the Pleistocene: mountain-adapted Asian species could disperse more easily along the mountain ridges of Arabia and the Sinai into northern Africa, while arid-adapted Saharan species could only disperse along a narrow corridor of sand dunes along the northern Sinai and had to cross the Aqaba-Levant Transform Fault (Ferguson 1981; Tchernov 1988; Delany 1989).

These geological events set the course for the evolution of the genus *Gazella* as it is known today. To study evolutionary pathways of gazelles a phylogeny of all present-day 'true gazelles'—*i.e.*, genera *Nanger*, *Eudorcas*, *Antilope* and *Gazella*—(*sensu* von Boetticher 1953; Groves 1988, 2000; Groves & Grubb 2011) was inferred using mitochondrial cytochrome *b* gene sequences of all species of 'true gazelles' while using the genera *Antidorcas* (springbok) and *Litocranius* (gerenuk) as outgroups. Here, a monophyly of gazelles (dating back 10.5-6.3 Ma) could be inferred but its sister group relationships remained unresolved (Fig. 2). Furthermore, the genus *Gazella* formed a monophyletic clade with most species splits receiving high statistical support. These results were comparable with findings by Rebholz and Harley (1999) and Hassanin *et al.* (2012) who also used mitochondrial markers, but as their studies showed less phylogenetic resolution or were focused on the evolution of Cetartiodactyla, respectively, no conclusions about possible scenarios explaining speciation within the genus *Gazella* were drawn.

Within *Gazella* two reciprocally monophyletic lineages were statistically well supported (Fig. 2, chapter 2). One clade comprised the species *G. dorcas*, *G. spekei*, *G. arabica* and *G. gazella* and shows a primarily African distribution ('African clade'), whereas the second clade comprised all Asian taxa (*G. subgutturosa* and *G. bennettii*) as well as *G. marica*, *G. leptoceros* and *G. cuvieri* and has a primarily Asian distribution ('Asian clade'). Both clusters emerged in the early Pleistocene, presumably in allopatry and comprised extant taxa from the "opposite" continent (chapter 2). For the African clade *G. dorcas*-like gazelles are hypothesized to represent the ancestral character state combination (Lowenstein 1986; Gentry 1992; Vassart *et al.* 1995) with *G. spekei*, *G. arabica* and *G. gazella* being derived forms that emerged at the edges of the ancestral distribution range, *i.e.*, at the horn of Africa (*G. spekei*) and on the Arabian Peninsula (*G. gazella* and *G. arabica*) (chapter 2). With respect to the Asian clade it is hypothesized that the diversification started in central Asia (forming a reciprocally monophyletic clade consisting of *G. subgutturosa* and *G. bennettii*), followed by a range extension westwards onto the Arabian Peninsula (*G. marica*) as far as into the Atlas Mountains (*G. leptoceros* and *G. cuvieri*), with the latter species forming yet another monophyletic clade (chapter 2).

Within both major clades species pairs exist that may have evolved adaptively in response to divergent ecological selection. These pairs uniformly consist of one species being a desert-adapted grazer, and forming herds (supposed to be the ancestral character state combination), and one species being a browser, more humid-adapted, sedentary and showing a territorial social organization (Fig. 2, chapter 2). From the phylogenetic analysis conducted three such species pairs could be inferred, *i.e.*, *G. dorcas* vs. *G. gazella* and *G. arabica*, *G. subgutturosa* vs. *G. bennettii*, and *G. leptoceros* vs. *G. cuvieri*. For these speciation events the concept of parallel speciation forwarded by Schluter and Nagel (1995) might be applicable. Schluter and Nagel (1995) introduced three criteria to be met for parallel speciation to occur: "(1) separate populations in similar environments must be phylogenetically independent [...], (2) ancestral and descendant populations [...] must be reproductively isolated, and (3) separate descendant populations inhabiting similar environments must not be reproductively isolated from one another". When applying this definition to potential parallel speciation events in the phylogeny of gazelles it must be interpreted in a broader sense, because adaptation to a more humid climate occurred independently from different ancestral lineages (*i.e*, the third criterion is not met). The oldest ecologically divergent species pair that could be inferred from the phylogenetic analysis was *G. dorcas* vs. *G. gazella* and *G. arabica* and emerged 2.8–1.6 Ma ago (chapter 2). *G. dorcas* are grazers, inhabit semi-arid gravel and sand deserts and migrate a considerable time of the year (Yom-Tov *et al.* 1995; Wacher *et al.* 2004), while *G. gazella* and *G. arabica* are sedentary and live in upland areas of broken terrain (Mendelssohn *et al.* 1995; Martin 2000). In case of *G. subgutturosa* vs. *G. bennettii*, species split ca. 2.4–1.3 Ma ago (chapter 2) with *G. bennettii* being sedentary and only occurring at the edge of deserts (Roberts 1977; Sharma 1977; Rahmani 1990; Karami *et al.* 2002), and *G. subgutturosa* being semi-nomadic while also occurring in desert regions (Kingswood & Blank 1996). The youngest species pair is that of *G. leptoceros* and *G. cuvieri*. It emerged 0.4–0.1 Ma ago (chapter 2) and provides a good example of how the Integrative Species Concept could be applied: during the short time of diversification of both species only few substitutions in neutral molecular markers accumulated, such that the species status for both taxa had been doubted (Hassanin *et al.* 2012). Nonetheless, both species show remarkable morphological and ecological differences, with *G. leptoceros* being a nomadic desert dweller and *G. cuvieri* being sedentary while inhabiting the dry forest of the Atlas Mountains (Gentry 1964; Groves 1969; Sellami & Bouredjli 1991; Saleh 2001; Smith *et al.* 2001; Beudels-Jamar, Lafontaine, *et al.* 2006; Groves & Grubb 2011; Louys *et al.* 2011). Following the Integrative Species Concept both taxa can be regarded as differed species when considering all lines of evidence available.

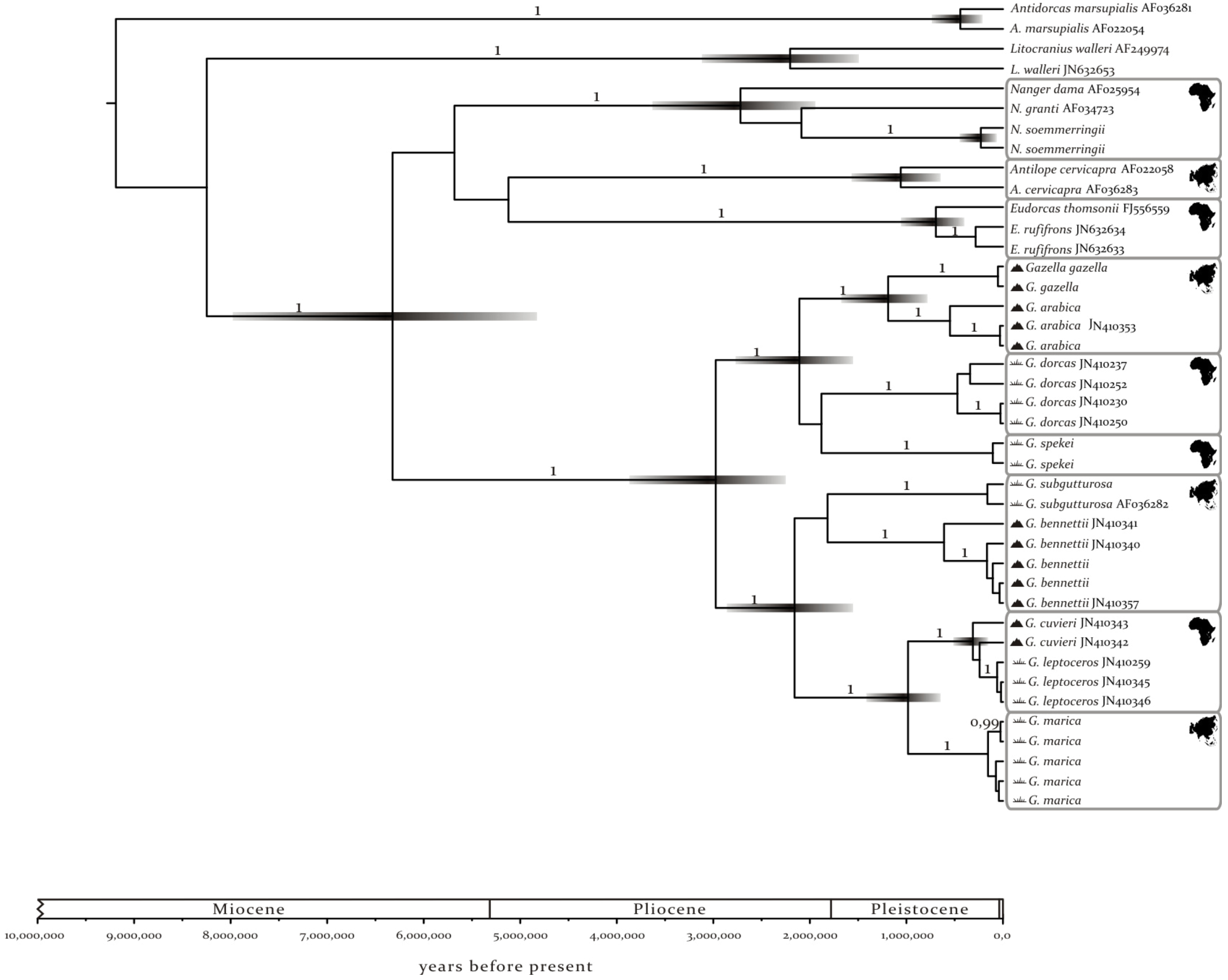

***Figure 2**: Phylogeny based on the alignment of the complete sequences of the cytochrome* b *gene. Bayesian analysis was performed with 41 sequences using the HKY + Γ substitution model. Only posterior probability values larger than 0.9 are reported. Node bars represent the 95% credibility intervals of the divergence times of statistically supported phylogenetic splits. Symbols of Africa and Asia indicate the occurrence of single species on that continent. Within the genus* Gazella, *a mountain symbol indicates a more humid and/ or mountainous habitat, whereas a grass symbol indicates open savannah/ desert habitat. Taken from chapter 2, not modified.*

Phylogenetic analysis of mitochondrial DNA sequences reveals polyphyly in the goitred gazelle (*Gazella subgutturosa*) (chapter 3)

In chapter 3 the question was considered as to whether what was once considered to be a single species—'*G. subgutturosa*'—in reality comprises more than one species. The commonly accepted view was that *G. subgutturosa* only comprises a single species whose large distribution range extends from the Arabian Peninsula in the west as far as Mongolia and China in the east (Kingswood & Blank 1996; Mallon & Kingswood 2001). Several subspecies have been described within this amazingly large distribution range. On the Arabian Peninsula the subspecies *G. s. marica* (sand gazelle) can be found in open habitats (Groves & Harrison 1967). Its range extends further into Jordan, Syria, southern Turkey and Iraq (Mallon & Kingswood 2001). Typically, *G. s. marica* has a pale body color with a white face, and females often bear long slender horns (Groves & Harrison 1967; Kingswood & Blank 1996). Further eastwards, *G. s. marica* gets replaced by *G. s. subgutturosa*, which is readily distinguishable from *G. s. marica* by a larger body weight, adult females mostly bearing no horns, and males showing a remarkable swelling on the larynx during the rutting season (Kingswood & Blank 1996). The taxonomy of sand gazelle (*G. s. marica*) has changed over the last century. Initially, it was described as full species (*G. marica*) by Thomas (1897), but became later synonymized with slender-horned gazelle (*G. leptoceros*) (Ellerman & Morrison-Scott 1951). More recently, it was assigned subspecies status (*G. subgutturosa marica*) based on karyological and morphological investigations (Groves & Harrison 1967; Kingswood *et al.* 1996, 1997).

Unfortunately, sand gazelles already disappeared from large parts of their natural distribution range because of hunting and over-grazing by domestic livestock (Mallon & Kingswood 2001). In Saudi Arabia the taxon can be found only in four protected areas of which two represent reintroduced populations (Haque & Smith 1996; Thouless *et al.* 1997; Cunningham & Wacher 2009). For successful captive breeding and reintroduction initiatives of both taxa knowledge about their phylogenetic relationships and easy identification of individuals is vital to avoid mixing of distantly related taxa and to detect suitable breeding stock allowing the maintenance of genetic variability and avoiding inbreeding. The aim of this study was to analyze the relationship of both taxa by sequencing the mitochondrial cytochrome *b* gene of specimens originating from the wild with known origin, from museum collections and from captive breeding stocks (chapter 3). Furthermore, my study aimed at providing an easy-to-use approach for breeding centers to test for the taxonomic affiliation of their stocks.

The phylogenetic tree obtained from the sequence data (a 333 bp long fragment of the cytochrome *b* gene) was constructed using the software RAxML 7.0.3 (Stamatakis 2006) and used the substitution model GTR + I + Γ. It could be shown that sand gazelles are more closely related to *G. leptoceros* than to *G. s. subgutturosa*. The grouping of '*G. s. marica*', *G. leptoceros* and *G. cuvieri* revealed high statistical support. The alternative hypothesis of *G. subgutturosa* being a monophylum (*i.e.*, comprising both *G. s. subgutturosa* and *G. s. marica*) was significantly rejected (*P* = 0.001) using the Approximately Unbiased-test implemented in CONSEL (Shimodaira & Hasegawa 2001, chapter 3). Mitochondrial '*G. s. marica*'-haplotypes were found in specimens from Saudi Arabia, Jordan, Syria, and Oman, while '*G. s. subgutturosa*'-haplotypes were found in Iraq, Iran, Afghanistan, Azerbaijan and Chinese Turkistan (chapter 2). The status of presumed sand gazelles in southern Turkey could not investigated as I lacked samples from that region, but it could be shown more recently that sand gazelles still exist in that region and exhibit '*G. s. marica*'-haplotypes (Kankilic *et al.* 2012). The findings of my phylogenetic analysis support the notion by Hammond *et al.* (2001) that *G. s. marica* and *G. s. subgutturosa* could actually be more distantly related than previously suggested. Furthermore, the close relationship of '*G. s. marica*' to *G. leptoceros*—firstly suggested by Ellerman and Morrison-Scott (1951)—could be corroborated and contradicts other classifications inferred from skull and horn analyses (Groves & Harrison 1967).

With respect to recommendations as to the taxonomy of the taxa under consideration—an important question, e.g., for conservation legislation—restoration of sand gazelles to full species level, *G. marica*, seemed most helpful (chapter 3). However, the results were based on mitochondrial markers only and could, therefore, be confounded by mitochondrial introgression or male dispersal combined with female philopatry (Ballard & Whitlock 2004; see Apio *et al.* 2010 for such a pattern in bushbuck). Indeed, a study investigating the morphology and mitochondrial haplotype variation of eastern Turkish gazelles found signs of maternal introgression of *G. marica* mitochondrial DNA into *G. subgutturosa* (Murtskhvaladze *et al.* 2012). Nevertheless, *G. subgutturosa* and *G. marica* show pronounced morphological differences: *G. marica* females normally bears long horns, whereas horns are mostly absent in *G. subgutturosa* (Groves & Harrison 1967). Furthermore, samples from Iraq—supposedly showing intermediate morphological character state combinations—clustered with *G. subgutturosa* in the phylogenetic analysis (chapter 3). Therefore, it is suggested that no range overlap or male-biased introgression exist, which contrasts earlier views that assumed a hybrid zone in Iraq (Groves & Harrison 1967; Groves 1997; Al-Robaae & Kingswood 2001).

For captive breeding purposes a method was established (chapter 3) that allows for fast assignment of samples to *G. marica* or *G. subgutturosa* haplotypes using amplified fragment length polymorphism (AFLP). Using PCR primers, restriction enzymes, a PCR thermocycler and subsequent gel electrophoresis the method enables the high-throughput identification of individuals while keeping costs relatively low. In chapter 3 a combination of four restriction enzymes is presented that allows for unambiguous assignment of the tested haplotypes. In total, 894 individuals could be tested with this method and biogeographical patterns of the phylogenetic tree could be confirmed.

A phylogeographic framework for the conservation of Saharan and Arabian Dorcas gazelles (chapter 4)

Chapter 4 focus on the species with the largest distribution area of the 'African Clade' (*sensu* chapter 2): the Dorcas gazelle (*Gazella dorcas*). It once occurred from Morocco over Israel and the Sinai Peninsula into Mauretania and Somalia (*e.g.*, Yom-Tov *et al.* 1995; East 1999; Wacher *et al.* 2004). On the Arabian Peninsula a local variety of *G. dorcas* showed a distinct morphology compared to African conspecifics (Groves 1988; Rebholz *et al.* 1991; Hammond *et al.* 2001). Consequently, this lineage was described as a distinct species: the Saudi gazelle (*G. saudiya*; Carruthers & Schwarz 1935). Dorcas gazelles occur in a variety of habitats from Sahelian savannahs to semi-arid gravel plains but avoid hyper-arid areas and the upper elevations of Hoggar and Tibesti Mountains (Charlisle & Ghobrial 1968; Ghobrial 1970, 1974; Baharav 1980, 1982). Like most larger mammalian species of the desert eco-regions of North Africa and the Arabian Peninsula, Dorcas gazelles are threatened by hunting, poaching, and to a lesser extent by habitat loss and desertification (Mallon & Kingswood 2001), which already led to the extinction of the Arabian form *G. saudiya* (Habibi & Williamson 1997; Thouless *et al.* 1997; Hammond *et al.* 2001). For African Dorcas gazelles a severe decline was reported for each country they inhabit (except Israel and Ethiopia) with a total loss of more than 30% of the individuals within three generations, and only 25% of the remaining animals live in protected areas (Mallon & Kingswood 2001). The fragmentation of the remaining populations strongly increased within only a few decades (UNEP/CMC 1998; Mallon & Kingswood 2001; Lafontaine *et al.* 2006). Taken together, those trends led to an IUCN Red List classification of 'vulnerable' for Dorcas gazelles (IUCN/SSC Antelope Specialist Group 2008a).

In order to stop this devastating trend, different conservation actions were established ranging from the prohibition of hunting and the establishment of protected areas to captive breeding initiatives (UNEP/CMC 1998; Lafontaine *et al.* 2006). If laws against hunting would be enforced, conservation efforts may still bear fruits in the near future. This is because most of the areas from which *G. dorcas* have already disappeared are still suitable sites for future reintroductions as they are not degraded through over-exploitation (Mallon & Kingswood 2001). However, conservation efforts have been hindered by an uncertain taxonomy, which is characterized by a lack of phylogenetic information, while at the same time pronounced phenotypic variability can be observed (Ryder 1986, 1987; Hammond *et al.* 2001). The latter—in the form of differences in horn length and shape, fur coloration and other morphological characteristics—provided the basis for the description of various subspecies (Groves 1969, 1985a; b, 1988; Yom-Tov *et al.* 1995) with uncertain genetic and no obvious ecological differentiation (Lafontaine *et al.* 2006). However, to successfully set captive breeding programs into action and to ensure the success of potential reintroduction initiatives, knowledge about phylogenetically appropriate populations (or management units) is mandatory (Avise 1989; Vogler & DeSalle 1994).

In order to infer the phylogeographic relationships between populations more than 70 individuals of *G. dorcas* and *G. saudiya* from the entire distribution range were sequenced for the mitochondrial cytochrome *b* gene and control region. Furthermore, I made an attempt to reconstruct the phylogeographic origin of the species and its historic population demography by using a coalescence approach.

A phylogenetic tree using the GTR + I + Γ substitution model for a Bayesian Inference approach based on these sequence data uncovered reciprocal monophyly for the genus *Gazella* comparable to the results presented in chapter 2. Within the genus all Dorcas gazelles (except one presumably misidentified individual from Israel) were grouped into one clade that gained maximum posterior probability support (PP = 1.00; chapter 4). For the first time my phylogenetic analysis of Dorcas gazelles from their entire distribution range (IUCN/SSC Antelope Specialist Group 2008a) could demonstrate the surprisingly low phylogeographic structure within this species despite of morphological variation between different populations (*e.g.*, Groves 1969, 1985b; Alados 1987; Yom-Tov *et al.* 1995). As I will discuss below, in other gazelle species closely related to *G. dorcas*, (*i.e.*, *G. gazella* and *G. arabica*; chapter 5), morphological traits were also weak indicators for genetic differentiation. Thus, results of phylogenetic analyses and morphological classifications schemes often lead to conflicting species boundaries by splitting morphologically diverse taxa with low genetic structure into several species or subspecies (*G. dorcas*) or by comprising morphologically similar taxa with a high genetic differentiation into one species (*G. gazella* and *G. arabica*; see below).

Within the clade of Dorcas gazelles three clusters were uncovered showing posterior probability values from 0.98 – 1.00. However, branch lengths within the Dorcas clade were low and a statistical parsimony network analysis using TCS (Clement *et al.* 2000) inferred a pronounced haplotype sharing between different sampling regions, which may be indicative of ongoing gene flow. In the latter analysis sequence data of *G. saudiya* could be included and two distinct haplotypes were detected [congruent with the findings of Hammond *et al.* (2001)]. Although both *G. saudiya* haplotypes were monophyletic, the genetic distance to other *G. dorcas* samples was low (two mutational steps) and ranged within the variability of Dorcas gazelles. Therefore, following the Integrated Species Concept (de Queiroz 2007) Dorcas gazelles, including *G. saudiya*, can be regarded as one species comprising no separately evolving metapopulations (*sensu* de Queiroz 2007). In terms of conservation Dorcas gazelles could be regarded as a single conservation unit (*sensu* Vogler & DeSalle 1994).

Another aim of this study was to infer the origin and past demography of Dorcas gazelles. Using a Bayesian Skyline model implemented in the software BEAST (Drummond & Rambaut 2007) the time to the most recent common ancestor could be estimated at 768,000 years before present. Therefore, the shallow genetic structure and the presumed gene flow within *G. dorcas* could be the result of a recent range expansion. The south-eastern and south-central groups of Dorcas gazelles could be inferred as the possible origin of the species. Here, haplotype diversity within and haplotype sharing between both groups was highest, and no significant population differentiation was observable (unlike in other group comparisons). I argue that *G. dorcas* later expanded their range into the western parts of North Africa and the Arabian Peninsula from the south eastern and central parts of its distribution range. How Dorcas gazelles crossed the Red Sea to enter the Arabian Peninsula could not be resolved in detail. Even though the results were not statistically significant, tests for Isolation-By-Distance using different migration scenarios suggested the northern route via the Sinai Peninsula rather than the southern route via the Bab al Mandab strait as the most likely dispersal corridor for Dorcas gazelles to reach the Arabian Peninsula (chapter 4). This result support the view of Fernandes *et al.* (2006) who analyzed palaeoceanographic and palaeoecological data and found no indication for a land bridge existing in the Bab al Mandab area for the last 470,000 years. Interestingly, gazelles originating from the Sinai Penisula and Israel showed a significant population differentiation to conspecifics from the western part of North Africa, indicating that the river Nile acts as a geographical barrier between both, hindering migration and, consequently, gene flow. However, to address this hypothesis in detail a much larger sampling, especially from the Western Desert in Egypt and Libya, would be needed in future studies (see below).

Concerning the population demography of Dorcas gazelles, a steep decline of the effective population size could be uncovered starting approximately 27,000 years before present and continuing until today (Lafontaine *et al.* 2006) (Fig. 3). The onset of this decline coincides with human hunting activities firstly involving spears as weapons (Nentwig 2007). Indeed, stony spear tips were often found together with large game in archaeological sites (Legge & Rowley-Conwy 1987; Nentwig 2007). Furthermore, hunting of gazelles was continued in Near East post-Neolithic societies—even after the domestication of cattle (Götherström *et al.* 2005)—and was a communal practice with religious connotations leading to a massive decline of population sizes (Bar-Oz *et al.* 2011). Thus, hunting that started already in ancient times had a much larger impact on *G. dorcas* populations than previously thought.

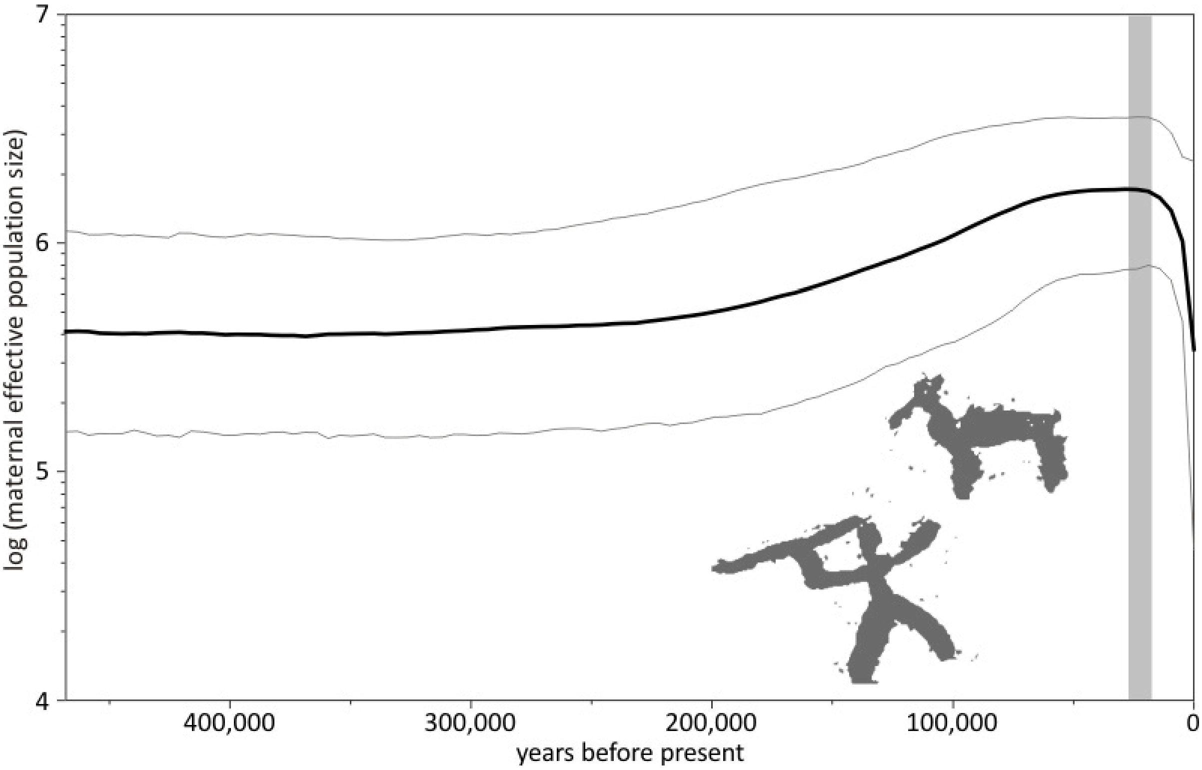

Figure 3 *Bayesian Skyline Plot showing maternal effective population size (mean and 95% confidence interval) based on 1,612 bp concatenated cytochrome* b *gene and mitochondrial control region sequences of 53 Dorcas gazelles over time (x-axis: years before present). The gray shaded area marks the onset of the decline in effective population size. Inserted figure shows a hunting scene from ancient Saharan rock art illustrating our interpretation of the cause of the observed population decline. Taken from chapter 4, unchanged.*

Phylogenetic and population genetic analyses suggest a potential species boundary between Mountain (*Gazella gazella*) and Arabian gazelles (*G. arabica*) in the Levant (chapter 5)

Chapter 5 deals with the species that created most confusion among taxonomists working with antilopine bovids: the Mountain gazelle (Groves & Harrison 1967; Groves 1969, 1985a, 1989; Lange 1972; Groves & Lay 1985; Vassart *et al.* 1995; Greth *et al.* 1996; Wronski *et al.* 2010). The taxonomy of mountain gazelles had a lively history, at times lumping all gazelles from the Arabian Peninsula and the Levant into one species (*G. gazella*) (*e.g.*, IUCN/SSC Antelope Specialist Group 2008b), while other authors considered up to four species (*G. gazella*, *G. bilkis*, *G. arabica*, and *G. erlangeri*) and up to eight subspecies (Groves 1996, 1997; Grubb 2005; Groves & Grubb 2011).

In the context of a phylogeny of the Antilopinae based on mitochondrial markers (cytochrome *b* and cytochrome *c* oxidase III), it was firstly suggested that two genetically distinct lineages might exist within Mountain gazelles; one occurring in the Levant and northern Turkey (henceforth called Mountain gazelle, *G. gazella*) and the other in the Negev desert and the entire Arabian Peninsula (henceforth called Arabian gazelle, *G. arabica*) (Rebholz & Harley 1999). The aforementioned study, however, was only based on five specimens and the question arose as to whether the observed pattern resulted from pronounced intraspecific variation or if two distinct species exist. Recently, a more detailed study comprising more specimens from the entire Arabian Peninsula provided further support for the hypothesis of two distinct species (Wronski *et al.* 2010). Unfortunately, molecular analyses were uniformly based on mitochondrial sequence data, and the spatial distribution pattern of maternally inherited haplolines could simply reflect female philopatry combined with male-biased dispersal (chapter 5).

In order to overcome the limitations of mitochondrial markers alone (*e.g.*, introgression, incomplete lineage sorting) (see Funk & Omland 2003 for a review) a study combining mitochondrial sequence and microsatellite marker data was conducted (chapter 5). To infer recurrent gene-flow between both lineages the study focused on Israel and the western parts of the Arabian Peninsula to cover the area where both lineages possibly co-occur and might hybridize. In total, 47 specimens could be included, 21 of which were reanalyzed from Wronski *et al.* (2010). The mitochondrial sequence data were analyzed under a HKY + Γ substitution model and molecular clock estimates inferred from chapter 4 using the software BEAST (Drummond & Rambaut 2007). Furthermore, the software MEGA (Tamura *et al.* 2011) and FSTAT (http://www2.unil.ch/popgen/softwares/fstat.htm) were used to infer Kimura-2-

parameter pairwise *p*-distances (K2P) to test for differences in intra- and interspecific sequence divergence (with K2P × 100) (see Tobe *et al.* 2010) using a Mantel-Test. Microsatellite data were analyzed using the software GENETIX (Belkhir *et al.* 2004) and STRUCTURE (Pritchard *et al.* 2000).

It could be shown that the phylogenetic split described by Rebholz and Harley (1999) and Wronski *et al.* (2010) also became apparent in this analysis. Of special interest were specimens collected in Central Israel that were assigned to the *G. gazella* clade, because previous analyses could only include samples from the Golan Heights as references for *G. gazella* (Wronski *et al.* 2010). The divergence time for both lineages could be estimated approximately one million years before present. Within-species K2P values (× 100) were found to fall into the intraspecific range (*sensu* Tobe *et al.* 2010) for both lineages, whereas between-species values were found to border the definite inter-specific value (*sensu* Tobe *et al.* 2010). A Mantel-test found significant support for stronger between- than within-species differentiation. However, it should be mentioned that K2P values should be discussed with caution and may be sufficient but not necessary for species delimitation (Srivathsan & Meier 2012).

The population genetic analyses also supported the genetic distinctiveness of Mountain and Arabian gazelles, since no recurrent gene-flow could be detected between groups (Fig. 4). GENETIX separated all individuals by species without using prior information on sampling origin (Fig. 4A), and STRUCTURE found $K = 2$ as the uppermost level of population differentiation with assignment scores for each individual being larger than 0.9 for assignment to the respective species (Fig. 4B, C). However, these results did not necessarily provide support for separate species status of both lineages and might also emerge in highly fragmented populations of the same species, particularly if one or both had undergone recent demographic bottlenecks. Nevertheless, when combining the results derived from mitochondrial sequence and microsatellite analyses both taxa—Mountain and Arabian gazelles—can be interpreted as separately evolving metapopulation lineages (*sensu* de Queiroz 2007), although inclusion of nuclear sequence markers is highly warranted in future studies to evaluate if these metapopulations refer to good species (chapter 5).

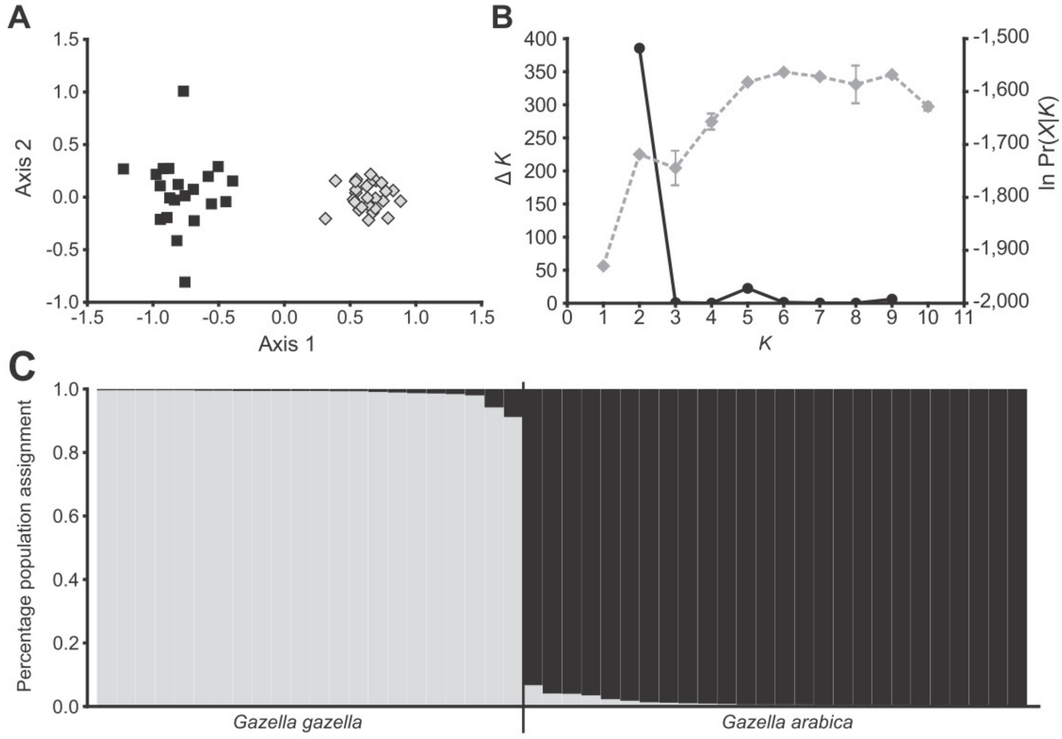

Figure 4 Number of genetically distinct groups in the data set. (A) Factor correspondence analysis of allele frequencies obtained from GENETIX (black squares and gray diamonds representing G. arabica *and* G. gazella*, respectively). (B) Estimated ln Pr (*X|K*) (grey diamonds) and ΔK (black circles) as a function of* K *inferred from STRUCTURE results. (C) Percentage population assignment to inferred genetic clusters for* K=2. *Taken from chapter 5, unchanged.*

Furthermore, both lineages can be separated by morphological and ecological characteristics. They differ in body size, fur coloration and horn characteristics (see Wronski *et al.* 2010) and are separated by the 500 mm-isohyet that divides the Eastern Mediterranean Region of the Levant and north Turkey—characterized by winter precipitation of 1.800 mm—from the Western and Southern Arabian Region—characterized by precipitation between 100 and 450 mm (Yom-Tov & Ilani 1987; Abdulsalam *et al.* 1988). It can be hypothesized that Mountain gazelles are adapted to Mediterranean climate corresponding with a broad-leaved diet (Baharav 1980, 1982), whereas Arabian gazelles are adapted to unpredictable rain fall corresponding with xeromorphic shrubs (Vesey-Fitzgerald 1952).

In terms of taxonomical nomenclature the nominate form refers to gazelles from the Levant and northern Turkey, as *G. gazella* was originally described as *Antilope gazella* (Buffon, 1764) from this region. But what name should the lineage from the Arabian Peninsula be assigned to? Recent molecular analyses using the cytochrome *b* gene could show that the type specimen of *G. arabica* (described as *Antilope arabica*, Lichtenstein, 1827) seems to be invalid, because skin and skull did not form a separate lineage, but clustered with *G. gazella* (skull) and with *G. arabica* (skin) (Bärmann, Börner, *et al.* 2013). When following the rules of precedence (priority rule, International Code of Zoological Nomenclature, ICZN) the gazelles of the Arabian Peninsula could be assigned to the name *G. arabica* (Bärmann, Börner, *et al.* 2013). However, as the sampling scheme was restricted to Western Arabia it could not be ruled out that Arabian gazelles originating from, *e.g.*, Oman or the United Arab Emirates would need to be given yet another species name as they could be another separately evolving metapopulation (but see chapter 6). So far, three proclaimed species of Arabian gazelles (Groves & Grubb 2011) could be included in chapter 5 and no genetic differentiation was detectable, casting doubt on the validity of those species.

For conservation and reintroduction efforts, the results of chapter 5 further support the advice to breed Mountain and Arabian gazelles separately (Wronski *et al.* 2010). Since the IUCN status 'vulnerable' was assigned to the combination of Arabian and Mountain gazelles (IUCN/SSC Antelope Specialist Group 2008c), it is easily conceivable that the situation is actually worse for both taxa. About 3,000 individuals of *G. gazella* were estimated suffering from a decreasing population trend in the last decades (IUCN/SSC Antelope Specialist Group 2008c). For *G. arabica* a total number of 11,000 specimens was estimated on the entire Arabian Peninsula (IUCN/SSC Antelope Specialist Group 2008c) with local populations sizes often equaling fewer than 100 individuals (T. Wronski, *pers. comm.*).

Utility of island populations in reintroduction programs—relationships between Arabian gazelles (*Gazella arabica*) from the Farasan Archipelago and endangered mainland populations (chapter 6)

Chapter 6 focuses on an island population of the Arabian gazelle (*G. arabica*) and its special phenotypic and genetic features. Generally, in mammals a pattern of altered (in- or decreased) body size in island populations is observable (Forster 1964), that later became known as the "Island Rule" (van Valen 1973). In ungulates island populations often show dwarfism (Raia & Meiri 2006). But how should conservationists deal with island populations of an endangered species when mainland populations diminish? The concept of "evolutionarily enlightened management" underlines the importance of considering the evolutionary history of a given population for sustainable conservation management decisions but also evolutionary consequences arising from those decisions (Ashley *et al.* 2003).

In Arabian gazelles (*G. arabica*) such an island population is known from the Farasan Archipelago, which is located in the Red Sea, 40 km off the coast of the Saudi Arabian harbor town Jizan (Fig. 5). This population is the largest known in Saudi Arabia and maintained stable individual numbers since 1988 (Cunningham & Wronski 2011). In contrast, all mainland populations showed a drastic downward trend of individual numbers (see chapter 2). This highlights the urgent need to clarify the conservation status of Farasan gazelles to assess the potential suitability of this population for future reintroductions.

Farasan gazelles are morphologically distinct from mainland conspecifics. Following the "Island Rule" body size is reduced and also island gazelles show unique morphological features that led to the description of those gazelles as a distinct subspecies (*Gazella gazella farasani*) (Thouless & Al Bassri 1991). A molecular analysis using mitochondrial markers revealed two lineages of Arabian gazelles occurring on the Farasan Island and thus casting doubt on the genetic uniqueness of those gazelles (Wronski *et al.* 2010). The authors hypothesized that one lineage resulted from an ancient colonization, whereas the other may have been introduced by man more recently (Wronski *et al.* 2010). Nevertheless, the resolution of the phylogenetic analysis was low and the study was based on maternally inherited markers only. Therefore, the question remained whether Farasan gazelles should be treated as distinct management unit.

In order to answer this question a population genetic study was conducted using 11 nuclear microsatellite markers (chapter 6). In total, 66 specimens from the Farasan Islands and 48 specimens from different mainland populations were genotyped (Fig. 5). The number of genetically distinct clusters was assessed using STRUCTURE (Pritchard *et al.* 2000) and STRUCTURE HARVESTER (Earl & VonHoldt 2011). Historical and recent migration, time since separation from the mainland and effective population sizes of island and mainland populations were estimated using an isolation-with-migration-model implemented in IMa2 (Hey 2010). Furthermore, skull measurements of Farasan and mainland gazelles were analyzed to quantify phenotypic differences between the groups.

Furthermore, animals confiscated by Saudi Arabian customs were genotyped that were intended to be illegally traded on Akhoba Market near Jizan (Fig. 5). They were tested against the microsatellite reference data base derived from genotyped individuals from the entire distribution range of *G. arabica* (chapter 6). The aim was to infer the origin of confiscated animals and gain information on possible trading routes.

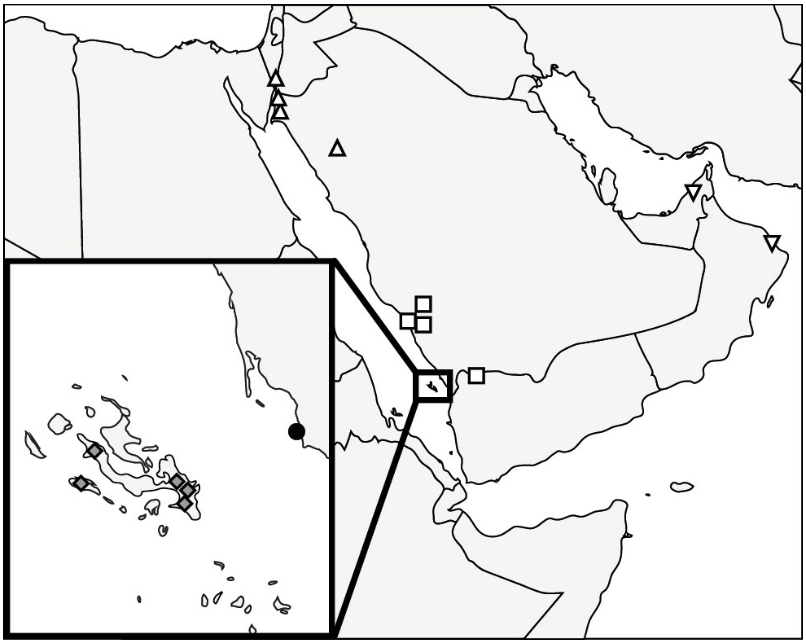

***Figure* 5** *Sampling locations of Arabian gazelles (*G. arabica*) with known provenance. Individuals included in the North group (△), the South-West group (□) and the East group (▽) are depicted as blank symbols in the main map, individuals from Farasan Islands (◆) and animals of unknown provenance confiscated at Akhoba Market in Jizan (●) are depicted on the inserted map. Taken from chapter 6, unchanged.*

The uppermost level of population differentiation (Evanno *et al.* 2005) was identified as *K*=2 and showed that all mainland specimens belong to one cluster (mainland cluster). The second genetic cluster inferred by STRUCTURE was exclusively found on the Farasan Islands with the majority of Farasan gazelles being assigned to it (Farasan cluster). Nevertheless, several individuals from Farasan were assigned to the mainland cluster and virtually all states of admixture between both clusters were observable. The isolation-with-migration-model revealed an estimate of effective population size on the Farasan Islands being 2.5-fold and 132-fold smaller than that of the reference population on the mainland (South-West group in Fig. 5) and the ancestral population of both, respectively. The divergence time of the populations, *i.e.*, the time of colonization of the Farasan islands was estimated between zero and 12,600 years before present (90% Highest-Posterior-Density interval). Migration between Farasan and mainland populations could not be assessed in detail, but the respective parameters converged at the upper limit of the prior distribution, indicating a much stronger migration than previously assumed. Differences in skull morphology between both groups were statistically significant with Farasan gazelles being generally smaller and having shorter horns than mainland conspecifics. Furthermore, males were significantly larger than females and bear larger horns. This sexual dimorphism was more pronounced in Farasan gazelles, mainly caused by disproportionately small horns in female Farasan gazelles.

Raia and Meiri (2006) argued that island dwarfism in ungulates is caused by the release from interspecific competition and predation: in habitats of high interspecific competition and predation animals are forced to allocate more resources into somatic maintenance and body growth. Release from these selective forces on islands could then allow for an increased investment into, and an earlier beginning of reproduction with a corresponding smaller body size (Raia & Meiri 2006). Indeed, mainland *G. arabica* compete with other ungulates, *e.g.*, Arabian Oryx (*Oryx leucoryx*) and feral camels (*Camelus dromedarius*) for resources, while on the Farasan Islands no competitors and only few predators of juvenile gazelles exist (Masseti 2010). Hence, the outlined argumentation seems to hold also for Arabian gazelles on the Farasan Islands. But a closer look at the population genetic patterns uncovers that about 30% of Farasan gazelles were not assigned to the Farasan cluster and that migration between the mainland and the Farasan archipelago occurred recently (maybe caused by continued translocation of gazelles by man; Groves 1997). Assuming that morphological differences are heritable and that Farasan gazelles assigned to the Farasan cluster represent locally adapted gazelles whereas the other Farasan gazelles are descendants from translocated mainland gazelles, one would expect up to 30% of Farasan gazelles to show morphological features of the mainland type, *i.e.*, a larger body size, fur coloration and more pronounced horns in females. However, all gazelles on the Farasan archipelago show dwarfism and even intense monitoring (Wronski *et al.* 2013) could not detect any mainland phenotype on the islands (T. Wronski *pers. comm.*).

Another possible explanation for island dwarfism is that the population suffers from inbreeding depression (Roldan *et al.* 1998), but in Farasan gazelles no signs of increased inbreeding could be detected—translocation of mainland animals even promotes outbreeding (chapter 6). Therefore, it can be argued that the dwarfism of Farasan gazelles reflects phenotypic plasticity (Pigliucci 2001). The Farasan Islands are an atypical habitat for Arabian gazelles as no surface water is available to them (Flamand *et al.* 1988). Also the high population density on the islands increases intraspecific competition for limited resources. From other ungulate species is known that body growth is hampered under resource limited conditions (Skogland 1983; Choquenot 1991).

Regarding the initial question of this study, the population genetic analyses support the conclusion that Farasan gazelles are not a distinct management unit (*sensu* Vogler & DeSalle 1994) and, therefore, would be suitable stock for reintroductions on the Arabian mainland. The genetic uniqueness of Farasan gazelles promoted by the separation of mainland and island gazelles (Farasan cluster) was confounded by the recent translocation of animals onto the archipelago accompanied by the introgression of genetic material from the Arabian mainland.

Another aim of this study was to investigate confiscated animals from a pet market near Jizan. It could be shown that some of the animals were captured alive at the Farasan Islands and brought to the market (chapter 6). Hunters responsible for these catches trespassed a protected area and violated Saudi Arabian and international law (Child & Grainger 1990). Furthermore, some of the confiscated gazelles were assigned to a genetic cluster not represented in the reference data base and could have been caught in Yemen as no samples from there could be included in the reference data set and the Saudi-Yemen border is close to Jizan.

Critical Discussion

The taxonomy of the genus *Gazella* is thought to be one of the least understood in mammals and, accordingly, several hypotheses were forwarded in the scientific literature based on skull morphometry, overall phenotypic appearance and genetic information (Lydekker & Blaine 1914; Ellerman & Morrison-Scott 1951; von Boetticher 1953; Gentry 1964; Groves & Harrison 1967; Groves 1969, 1985a, 1988; Rostron 1972; Lange 1972; Rebholz & Harley 1999; Groves & Grubb 2011; Hassanin *et al.* 2012). Indeed, no other taxon of large mammals has created confusion regarding its classification as profound as in *Gazella*.

The combination of the two parts of this thesis, *i.e.*, the phylogeny of genus *Gazella* on the one hand and the phylogeography and population genetics of Arabian gazelle species on the other, allows for a deeper understanding of speciation and radiation processes within the genus *Gazella* on the Arabian Peninsula. In terms of conservation of the threatened fauna of Arabia those studies in my thesis focusing on Arabian gazelle species provide new insights into the colonization histories and patterns of genetic viability of those species. Nowadays, the survival of several gazelle taxa is secured by captive breeding programs, but conservation efforts have been plagued by a largely uncertain taxonomy and a lack of robust phylogenetic background knowledge. Although a more comprehensive sampling and the application of a large number of recombinant markers in some cases was not feasible, the presented studies allow for improved ("evolutionary enlightened"; Ashley *et al.* 2003) conservation actions by clarifying the status of some critical taxa and could serve as a basis for future work, *e.g.*, on the evolution of *G. leptoceros* and *G. cuvieri* or on taxonomically uncertain (sub-)species of *G. arabica* (*e.g.*, *G. erlangeri* or *G. bilkis*). However, some questions remained unresolved and further investigations are recommended to achieve a better understanding of the complex history of the Arabian gazelle species.

In chapter 2 a phylogeny of the genus was inferred, providing new insights in the evolution of this genus and, thus, allowing for some taxonomic revisions. It could be shown that the genus *Gazella* is a reciprocally monophyletic taxon with nine extant species. This contradicts earlier findings of a paraphyletic genus *Gazella* including *G. subgutturosa* that was thought to be more closely related to *Antilope cervicapra* than to other members of the genus *Gazella* (Vassart *et al.* 1995). The inferred monophyly of *Gazella* could also be uncovered in other studies using mitochondrial markers (Rebholz & Harley 1999; Hassanin *et al.* 2012) and, more recently, also in a study including nuclear markers (Bärmann, Rössner, *et al.* 2013).

In a recent study by Bärmann, Rössner, *et al.* (2013) exact species relationships could not be resolved as sequence variability in the markers employed in that study was too low. My own phylogenetic studies (including chapter 2) were based on mitochondrial markers only and, therefore, incorporate only maternally inherited information. To overcome these limitations I started a study including more nuclear markers. Another aim of this study will be to gain insights on character evolution within the genus *Gazella*—which remained largely speculative in chapter 2 due to the outlined limitations—while reconstructing the ancestral character combination and the historical biogeography of *Gazella*. Unfortunately, I could not finish this study yet, but data collection is underway. Preliminary results suggest that gazelles (including the genera *Gazella*, *Eudorcas*, *Nanger* and *Antilope*) are characterized by a high degree of conservation of their 18S gene sequence as in a total of 1,129 base pairs only one variable position could be detected. Other nuclear markers that should be included in this study were all intron sequences derived from the complete genomes of cow (*Bos taurus*) and sheep (*Ovis aries*), because exon or mixed exon-intron sequences showed up to one order of magnitude fewer parsimony-informative sites than mitochondrial protein-coding markers (Bärmann, Rössner, *et al.* 2013).

The second part of the thesis focused on the phylogeography and populations genetics of Arabian gazelle species. As the Arabian Peninsula acted as a contact zone and migration corridor for African and Asian faunal elements it also became important for the evolution of gazelles, because extant representatives of different clades within the genus *Gazella* are distributed on both continents. Moreover, different species from the African and Asian clade (*sensu* chapter 2) occur on the Arabian Peninsula forming a secondary contact zone between both clades that diverged 3.9 – 2.3 Ma ago (chapter 2). Therefore, closer investigation of species living (or becoming recently extinct) on the Arabian Peninsula is certainly fully justified. This becomes even more imperative when considering the conservation status of those species combined with the lack of studies investigating genetic variability of Arabian mammals (but see Wronski *et al.* 2010). Three gazelle species are recognized on the Arabian Peninsula and assigned the conservation status 'vulnerable' by the IUCN, namely *G. marica*, *G. gazella* and *G. arabica*, with the latter two currently still being treated as a single species (IUCN/SSC Antelope Specialist Group 2008b; c). In the case of *G. dorcas*, the species' representative on the Arabian Peninsula has already gone extinct (Hammond *et al.* 2001).

For *G. marica* the study presented in chapter 3, making use of cytochrome *b* sequences and maximum-likelihood phylogenetic analyses, was the first to quantify the degree of genetic distinctiveness in maternal haplotypes compared to those of *G. subgutturosa*. These finding were later corroborated by the study presented in chapter 2 and Hassanin *et al.* (2012) using other individuals and more mitochondrial sequence data. Furthermore, the data published in chapter 3 were used by Kankilic *et al.* (2012) as reference for genotyping individuals from southern Turkey, where persistence of *G. marica* could be confirmed. This underlines the importance to improve conservation efforts in this region (Kankilic *et al.* 2012). However, whether or not the supposed polyphyly of both species is the reason for the observable differences in mitochondrial DNA sequences could not be resolved in detail. Especially with respect to the regions where morphologically "intermediate" forms of *G. marica* and *G. subgutturosa* could be found (Groves & Harrison 1967; Groves 1997; Mallon & Kingswood 2001), sampling was insufficient, comprising only 13 individuals from two sampling sites in Iraq (chapter 3). More recently, another population from this supposed hybrid zone, *i.e.*, from eastern Turkey, was investigated morphologically and genetically by Murtskhvaladze *et al.* (2012). Even though animals in that region clearly display *G. subgutturosa* morphology the four samples that originated from a breeding farm had *G. marica* haplotypes. The authors interpreted their findings as ongoing maternal gene introgression from *G. marica* into *G. subgutturosa*. In order to distinguish both species unequivocally they suggested an approach combining morphology and recombinant markers rather than only mitochondrial sequence data (Murtskhvaladze *et al.* 2012). When applying the Integrative Species Concept in this context, the need for different lines of evidence again becomes apparent, because a single line of evidence—*i.e.*, mitochondrial sequence data only—could obviously be interpreted falsely (de Queiroz 2007). To further investigate the relationship between *G. marica* and *G. subgutturosa*, including potential hybridization, future studies should also consider morphometric data and analyze recombinant nuclear sequences on a quantitative basis in animals originating from this zone of "intermediate" morphology.

In case of Dorcas gazelles the data presented in chapter 4 provided the first assessment of mitochondrial sequence diversity for this species (IUCN/SSC Antelope Specialist Group 2008a). Interestingly, only shallow genetic structuring was found throughout the distribution range of *G. dorcas* with a high degree of gene flow between different areas (chapter 4). Furthermore, the center of origin of the species could be inferred as the south-eastern to south-central part of its extant distribution range (chapter 4). In this region, gene flow was especially pronounced showing the ability of the species to cross the river Nile. On the other hand, a significant population differentiation could be observed downstream the Nile between north-eastern and western populations (chapter 4). Whatever caused this differentiation could not be investigated in detail in the course of my dissertation due to the lack of samples that were available from this region. Future studies that aim at answering this question should, therefore, include samples from the Western Desert of Egypt and Libya as well as from east and west of the Nile in Sudan. Regarding the population of Dorcas gazelles from North Morocco—taxonomically addressed as *G. dorcas massessyla*—only little sequence information was available for the analyses outlined in chapter 4 and the question of whether or not these animals should be treated as a distinct conservation unit could not be answered conclusively. However, a recent study could show that the mitochondrial haplotype found in North Morocco was new and distinct from all other *G. dorcas* haplotypes inferred in chapter 4 (by four mutational steps) and it was argued that specimens were isolated by the Atlas Mountains and might represent the last existing *G. d. massessyla* (Godinho *et al.* 2012).

In the study presented in chapter 5 it was possible to apply microsatellites to *G. arabica* and *G. gazella* that were previously applied to other antelope species. The results from the population genetic analyses were congruent with mitochondrial sequence data obtained in this study and to the results of Wronski et al. (2010). No recurrent gene flow could be detected between *G. gazella* and *G. arabica*, providing another line of evidence in favor for the classification of both taxa as good species. However, the absence of gene flow could also be a signature of highly fragmented populations of the same species, particularly if one or both had undergone recent demographic bottlenecks. Especially when two taxa do not occur in sympatry, gene flow patterns should not be used as the sole criterion for species delimitations. Here, the application of nuclear sequence markers is recommended as those markers would allow for estimating the degree of differentiation between both taxa (Brito & Edwards 2009). Nevertheless, the results for the central Israeli samples included in chapter 5 indicated that the southernmost populations of *G. gazella* showed no signs of hybridization with neighboring *G. arabica* populations. Hence, it could be hypothesized that both taxa are separated ecologically with *G. gazella* inhabiting forests with Mediterranean climate and *G. arabica* occurring in more arid environments of the Arabian Peninsula and Southern Israel. Considering morphology, both taxa could be clearly distinguished especially due to horn characteristics. In *G. gazella* males horns are straight and not as long-limbed as in *G. arabica* (Wronski *et al.* 2010). Recently, a study using 34 skull measurements could further support the separation of the species at least for males and found no cases of misclassification in a discriminant function analysis (Bärmann, Azanza, *et al.* 2013).

The same set of microsatellites could be applied in a study dealing with the problem of the utility of an island population of Arabian gazelles for future reintroductions (chapter 6). Animals occurring on the Farasan Islands uniformly show dwarfism, irrespective of their genotype (even though unfortunately no overlapping datasets for phenotypic and genetic datasets could be used). Most Farasan gazelles were completely assigned to a genetic cluster that could only be found on the archipelago, but in other cases assignment to the mainland cluster was found, and all states of admixture occurred. From these results it was concluded that the dwarfism results from phenotypic plasticity and, therefore, Farasan gazelles could provide a source for future reintroductions (chapter 6). However, the question of whether smaller, short-horned Farasan gazelles would survive in a mainland environment, where interspecific competition and predation is increased, remains unclear. In order to further evaluate the genetic distinctiveness of the Farasan cluster private alleles could also be taken into account in the future. However, this study provides insights into the genetic variability of wild populations of *G. arabica* and is the first population genetic approach to quantitatively assess the taxonomical status of Farasan gazelles. Since other mainland populations suffer from a high degree of inbreeding (chapter 6) the optimal solution might be to breed gazelles from different populations together in a breeding center to improve the genetic variability and later release their descendants. In general, the conservation of mainland populations must be given high priority in order to preserve this species on the entire Arabian Peninsula. Moreover, the evidence for human induced changes of an insular fauna, *i.e.*, the translocation of mammals between an archipelago and the mainland, highlights the need for a stronger protection of wildlife on Farasan Islands and an advanced management plan that aims at the conservation of genetic variability of the entire species in general.

In conclusion, the presented studies included in my thesis provided new insights in the evolution of the genus *Gazella* and contribute important findings to the highly debated taxonomy of mammals (see Groves 2013; Zachos *et al.* 2013). Even though problems in acquiring material from politically instable countries (like Somalia or Yemen) constitute a major challenge to studies of this kind, the studies presented here often were the first approaches (*G. dorcas* and *G. marica*) or were the first to determine genetic variability quantitatively (*G. arabica* and *G. gazella*) for the respective species. Most importantly, the results gained from my thesis are important in terms of management recommendations for future conservation actions for some endangered gazelle species and could, therefore, make a contribution for conservationists (also through publications in the newsletter of the Antelope Specialist Group of the IUCN; Lerp, Butynski, *et al.* 2012; Lerp, Wacher, *et al.* 2012) to preserve the world's biodiversity as claimed in the UN Convention on Biological Diversity.

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Part I

Phylogeny of the Genus *Gazella*

Chapter 2

by Hannes Lerp, Torsten Wronski, Thomas M. Butynski and Martin Plath

Speciation of Arabian Gazelles

The following manuscript was published as book chapter (pp. 59–82) in *Speciation: Natural Processes, Genetics and Biodiversity* edited by Pawel Michalak. The manuscript was peer-reviewed and I conducted the analyses, wrote the parts *Introduction*, *Major clades of gazelles*, *Extant species of smaller gazelles* and *Parallel, adaptive speciation of species pairs*. I co-wrote the part *A taxonomic review of the genus Gazella*. More details on my contribution to this collaborative work are given below in the form *Anlage 1* of the *Ausführungsbestimmungen für Dissertationen im Promotionsfach Biologie, die bereits veröffentlichte Teile oder eingereichte Manuskripte enthalten*.

Anlage 1

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts: Speciation of Arabian gazelles

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen[#]	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	Hannes Lerp: 70% Co-Autoren: 30%	Martin Plath, Torsten Wronski
(2) Durchführung der einzelnen Untersuchungen/ Experimente	Hannes Lerp: Sequenzierung des Cytochrom-*b*-Gens	
(3) Erstellung der Daten-sammlung und Abbildungen	Hannes Lerp: Veröffentlichung der Sequenzen bei Genbank, Erstellen der Abbildung	
(4) Analyse/Interpretation der Daten	Hannes Lerp: phylogenetische Analyse mit BEAST Hannes Lerp und Co-Autoren: Interpretation der Hauptkladen und der Artenpaare	Martin Plath
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	Hannes Lerp: 70% Co-Autoren: 30%	Martin Plath, Torsten Wronski, Tom M. Butynski

[#] Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

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Datum/Ort

Datum
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Chapter

SPECIATION OF ARABIAN GAZELLES

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ABSTRACT

Gazelles are distributed across Africa and Asia and are adapted to arid and semi-arid environments. In this chapter, we discuss potential factors promoting the divergence of lineages within this group (i.e., speciation events). The most recent common ancestor of gazelles is thought to have emerged during the Miocene (12-14 Ma) and to have split into the extant genera Nanger and Eudorcas (both endemic to Africa), Antilope (endemic to Asia), and Gazella (present in Africa, the Middle East and Asia). Within Gazella, two major clades are thought to have evolved allopatrically: (1) a predominantly Asian Clade (G. bennettii, G. subgutturosa, G. marica, G. leptoceros, and G. cuvieri) and (2) a predominantly African Clade (G. dorcas/ G. saudiya, G. spekei, G. gazella, and G. arabica). At present, both clades meet in North Africa and, especially, in Arabia. Other splits in this group are better explained by adaptive speciation in response to divergent ecological selection. In both clades, parallel evolution of sister species pairs (a desert-adapted form and a humid mountain-adapted form) can be inferred; desert-dwelling G. dorcas in Africa and G. saudiya in Arabia have a sister group relationship with mountain-dwelling G. gazella in the Levant and

G. arabica in Arabia. This relationship exists within Africa between the desert-dwelling slender-horned gazelle (G. leptoceros) and the mountain-dwelling Cuvier's gazelle (G. cuvieri) of the Atlas Mountains. A third species pair occurs in Asia; desert-dwelling goitred gazelle (G. subgutturosa) and mountain-dwelling chinkara (G. bennettii). These (ecological) speciation events correlate with ecology and behavior: the mountain forms being browsers, sedentary, territorial, and living in small groups, while the desert forms are grazers, migratory/ nomadic, non-territorial, and living in herds. Furthermore, cryptic sister species (G. gazella, G. arabica), with strikingly similar phenotypes, exist within presumed 'G. gazella', alluding to a possible allopatric origin of this divergence following an isolation of humid mountain regions during hyper-arid phases. On the other hand, phenotypes within G. arabica tend to be variable, but are difficult or impossible to distinguish genetically.

INTRODUCTION

The earliest known fossil antelope, found in the Baringo Basin, Kenya, is of early Miocene origin (Thomas 1981), but it is uncertain where gazelline antelopes first emerged: in Africa, as proposed by Kingdon (1988), or in Asia, as suggested by Vrba and Schaller (2000). One of the four extant genera (Antilope) that evolved from these ancestors is in Asia, two (Nanger and Eudorcas) are in Africa, and one (Gazella) is on both continents as well as on the Arabian Peninsula. To understand the present distribution of gazelles (i.e., Antilope, Gazella, Nanger and Eudorcas) it is important to interpret results from phylogenetic analyses in light of the geological and climatological history of the entire historic ranges of these genera.

The Arabian Peninsula is a prime example of a biogeographic transition zone, as it connects the floral and faunal regions of Africa and Asia (Vincent 2008). The present pattern is predominantly the result of the Afro-Eurasian species interchanges following the joining of the northern edge of the Afro-Arabian continent with Eurasia in the mid-Oligocene (ca. 30 Ma; Tchernov 1988; Bosworth et al. 2005; Vincent 2008). At the beginning of the Neogene (23 Ma), the Tethys acted as a substantial geographic barrier between Eurasian and Afro-Arabian faunas (Bernor 1983), leading to great divergence between these two realms and the evolution of two unique biotas (Tchernov 1988). The first major faunal interchange between Eurasia and Africa took place at the Proboscidean Datum Event (ca. 20 Ma; Madden and Van Couvering 1976), when a new land bridge (i.e., the Gomphotherium Land Bridge; Rögl 1999a) connected Africa and Asia, and the Mediterranean Sea was isolated from the Indian Ocean for the first time.

The following faunal exchange was not continuous though, and was intensified during two main dispersal events in the Miocene at ca. 18–19 Ma and ca. 16–17 Ma (Thomas 1985; Rögl 1999b), interrupted by a re-opening of the seaway between Arabia and South Anatolia (Rögl 1999a). Evidence for faunal exchange during the first phase can be found in the Jibal Hadrukh formation in Saudi Arabia (about 19 Ma) which contains fossil representatives of north-Tethyian fauna (Rögl and Steininger 1983). Before the connection to Eurasia was formed, the Arabian Peninsula supported an African fauna. After connection, Palaearctic faunal elements appear in Arabia (Tchernov 1988; Delany 1989). During the early Miocene, extensive rifting of the Rift Valley resulted in a dramatic increase in water depth of the Red Sea, thus separating Arabia from Africa (Bosworth et al. 2005). During this period, savannah and steppe ecosystems expanded, leading to a radiation of grasses (Poaceae) followed by the rise of hypsodont ungulates (Strömberg 2011) and a rapid radiation of several tribes of bovids (Matthee and Robinson 1999). Although Africa became seemingly isolated from the northern hemisphere by the Saharo-Arabian arid belt in the late Miocene, faunal exchange of mammals increased once savannah- and desert-adapted forms evolved and the arid belt became a less effective barrier to the dispersal of such species (Thomas 1979; Tchernov 1988).

In the late Miocene/ early Pliocene era, the “savannah-mosaic” assemblages of Mesopotamia were already populated with representatives of the tribe Antilopini (e.g., Gazella deperdita and G. rodleri) and other ungulates (Bernor 1986). The Miocene/ Pliocene boundary was characterized by the onset of the Messinian Salinity Crisis (6 Ma), when the Mediterranean Sea became isolated from the Atlantic ocean and water levels regressed dramatically (Krijgsman et al. 1999). This resulted in an accelerated faunal interchange between Africa and Eurasia (e.g., Agusti et al. 2006), especially of savannah-adapted species (Hassanin and Douzery 1999).

Following the Messinian Salinity Crisis the Mediterranean Sea reconnected to the Atlantic Ocean (Hilgen and Langereis 1988). At the same time, there was inflow of marine water into the Red Sea through the Bab el-Mandeb Strait, severing the connection between Arabia and the Horn of Africa (Bosworth et al. 2005). Furthermore, the orogeny of the Zagros Mountains—as part of the Alpine-Himalayan Mountain Belt—hampered biotic exchanges between Arabia and Asia (Tchernov 1988). All these factors led to an increasing isolation of Arabian fauna from Africa and Eurasia.

Moreover, the Afro-Arabian land bridge via the Sinai Peninsula became less permeable to faunal exchange due to a pull-apart basin development along the Aqaba-Levant Transform Fault (Bosworth et al. 2005). It remains uncertain as to whether there was a reconnection of both regions via the Bab el-Mandeb after the Miocene (Wildman et al. 2004; Winney et al. 2004; Fernandes et al. 2006; Bailey et al. 2009; Fernandes 2009). Climatic conditions during this time are thought to have caused a small-scale mosaic of ecosystems in the region (Tchernov 1988). Especially in Africa, faunal and palaeo-climatic records indicate shifts towards increasingly variable (and, on average, drier) conditions during the Plio-/ Pleistocene (2.8 Ma), allowing arid-adapted taxa to become more abundant (Thomas 1979; DeMenocal 2004).

In the Pleistocene the biotic interchange between Arabia and the Sahara was more asymmetric. Asian species, being more adapted to moister (mountainous) conditions, dispersed more easily into Arabia and North Africa along the mountain ridges of Arabia and the Sinai. By contrast, for arid-adapted Saharan species it was more difficult to invade the more humid parts of Asia (Delany 1989). Firstly, Saharan species on their way to Asia needed to cross the Nile Delta, which developed after the Messinian Salinity Crisis (Stanley and Warne 1998). Secondly, only the narrow stretch of sand dunes along the northern Sinai served as a suitable dispersal corridor for species adapted to hyper-arid conditions (Ferguson 1981). In addition, dispersing species would have needed to cross the Aqaba-Levant Transform Fault on their passage from the eastern Mediterranean towards Asia (Tchernov 1988).

The coastal plains of Arabia and the Sinai Peninsula experienced eustatic sea-level fluctuations, and large parts were submerged during inter-glacial periods (Chappell and Shackleton 1986; Shackleton 1987; van Andel 1989; Lambeck and Chappell 2001). During the Holocene (i.e., after the glacial cycles) the geological situation remained more or less stable, and mammalian species in Arabia and surrounding areas—particularly gazelles—were increasingly impaired by human activities. Archeological evidence suggests that hunting by humans in prehistoric times was already having a major impact on populations of gazelles (Legge and Rowley-Conwy 1987; Bar-Oz et al. 2011).

In this section, we have provided a brief overview of the geological and climatological setting in which the evolution of extant gazelle species took place. In the following, we consider the question of how the above-mentioned factors influenced speciation in this group. We concentrate on possible scenarios for the modes of speciation, and discuss evidence for both allopatric and ecological speciation.

Table 2. List of specimens included in the phylogenetic analyses, their collectors/ accession numbers, and source of sequence. Abbreviations: EEZA – Estación Experimental de Zonas Áridas, Almeria, Spain; KKWRC – King Khalid Wildlife Research Centre; OCE – Office for Conservation of the Environment, Muscat, Oman; WASWC - Wadi Al-Safa Wildlife Centre, Dubai, United Arab Emirates.

Species	Origin	Collector/ accession number	Source	Group

Gazella arabica	Oman, Muscat – Sur	OCE	tissue	African Gazella
G. arabica	Farasan Islands, Saudi Arabia	JN410353	GenBank (Lerp et al. 2011)	African Gazella
G. arabica	Israel, A'rava Valley	KC188759	GenBank (Lerp et al. 2013)	African Gazella
G. bennettii	KKWRC (ancestors from Pakistan)	JN410340	GenBank (Lerp et al. 2011)	Asian Gazella
G. bennettii	KKWRC (ancestors from Iran)	JN410341, JN410357	GenBank (Lerp et al. 2011)	Asian Gazella
G. bennettii	Pakistan	KKWRC	blood, hairs	Asian Gazella
G. cuvieri	EEZA	JN410342, JN410343	GenBank (Lerp et al. 2011)	Asian Gazella
G. dorcas	Israel, A'rava Valley	JN410230	GenBank (Lerp et al. 2011)	African Gazella
G. dorcas	Chad	JN410237	GenBank (Lerp et al. 2011)	African Gazella
G. dorcas	Sudan, Mashail	JN410250	GenBank (Lerp et al. 2011)	African Gazella
G. dorcas	Algeria, Hoggar Mountains	JN410252	GenBank (Lerp et al. 2011)	African Gazella
G. gazella	Israel, Yehuda Mountains	KC188775	GenBank (Lerp et al. 2013)	African Gazella
G. gazella	Israel, Shomeron	KC188774	GenBank (Lerp et al. 2013)	African Gazella
G. leptoceros	Hoggar Mountains, Algeria	JN410259	GenBank (Lerp et al. 2011)	Asian Gazella
G. leptoceros	Tunisia	JN410345	GenBank (Lerp et al. 2011)	Asian Gazella
G. leptoceros	Western Desert, Egypt	JN410346	GenBank (Lerp et al. 2011)	Asian Gazella
G. marica	Syria, Dara Region	K. Habibi	hairs	Asian Gazella
G. marica	Saudi Arabia, Khunfah	KKWRC	tissue	Asian Gazella

Table 2. (continued).

Species	Origin	Collector/ accession number	Source	Group
G. marica	Saudi Arabia, Uruq Bani ma'Arid	S. Ostrowski	hairs	Asian Gazella
G. spekei	WASWC	D. O'Donovan	hairs	African Gazella
G. subgutturosa	Mongolia, south	D. Maaz	tissue	Asian Gazella
G. subgutturosa	unknown	AF036282	GenBank (Hassanin and Douzery 1999)	Asian Gazella
Antilope cervicapra	unknown	AF022058, AF036283	GenBank (Matthee and Robinson 1999; Hassanin and Douzery 1999)	Larger gazelles
Eudorcas thomsoni	unknown	FJ556559	GenBank (Tungsudjai et al. unpublished)	Larger gazelles
E. rufifrons	Sudan	JN632633, JN632634	GenBank (Hassanin et al. 2012)	Larger gazelles
Nanger dama	unknown	AF025 954	GenBank (Matthee and Robinson 1999)	Larger gazelles
N. granti	unknown	AF034723	GenBank (Hassanin et al. 1998)	Larger gazelles
N. soemmerringii	Egypt, Cairo, Giza Zoo	KC188777	GenBank (Lerp et al. 2013)	Larger gazelles
N. soemmerringii	Saudi Arabia, Jenadriyah, private collection	H. Tatwany	blood	Larger gazelles
Litocranius walleri	unknown	AF249974	GenBank (Matthee and Davis 2001)	outgroup
L. walleri	Somalia	JN632653	GenBank (Hassanin et al. 2012)	outgroup
Antidorcas marsupialis	unknown	AF022054, AF036281	GenBank (Matthee and Robinson 1999; Hassanin and Douzery 1999)	outgroup

MAJOR CLADES OF GAZELLES

Gazelles are members of the tribe Antilopini. Although the other members of this tribe are not part of this review, they are worth mentioning since they are considered to represent highly derived descendants of gazelle-like ancestors (Gentry 1992). Today the tribe Antilopini comprises as many as 13 genera (Raphiceros, Ourebia, Madoqua, Dorcatragus, Saiga, Litocranius, Ammodorcas, Antidorcas, Procapra, Eudorcas, Nanger, Antilope and Gazella; Effron et al. 1976; Gentry 1992; Rebholz and Harley 1999; Groves 2000; Grubb 2005; Groves and Grubb 2011; Hassanin et al. 2012), four of which are traditionally labeled 'true gazelles', i.e., the genera Gazella, Antilope, Nanger, and Eudorcas (von Boetticher 1953; Groves 1985, 1988, 2000; Groves and Grubb, 2011).

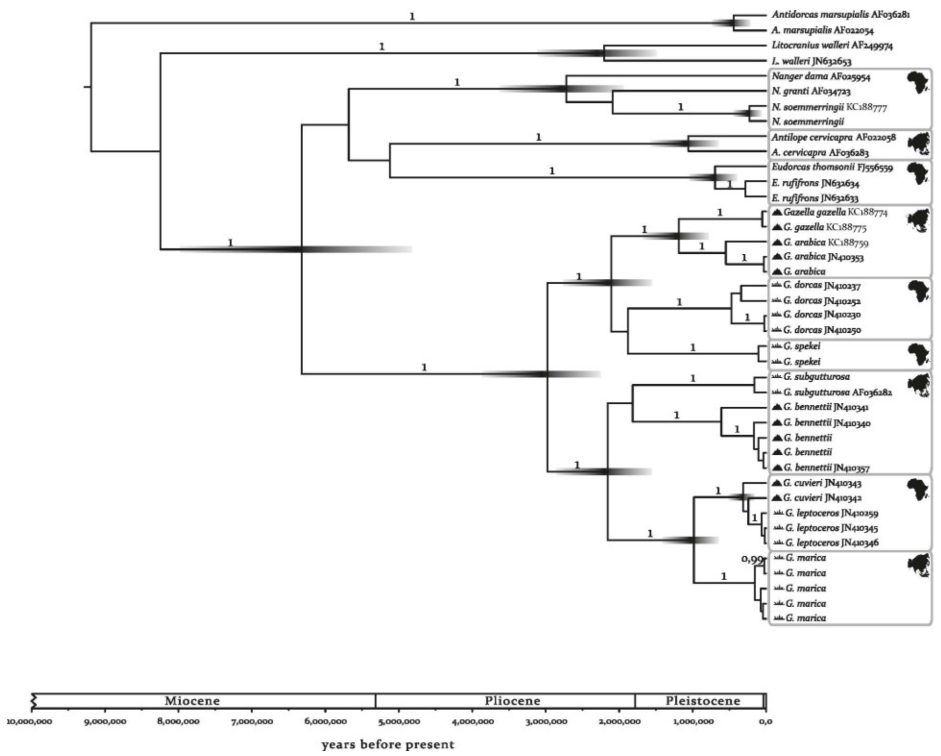

Figure 8. Phylogeny based on the alignment of the complete sequences of the cytochrome b gene. Bayesian analysis was performed with 41 sequences with the HKY + Γ substitution model. Only posterior probability values larger than 0.9 are reported. Node bars represent the 95% credibility intervals of the divergence times of statistically supported phylogenetic splits. Symbols of Africa and Asia indicate the occurrence of single species on that continent. Within the genus Gazella, a mountain symbol indicates a more humid and/or mountainous habitat, whereas a grass symbol indicates open savannah/ desert habitat.

To infer the phylogeny of gazelles, we investigated sequence variation of the mitochondrial cytochrome b gene of 17 taxa (including newly sequenced and already published data, see Table 2) covering all four gazelle genera, as well as the genera Antidorcas (springbok) and Litocranius (gerenuk). Bayesian analysis was performed in BEAST 1.5.2 (Drummond and Rambaut, 2007); no outgroup was defined beforehand. We used molecular clock data estimates inferred for Gazella dorcas. For methodological details see Lerp et al. (2011). jModelTest 0.1.1 (Posada 2008) uncovered HKY + Γ as the best fitting substitution model. We ran a Metropolis coupled Monte Carlo Markov chain (MC3) for 15 million generations with a burn-in phase of 1.5 million generations.

The phylogenetic tree inferred from this analysis is shown in Figure 8. High statistical support [i.e., posterior probability (PP) greater than 0.9] was found for the monophyly of gazelles (i.e., the genera Antilope, Eudorcas, Gazella and Nanger), but our analysis could not unambiguously resolve whether Antidorcas or Litocranius is the extant sister genus to the gazelles. Our findings are congruent with the results from a recent phylogenetic investigation of the order Cetartiodactyla by Hassanin et al. (2012), who analyzed the complete mitochondrial DNA sequence information, but included fewer gazelle taxa. Within the gazelles, all four genera were well supported as forming monophyletic clades, although the exact relationship among those genera could not be resolved. Time estimates for the first emergence of gazelles (95% credibility interval: 10.5–6.3 Ma), based on a molecular clock, were statistically not well supported (PP = 0.68), but are comparable to those provided by Hassanin et al. (2012), who estimated 8.5 ± 1.3 Ma (mean ± SD) for the corresponding phylogenetic split. During this time (i.e., in the late Miocene) savannah and steppe ecosystems with xerophytic shrub-land expanded into eastern and northern Africa and onto Arabia (Pound et al. 2011). This expansion of grasslands, together with the subsequent diversification of grasses (Strömberg 2011), probably facilitated the remarkable diversification (i.e., radiation) of antelopes at this time.

In contrast to paleontological studies describing the earliest fossil Antilopini from the middle Miocene in Africa (14 Ma; Vrba 1985) our molecular estimates for the first appearance of gazelles are considerable younger (10.5–6.3 Ma). How can these contrasting findings be reconciled? First of all, phylogenetic analyses through the analysis of sequence variation are based on extant taxa only, so extinct clades typically go undetected unless analyses of ancient DNA are feasible. Also, inference of time estimates from molecular phylogenetic approaches—as was done in this study—depend on the settings (i.e., substitution model and rates) for the molecular clock. Here, we used no fossil calibration points as constraints for our analysis (see below), but found similar time estimates as described by Hassanin et al. (2012), who used six calibration points from the fossil record for estimating the diversification of the entire order Cetartiodactyla. We are, therefore, confident that the settings of the molecular clock used in this study were realistic. Secondly, the classification of fossils is based on morphological measurements, especially with respect to skull and horn morphology. Gazelles show character state combinations that are likely plesiomorphic for the entire subfamily Antilopinae or, perhaps, even for the entire family Bovidae, which first appeared in the early Miocene (Gentry 1992; Vrba and Schaller 2000). Such morphological parallelisms and the incomplete fossil record render taxonomy within the Bovidae difficult (Vrba 1985; Gentry 1992). In addition, some bovid fossils showing this plesiomorphic character state combination are likely misclassified and falsely described as belonging in the vicinity of the genus Gazella.

The divergence of gazelles (PP = 1; 95% credibility interval: 8.0–4.8 Ma)—ultimately leading to the four extant genera in a relatively short period of time (Fig. 8)—could have been promoted by climate change following the Messinian Salinity Crisis (~6 Ma). Conditions were generally dryer (DeMenocal 2004), and new and larger areas became inhabitable for arid-adapted antelopes. The ancestors of the genus Antilope seem to have reached Asia by this time (Khan et al. 2006). The occurrence of blackbuck (Antilope cervicapra)—the only extant species of this genus—is still restricted to the Indian subcontinent and might be a descendant of this first expansion wave. Today, the descendants of Eudorcas and Nanger occur exclusively in Africa, and it remains doubtful if these genera ever occurred outside Africa.

The situation within genus Gazella, however, is more complex, because extant species occur both in Africa and Asia, as well as in Arabia (Kingdon 1988; Gentry 1992). Two major clades, with a well-supported monophyly, are inferred by our present study; their split is estimated at 3.9–2.3 Ma, i.e., in the Pliocene (Fig. 8). The 'African Clade', comprises more species, endemic to Africa, whereas the 'Asian Clade' is predominantly in Asia. Both clades, however, comprise taxa that occur on the "opposite" continent (Fig. 8).

The African Clade contains Speke's gazelle (G. spekei), which is endemic to the Horn of Africa in Somalia (East 1999), dorcas (G. dorcas), mountain (G. gazella) and Arabian gazelles (G. arabica; Effron et al. 1976; Rebholz and Harley 1999; Wronski et al. 2010; Bärmann et al. 2012; this study). The diversification of the African Clade started 2.8–1.6 Ma ago (early Pleistocene; Fig. 8). By far the widest distribution range within this clade is that of G. dorcas, which includes large parts of northern Africa and, once, much of Arabia (where described as Saudi gazelle G. saudiya; Carruthers and Schwarz 1935; Rebholz et al. 1991;

Rebholz and Harley 1997; Hammond et al. 2001). Together with G. gazella and G. arabica—which also inhabit the Arabian Peninsula—this is the most eastern extent of the range of the African Clade. We hypothesize that G. dorcas represents the ancestral character state combination of the African Clade because cytogenetic and morphological data showed G. dorcas to be basal to several species within Gazella (Lowenstein 1986; Gentry 1992; Vassart et al. 1995b). Moreover, it is suggested that the Antilopini evolved as grazers in the open, semi-desert and desert habitats of Africa (Kingdon 1988; Hassanin et al. 2012) and that the dispersal into mountainous and more humid habitats represents a shift associated with speciation events. At the edges of its distribution range, G. dorcas seems to have split rapidly into G. spekei and G. gazella, leaving sister group relations of these three species unresolved. This diversification was probably the result of 'ecological speciation' (see below). Lerp et al. (2011) found support for the idea that G. dorcas colonized Arabia via the Sinai and not via the Bab el-Mandeb. Thus, great distance and the Red Seas likely separated the ancestors of today's G. arabica, G. gazella and 'G. saudiya' of Arabia from Africa's G. spekei and G. dorcas.

Within the Asian Clade the majority of species are distributed on the Asian continent. The divergence time of this clade is estimated as 2.9–1.6 Ma ago and is comparable with the diversification of the African Clade of the genus Gazella. Therefore, the early Pleistocene is when most of today's species of Gazella emerged. We hypothesize that the diversification of the Asian Clade occurred in central Asia after the first (smaller) gazelles appeared, probably in the late Pliocene. The Asian Clade consists of G. subgutturosa and G. bennettii, both forming a reciprocally monophyletic clade in our present phylogeny. Both species occur in central Asia and India. Other members of the Asian Clade include G. marica and the African G. cuvieri and G. leptoceros, which together form a highly supported monophylum (Hammond et al. 2001; Wacher et al. 2010; Fig. 8). Changing climatic and geological conditions at the beginning of the Pleistocene could have enabled the ancestors of G. marica to cross the Zagros Mountains and invade the Middle East, where they occurred sympatrically with gazelles from the African Clade. Pliocene and early Pleistocene fossils of gazelles found in Turkey support this hypothesis, because they are distinct from fossils of G. gazella from the same period (Sickenberg 1975). Later (1.4–0.7 Ma ago) members of the Asian Clade crossed the Sinai Peninsula and Nile River to enter Africa and evolve into G. leptoceros which, today, occupies a habitat type similar to that of G. marica (i.e., the sand dunes and gravel plains of northern Africa; Harrison 1968; Devillers et al. 2005).

EXTANT SPECIES OF SMALLER GAZELLES

Before we elaborate on the mechanisms of speciation within the group of smaller gazelles (genus Gazella), we provide a brief overview of the historical and current distribution patterns as well as the current threats to the survival of the nine extant species in this group.

Dorcas gazelles (G. dorcas) were originally distributed from Morocco and Mauretania eastwards to the Horn of Africa, Sinai Peninsula, the Levant (Yom-Tov et al. 1995; East 1999; Hammond et al. 2001) to east of the Hejaz and Asir Mountains of western Arabia. This species was extirpated from Arabia about 30-40 years ago (Vesey-Fitzgerald 1952; Thouless et al. 1991; Habibi and Williamson 1997). With the exception of Israel and Ethiopia, numbers are decreasing rapidly and populations are increasingly fragmented (Smith 1999; Mallon and Kingswood 2001; Lafontaine et al. 2005). This decline is estimated at >30% over three generations, with less than 25% of the remaining animals living in protected areas, resulting in the IUCN status ‘Vulnerable’ (Mallon and Kingswood 2001; IUCN/SSC Antelope Specialist Group 2008a).

Mountain gazelles (G. gazella) are distributed from the eastern Turkey and Lebanon, through Palestine, Golan and western Jordan. Previously lumped with the Arabian gazelle (which we refer to as G. arabica—see below), which ranged over the Arava Valley in southern Israel, western Saudi Arabia, Yemen, Oman and United Arab Emirates. The number of G. arabica has declined dramatically during the past 50 years (Thouless and Al Bassri 1991; Magin and Greth, 1994; Mallon and Kingswood, 2001). Extensive hunting, habitat loss, and population fragmentation are principal causes of decline (Thouless et al., 1991; Magin and Greth 1994; Mallon and Kingswood 2001). The IUCN category is ‘Vulnerable’ based on G. gazella plus G. arabica (Mallon and Kingswood 2001; IUCN/SSC Antelope Specialist Group 2008b). The situation for G. gazella from northern and central Israel is less critical (Clark and Frankenberg 2001).

Speke’s gazelle (G. spekei) is endemic to the Horn of Africa, occurring in Somalia from the Indian Ocean westwards to the Gulis Range (Heckel et al. 2008). Although traditionally not hunted by people, numbers have collapsed over the last 20 years due to uncontrolled hunting by soldiers (Heckel et al. 2008). Probably the species is extirpated from Ethiopia. No effective protection is in place for G. spekei. The IUCN status is ‘Endangered’ (Heckel et al. 2008).

Goitred gazelle (G. subgutturosa) occurs east of the Tigris/ Euphrates Basin, north into the Caucasus and across Iran into Turkmenistan. Following the steppes of central Asia, G. subgutturosa inhabits the Takla Makan, Tarim Basin and Sianking of China and extends farther eastwards to central Mongolia, where it is replaced by the Mongolian gazelle (Procapra gutturosa; Groves 1985; Kingswood and Blank 1996; Mallon and Kingswood 2001; Zachos et al. 2010). Large populations occurred over a vast area until recently with ca. 100,000 individuals in the early 1990's (Mallon and Kingswood 2001). Hunting and habitat loss have caused a decline of >30% over the last ten years in many populations, resulting in the IUCN status 'vulnerable' (Mallon 2008a). The example of Mongolia should be highlighted, since a substantial proportion of the global population of G. subgutturosa once lived there, but heavy poaching, following collapse of the communist regime, has eliminated most of the large herds, resulting in a population decline >50% (Mallon 2008a).

The chinkara (G. bennettii) occurs in western and central India (especially in the Thar Desert), in the arid regions of Baluchistan and Sindh Provinces in Pakistan, south-western Afghanistan and north-central Iran (Rahmani 1990, 2001; Habibi 2001; Karami et al. 2002; Mallon 2008b). Scattered populations are also found in the sub-mountainous tracts of Punjab (Roberts 1977; Habibi 2001). Although numbers in Pakistan and Iran are decreasing due to overhunting (Mallon 2008b), the population in India is >100,000 (Rahmani 2001). Despite the large number of people in India, antelope populations there are relatively stable. This is mainly the result of an extensive network of protected areas coupled with low hunting pressure (Mallon and Kingswood 2001). G. bennettii, in particular, is secure in the Thar Desert with 80,000 individuals (Rahmani 2001) and, furthermore, is protected in reserves or by local people (Mallon and Kingswood 2001). For these reasons, G. bennettii is the only Gazella sp. that is not threatened (IUCN status 'least concern'; Mallon 2008b).

The sand gazelle (G. marica) is found in open habitats of the Middle East from the Tigris/ Euphrates Basin in Iraq, through Jordan and Syria into southern Turkey, and southwards through much of Arabia (Wacher et al. 2010). Current distribution is limited to a few (protected) areas in the United Arab Emirates, Oman, Syria, Turkey, probably in Jordan and perhaps western Iraq (Kasparek 1986; Mallon and Kingswood 2001; Massolo et al. 2008). In Saudi Arabia, G. marica is probably extinct outside of two protected areas Mahazat as-Sayd and Uruq Bani Ma'arid, both of which harbor reintroduced populations (Cunningham and Wacher 2009). There are probably <10,000 mature individuals and the population trend is downwards (IUCN/SSC Antelope Specialist Group 2008c). A good number of G. marica occur in captivity and are available for re-introductions (Cunningham and Wacher 2009). The current IUCN status is 'Vulnerable' (IUCN/SSC Antelope Specialist Group 2008c).

Slender-horned gazelle (G. leptoceros) is endemic to the sand dunes (ergs) of the Sahara, west of the Nile River (Devillers et al. 2005). Until recently, two subspecies were distinguished, i.e., G. l. loderi from the sand deserts of Tunisia, Algeria and Libya, and G. l. leptoceros from the Western Desert in Egypt (Devillers et al. 2005). However, phylogeographic analyses for validating these subspecies are lacking (Mallon et al. 2008). Numbers of G. leptoceros have decreased severely in the past decade due to hunting, especially in Egypt (Saleh 1987; Mostafa 2005), but also to habitat loss (Devillers et al. 2005). The conservation status of G. leptoceros in Mali, Niger, Chad and Libya is not known, but numbers are probably low (Devillers et al. 1999, 2005). All known populations are small to very small. The IUCN degree of threat status is 'Endangered' (Mallon et al. 2008).

Finally, Cuvier's gazelle (G. cuvieri) is endemic to the Atlas Mountains and neighboring ranges in Morocco (including the lowlands in the west), Algeria and Tunisia (Lafontaine et al. 1999; Beudels-Jamar et al., 2005). As for most Gazella spp., hunting is the major threat to the species and has caused a sharp population decline since the 1930's (Lafontaine et al. 1999; Beudels-Jamar et al. 2005). Habitat loss and degradation have also contributed to this decline (Sellami et al. 1990; de Smet 1991, 1994; Beudels-Jamar et al. 2005). There are currently ca. 2,500 individuals in several fragmented populations. The IUCN status is 'Endangered' (Mallon and Cuzin 2008). Some populations recently reported to be stable or even increasing (Mallon and Kingswood 2001; Mallon and Cuzin 2008).

PARALLEL, ADAPTIVE SPECIATION OF SPECIES PAIRS

Within both clades of genus Gazella, species pairs exist that exhibit parallel specializations in trophic ecology and social organization: On-the-one-hand, there are species more adapted to open, hot dry deserts. These species likely represent the ancestral character state combination. These species tend to be grazers, form herds and migrate. On-the-other-hand, species adapted to a more humid climate, are browsers that live in small groups and are sedentary and territorial. Our phylogenetic analysis infers three such species pairs (i.e., G. dorcas vs. G. gazella plus G. arabica; G. subgutturosa vs. G. bennettii, and G. leptoceros vs. G. cuvieri), where three lineages of desert-adapted forms independently diverged into a browsing, mountain-dwelling form, and a grazing, desert- or savannah-dwelling form. Even though we lack a plausible explanation as to how adaptation to different habitat types promoted reproductive isolation in gazelles, we argue that these three splits represent ecological speciation events.

Schluter and Nagel (1995) presented three—rather strict—prerequisites for parallel ecological speciation to occur; "(1) separate populations in similar environments must be phylogenetically independent […], (2) ancestral and descendant populations […] must be reproductively isolated, and (3) separate descendant populations inhabiting similar environments must not be reproductively isolated from one another". This concept is particularly useful when considering contemporary parallel speciation within the same species, as indicated by the third criterion. When trying to apply the concept of parallel speciation to the phylogeny of gazelles it needs to be interpreted in a slightly broader sense. Point (3) of Schluter and Nagel's (1995) definition is not met, as speciation in response to adaptation to a more humid climate occurred, independently, three times, and at different times, in different geographical regions, and from different ancestral species.

The oldest split of an ecologically divergent species pair inferred from our phylogenetic analysis is between G. dorcas and G. gazella/ G. arabica. This split occurred 2.8–1.6 Ma (late Pliocene; Fig. 8). G. dorcas are grazers that inhabit Sahelian savannahs as well as semi-arid gravel and sand deserts, while avoiding hyper-arid areas and the upper elevations of the central-Saharan massifs (Yom-Tov et al. 1995; Wacher et al. 2004). This species usually forms small family groups of 5–12 individuals (Yom-Tov et al. 1995), but during migration form herds of more than 100 individuals (Haltenorth and Diller 1977). G. gazella and G. arabica, by contrast, are sedentary, live in very small groups (two to maximal 20 individuals), live in upland areas of broken terrain on the Arabian Peninsula and the Levant, and adult males defend territories (Walther et al. 1983; Mendelssohn et al. 1995; Martin 2000; Wronski and Plath 2010). G. dorcas can cope without surface water by relying on hygroscopic food and respiratory water (Yom-Tov et al. 1995), whereas G. gazella and G. arabica prefer to drink on a regular basis (Mendelssohn et al. 1995). G. dorcas are reproductively isolated from G. gazella, and their hybrids are sterile or at least sub-fertile (Mendelssohn et al. 1995).

The second ecologically diverged species pair is G. subgutturosa and G. bennettii. Divergence probably occurred ca. 2.4–1.3 Ma ago (late Pliocene/ early Pleistocene) but statistical support for this date is weak (PP=0.89). G. bennettii are adapted to sand dune areas, regolith plains and hilly regions up to 1,500 m above sea level. This species avoids flat and steep terrain, and is typically on the edge of deserts (Roberts 1977; Sharma 1977; Rahmani 1990; Karami et al. 2002). G. bennettii are sedentary and live in groups of one to three individuals, but sometimes in larger herds (Rahmani 1990; Bagchi et al. 2008). Males form territories that they defend vigorously (Walther et al. 1983). The species is typically a browser, but during the rainy season they also graze (Sharma 1977; Habibi 2001). Compared to G. subgutturosa—which meet their water needs entirely from hygroscopic food plants—G. bennettii are independent of surface water only in winter. In the hotter months, when temperatures are >40°C, they have to drink regularly (Habibi 2001). G. subgutturosa are grazers that can also browse on xerophytic bushes (Roberts 1977; Kingswood and Blank 1996; Karami et al. 2002). This species is semi-nomadic with males forming territories only during the rut (i.e., October-December; Kingswood and Blank 1996; Blank 1998; Bekenov et al. 2001).

The youngest split of an ecologically divergent species pair is between G. leptoceros and G. cuvieri and dates to 420,000–110,000 years ago (middle Pleistocene). The young age of this split (i.e., the small genetic divergence between them) raises doubt concerning their species status (Hassanin et al. 2012). Nonetheless, both species are morphologically readily distinguished (Gentry 1964; Groves 1969; Groves and Grubb 2011). G. leptoceros are desert-dwelling grazers (Louys et al. 2011; Smith et al. 2001), that occasionally browse on Acacia (Saleh 2001). The species is nomadic, crossing vast areas of flat, open desert in search of sparse, ephemeral grasses (Kingdon 1997; Saleh 2001; Smith et al. 2001). The typical group size is <15 individuals (Smith et al. 2001). In contrast, G. cuvieri inhabit dry forests and maquis of the semi-arid Mediterranean type (Sellami and Bouredjli 1991; Beudels-Jamar et al. 2005), browse on acorns and young leaves of legumes, but also graze (Kingdon 1997; Smith et al. 2001). They live up to 2,600 m above sea level where they are limited by snow in winter (Aulagnier et al. 2001; Beudels-Jamar et al. 2005). G. cuvieri need to drink on a regular basis (Smith et al. 2001; Beudels-Jamar et al. 2005). This species lives in groups of 5-8 individuals, but solitary individuals are common (Sellami and Bouredjli 1991; Kingdon 1997; Beudels-Jamar et al. 2005). Males are territorial during the rut (in winter; Sellami and Bouredjli 1991; Kingdon 1997; Smith et al. 2001).

A TAXONOMIC REVIEW OF THE GENUS GAZELLA

It has been repeatedly emphasized that the taxonomy of gazelles is one of the least resolved among mammals (Groves and Harrison 1967; Groves, 1969). No other genus of large mammals creates such problems with regards to its classification based on skull morphometry, phenotypic appearance and genetic information, as does Gazella. As such, many taxonomic revisions of this genus have been put forth (Lydekker and Blaine 1914; Ellerman and Morrison-Scott 1951; von Boetticher 1953; Gentry 1964; Groves and Harrison 1967; Groves 1969, 1985, 1988; Lange 1972; Rostron 1972).

While the taxonomy of Antilope and Nanger has not changed substantially in recent decades (von Boetticher 1953; Gentry 1964; Lange 1972; Groves and Grubb 2011) the taxonomy within Eudorcas and Gazella remains uncertain, and with recent molecular findings casting doubt on earlier classifications that are based on morphological and cytogenetic traits. Our phylogenetic analysis of Gazella supports the existence of nine species (G. gazella, G. arabica, G. dorcas, G. spekei, G. bennettii, G. subgutturosa, G. marica, G. leptoceros and G. cuvieri), most of which require further taxonomic clarification.

Gazella marica (Thomas 1897), was subsumed within Gazella leptoceros by Ellerman and Morrison-Scott (1951). Subsequently, G. marica was considered a subspecies of G. subgutturosa based on morphological and karyological similarity (Groves and Harrison 1967; Kingswood et al. 1996, 1997). In more recent studies the phylogenetic relationships between G. subgutturosa from east of the Euphrates/ Tigris Basin and from the Arabia (G. marica) were reanalyzed based on molecular genetic information (Hammond et al. 2001; Wacher et al. 2010) and supported G. marica as a species. This conflicted with the grouping pattern inferred from skull structure and horn conformation (Groves and Harrison 1967). G. marica appears to be most closely related to the North African species G. leptoceros and G. cuvieri (Hammond et al. 2001; Wacher et al. 2010; see above).

In case of G. subgutturosa, Vassart et al. (1995b) state that Gazella will be paraphyletic when this species is included, because G. subgutturosa could be a sister taxon of Antilope. Both taxa share two unique centric fusions in their chromosomes causing the need to revive the genus Trachelocele (Ellerman and Morrison-Scott 1951; Groves 1969). Other studies investigating morphology or mitochondrial sequence variation placed G. subgutturosa within Gazella and refute Trachelocele (Grubb 2005; Groves and Grubb 2011; Hassanin et al. 2012; this study). Due to morphological variation within G. subgutturosa, up to three species are proposed by Groves and Grubb (2011), but there are no empirical data to support this position.

Early classifications place G. bennettii as either a subspecies of G. gazella (Haltenorth and Diller 1977; Roberts 1977) or as a subspecies of G. dorcas (Gentry 1964; Groves 1969; Lange 1972). Karyological data, however, found G. bennettii to be unrelated to G. gazella (Furley et al. 1988; Kumamoto et al. 1995). Within G. bennettii, up to six species are proposed on the basis of morphological divergence (Hemami and Groves 2001; Groves and Grubb 2011), but, again, evidence justifying this division is lacking. In this study—where two of the proposed G. bennettii taxa were included—there was no indication of more than one species. Nevertheless, a phylogeographic study with individuals from the entire distribution range is highly warranted.

In the cases of G. cuvieri and G. leptoceros the taxonomic classification remains confusing. Lange (1972) classified G. cuvieri under G. gazella, while G. leptoceros was considered a subspecies of G. subgutturosa. Later, a karyological study showed that G. cuvieri is unrelated to G. gazella (Kumamoto and Bogart 1984). Furthermore, a division of G. leptoceros into two subspecies (G. l. loderi and G. l. leptoceros) was suggested based on differences in distribution ranges and ecology (Devillers et al. 2005). In contrast, G. marica and G. leptoceros are recently proposed to be subspecies of G. cuvieri because of their relatively low mitochondrial sequence divergence (Hassanin et al. 2012).

Within G. dorcas, several subspecies are described on the basis of phenotypic variation, such as coat coloration and horn shape and length (Groves 1969, 1981; Alados 1987; Yom-Tov et al. 1995; Groves and Grubb 2011). A phylogeographic study based on sequence variation of the mitochondrial cytochrome b gene and control region recently indicates that G. dorcas—including ‘G. saudiya’ and ‘G. pelzelni’—represent a reciprocally monophyletic group with a sister-group relationship to G. gazella and G. arabica (Lerp et al. 2011). No statistically significant support was found for any geographic structure within the distribution range of G. dorcas. Nevertheless, keeping G. dorcas, ‘G. saudiya’ and ‘G. pelzelni’ separated at captive breeding centers is warranted as low genetic divergence at neutral markers does not preclude the potential existence of local adaptations (Hammond et al. 2001; Lerp et al. 2011).

Confusion over taxonomy and nomenclature at the species level has reached a maximum in G. gazella and G. arabica (Groves and Harrison 1967; Harrison 1968; Groves 1969, 1983, 1989, 1996, 1997; Lange 1972; Groves and Lay 1985; Vassart et al. 1995a; Greth et al. 1996; Vassart et al. 1996; Kingswood et al. 1997; Rebholz and Harley 1999; Wronski et al. 2010). At least four species (G. gazella, G. bilkis, G. arabica, and G. erlangeri) and eight subspecies have been named (Groves 1996, 1997; Grubb 2005; Groves and Grubb 2011). Based on the analysis of cytochrome b sequences of five G. gazella in the context of a phylogeny of the Antilopinae, Rebholz and Harley (1999) suggested that two genetically distinct lineages might exist: one from the Levant (Galilee to Turkey) and one from Negev and Arabia. Those findings have been confirmed in an analysis comprising more individuals from a larger area and more mitochondrial and microsatellite markers (Wronski et al. 2010; Lerp et al. 2013). This supports recognition of two ‘cryptic’ species in this clade, which may have evolved due to prolonged isolation or local adaptations to divergent environments (Wronski et al., 2010; Lerp et al. 2013). The nominate G. gazella was originally described as Antilope gazella (Buffon 1764) from the Levant. This raises the question of which species name to assign to the populations in Arabia. Recent molecular analyses of the cytochrome b gene from the type G. arabica (described as Antilope arabica Lichtenstein 1827) indicate that this taxon is invalid, because skin and skull of the type specimen of G. arabica did not form a separate lineage, but clustered with G. gazella (skin) and with G. arabica (skull; Bärmann et al. 2012). Following the rules of precedence (priority rule, International Code of Zoological Nomenclature, ICZN) the name G. arabica is available for gazelles in Arabia.

Within G. arabica, however, much taxonomic uncertainty remains. One of the most challenging questions is the status of G. erlangeri. Neumann (1906) described specimens from Lahadsch (Lahej), north of Aden, as a greyer form of G. arabica. He introduced a new subspecies name to account for this difference and cited the illustration labeled G. arabica in Sclater and Thomas (1898) as an accurate representation of what he was describing. Due to its putative sympatric distribution with G. arabica, Groves (1996) suggested full species status for G. erlangeri. Gazelles currently kept in captivity at King Khalid Wildlife Research Centre in Saudi Arabia and at Al Wabra Wildlife Preservation in Qatar show the described combination of diagnostic features and thus, were considered to represent G. erlangeri (Groves 1996)—even though the provenance of these gazelles is not known. Phylogenetic studies (using mitochondrial markers) on these putative G. erlangeri cluster them amongst other G. arabica from all over Arabia (Hammond et al. 2000; Blacket et al. 2001; Hundertmark and Omer 2004; Wronski et al. 2010). In summary, it remains unsolved whether Neumann's (1906) G. erlangeri is a distinct taxon and how it relates to other gazelles.

Finally, the most enigmatic gazelle described from Arabia should be mentioned: the Queen of Sheba's gazelle (Gazella bilkis). Specimens shot in the Taizz Mountains of southern Yemen in 1951 (now stored at Chicago FMNH) were originally identified as G. arabica erlangeri by the collector Hoogstraal. They were, however, re-evaluated retrospectively based on skull morphology and described as Gazella bilkis (Groves and Lay 1985; Groves and Grubb 2011). Even though the taxonomic status of these gazelles remains unclear, there is no doubt that G. bilkis is extinct (Mallon and Al-Safadi 2001).

CONCLUSION

Gazelles comprise four monophyletic genera (Antilope, Nanger, Eudorcas and Gazella) and emerged in the early Miocene (10.5–6.3 Ma). While three genera are restricted to the continent on which they probably evolved (Antilope to Asia, Nanger and Eudorcas to Africa), the situation in Gazella is more complex, with extant species in Africa, the Middle East, and Asia. Different modes of speciation are apparent within Gazella: (1) allopatric speciation in two major clades, with one predominantly Asian Clade and the other a predominantly African Clade; (2) parallel, adaptive speciation of three species pairs in parapatry, with one representative being a grazing, desert- or savannah-dwelling, (semi-) nomadic form, and the other being a browsing, mountain-dwelling and mostly sedentary form; and (3) cryptic speciation following phases of geographic isolation, where two genetically distinct forms with similar phenotypes can be seen (G. gazella and G. arabica). In general, gazelles are characterized by pronounced phenotypic variability that is not always mirrored by molecular sequence divergence, and a part of this variation may be due to phenotypic plasticity. This led to taxonomical incongruence plainest expressed in the number of described species that reached a maximum in a recent book by Groves and Grubb (2011), with 36 extant gazelle species (including 1 species in the genus Antilope, 5 in Nanger, 6 in Eudorcas and even 24 in Gazella) being listed. In terms of conservation this situation is unfortunate. The taxonomical incongruence hampers conservation efforts regarding captive breeding or re-introduction programs, as it remains confusing which gazelles should be bred separately to preserve natural biodiversity. Further investigations using nuclear DNA markers of the extant taxa will be helpful to clarify the situation for critical taxa.

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Part II

Phylogeography and Population Genetics of Arabian Gazelle Species

Chapter 3

by Timothy Wacher, Torsten Wronski, Robert L. Hammond, Bruce Winney, Mark J. Blacket,
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Phylogenetic analysis of mitochondrial DNA sequences reveals non-monophyly in the Goitered gazelle (*Gazella subgutturosa*)

The following manuscript was published as short communication in *Conservation Genetics* 12 (pp. 827–831). The manuscript was peer-reviewed and I co-wrote and revised the manuscript. More details on my contribution to this collaborative work are given below in the form *Anlage 1* of the *Ausführungsbestimmungen für Dissertationen im Promotionsfach Biologie, die bereits veröffentlichte Teile oder eingereichte Manuskripte enthalten*.

Anlage 1

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts: Phylogenetic analysis of mitochondrial DNA sequences reveals non-monophyly in the Goitered gazelle (*Gazella subgutturosa*)

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen[#]	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	Hannes Lerp: 0% Co-Autoren: 100%	Tim Wacher, Torsten Wronski, Martin Plath, Robert Hammond, Christoph Bleidorn
(2) Durchführung der einzelnen Untersuchungen/ Experimente	Co-Autoren: Sequenzierung des Cytochrom-*b*-Gens RFLP-Analysen	William Macasero, Kris Hundertmark, Osama B. Mohammed, Bruce Winney, Mark J. Blacket
(3) Erstellung der Daten-sammlung und Abbildungen	Hannes Lerp und Co-Autoren: Erstellung der Abbildung Co-Autoren: Veröffentlichung der Sequenzen bei Genbank	Christoph Bleidorn, Torsten Wronski
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SHORT COMMUNICATION

Phylogenetic analysis of mitochondrial DNA sequences reveals polyphyly in the goitred gazelle (*Gazella subgutturosa*)

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Abstract Goitred gazelle (*Gazella subgutturosa*) rank among the most endangered mammals on the Arabian Peninsula and the Asian steppes. Past conservation efforts have been plagued by confusion about the phylogenetic relationship among various—phenotypically discernable—populations, and even the question of species boundaries was far from being certain. This lack of knowledge had a direct impact on conservation measures, especially ex situ breeding programmes, hampering the assignment of captive stocks to potential conservation units. Here, we provide a phylogenetic framework, based on the analysis of mtDNA sequences of a number of individuals collected from the wild and captivity throughout the species' natural range. Our analyses revealed a polyphyly within the presumed species of *G. subgutturosa* resulting in two distinct clades: one on the Arabian Peninsula, Iraq, Jordan, Syria and Turkey (*Gazella marica*; sand gazelle) and one genetically diverse larger clade from the rest of its Asian range (*G. subgutturosa*; goitred gazelle). Additionally, we provide a quick method (PCR-RFLP) to analyse the taxonomic affiliation of captive gazelles that will be used for re-introductions into the wild.

Keywords Arabian Peninsula · Cryptic species · *Gazella subgutturosa* · Phylogeny · Conservation units

Introduction

Conservation genetics has major implications for the conservation of biodiversity by clarifying taxonomic

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relationships (Avise 1989), and determining stocks or individuals from captive breeding programs for future reintroductions (Vogler and DeSalle 1994). Gazelles are a very diverse group of mammals, with phylogenetic relationships within this group being largely unexplored (Groves 1989, 1997), a fact that has hampered and complicated conservation efforts (Ryder 1987; Hammond et al. 2001). Here, we ask whether or not goitred gazelles (*Gazella subgutturosa*) consist of more than one species, and second, we investigate the taxonomic affiliation of a large number of captive individuals that have been bred for reintroduction.

The conventional view is that *G. subgutturosa* occurs over a very wide range from Oman across the Arabian Peninsula to southern Turkey (Mallon and Kingswood 2001), following the steppes of central Asia eastwards into central Mongolia (Kingswood and Blank 1996; Mallon and Kingswood 2001). Several subspecies have been described across this range, with two occurring in the Middle East:

(1) The sand gazelle or 'reem' (currently recognized as *G. s. marica*; Groves and Harrison 1967), is found in open habitats of the Arabian Peninsula, ranging through Iraq, Jordan and Syria into southern Turkey (Mallon and Kingswood 2001). Sand gazelles are characterized by pale body colour and a white face. Females bear long slender horns, which can be also much reduced. Adult males are heavier-bodied, with a noticeably thickened neck in breeding condition (though typically with a less obviously developed goitre than in continental Asian forms) and much longer, lyrate horns (Groves and Harrison 1967; Kingswood and Blank 1996).

(2) The Persian goitred gazelle (*G. s. subgutturosa*) differs from *G. s. marica* by having a larger body weight (Kingswood and Blank 1996), and by adult females usually lacking horns or sometimes growing short horns. Adult males develop a prominent swelling on the larynx during the rutting season, the so-called 'goitre'.

The sand gazelle was first described as a full species (*G. marica*; Thomas 1897) and later synonymized with the closely related slender-horned gazelle (*Gazella leptoceros*) of the sand dune systems of northern Africa (Ellerman and Morrison-Scott 1951). More recently, it has been considered a subspecies of *G. subgutturosa* based on morphological and karyological similarity (Groves and Harrison 1967; Kingswood et al. 1996, 1997). The disappearance of the sand gazelle from large parts of its range in Arabia can be attributed to a combination of over-hunting and intense over-grazing by domestic livestock (Thouless et al. 1997). The modern distribution of wild sand gazelles in Saudi Arabia is limited to the two original populations in the northern protected areas (Seddon et al. 1997; Thouless et al. 1997), and two reintroduced populations at Mahazat as-Sayd and Uruq Bani Ma'arid (Haque and Smith 1996; Cunnigham and Wacher 2009).

A primary objective of our present study was to analyse the phylogenetic relationships between *G. s. subgutturosa* from east of the Euphrates-Tigris basin and those from the Arabian Peninsula (*G. s. marica*). To do so, we analysed sequence variation of a mitochondrial marker (cytochrome *b*) of samples obtained from wild sand (*G. s. marica*) and Persian goitred gazelles (*G. s. subgutturosa*) of known origin, from museum specimens, as well as from captive-breeding stocks. In addition, we used PCR-RFLP analysis to investigate the taxonomic affiliation of a large number of individuals from a series of captive groups of gazelles.

Material and methods

The origin of samples, provenance, collector and the kind of material sampled (skin, blood, hairs) are summarized in Table 1. Sequences from other gazelle taxa were obtained from GenBank (7 sequences, Rebholz and Harley 1999; Hassanin and Douzery 1999; Hammond et al. 2001), the Estación Experimental de Zonas Áridas in Almeria, Spain (*G. cuvieri*), Wadi al-Safa Wildlife Centre, UAE (*G. bennetti*), Prince Ahmed bin Abdulaziz Farm, Saudi Arabia (*G. leptoceros loderi*; reported to originate from Tunisia), or from the wild (*G. leptoceros leptoceros*: Hatiyat Umm Ghuzlan, western Egypt; *G. cuvieri*: Chambi N.P., Tunisia). Sequences from '*G. gazella*' were reanalysed from another study (Wronski et al. 2010).

DNA extraction of hair and blood samples was carried out as described in Wronski et al. (2010). The 5′-region of the cytochrome *b* gene was PCR-amplified using the versatile primers L14724 and H15149 (Kocher et al. 1989). Double stranded PCR products were sequenced with a Thermosequenase-based cycle-sequencing kit using an end-labelled primer protocol using γ P^{33} as a label (Amersham-Pharmacia Biotech). Both L14724 and H15149 were used as sequencing primers. Sequence reactions were run on standard polyacrylamide sequencing gels and visualised by autoradiography. For museum samples, each sample was amplified and sequenced from at least two separate DNA extractions. Autoradiographs were scored by eye, with each individual sequence being read at least twice. All unique haplotypes have been deposited in GenBank (accession numbers HQ316150–HQ316165).

Sequences were aligned using MAFFT version 6 (Katoh et al. 2005) using the iterative refinement method E-INS-i. A χ^2-test as implemented in PAUP 4.0b10 (Swofford 2002) was used to test for compositional heterogeneity of base frequencies. Maximum likelihood (ML) analysis of the dataset was conducted using RAxML version 7.0.3

Table 1 List of specimens (wild and captive) of *G. s. subgutturosa* and *G. s. marica* included in the phylogenetic analyses, their collectors, accession numbers, and sample types

Species	Origin	Wild/captive	Collector/accession number	Sample type
G. s. subgutturosa	Aksu, Chinese Turkistan	Wild	Cumberland, BMNH London/HQ316159	Tissue
G. s. subgutturosa	Al-Areen, Bahrain*	Captive	Hundertmark, Mohammed, KKWRC	Blood, hairs
G. s. subgutturosa	Kabul Zoo, Afghanistan	Captive	Lindsay, ZSL	Hairs
G. s. subgutturosa	Samarra, Iraq	Wild	Pitman, BMNH London	Tissue
G. s. subgutturosa	Unknown*	?	MNHN Paris/AF036282	Sequence
G. s. subgutturosa	San Diego Zoo, originally Teheran Zoo, Iran (2)	Captive	Hammond, KKWRC/HQ316157	DNA
G. s. subgutturosa	Azerbaijan	Wild	Lindsay, ZSL/HQ316158	Tissue
G. s. subgutturosa	Warsan Farm, Abu Dhabi, UAE (2)*	Captive	Hoy, Warsan Farm/HQ316156	Hairs, tissue
G. s. marica	Harrat Al Harrah/Iraqi border*	?	Rangers, SWC	Tissue
G. s. marica	Ramlat Fasad, Oman	Wild	Butler, Harrison Museum/HQ316160	Tissue
G. s. marica	Abu Al Jir, Iraq	Wild	Harrison, Harrison Museum/HQ316162	Tissue
G. s. marica	Al Khunfah Protected Area, Saudi Arabia (3)	Wild	Rangers, SWC	Blood, tissue
G. s. marica	King Khalid Wildlife Research Centre, Saudi Arabia*	Captive	KKWRC staff members	Blood, tissue
G. s. marica	Harrat Al Harrah, Saudi Arabia	Wild	Wacher, KKWRC	Hairs, tissue
G. s. marica	Harrat Al Harrah, Saudi Arabia (4)	Wild	Rangers, SWC/HQ316164	Tissue
G. s. marica	Syria	Wild	Ginani	Tissue
G. s. marica	Wadi Al-Safa Wildlife Centre, Sharjah, UAE (6)*	Captive	Nader, KKWRC/HQ316161	Tissue
G. s. marica	Warsan Farm, Abu Dhabi, UAE (2)*	Captive	Hoy, Warsan Farm	Skin
G. s. marica	Syria/Jordan	Captive	Williamson, KKWRC	Hairs
G. s. marica	Qarn Sahma, Oman	Wild	Harrison, Harrison Museum	Tissue
G. s. marica	Warsan Farm, Abu Dhabi, UAE (4)*	Captive	Hoy, Warsan Farm/HQ316163	Hairs, tissue
?	Rutba region, Iraq (5)*	Captive	Hammond, Al-Aqeel, Mubarak, KKWRC/HQ316165	Tissue

Numbers in *brackets* indicate the number of samples obtained from that location, while *asterisks* indicate unknown provenance and ? unknown whether an individual was wild or captive

(Stamatakis 2006), using the GTR+Γ+I model. Parsimony analysis was performed in PAUP*v.4.0b10 (Swofford 2002) using a heuristic search with the TBR (tree bisection-reconnection) option and 1,000 random sequence additions. Bootstrap support was estimated by 1,000 replicates. Hypothesis testing using the Approximately Unbiased (AU)-test as implemented in CONSEL (Shimodaira and Hasegawa 2001) was performed under the ML-criterion to compare constrained monophyly of *G. subgutturosa* against the best tree.

To rapidly assess the cytochrome *b* haplotype of a large number of captive *G. s. marica* and *G. s. subgutturosa* the same PCR product used for sequencing (see above) was digested with HaeII, HinfI, NlaIII, RsaI and DdeI. These restriction enzymes identified 5 nucleotides that diagnosed membership of either the *G. s. marica*-clade or *G. s. subgutturosa*-clade (Table 2). PCR products were digested following manufacturer's instructions (New England Biolabs), separated on 1% agarose gels, and bands were visualized under UV after ethidium bromide staining. In total, 894 samples were RFLP typed from the following captive populations: Prince Mohammed Al Sudairy Centre, Qassim, Saudi Arabia ($n = 424$), KKWRC ($n = 253$), KKWRC animals released in the Urug Bani Ma'arid

Table 2 PCR-RFLP haplotypes of *G. s. marica* and *G. s. subgutturosa*

Taxon	HaeIII 193	HaeIII 352	HinfI 163	HinfI 280	NlaIII 139	NlaIII 300	NlaIII 342	NlaIII 388	RsaI 369	RsaI 277	DdeI 377
G. s. marica	0	1	0	1	1	1	1	1	1	1	1
G. s. subgutturosa	1	1	1	1	0	1	1	1	0	0	0

The presence of a restriction site is indicated by 1, while absence of a is indicated by 0. *Numbers* given indicate the position of the restriction site in base pairs. This applies to a region of the cytochrome *b* gene amplified using the primers L14724 and H15149

Protected Area, Saudi Arabia ($n = 105$), Al Areen Wildlife Park, Bahrain ($n = 99$), and gazelles confiscated at a Saudi Arabia/Iraq border post, which were said to have originated from the Rutba region of Iraq ($n = 13$).

Results and discussion

Our final dataset included 27 Operational Taxonomic Units (OTUs) and 333 aligned nucleotide positions with 46 parsimony-informative sites. A χ^2-test showed no significant deviation from stationarity for the nucleotide composition.

Maximum likelihood (ML) and maximum parsimony (MP) analyses resulted in congruent topologies (Fig. 1). Both analyses recovered high bootstrap support for the monophyly of the OTUs assigned to *G. s. marica* [ML-bootstrap (MLB): 92%; MP-bootstrap (MPB): 94%] and *G. s. subgutturosa* (MLB: 97%; MPB: 97%). However, these taxa do not show a sister group relationship, as *G. s. marica* shows a closer relationship to individuals from *G. cuvieri* and *G. leptoceros* (Fig. 1). Monophyly of a clade comprising the latter three taxa is strongly supported (MLB: 94%; MPB: 97%).

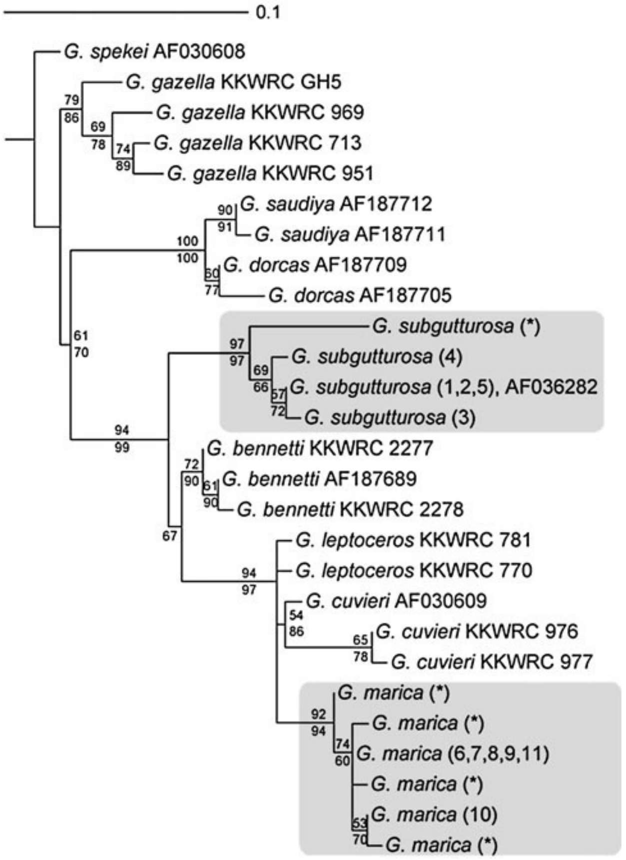

Fig. 1 Maximum likelihood tree of the cytochrome *b*-dataset with the GTR+Γ+I model. Likelihood bootstrap-support (*below*) and parsimony bootstrap support (*above*) were estimated from 1,000 replicates and are given at the branches. The *numbers* given in *brackets* correspond with the known provenances as shown in Table 1; *asterisks* indicate unknown provenance. For sequences obtained from GenBank accession numbers are given; for sequences obtained from KKWRC, sample numbers are provided

Crucially, a monophyletic clade comprising both *G. subgutturosa*-subtaxa was significantly rejected by hypothesis testing using an AU-test ($P = 0.001$). OTUs from wild caught animals belonging to the *G. s. marica*-clade originated from Saudi Arabia, Jordan, Syria, and Oman. All animals with known provenance in the *G. s. subgutturosa*-clade are from Iraq, Iran, Afghanistan, Azerbaijan and Chinese Turkistan. Our data, therefore, reinforce the observation made by Hammond et al. (2001) that the two "subspecies" *G. s. subgutturosa* and *G. s. marica* are more distantly related than previously thought. The Arabian subspecies (*G. s. marica*) is clearly more closely related to the north-African species *G. leptoceros* and *G. cuvieri*, suggesting that *G. s. marica* and *G. s. subgutturosa* have evolved independently. Our findings support the earlier interpretation of Ellerman and Morrison-Scott (1951) while conflicting with the grouping pattern inferred from a comparison of skull structure and horn conformation (Groves and Harrison 1967).

We are aware of the limitations of analysing mtDNA data only, but based on the marker examined here, the sand gazelle appears to form a more distinct conservation unit than might have been expected; in this sense restoration to full species status with name *G. marica* (Thomas 1897) may be the most helpful solution with respect to future conservation management and legislation for the *marica*-, *leptoceros*- and *subgutturosa*-grouping. It remains to be studied if other (i.e. nuclear) markers support our claim of polyphyly. However, Zink and Barrowclough (2008) demonstrated for birds that mitochondrial markers proved to be robust indicators of population histories and species boundaries. Theoretically, *G. s. subgutturosa* samples of unknown provenance, i.e. captive specimens, might have had a misleading effect on our interpretation, but our discussion of polyphyly is based primarily on individuals of known provenance.

Female *G. s. marica* show consistently long horns unlike generally hornless *G. s. subgutturosa* females (Groves and Harrison 1967), while specimens from the region between the Euphrates-Tigris basin and the Zagros Mountains of Iran were found to present a mix of characters. Consequently, this zone has been considered a hybrid zone (Groves and Harrison 1967; Kingswood and Kumamoto 1988; Groves 1997; Al-Robaae and Kingswood 2001). Sampling from the putative hybrid zone is very limited (Rutba region, Iraq in Table 1) but samples clustered with specimens from Chinese Turkistan and Afghanistan (Table 1) suggesting that either no overlap zone exists, or only male-biased introgression occurs.

The RFLP-analysis confirmed the biogeographical patterns we found from our sequence analysis as all captive animals from Saudi Arabia and Bahrain showed *G. s. marica* haplotypes, whilst gazelles of probable Iraqi origin were largely *G. s. subgutturosa* (see Table 1) with a minority of *G. s. marica* haplotypes (3 out of 13). All KKWRC animals, including those released in the Uruq Bani Ma'arid Protected Area, had *G. s. marica* mtDNA.

The genetic differences described here show that the original concern (Greth et al. 1996) to avoid mixing these putative 'subspecies' in captivity was fully justified. Further enlargement of the genetic sample base, especially of Asian *G. s. subgutturosa* and African *G. leptoceros*, and re-examination of the morphology of the entire group, is certainly indicated.

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Chapter 4

by Hannes Lerp, Torsten Wronski, Markus Pfenninger and Martin Plath

A phylogeographic framework for the conservation of Saharan and Arabian dorcas gazelles

The following manuscript was published in *Organisms Diversity & Evolution* 11 (pp. 317–329). The manuscript was peer-reviewed and I sequenced the cytochrome *b* gene and the control region of the used samples. I conducted the analyses together with Markus Pfenninger. All co-authors and I wrote and revised the manuscript. More details on my contribution to this collaborative work are given below in the form *Anlage 1* of the *Ausführungsbestimmungen für Dissertationen im Promotionsfach Biologie, die bereits veröffentlichte Teile oder eingereichte Manuskripte enthalten*.

Anlage 1

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts: A phylogeographic framework for the conservation of Saharan and Arabian dorcas gazelles

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen#	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	Hannes Lerp: 50% Co-Autoren: 50%	Torsten Wronski, Martin Plath
(2) Durchführung der einzelnen Untersuchungen/ Experimente	Hannes Lerp: Sequenzierung des Cytochrom-*b*-Gens und der mitochondrialen Kontrollregion	
(3) Erstellung der Daten-sammlung und Abbildungen	Hannes Lerp: Veröffentlichung der Sequenzen bei Genbank, Veröffentlichung des Stammbaums bei Treebase, Erstellen der Abbildungen	
(4) Analyse/Interpretation der Daten	Hannes Lerp: Haplotypennetzwerk mit TCS Hannes Lerp und Co-Autoren: Szenarien testen, phylogenetische Analyse mit BEAST, Interpretation der Daten Co-Autoren: Analyse des Bayesian Skyline Plots mit BEAST	Markus Pfenninger, Martin Plath
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	Hannes Lerp: 65% Co-Autoren: 35%	Martin Plath, Torsten Wronski, Markus Pfenninger

[#] Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

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Datum
zustimmende Bestätigung der vorgenannten Angaben

Unterschrift Promovend/Promovendin

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ORGANISMS DIVERSITY & EVOLUTION

A phylogeographic framework for the conservation of Saharan and Arabian Dorcas gazelles (Artiodactyla: Bovidae)

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Abstract Many species of gazelles (*Gazella* spp.) are nowadays threatened by hunting, poaching, habitat loss and habitat deterioration. Conservation efforts for this group not only face the problem of maintaining remnant populations, but often natural populations have been extirpated from the wild. In some cases, though, captive breeding programs exist that might provide a valuable source for future reintroductions. A major problem in this context is that phylogeographic relationships among different (potentially locally adapted) populations, and even basic phylogenetic relationships between species, are poorly understood, thus hampering the assignment of management units, breeding groups or stocks for reintroduction projects. Our present study focused on Dorcas gazelles (*G. dorcas* and *G. saudiya*) from the species' entire distribution range, with samples originating from western Saharan Africa into Saudi Arabia. In stark contrast to previous studies reporting on pronounced genetic structure in taxa such as Mountain gazelles (*G. gazella*), we detected low genetic diversity and no evidence for major phylogenetic lineages when analyzing two mitochondrial genetic markers. Using a coalescent approach we infer a steep population decline that started approximately 25,000 years before present and is still ongoing, which coincides with human activities in Saharan Africa. Our phylogenetic analyses, statistical parsimony network analysis and inferred colonization patterns shed doubt on the validity of various described subspecies of *G. dorcas*.

Keywords Desert ungulate · *Gazella dorcas* · Conservation unit · Phylogeography · Range expansion

Introduction

A number of ungulate species inhabiting the arid savannah and desert eco-regions of northern Africa and the Arabian Peninsula are nowadays threatened by extinction. The primary reason is certainly intensified hunting, which has become far more efficient since the introduction of firearm-based hunting from motorized vehicles (Newby 1988, 1990; Smith 1998; Mallon and Kingswood 2001). Furthermore, habitat loss or deterioration as well as competition with domestic livestock has had a major impact on many migratory ungulates (Smith 1998; Mallon and Kingswood 2001; Beudels-Jamar et al. 2006). These threats already led to the IUCN (2010) classification of scimitar-horned Oryx (*Oryx dammah*) as 'extinct in the wild' and 'critically endangered' in the case of addax (*Addax nasomaculatus*)

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and Dama gazelles (*Nanger dama*). The smaller antelopes of the genus *Gazella* are also affected and all of them are endangered.

The Dorcas gazelle (*Gazella dorcas*), which was once common throughout peri-Saharan North Africa, is no exception in this regard. Dorcas gazelles are thought to exist in a wide variety of habitats (Carlisle and Ghobrial 1968; Ghobrial 1970, 1974; Ghobrial and Cloudsley-Thompson 1976; Baharav 1980, 1982, 1983; Yom-Tov et al. 1995; Lafontaine et al. 2006), from Sahelan savannahs to semi-arid gravel and sand deserts, while avoiding hyper-arid areas and the upper elevations of the central-Saharan massifs (Haltenorth and Diller 1977; Dorst and Dandelot 1993; Yom-Tov et al. 1995; Wacher et al. 2004). Originally, Dorcas gazelles were distributed from Morocco and Mauretania in the west to the Horn of Africa in the east, and also occurred on the Sinai Peninsula and the Levant (e.g., Dorst and Dandelot 1993; Yom-Tov et al. 1995; East 1999; Fig. 1). On the Arabian Peninsula, representatives of this taxon were described as a separate species, the Saudi gazelle (*G. saudiya*). *Gazella saudiya* was thought to be isolated and distinct from African continental *G. dorcas* (Groves 1988; Rebholz et al. 1991; Hammond et al. 2001), but has most probably gone extinct (Thouless et al. 1997; Habibi and Williamson 1997). With the exception of Israel and Ethiopia (Yom-Tov and Ilani 1987; Mallon and Kingswood 2001), population numbers of Dorcas gazelles are decreasing at an alarming pace, and the remaining populations are even more fragmented than only a few decades ago (UNEP/CMS 1998; Smith 1998, 1999; Mallon and Kingswood 2001; Lafontaine et al. 2006). This decline is continuing and is estimated to have exceeded 30% over three generations (IUCN 2010), with less than 25% of the remaining animals living in protected areas (Mallon and Kingswood 2001).

Conservation efforts for Dorcas gazelles in different countries include the prohibition of hunting and establishment of protected areas (UNEP/CMC 1998; Smith 1998, 1999; Lafontaine et al. 2006). Most habitats across the species' natural range have not been degraded through over-exploitation, so reintroduction programs remain a feasible option given that poaching is prevented (Mallon and Kingswood 2001; Abáigar and Cano 2007a). Various breeding centers have started actions to preserve Dorcas gazelles (Abáigar and Cano 2007a, b), but a general lack of phylogenetic and phylogeographic information hampers progress (Ryder 1986, 1987; Hammond et al. 2001). Several subspecies of Dorcas gazelles were described on the basis of phenotypic variation, such as fur coloration, horn shape and length, and other morphometric measures (e.g., Alados 1987; Groves 1969, 1981, 1985a, b, 1988; Yom-Tov et al. 1995), but genetic differentiation is generally not well documented, and the presumed taxa seem to show no obvious ecological differences congruent with the proposed taxonomic classification (Lafontaine et al. 2006). The identification of taxonomically correct and phylogeographically appropriate populations of *G. dorcas* using a conservation genetic framework for the purpose of captive breeding and reintroduction programs is pivotal to the success of those programs (Avise 1989; Vogler and DeSalle 1994).

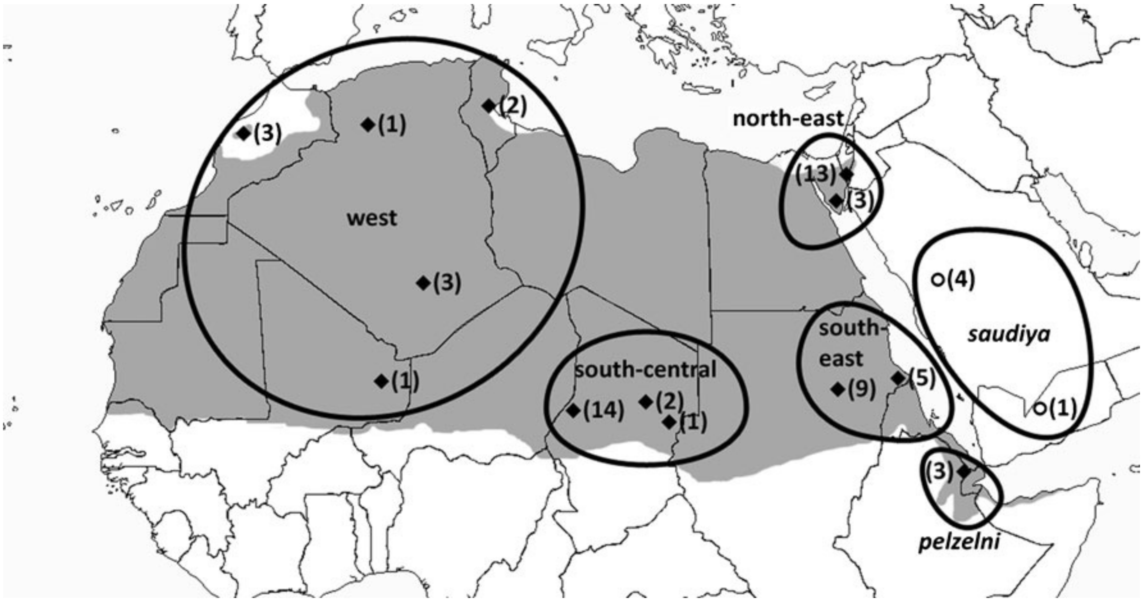

Fig. 1 Potential distribution (*gray shaded*) of Dorcas gazelles (*G. dorcas*) according to IUCN antelope survey reports (East 1988a, b; Mallon and Kingswood 2001) including sampling locations of wild specimens of Dorcas (♦) and Saudi gazelles (○) used in this study. Numbers in *brackets* indicate how many samples were obtained from that region, whereby nearby sampling points were pooled. *Circles* delineate groups according to the most likely grouping scenario as tested by AMOVA (scenario *a* in Table 2)

Here, we investigate sequence variation of the mitochondrial cytochrome *b* gene and control region of 73 Dorcas (*G. dorcas*) and Saudi gazelles (*G. saudiya*) throughout the entire distribution range to make inferences related to the following questions: (1) Is there evidence for genetic structure across the species' distribution range? (2) Did some populations diverge from (and show reduced gene-flow towards) neighboring ones, suggesting that they would need to be given priority in conservation programs as distinct management units? (3) Can we infer the phylogeographic origin of different populations or lineages? (4) Finally, can we find evidence for possible past and present population declines in the demographic history of the species using a coalescent approach?

Materials and methods

Study area and sample collection

We obtained samples from wild specimens from Mali and Algeria in the west to Sudan and Israel in the east (Fig. 1), thus covering the species' entire distribution range. Table 1 summarizes origins, collectors and the kind of material sampled. Additionally, we included samples from the Arabian Peninsula referred to as *G. saudiya*. We also included sequences of several other antelope taxa [genus *Gazella*: chinkara (*G. bennettii*), Mountain gazelle (*G. gazella*), Cuvier's gazelle (*G. cuvieri*), Slender-horned gazelle (*G. leptoceros*) and Goitered gazelle (*G. subgutturosa*); genus *Eudorcas*: Thomson's gazelle (*E. thomsonii*); genus *Nanger*: Grant's gazelle (*N. granti*), Dama gazelle (*N. dama*), genus *Antidorcas*: springbok (*A. marsupialis*); Table 1].

DNA extraction, sequencing and alignment

Samples used to extract DNA consisted of tissue, hairs, blood or fecal material. We extracted DNA by using the Qiagen Blood and Tissue Kit® for hair, skin or tissue samples by following the manufacturer's instructions. In the case of fecal samples we used the Qiagen Mini Stool Kit®; for museum samples we followed the phenol/chloroform extraction protocol described by Ausubel et al. (1995).

We amplified the left domain of the mitochondrial control region (Saccone et al. 1991) between the PRO-tRNA and the conserved central domain using the universal primer HL15926 (Kocher et al. 1989) and the gazelle-specific primer HH16397 (Arctander et al. 1996), or, alternatively, specific primers developed at the King Khalid Wildlife Research Centre (KKWRC; L15767: 5'-CCC ACT ATC AAC ACC CAA AGC TG-3'; H16220: 5'-CCC CAC GAT TTA TGG GCG T-3'; Hundertmark 2005). The complete coding region of the cytochrome *b* gene was PCR-amplified using KKWRC primers (L14724: 5'-TGA CTA ATG ATA TGA AAA ACC ATC GTT G-3'; H15915: 5'-TGC TCT CCT TCT CTG GTT TAC AAG AC-3'; Hundertmark 2005). In case of repeated amplification failure of the complete cytochrome *b* gene, we analyzed only a shorter (412 bp), upstream (5'-end) part of it using alternative primers developed at KKWRC [L14724 (see above); H15149: 5'-TAA CTG TTG CTC CTC AAA AAG ATA TTT GTC CTC A-3'; Hundertmark 2005]. All PCRs were performed in a 12.5-µl reaction volume with *Taq* PCR Core Kit® (Qiagen) using 2.5 units of *Taq* polymerase, 1x Q-Solution®, 200 µM of each dNTP and 0.16 µM of each primer. Amplifications of both markers were performed under the following conditions: initial denaturation (180 s at 95°C), followed by five cycle steps of 60 s at 94°C (denaturation), 90 s at 45°C (primer annealing) and 90 s at 72°C (elongation), then 40 cycle steps of 60 s at 94°C, 60 s at 50°C and 90 s at 72°C, and lastly, a final extension step (600 s at 72°C).

Sequencing of PCR fragments was conducted using the same PCR primers as used for PCR amplification using the BigDye Terminator Kit® (Applied Biosystems, Foster City, CA, USA). Sequences were run on a capillary ABI 3730 DNA Analyzer sequencer® (Applied Biosystems, Foster City, CA, USA). Chromatograms were analyzed with Geneious Pro v5.1.7 (Drummond et al. 2010) and sequences aligned with the ClustelW algorithm implemented in the software with a final correction done by eye. All new sequences have been submitted to GenBank (accession numbers JN410219-JN410357).

As not all samples delivered sequences from both markers, we composed different alignments for the phylogenetic and phylogeographic analyses: (1) the complete cytochrome *b* gene sequence including all outgroup samples, (2) the 412 bp fragment of the cytochrome *b* gene including all Dorcas and Saudi gazelle samples, and (3) a concatenated alignment of 1,612 bp length, consisting of the left domain of the mitochondrial control region and the complete cytochrome *b* gene in a subset of samples.

Mitochondrial haplotype phylogeny

The phylogeny of alignment (1) was inferred by Bayesian analyses performed in BEAST 1.5.2 (Drummond and Rambaut 2007). We chose a GTR + Γ + I model because it is the most conservative one (resulting in the widest confidence interval), with a gamma-shaped rate variation of 1.24% substitutions per million years (estimated from the Bayesian Skyline Analysis, see below). We ran four Metropolis coupled Monte Carlo Markov chains (MC^3) for 10 million generations. After a burnin phase of 1 million generations, trees were sampled every 1,000 generations. A majority consensus tree was computed from the sampled trees.

Table 1 List of specimens (wild and captive) of *G. dorcas* and *G. saudiya* and outgroups included in the phylogenetic analyses, their collectors, accession numbers and sample types. Numbers in brackets indicate the number of samples obtained from that location. Group assignment is in accordance with 'scenario *a*' (see Fig. 1)

Species	Origin (coord.)	Wild/ captive	Collector/ accession number (number of samples)	Sample type	Group
G. dorcas	Morocco, Rmila	Unknown	EU723704	GenBank	West
G. dorcas	Morocco, Sidi Chiker	Unknown	EU723705	GenBank	West
G. dorcas	Morocco, Bouznika	Unknown	EU723706	GenBank	West
G. dorcas	Mali, South Tamesna (N 17.080, E 1.934)	Wild	Unknown (1)	Feces	West
G. dorcas	Algeria, Hoggar Mountains	Wild	K. de Smet (3)	Tissue, hair	West
G. dorcas	Algeria, El Bayadh	Wild	A. Fellous, D. Koen (1)	Tissue, hair	West
G. dorcas	Tunisia	Wild	Powell Cotton Museum Birchington, Kent, UK (1); unknown (1)	Tissue, blood	West
G. dorcas	Chad (N 15.096, E 15.302)	Wild	T. Wacher (1)	Feces	South-central
G. dorcas	Chad (N 15.106, E 15.296)	Wild	T. Wacher (1)	Tissue	South-central
G. dorcas	Chad (N 15.393, E 15.306)	Wild	T. Wacher (11)	Tissue	South-central
G. dorcas	Chad (N 15.600, E 14.810)	Wild	T. Wacher (1)	Tissue	South-central
G. dorcas	Chad (N 15.488, E 14.653)	Wild	T. Wacher (1)	Tissue	South-central
G. dorcas	Chad (N 15.582, E 18.706)	Wild	T. Wacher (1)	Tissue	South-central
G. dorcas	Chad (N 15.102, E 20.557)	Wild	T. Wacher (1)	Tissue	South-central
G. dorcas	Sudan, Hafta (N 18.029, E 37.988)	Wild	Powell Cotton Museum Birchington, Kent, UK (4)	Tissue	South-east
G. dorcas	Sudan, Mashail (N 17.750, E 38.083)	Wild	Powell Cotton Museum Birchington, Kent, UK (1)	Tissue	South-east
G. dorcas pelzelni	Ethiopia, Danakil (N 14.000, E 40.500)	Wild	Natural History Museum (3)	Tissue	*Pelzelni*
G. dorcas	Israel, southern Arava Valley	Wild	R. Hammond, D. Blank (10)	DNA, tissue	North-east
G. dorcas	Israel, Haibar	Wild	D. Blank (3)	Tissue	North-east
G. dorcas	Israel, Eilat region	Wild	D. Blank (1)	Tissue	North-east
G. dorcas	Egypt, Sinai (N 28.097, E 34.398)	Wild	T. Wacher (1)	Tissue	North-east
G. dorcas	Egypt, Sinai (N 28.402, E 33.705)	Wild	T. Wacher (1)	Feces	North-east
G. dorcas	Egypt, Sinai (N 27.935, E 34.023)	Wild	T. Wacher (1)	Tissue, hairs	North-east
G. dorcas	Sudan, east of Nile	Captive	Al-Wabra Wildlife Preservation (2), Breeding Center Sharjah (2), S. Muhammad (3), M. Sandouka (1)	Tissue, blood	South-east
G. dorcas	Saudi Arabia	Captive	King Khalid Wildlife Research Center, Thumamah (2)	Blood	-
G. dorcas	Qatar	Captive	Al-Wabra Wildife Preservation (1)	Blood	-
G. dorcas pelzelni	Qatar	Captive	Al-Wabra Wildife Preservation (4)	Tissue, hair	*Pelzelni*
G. dorcas	Sudan	Unknown	Unknown	Blood	South-east
G. saudiya	Saudi Arabia, Dhalm (N 22.500, E 41.400)	Wild	Natural History Museum (2)	Tissue	*Saudiya*

Table 1 (continued)

Species	Origin (coord.)	Wild/ captive	Collector/ accession number (number of samples)	Sample type	Group
G. saudiya	Saudi Arabia, Sirr Al Yamani (N 16.200, E 46.500)	Wild	AF187710	GenBank (Hammond et al. 2001)	*Saudiya*
G. saudiya	Saudi Arabia, Wadi Markha (N 14.570, E 46.350)	Wild	AF187722	GenBank (Hammond et al. 2001)	*Saudiya*
G. saudiya	unknown	Wild	AF187711	GenBank (Hammond et al. 2001)	*Saudiya*
			Outgroups		
G. gazella			Farasan Islands, King Khalid Wildlife Research Center (11), AJ222682	Tissue, blood, GenBank (Hassanin and Douzery 1999)	
G. bennettii			King Khalid Wildlife Research Centre, Thumamah (3)	Blood	
G. leptoceros			Tunisia, Algeria, Western Desert Egypt, T. Wacher (5)	Hair	
G. cuvieri			Estación Experimental de Zonas Áridas in Almeria, Spain (2)	Hair	
G. subgutturosa			AF036282	GenBank (Hassanin and Douzery 1999)	
Eudorcas thomsoni			FJ556559	GenBank	
Nanger dama			AF025954	GenBank (Matthee and Robinson 1999)	
Nanger granti			AF034723	GenBank (Hassanin et al. 1998)	
Antidorcas marsupialis			AF022054, AF036281	GenBank (Hassanin and Douzery 1999; Matthee and Robinson 1999)	

A statistical parsimony (SP) network was constructed using TCS v1.21 (Clement et al. 2000) with alignments (1) and (2). The connection limit was set at 95% such that no outgroup animal would be connected. Gaps were treated as fifth character state.

The 32 sampling sites were pooled into 13 different geographic scenarios (see Fig. 1 and Online Resource Fig. S1 for details) and tested with a series of AMOVAs (using Arlequin 3.5.1.2; Excoffier and Lischer 2010) with the aim to detect the most likely natural grouping. We used an information theoretical approach to discriminate between the different models based on their Akaike weight. Using the inferred best grouping, we determined the exact population differentiation (based on haplotype frequencies) as implemented in Arlequin 3.5.1.2 (Excoffier and Lischer 2010) with 1 million Markov Chain steps and a burnin of 100,000 steps. Different groups were treated as separate populations.

The program Migrate-n 3.2.6 (Beerli and Palczewski 2010) was used to investigate past and present gene-flow patterns. We ran an analysis with an unconstrained migration model using the Bayesian Inference option to obtain probability estimates. As starting parameters, we used an UPGMA tree as first genealogy, initial theta values were generated randomly, and M values (number of migrants) were generated from F_{ST}-calculation. We used a migration matrix model with variable theta and constant mutation rate. We ran four MC^3 analyses with a static heating scheme. The chain was run for 20 million generations while sampling every 1,000 generations. The first 10 million generations were discarded as burnin. We replicated the analysis multiple times but obtained no stable results (see results). For this analysis alignment (3) was used. Finally, based on the most likely structure among populations (see above) we tested for isolation-by-distance (IBD) using Mantel's tests (as implemented in GenAlEx 6.4; Peakall and Smouse 2006), while contrasting three potential dispersal scenarios that differed in the route(s) by which the Red Sea was assumed to have been crossed (i.e., via the Sinai, the Bab al-Mandab strait or both). Pairwise F_{ST} values were obtained from Arlequin 3.5.1.2 (Excoffier and Lischer 2010), and geographic distances were determined in DIVA-GIS 7.1.7.2 (Hijmans et al. 2005), whereby potential migration routes were inferred from the gene-flow analyses.

Past demography of the mitochondrial *G. dorcas* clade

Past population demography for Dorcas gazelles was inferred with alignment (3) using a Bayesian Skyline Plot model (Drummond et al. 2005) as implemented in BEAST 1.5.2 (Drummond and Rambaut 2007) and visualized in Tracer 1.5. This individual-based coalescent approach uses standard MC^3 sampling procedures to estimate a posterior distribution of gene genealogies and population parameters under a GTR+Γ+I substitution model used for the same reasons as mentioned above. Because only intraspecific data were used in this analysis, a strict clock model was employed (Drummond et al. 2006). These distributions were then used to generate confidence intervals that represent model, phylogenetic and coalescent uncertainty (Drummond et al. 2005). The hyperparameter *m* (number of grouped intervals) was set to 100. Four independent MC^3 analyses of 10^7 steps each were performed while sampling every 1,000 generations with the burnin set at 10% of the run (we checked with Tracer 1.5 that stationarity was reached). *A priori* information on the mean substitution rate per year was available for both markers (0.015 substitutions per million years for the cytochrome *b* gene, Ho et al. 2005; 0.15 substitutions per million years for the control region, Guo et al. 2006). Both rates were not estimated specifically for Dorcas gazelles, and we are aware of potential caveats (e.g., taxon-specific differences). We used normally distributed priors with means set to the aforementioned values and standard deviations of 0.005 and 0.05, respectively, i.e., 95% confidence intervals of 0.005–0.025 and 0.05–0.25, respectively.

Results

Phylogeny of *Gazella dorcas*

The alignment of the complete cytochrome *b* gene [alignment (1)] comprised 56 sequences of Dorcas gazelles (*G. dorcas*) and 27 sequences of other antelope taxa; the resulting phylogenetic tree from the Bayesian Inference analysis is shown in Fig. 2 (TreeBASE submission ID 11478, http://purl.org/phylo/treebase/phylows/study/TB2:S11758). High support [i.e., posterior probability (PP) greater than 0.9] for the monophyly of the genus *Gazella sensu stricto* was uncovered. Within the genus *Gazella* two different lineages were well supported: one comprising *G. dorcas* plus *G. gazella* and one including *G. bennettii*, *G. subgutturosa*, *G. leptoceros* and *G. cuvieri*. All but one sample of Dorcas gazelles formed a reciprocally monophyletic clade with a sister group relationship to *G. gazella* (PP=1). One presumed *G. dorcas* sample from the Arava valley in Israel clustered within *G. gazella* and hence seems to have been misidentified. Indeed, *G. dorcas* and *G. gazella* co-occur sympatrically in this region. We could not include any Speke's gazelle (*G. spekei*)—which is also closely related to Dorcas gazelles (Wacher et al. 2011)—into our analysis, so the exact sister group relationships of Dorcas gazelles remain to be determined in more detail.

Population differentiation and phylogeographic analyses

We compared different scenarios of the most likely population structure. For this analysis we used alignment (2) as more samples from a wider geographic range could be included in this alignment (e.g., *G. saudiya* was included). The scenario that received the best support was 'scenario *a*' (Table 2; relative AIC weight=0.43) and comprised the groups: 'west,' 'south-central,' 'north-east,' 'south-east,' '*saudiya*' and '*pelzelni*' (with the latter two corresponding with taxa described as sister group to and subspecies of Dorcas gazelles, respectively; Alados 1987; Groves, 1969, 1981, 1985a, b, 1988; Fig. 1, Table 1). The scenario with the second best support (scenario *b*, relative AIC weight=0.22) was a slight modification of the first, the only difference being that the groups 'north-east' and 'south-east' were pooled (see Online Resource Fig. S1). A test of exact population differentiation, in which the groups under scenario *a* were treated as separate populations, found significant differentiation toward all other groups in the case of the groups 'west,' 'north-east,' '*pelzelni*' and '*saudiya*' (Table 3). This implies that the locations of the groups 'south-east' and 'south-central' are the most likely center of origin of the species from where it must have dispersed, e.g., onto the Arabian Peninsula. We tested for

Fig. 2 Phylogeny based on the complete cytochrome *b* gene, Bayesian analyses of 83 sequences under the GTR + Γ + I parameters. Posterior probability values larger than 0.9 are reported. Dorcas gazelles are *gray shaded* except for one presumed *G. dorcas* sample (*) from the Arava valley in Israel, which clustered within the *G. gazella* 'south' clade (the sample seems to have been misidentified). The phylogeny can be found in TreeBase (submission ID 11748, http://purl.org/phylo/treebase/phylows/study/TB2:S11758)

Table 2 Results of AMOVAs for the different population groupings (*a*—*m*)

Scenario[1]	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)	(*j*)	(*k*)	(*l*)	(*m*)
Number of local groups	6	5	3	4	5	5	4	4	3	2	6	5	5
	% Variation:												
Among groups	37.78	41.89	12.80	41.51	32.02	41.65	37.23	32.53	24.61	−13.42	40.39	36.84	36.59
Among populations within groups	19.24	17.31	45.03	19.56	25.46	19.22	23.60	27.23	35.43	66.05	14.70	18.17	18.42
Within populations	42.99	40.79	42.18	38.93	42.51	39.13	39.17	40.23	39.97	47.37	32.60	32.68	32.68
Relative AIC weight	0.43	0.22	0.00	0.05	0.05	0.06	0.01	0.00	0.00	0.00	0.12	0.03	0.03

Partitioning of molecular variance within and among sampling sites; significance of variance components was tested by 1,000 permutations. Grouping (*a*) yielded a maximum relative Akaike information criterion weight (AIC).

[1] See Fig. 1 and Online Resource Fig. S1

Table 3 Probability (*P*-)values of exact population differentiation according to the most likely grouping ('scenario *a*'; Fig. 1). Significant values ($P<0.05$) are highlighted bold

Group	West	South-central	North-east	South-east	*Saudiya*
South-central	0.266±0.0035				
North-east	**0.017±0.0008**	0.053±0.0015			
South-east	0.245±0.0036	1.000±0.0000	0.052±0.0016		
Saudiya	**0.033±0.0007**	0.249±0.0031	**0.025±0.0007**	0.209±0.0025	
Pelzelni	**0.026±0.0007**	0.178±0.0028	**0.012±0.0005**	0.149±0.0021	**0.010±0.0003**

isolation-by-distance (IBD) based on three scenarios of how the Red Sea could have been crossed: range expansion via (1) the Sinai Peninsula, (2) across the Bab al-Mandab strait in the southern part of the Red Sea or (3) via both routes. No significant correlation was found for any of the tested scenarios, but scenario (1) explained more variance than the other two [scenario (1), r=0.543, P=0.091; scenario (2), r= 0.429, P=0.168; scenario (3), r=0.407, P=0.168].

Statistical parsimony (SP) networks were constructed using alignments (1) and (2), and are shown in Fig. 3. In both networks we found pronounced haplotype sharing, especially between the groups 'south-central' and 'south-east'. In the network shown in Fig. 3b, which comprises considerably more genetic information but fewer samples, shared haplotypes are seen in the case of the groups 'north-east' and 'south-east' as well as between the groups 'south-east' and 'south-central'. Haplotypes found in the '*pelzelni*' group are all private and comprise two distinct lineages. In the case of the Saudi gazelle, which could only be included in alignment (2), we found two different haplotypes that are distinct from all other samples (Fig. 3a), but this separation is the result of only two mutational steps.

For the analyses of past and present gene-flow patterns we used the alignment with the most sequence information [alignment (3)]. Even extended runs of the program Migrate-n did not provide stable results, in particular with respect to the direction of gene flow between the 'west' and the two southern groups. We attribute this to a lack of a clear-cut signal in the data set, and/or insufficient sample size in individuals and/or markers, respectively. Some features of the gene-flow network, however, were stable in all runs: we found high gene-flow between the groups 'south-central' and 'south-east'. This corresponds with the finding of shared haplotypes in both groups in the SP cladogram and the exact population differentiation test. Furthermore, we could not detect any gene flow between the groups 'west' and 'north-east'.

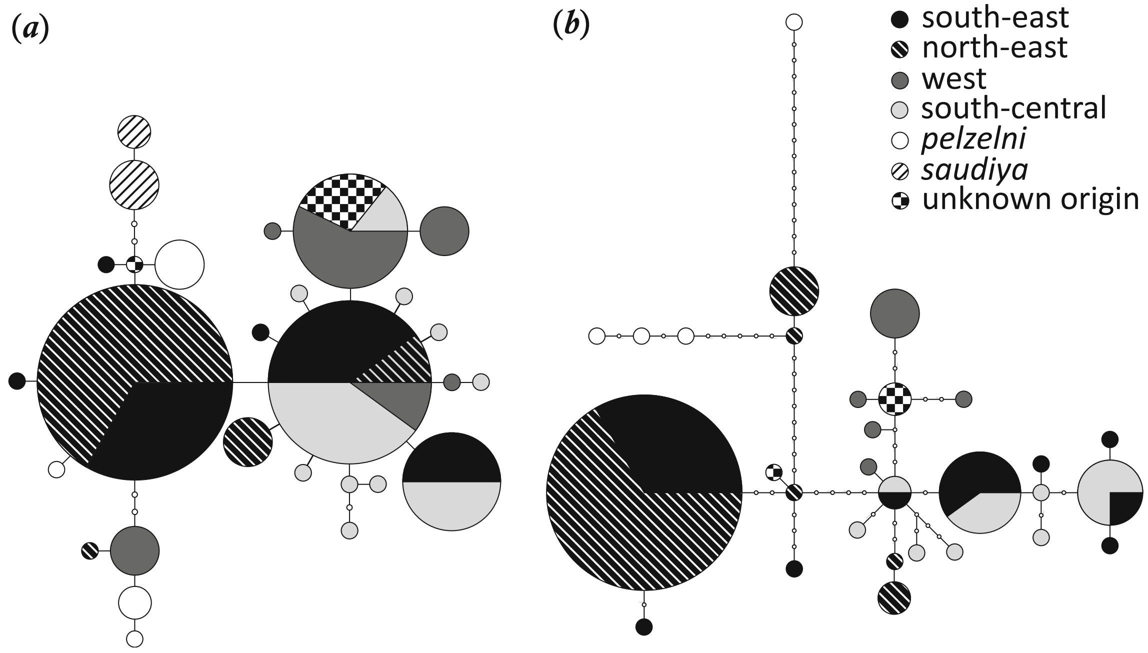

Fig. 3 Statistical parsimony network based on (**a**) a 412 bp fragment (73 sequences) and (**b**) the complete cytochrome *b* gene (57 sequences). Each *circle* represents a different haplotype, whereby circle size is proportional to the number of individuals in our data set showing that haplotype (legend size represents one animal). The *color code* indicates the origin of the samples (see legend). Smaller *open circles* represent missing haplotypes, and *connecting lines* correspond to one mutational step

Bayesian Skyline Plot

Estimated mean substitution rates for the mitochondrial control region and cytochrome *b* gene were 0.156 (95% confidence interval: 0.121–0.195) and 0.0124 (0.008–0.017) substitutions per million years, respectively. Both posterior estimates corresponded well with their respective priors. Furthermore, the data changed the prior information and narrowed the 95% confidence interval estimates for both markers, further justifying the use of priors as described before. The inferred time to the most recent common ancestor was 768,000 years before present with a 95% confidence interval of 1,110,000–468,000 years before present. The past population demography (Fig. 4) shows a long period of stability followed by an exponential increase in population size (starting approximately 200,000 years before present), until a steep population decline started about 27,175 to 17,500 years before present (gray shaded area in Fig. 4), which continues until the present day.

Discussion

Lack of detailed information about phylogenetic relationships among and within threatened groups of animals can hamper conservation efforts (Avise 1989; Vogler and DeSalle 1994). For instance, unrecognized differentiation within a putative species can lead to admixture of independent evolutionary entities in captivity. In this context, conservation genetic approaches are valuable tools for captive breeding and in situ conservation programs as morphologically indiscernible (cryptic) animal species appear to exist throughout taxonomic groups and biogeographic regions (Pfenninger and Schwenk 2007; Trontelj and Fišer 2009; Rueness et al. 2011). Investigations of other gazelle species revealed that phenotypic differences in this group do not always correspond with phylogenetic splits (in other words: morphological differences are sometimes poor indicators of species boundaries or genetic differentiation among populations). For example, a recent phylogenetic study on sequence divergence of mitochondrial cytochrome *b* and the control region of Mountain gazelles (*G. gazella*)—which are closely related to *G. dorcas*—found evidence for two reciprocally monophyletic lineages, one of which is restricted to a small area on the Golan Heights and may be considered as a separate species (Wronski et al. 2010). In another study comparing cytochrome *b* sequences of Goitered gazelles (*G. subgutturosa*), it was shown that the presumed species is clearly polyphyletic, and the Sand gazelle (*G. marica*) from the Arabian Peninsula—which was until then regarded as a subspecies of Goitered gazelles—is an evolutionarily significant unit (ESU) and should therefore be treated as a distinct management unit (Wacher et al. 2011).

No thorough phylogenetic or phylogeographic analyses focusing on *Gazella dorcas* have been conducted until now (IUCN 2010). Our present study was designed to fill this gap of knowledge. We are aware of the limitations of analyzing mitochondrial DNA data only [e.g., introgression, incomplete lineage sorting; see Funk and Omland (2003) for a review], but the kind of samples from which we could extract DNA ranged from dried skin of museum specimens to feces, or blood droplets on sand, and amplifying mitochondrial markers was the most feasible and promising option given the often very poor quality of

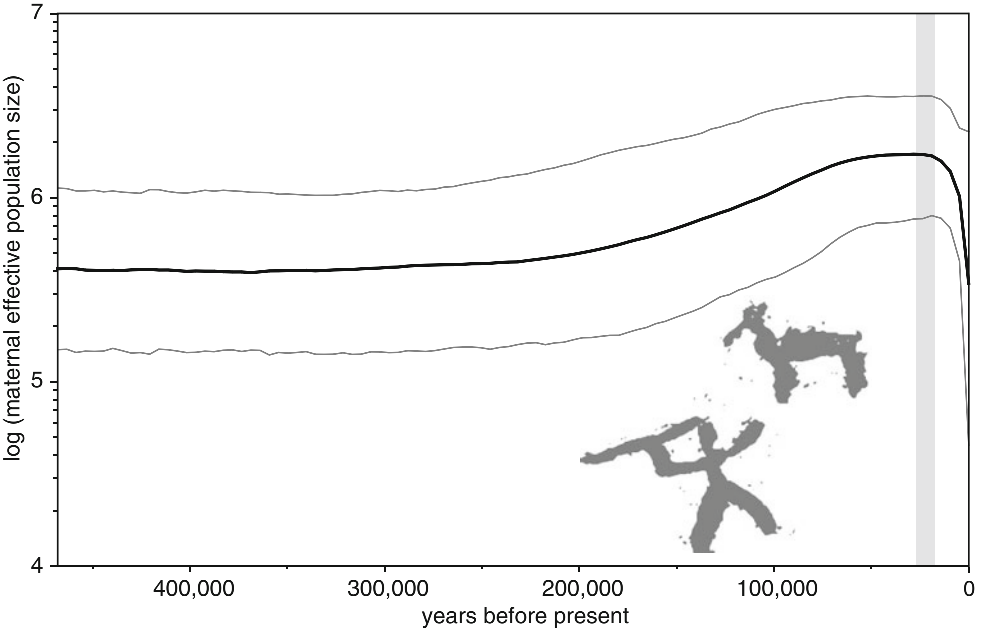

Fig. 4 Bayesian Skyline plot showing maternal effective population size (mean and 95% confidence interval) based on 1,612 bp concatenated cytochrome *b* gene and mitochondrial control region sequences of 53 Dorcas gazelles over time (x-axis: years before present). The *gray shaded area* marks the onset of the decline in effective population size. *Inserted figure* shows a hunting scene from ancient Saharan rock art illustrating our interpretation of the cause of the observed population decline

DNA obtained from those samples. We found Dorcas gazelles to form a reciprocally monophyletic clade consistent with the idea that all individuals included in our present study are members of one species with little intraspecific genetic structure. Following the integrative species concept (see de Queiroz 2007 for a review), we did not find evidence for separately evolving metapopulation lineages (*sensu* de Queiroz 2007). The admixture of *G. dorcas* samples of different geographic origins in several clades of our phylogeny (Fig. 2) could—theoretically—indicate "cryptic" metapopulations occurring in sympatry. This scenario seems very unlikely though, as no ecological or behavioral data suggest that two forms of Dorcas gazelles occur sympatrically. Furthermore, branch lengths within the *G. dorcas* clade in our phylogenetic tree were relatively short compared to other gazelle taxa (Fig. 2). Unlike the other gazelle taxa mentioned above, Dorcas gazelles, therefore, can be regarded as one evolutionarily significant unit (ESU). Low genetic differentiation among the different groups of Dorcas gazelles, with samples stemming from sites as distant as Mali and the Sinai, may be indicative of high ongoing gene flow because of migration or a recent range expansion. Our analysis of the species' past demography, in which the time to the most recent common ancestor of Dorcas gazelles was estimated as approximately 768,000 years before present, argues in favor of the latter hypothesis.

Gentry (1964) suggested that the origin of *G. dorcas* is palaearctic, extending into North Africa. Our present data, however, designate the south-central and south-eastern parts of the species' current distribution range as the likely center of origin as we found the largest haplotype diversity in that region, along with pronounced haplotype sharing among the two groups (Fig. 3). Furthermore, when testing for exact population differentiation we found no differentiation between the south-central and south-eastern groups ($P = 1.000 \pm 0.0000$), From here, the species could have colonized the other parts of its present distribution range, including the Arabian Peninsula. The weak gene flow between animals from the Sinai and the Arava valley to the 'west' group indicates some form of geographic barrier between those groups, preventing migration; most probably this can be attributed to the Red Sea in combination with the Nile delta. In order to investigate this phenomenon in more detail, Dorcas gazelles from Libya and Egypt should be included in future studies.

Rebholz et al. (1991) proposed that Saudi gazelles might be more distantly related to Dorcas gazelles than previously assumed (see also Rebholz and Harley 1999), but their analysis was partly based on samples from captive breeding groups, and it remains doubtful whether those samples were truly Saudi gazelles (Hammond et al. 2001). While probably extinct in the wild, presumed Saudi gazelles held in captivity appear to be the product of repeated hybridization with other gazelle taxa such as chinkara (*G. bennetti*) and Goitered gazelle (*G. subgutturosa*; Rebholz and Harley 1997; Hammond et al. 2001). Accordingly, the IUCN status of *G. saudiya* had to be changed from 'extinct in the wild' to 'extinct' (IUCN 2010). Our present study clearly places Saudi gazelles in the *Dorcas* clade, and genetic distances to other representatives of this clade were small.

We tested for isolation-by-distance (IBD) based on different migration scenarios to determine the route(s) by which Saudi gazelles may have reached the Arabian Peninsula. Even though tests for IBD were not statistically significant, most support was found for a scenario where Saudi gazelles arrived on the Arabian Peninsula via the Sinai Peninsula rather than by crossing the Bab al Mandab strait, which is congruent with the findings of Fernandes et al. (2006) that no land bridge existed in the area of the Bab al Mandab strait for at least 470,000 years. Ferguson (1981) proposed that another immigration event across the Nile and the Sinai Peninsula may have occurred recently (6–8,000 years ago). Palaeogeographic data indeed suggest that Dorcas gazelles expanded their range into the Mediterranean domain during the post-Neolithic period, thus replacing *G. gazella*—a species adapted to a more humid climate—from the drier parts of its former range (Davis 1980; Tchernov et al. 1986; Yom-Tov and Tchernov 1988). Ferguson's (1981) hypothesis implies that north African *G. d. dorcas* and coastal *G. d. isabella* may have migrated onto the Sinai at different points in time. Contrary to the predictions from this hypothesis, our data indicate a close relationship between *G. dorcas* from Sinai and the Levant and Pelzeln's gazelle ('*pelzelni*' group in Fig. 3b), suggesting that there may have been a continuous population east of the river Nile reaching from Israel to Somalia, probably during the more humid period following the last glacial period. It needs to be stressed though that genetic differences among groups were generally low, so this hypothesis remains speculative.

A steep decline in effective population size—calculated through a coalescent approach—started about 27,000 years ago and is still ongoing (Lafontaine et al. 2006). This date coincides with first records of spear hunting in northern Africa and the Middle East (Nentwig 2007). The typical stone projectile tips of such spears were found associated with large game species (Legge and Rowley-Conwy 1987; Uerpmann 1987; Nentwig 2007). Furthermore, Bar-Oz et al. (2011) recently reported that hunting strategies of post-Neolithic human societies played a major role in mass kills of gazelles in the ancient Near East. On the other hand, the onset of the population decline also coincides with the minimum sea level during the last glacial maximum (Fleming et al. 1998). Our Bayesian Skyline analysis, however, does not support the idea that changes between glacial periods (associated with a hyperarid Sahara) and interglacials (corresponding with more humid climate) had a major influence on the population dynamics, as no effect of other Pleistocene climate cycles on the population size was discernible (compare the long phase of stationarity in Fig. 4). Hunting—starting in ancient times—thus seems to have had a more drastic effect on Dorcas gazelles than previously thought.

Taxonomical implications of our phylogenetic analyses

Several subspecies of Dorcas gazelles from different regions of their distribution range have been described on the basis of phenotypic variation (e.g., Lange 1970; Rostron 1972; Alados 1987; Groves 1969, 1981, 1985a, b, 1988; Yom-Tov et al. 1995). Our phylogeographic analysis, however, found no evidence for any clear-cut geographic pattern of genetic structure and thus sheds doubt on the validity of the proposed subspecies. For example, we tested a scenario corresponding to a subspecies grouping forwarded by Alados (1987; Fig. S1*k*), but this model received little support (relative AIC weight=0.12). The hypothesis of a northern Saharan subspecies (*G. d. dorcas*), a southern Saharan subspecies (*G. d. osiris*) plus *G. d. massaesyla* and *G. d. isabella*, as suggested by Groves (1981), as well as the hypothesis of only a single subspecies inhabiting the entire southern Saharan region, being isolated from the northern Saharan populations, as suggested by Lafontaine et al. (2006), could also be rejected.

The question of whether *G. saudiya* is a separate species as suggested by some authors (Rebholz et al. 1991; Rebholz and Harley 1999) or just another form of Dorcas gazelles could not be resolved in detail. The private haplotypes found in this study are congruent with those detected by Hammond et al. (2001). The close connection (two mutational steps) to the next relative of Dorcas gazelles in the SP network (Fig. 3a), however, clearly shows the germane relationship of both forms. Also in the case of Pelzeln’s gazelle (‘*pelzelni*’ clade in Fig. 1), our data support the inclusion into *G. dorcas* firstly recommended by Groves (1969). In the SP network analyses we found two divergent mt-haplotype lineages, a pattern that became most obvious when using the complete cytochrome *b* sequence (Fig. 3b). Nevertheless, samples used in the latter analysis were obtained from Al Wabra Wildlife Preservation, not from the wild, and interpretations regarding natural hybridization or inbreeding should be made carefully. The described morphological distinctiveness of Pelzeln’s gazelles may be attributable to local adaptation to habitats with higher humidity (Alados 1987) coupled with incomplete lineage sorting. Keeping Dorcas and Pelzeln’s gazelles separately in captivity would be the most adequate solution regarding conservation until future studies using nuclear markers (e.g., SNPs) have been conducted to answer this question conclusively.

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Chapter 5

by Hannes Lerp, Torsten Wronski, Martin Plath, Anne Schröter and Markus Pfenninger

Phylogenetic and population genetic analyses suggest a potential species boundary between Mountain (*Gazella gazella*) and Arabian Gazelles (*G. arabica*) in the Levant

The following manuscript was published as short communication in *Mammalian Biology* 78 (pp. 383-386). The manuscript was peer-reviewed and I analysed the data. I wrote the manuscript that was revised by myself and all co-authors. More details on my contribution to this collaborative work are given below in the form *Anlage 1* of the *Ausführungsbestimmungen für Dissertationen im Promotionsfach Biologie, die bereits veröffentlichte Teile oder eingereichte Manuskripte enthalten.*

Anlage 1

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts: Phylogenetic and population genetic analyses suggest a potential species boundary between Mountain (*Gazella gazella*) and Arabian Gazelles (*G. arabica*) in the Levant

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen[#]	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	Hannes Lerp: 90% Co-Autoren: 10%	Martin Plath
(2) Durchführung der einzelnen Untersuchungen/ Experimente	Hannes Lerp und Co-Autoren: Amplifizieren der Mikrosatelliten Co-Autoren: Sequenzierung des Cytochrom-*b*-Gens und der mitochondrialen Kontrollregion	Anne Schröter
(3) Erstellung der Daten-sammlung und Abbildungen	Hannes Lerp: Veröffentlichung der Sequenzen bei Genbank, Erstellen der Abbildungen	
(4) Analyse/Interpretation der Daten	Hannes Lerp: phylogenetische Analyse mit BEAST, Analyse der Mikrosatelliten mit STRUCTURE und GENETIX Hannes Lerp und Co-Autoren: Interpretation der Daten	Markus Pfenninger, Martin Plath
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	Hannes Lerp: 85% Co-Autoren: 15%	Torsten Wronski, Martin Plath, Markus Pfenninger

[#] Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

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Short communication

Phylogenetic and population genetic analyses suggest a potential species boundary between Mountain (*Gazella gazella*) and Arabian Gazelles (*G. arabica*) in the Levant

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ABSTRACT

Two cryptic lineages of 'Mountain Gazelles' have been reported based on molecular phylogenetic analyses using maternally inherited (mitochondrial) sequence markers, namely *Gazella gazella* in the Levant and *G. arabica* south of the Arava Valley into the Arabian Peninsula. Here, we provide a rigorous test for the existence of two distinct lineages based on bi-parentally inherited (nuclear microsatellite) markers. Our study confirms two genetically distinct clusters in the Levant and detected no gene-flow between them. Divergence time (inferred from a cytochrome *b*-based phylogeny) was approximately one MYA. Treating and breeding both lineages separately in future conservation and captive breeding programmes is highly recommended.

'Mountain Gazelles' inhabit broken or undulating terrain in the Levant and the Arabian Peninsula. They were formerly described as a single species [*Gazella gazella* (Pallas, 1766)] with a number of presumed subspecies of doubtful taxonomic validity (Groves 1996). A recent phylogenetic analysis by Wronski et al. (2010) suggested that gazelles originating from the Golan Heights are reciprocally monophyletic to representatives from southern Israel and the Arabian Peninsula, alluding to the existence of two cryptic species. Bärmann et al. (in press) found the lectotype skin of *G. arabica* (Lichtenstein, 1827) to be nested within the Arabian clade of Mountain Gazelles (here called Arabian Gazelles), hence the name *G. arabica* is available for this taxon (provided that the lectotype skull – belonging to *G. gazella* – is excluded from the type). However, the results obtained by Wronski et al. (2010) were based on mitochondrial DNA only, and in theory the spatial distribution pattern of maternal haplolines could merely reflect female philopatry along with male-biased dispersal. To overcome limitations of mtDNA-based phylogeny we used bi-parentally inherited, nuclear microsatellite loci for multi-locus genotyping of both taxa and for unravelling possible gene-flow between them, while focussing on Israel as a region where both taxa supposedly co-occur (Mendelssohn et al. 1997).

We analysed 47 specimens of both putative taxa originating from the Levant and the western Arabian Peninsula (Fig. 1A, Suppl. material). Twenty-one specimens from Wronski et al. (2010) were re-analysed (i.e. re-sequenced); all others were obtained from the Steinhardt National Collection, Tel-Aviv (18 specimens) and other collectors (eight specimens). For phylogenetic analysis, we sequenced the cytochrome *b* gene of 39 specimens following Lerp et al. (2011) while including *G. dorcas* and the genera *Nanger* and *Eudorcas* in the final alignment (Genbank accession numbers are given in Fig. 1B). Bayesian analysis was performed in BEAST 1.6.1 (Drummond and Rambaut 2007); no outgroup was defined beforehand. jModelTest 0.1.1 (Posada 2008) identified HKY + Γ as the best fitting substitution model. We used molecular clock data estimates inferred for *G. dorcas* (Lerp et al. 2011) and ran MC^3 simulations with 10^7 generations, discarding the first 10% of the runs as burn-in. To compare sequence divergence within *G. gazella*, within *G. arabica*, and between both taxa, respectively, we calculated pairwise Kimura 2-Parameter *p*-distances (K2P) using MEGA 5 (Tamura et al. 2011) and conducted a Mantel-Test with K2P values (multiplied by 100, see Tobe et al. 2010) as the dependent variable and a binary independent variable that differentiated

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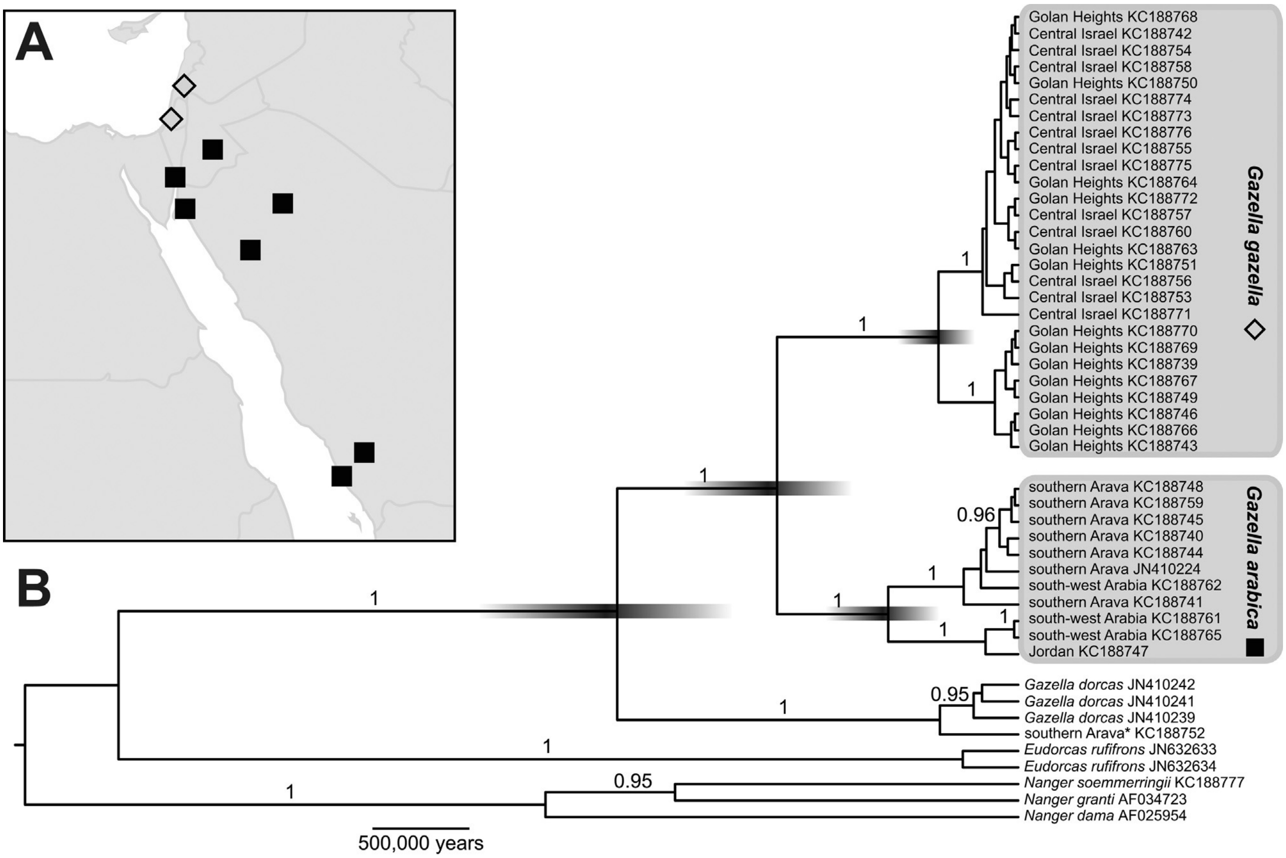

Fig. 1. Phylogeny based on cytochrome *b* sequences. (A) Sample origin. (B) Bayesian analysis was performed with 47 sequences with the HKY + Γ substitution model. Only PP-values ≥0.95 are reported. Node bars represent the 95% credibility intervals of divergence times of statistically supported phylogenetic splits. One presumed *G. arabica* sample (*) from the Arava Valley in Israel clustered within *G. dorcas* and seems to have been misidentified.

between comparisons within species ('0') and comparisons between species ('1'). This analysis was conducted using FSTAT 2.9.3.2 (http://www2.unil.ch/popgen/softwares/fstat.htm).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2012.11.005.

For the population genetic analyses we amplified microsatellite loci BM302, BM415, BM4505 (Bishop et al. 1994); CSSM043 (Moore et al. 1994); INRA40 (Vaiman et al. 1994); MCM38 (Hulme et al. 1994); OarFCB304 (Buchanan and Crawford 1993), RM088 (Kossarek et al. 1995); SRCRSP-6 (Bhebhe et al. 1994); TEXAN6 (Burns et al. 1995a) and TEXAN19 (Burns et al. 1995b) with dye-labelled forward primers (Cy5 and IRD700 obtained from Metabion; Dy-751 from Biomers) for visualization on a Beckman Coulter capillary sequencer CEQ 2000. Different dye-labelled primer pairs were arranged in three multiplex reactions using the Type-it Microsatellite PCR Kit® (Qiagen, Hilden, Germany).

We used GENETIX 4.05.2 (Belkhir et al. 2004) to visualize genetic differences by means of a multidimensional factorial correspondence analysis (FCA). STRUCTURE 2.3.3 (Pritchard et al. 2000) was employed to identify the number of genetically distinct clusters (K) according to Evanno et al. (2005) using the web-based tool STRUCTURE HARVESTER 0.6.8 (Earl and vonHoldt 2011). For each value of K, five iterations were run with a burn-in period of 10^5 generations, followed by 10^6 generations for values of $K = 1$ through 10. Each simulation was performed using an ancestry model incorporating admixture, a model of correlated allele frequencies, but no prior information on sample origins.

Our phylogenetic analysis uncovered high posterior probabilities (i.e. PP = 1) for the split between Mountain (*G. gazella*) and Arabian Gazelles (*G. arabica*), confirming the findings of Wronski et al. (2010) with new sequences (Fig. 1B). The divergence time of both taxa was estimated 1.73 to 0.86 MYA, i.e. in the Calabrian (middle Pleistocene). Samples originating from central Israel clustered together with those from the Golan Heights (i.e. *G. gazella*). One presumed *G. arabica* sample from the Arava Valley clustered within *G. dorcas.* Both species occur sympatrically in this region, so the sample seems to have been misclassified and was excluded from subsequent analyses. No further genetic structure was detectable in both taxa along their distribution ranges (Fig. 1B), which is of special interest in the case of *G. arabica* as Groves and Grubb (2011) claimed that three different species would exist within the sampled range. Mean (±s.d.) K2P-values (×100) were rather low for comparisons within taxa (*G. gazella*: 0.167 ± 0.173; *G. arabica*: 0.856 ± 0.694) but higher between taxa 2.270 (±0.205); the Mantel-Test found this difference to be statistically significant ($r_P = 0.989$, $P < 0.001$).

Population genetic analyses support genetic distinctiveness of both taxa. FCA clearly clustered samples into two groups corresponding with *G. gazella* and *G. arabica* even though no prior sample information was provided (Fig. 2A). The uppermost hierarchical level of population differentiation inferred from STRUCTURE was $K = 2$ (Fig. 2B). All individuals included were assigned to a genetic cluster with an estimated group membership of $Q > 0.9$ (Fig. 2C), i.e. no recurrent gene-flow could be detected.

Our phylogenetic analysis of cytochrome *b* sequences and our population genetic analyses support the hypothesis of two reciprocally monophyletic lineages within presumed 'Mountain Gazelles' (Wronski et al. 2010). The lack of recurrent gene-flow between both suggests complete (at least recent) reproductive isolation in the wild. In the light of the Integrative Species Concept (de

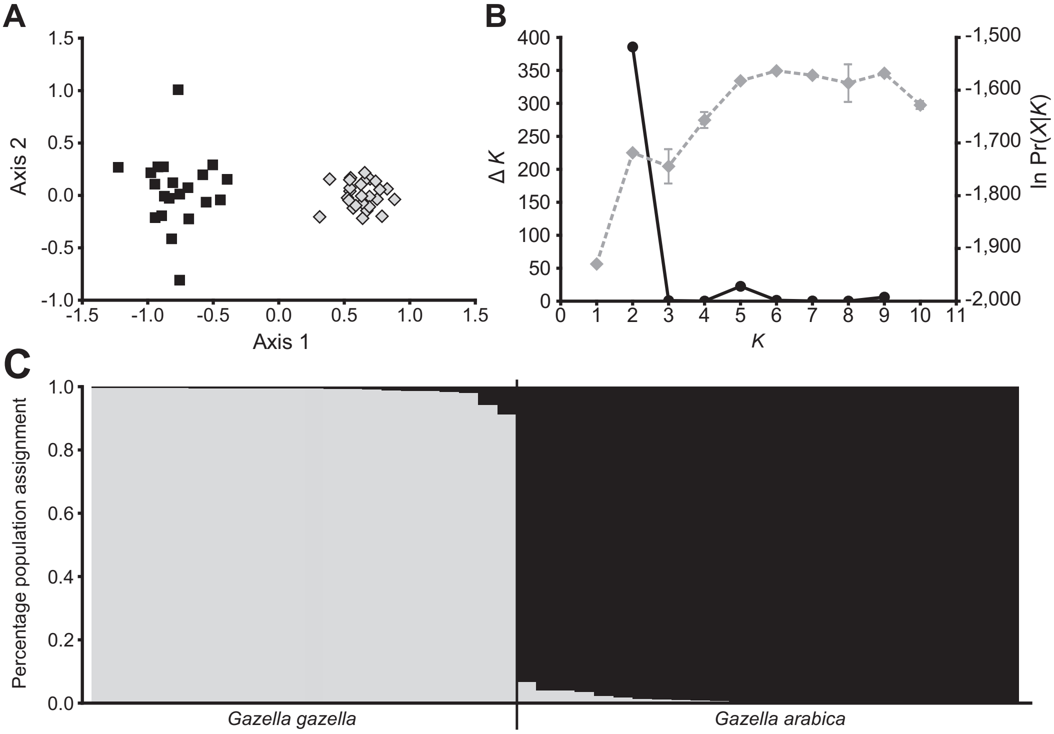

Fig. 2. Number of genetically distinct groups in the data set. (A) Factor correspondence analysis of allele frequencies obtained from GENETIX (black squares and grey diamonds representing *G. arabica* and *G. gazella*, respectively). (B) Estimated ln Pr (*X*|*K*) (grey diamonds) and Δ*K* (black circles) as a function of *K* inferred from STRUCTURE results. (C) Percentage population assignment to inferred genetic clusters for *K* = 2.

Queiroz 2007) these results can be seen as evidence for separately evolving metapopulation lineages (*sensu* de Queiroz 2007), although future studies should include nuclear sequence markers to further evaluate if these metapopulations refer to good species. However, pairwise genetic distances (i.e. K2P-values) within both taxa clearly indicate intraspecific variation, whereas distances between the two taxa are of a magnitude that can be interpreted as interspecific (Tobe et al. 2010). In the case of *G. arabica* our sampling scheme only comprises specimens from the western Arabian Peninsula and we can, therefore, only speculate about the taxonomic position of populations occurring in Oman and the United Arab Emirates.

Our results are in line with morphological differences and paleontological findings. Beside different body size and fur colouration, both taxa show distinct skull and horn features (see Wronski et al. 2010). Furthermore, mammalian faunas from late Pliocene and Pleistocene beds in the Levant comprise remnants of two distinct *Gazella* species (Sickenberg, 1975; Tchernov, 1988). The common ancestor supposedly immigrated into the Levant across the Saharo-Sahelian desert belt during the late Pliocene (Tchernov 1986). Within the Palaearctic the Levant was a centre of adaptive radiations as it was a crossroad of biotic interchanges between Africa and Eurasia and comprised extreme biogeographical heterogeneity due to the admixture of Palaearctic, Palaeotropic and Saharo-Arabian elements (Tchernov 1988). Here, immigrating taxa (such as *Gazella*) underwent extensive speciation especially during the late Neogene (Tchernov 1988). Some of the species that evolved in the Levant invaded areas such as southern Turkey and the Arabian Peninsula (Tchernov 1988). In the late Pleistocene Turkish gazelles disappeared and the more humid-adapted *G. gazella* settled in the northern Levant and southern Turkey (Kasparek 1986), while the more arid-adapted *G. arabica* colonized the Arabian Peninsula. The divergence between both taxa was further promoted when the local climate reached its extant conditions in the middle to late Palaeolithicum (Gibbard and van Kolfschoten 2004; Roy et al. 2004). In particular, the 500 mm-isohyet separates the Levant ecologically from Negev Desert and Sinai Peninsula (Yom-Tov and Ilani 1987), corresponding also to differences in vegetation and soil characteristics (Abdulsalam et al. 1988). In the north (Eastern Mediterranean Region) high and subdued, heavily karstified mountains prevail, with coniferous forests and shrublands representing the typical *G. gazella* habitat. The climate is Mediterranean with winter precipitation of up to 1800 mm. Further south (Western & Southern Arabian Region), where *G. arabica* occurs, high volcanic massifs and mountain chains with xeromorphic shrub and woodlands prevail. The climate is tropical with two rainy seasons (at least in the south) and precipitation between 100 and 450 mm. Therefore, we hypothesize an ecological species boundary between southern *G. arabica* adapted to unpredictable rainfall patterns and poor needle- and feather-leaved shrubs (Vesey-Fitzgerald 1952) and northern *G. gazella* adapted to Mediterranean climate enabling a more broad-leaved diet including considerable quantities of grass (Baharav 1981, 1983).

In terms of conservation and re-introduction efforts the advice of breeding both taxa separately (Wronski et al. 2010) was fully justified. Today, the situation for both taxa is critical. The total number of *G. gazella* is estimated at 3000 individuals and has drastically declined within the last two decades (IUCN 2008), whereas the total number of *G. arabica* left on the Arabian Peninsula is estimated at approx. 11,000 individuals (IUCN 2008).

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Chapter 6

by Hannes Lerp, Martin Plath, Torsten Wronski, Eva V. Bärmann, Anna Malczyk,
Revina-Rosa Resch, Bruno Streit and Markus Pfenninger

Utility of island populations in reintroduction programs—relationships between Arabian gazelles (*Gazella arabica*) from the Farasan Archipelago and endangered mainland populations

The following manuscript was submitted to *Molecular Ecology* and was peer-reviewed. Based on the reviews the editorial board decided to reject the manuscript in its current form but encouraged re-submission. For the manuscript I collected and analyzed the data together with my co-authors. I wrote a first draft of the manuscript and all co-authors revised it. More details on my contribution to this collaborative work are given below in the form *Anlage 1* of the *Ausführungsbestimmungen für Dissertationen im Promotionsfach Biologie, die bereits veröffentlichte Teile oder eingereichte Manuskripte enthalten*.

Anlage 1

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts: Utility of island populations in reintroduction programs—relationships between Arabian gazelles (*Gazella arabica*) from the Farasan Archipelago and endangered mainland populations

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen[#]	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	Hannes Lerp: 60% Co-Autoren: 40%	Torsten Wronski, Martin Plath
(2) Durchführung der einzelnen Untersuchungen/ Experimente	Hannes Lerp und Co-Autoren: Amplifizieren der Mikrosatelliten Co-Autoren: Vermessung der Gazellenschädel	Anna Malczyk, Revina-Rosa Resch, Eva Bärmann, Torsten Wronski
(3) Erstellung der Daten-sammlung und Abbildungen	Hannes Lerp: Sammlung der Mikrosatellitendaten, Erstellen der Abbildungen	
(4) Analyse/Interpretation der Daten	Hannes Lerp: Analyse der Mikrosatelliten mit MICROCHECKER, ARLEQUIN, STRUCTURE und GENETIX; Analyse der morphologischen Daten Hannes Lerp und Co-Autoren: Anwendung des Isolation-Migration-Modells auf die Mikrosatelliten, Interpretation der Daten	Markus Pfenninger, Martin Plath
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	Hannes Lerp: 70% Co-Autoren: 30%	Martin Plath, Markus Pfenninger, Torsten Wronski

[#] Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

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Datum/Ort

Datum
zustimmende Bestätigung der vorgenannten Angaben

Unterschrift Promovend/Promovendin

Unterschrift Betreuer/Betreuerin

Utility of island populations in reintroduction programs—relationships between Arabian gazelles (*Gazella arabica*) from the Farasan Archipelago and endangered mainland populations

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Running title: Island-mainland relationships of Arabian gazelles

Abstract

Understanding local adaptation and population differentiation is vital to the success of reintroduction initiatives. Like other mammals living on islands, Arabian gazelles (*G. arabica*) show reduced body size on the Farasan archipelago, which we corroborated in this study through morphometric analyses of skulls. In light of the steep population decline on the Arabian Peninsula—but stable population development on the archipelago—we tested the potential suitability of Farasan gazelles as a source for reintroductions on the mainland. We, therefore, investigated genetic differentiation between Farasan and mainland populations using eleven nuclear microsatellite loci and detected a genetic cluster being endemic to the archipelago, which we inferred to be separated for less than 2,000 years. About 30% of sampled individuals from Farasan islands showed assignment to a mainland cluster with signs of ongoing introgression. Analyses using the Isolation-with-Migration model confirmed recent (probably human induced) bi-directional exchange of gazelles between mainland and island populations. Hence, the surprisingly uniform island dwarfism most likely reflects phenotypic plasticity, *i.e.*, altered morphology as a direct consequence of harsh environmental conditions and resource limitation on the archipelago. Should a further decline of Arabian gazelles on the mainland necessitate restocking in the future, Farasan gazelles may thus become a valuable source for reintroduction.

Key words: Island Rule; Isolation-with-Migration; repatriation; phenotypic plasticity

Introduction

Changes in body size of mammalian island populations were first identified by Forster (1964) and became later known as the ‘Island Rule’ (Van Valen 1973). As a rule of thumb, small taxa tend to have an increased body size on islands, while large taxa—especially mammalian herbivores—show reduced body size. This phenomenon seems to hold for vertebrates in general (Lomolino 2005). In endangered species, island populations often raise questions regarding their conservation status; *e.g.*, do these populations deserve special protection and, more specifically, shall they be treated as separate conservation and management units (Ryder 1986)? This question is even more imperative when population sizes on the mainland diminish at an alarming pace. Is it appropriate to use remnant island populations for future reintroductions on the mainland? Management decisions should be based on the ecological and evolutionary history of island and mainland populations—considering the possibility that local adaptation to divergent ecological conditions can drive rapid divergence in phenotypic traits even in absence of strong overall genetic divergence (*e.g.*, Dieckmann *et al.* 2004)—and should consider the consequences emerging from those decision ('evolutionarily enlightened management'; Ashley *et al.* 2003). Such management decisions should be underpinned by population genetic studies to infer the colonization history of island populations (Lomolino *et al.* 2006). Here, we report on a study that aims to resolve the colonization history, gene flow patterns, and possible morphological divergence according to the 'Island Rule' in populations of endangered Arabian gazelles (*Gazella arabica*) from the Farasan Archipelago in the Red Sea. We exemplify how the application of the isolation-with-migration model (Nielsen & Wakeley 2001) allows uncovering the colonization history and recurrent gene flow between island and (endangered) mainland populations. Finally, this allows answering questions regarding the conservation status of those populations, and the suitability of island populations for future reintroduction programs on the mainland.

The antelope fauna of the Arabian Peninsula once comprised at least three species of gazelles (IUCN 2012); all of them experienced drastic declines due to hunting and competition with domestic livestock (Thouless *et al.* 1991; Magin & Greth 1994). In case of Arabian gazelles (*G. arabica*)—until recently thought to be synonymous to mountain gazelles (*G. gazella*; Wronski *et al.* 2010a; Bärmann *et al.* 2012; Lerp *et al.* 2012)—and sand gazelles (*G. marica*)—previously thought to be synonymous with goitred gazelles (*G. subgutturosa*; Wacher *et al.* 2010)—this led to isolated and widely scattered relict populations (Mallon & Kingswood 2001; Cunningham & Wacher 2009). Saudi gazelle (*G. dorcas saudiya*) and possibly 'Queen of Sheba's gazelle' (*G. bilkis*; a species of uncertain taxonomic validity) became already extinct (Mallon & Al-Safadi 2001; Hammond *et al.* 2001). Legislations to protect gazelles exist in most countries throughout the species' distribution ranges, however, laws are often inadequate or incomplete, and a lack of political will to enforce them is eminent (Mallon & Kingswood 2001).

The situation of *G. arabica* is alarming: once distributed from the Arava Valley in southern Israel through western Saudi Arabia and Yemen into Oman and the United Arab Emirates, the species disappeared from large parts of its former distribution range during the past 50 years (Thouless & Al Bassri 1991; Magin & Greth 1994; Thouless *et al.* 1997; Mallon & Kingswood 2001). At present, few disjunctive populations persist, with remaining population sizes of often less than 100 individuals (Magin & Greth 1996; Thouless *et al.* 1997; Wronski & Butynski 2013). For example, the world's largest wild population in Jiddat al Harasis in Oman declined from 10,000 individuals in 2001 to only 2,800 in 2009 (Strauss *et al.* 2009). Currently, the total number of *G. arabica* on the Arabian Peninsula is estimated to be less than 11,000 individuals (Mallon & Kingswood 2001; IUCN/SSC Antelope Specialist Group 2008; Strauss *et al.* 2009), leading to an IUCN red list classification as 'vulnerable' (IUCN/SSC Antelope Specialist Group 2008).

The only known exception to this detrimental development is the population occurring on the Farasan Islands. Nowadays, it represents the largest natural population in Saudi Arabia that remained stable at approximately 800 to 1,000 individuals since 1988 (Cunningham & Wronski 2011). The Farasan Islands are an archipelago formed from a raised coral reef with a maximum altitude of 50 m above sea level, located in the Red Sea, 40 km off the town of Jizan (Fig. 1). Farasan gazelles are morphologically distinguishable from animals found on the mainland, showing smaller body size, a rounded upper tooth row, and more grayish body color, with a coppery tone on legs and neck, and were even described as a distinct subspecies (*Gazella gazella farasani*) by Thouless and Al Bassri (1991). Moreover, almost all females are hornless or show minute, deformed horns (T. Wronski *unpubl. data*), while mainland gazelles possess horns in both sexes (Mendelssohn *et al.* 1995).

Morphological distinctiveness raised questions regarding the conservation status of Farasan gazelles. Generally, past conservation efforts of Arabian gazelles have been plagued by confusion about phylogenetic relationships among various—phenotypically discernible—populations, and even the question of species boundaries is far from being certain (Lerp *et al.* 2013). A molecular study based on mitochondrial sequence data (Cytochrome *b* and Control Region) revealed different genetic lineages in Farasan gazelles (Wronski *et al.* 2010a), and the authors hypothesized that one lineage resulted from an ancient colonization whereas others were later introduced by man. Nevertheless, resolution of the phylogenetic analyses was low, and only maternally inherited markers were employed. This left the question unanswered as to whether Farasan gazelles should be treated as a distinct management unit. Here, we provide a population genetic framework for *G. arabica* using 11 nuclear microsatellite loci to answer this question. We compared specimens from the Farasan Archipelago with those from different reference populations on the mainland, inferred distinct genetic clusters and estimated historical and recent migration, time since separation from the mainland and effective population sizes of island and mainland populations. Furthermore, we investigated morphological features (*i.e.*, skull measurements) of Farasan and mainland gazelles to provide a thorough quantitative analysis of phenotypic differences.

Another aim of this study was to identify the origin of illegally traded gazelles. Although strictly forbidden by international and national laws (Child & Grainger 1990) trading of wildlife is common in the Middle East (*e.g.*, Bachmann 2010; Stanton 2009). Gazelles are traditionally held as pets (*e.g.*, *G. subgutturosa*, Kingswood & Blank 1996; *G. dorcas*, Mallon & Kingswood 2001) or bred in private collections (IUCN/SSC

Antelope Specialist Group 2008). Living gazelles designated to be traded at Akhoba Market in Jizan (Saudi Arabia) were repeatedly confiscated by customs and brought to the King Khalid Wildlife Research Centre. In order to infer their origin and to obtain insights into the patterns of illegal gazelle trading, we tested the confiscated specimens against the microsatellite reference data base derived from the entire distribution range of *G. arabica*.

Materials and Methods

Study area and sample collection

Animals used in this study originated from the Arabian Peninsula and correspond to a reciprocally monophyletic mitochondrial clade with a sister group relationship to mountain gazelles (*G. gazella*) from the Levant (Wronski *et al.* 2010a; Lerp *et al.* 2012); following Bärmann *et al.* (2012) we refer to them as *G. arabica*. Samples included in this study originated from (1) the wild, obtained through non-invasive sampling techniques (hairs and feces from bedding sites or dried skins of dead animals; Fig. 1), (2) captive animals held in breeding centers, or (3) animals confiscated at Akhoba Market near Jizan (Table S1). Akhoba Market is one of the numerous pet markets in Saudi Arabia, situated close to the Yemen border, trading all kind of wild animals from the Middle East and Africa.

In total we included 137 specimens, the majority (75.9%) of which was collected in the wild during several years and by various collectors (Table S1). Those samples cover most of the species' extant distribution range except for Yemen where the conservation status of gazelles is unclear (Mallon & Kingswood 2001) and sampling was impossible due to political constraints. In order to compare mitochondrial with microsatellite information we genotyped 20 specimens already included in Wronski *et al.* (2010a).

DNA extraction and microsatellite amplification

We extracted DNA by using the Qiagen DNeasy Blood & Tissue Kit® and in case of fecal samples the Qiagen QIAamp DNA Stool Mini Kit® following the manufacturer's instructions. We amplified 11 microsatellite loci (Table S2) with dye-labeled forward primers (Cy5 and IRD700 obtained from Metabion; Dy-751 from Biomers) for visualization on a Beckman Coulter capillary sequencer CEQ 2000 (Table S2). The primer pairs were arranged into three separate multiplex reactions (Table S2) and amplified using the Type-it Microsatellite PCR Kit® (Qiagen, Hilden, Germany). PCR included an initial denaturation step for 5:00 min at 95°C, 30 cycles of 1:30 min at 57°C and 0:30 min at 72°C, followed by a final extension step for 30:00 min at 60°C. The 12.5 µl reaction mix included 6.25 µl Type-it master mix, 1 µl primer mix [containing primer pairs (5 pmol/l each) according to the multiplex reaction as seen in Table S2], 1 µl Q-solution, 2.25 µl RNase-free water and 2 µl template DNA. In case of a low fluorescence signal we repeated the PCR with 3 µl template DNA and 1.25 µl RNase-free water, respectively.

PCR products were electrophoresed on a CEQ 2000 (Beckman Coulter; denaturation at 90°C for 2 min; injection at 2.0 kV for 30 s; separation at 6.0 kV for 45 min) together with DNA Size Standard Kit – 400 (Beckman Coulter). Samples were screened using Genome Lab GeTX 10.2 software (Beckman Coulter) and alleles were called manually. All samples were independently genotyped two times and in case of conflicting results two additional amplifications were conducted. The correct genotype was inferred from the majority of the four replicates.

HWE, F_{ST}, and F_{IS}

ARLEQUIN v3.5 (Excoffier & Lischer 2010) was used to calculate expected (H_E) and observed (H_O) heterozygosity, to test for deviations from Hardy-Weinberg-Equilibrium (HWE), to calculate pairwise F_{ST}-values between wild specimens from Farasan Islands and from different parts of the Arabian Peninsula (see Fig. 1 for grouping), and to compute locus-wise inbreeding coefficients (F_{IS}-values) for each group. To test for differences in F_{IS}-values between groups we used a non-parametric Friedman test, treating the $k = 4$ groups as the repeated measurement for the $N = 11$ independent loci. Based on our observation of a genetic cluster being endemic to the Farasan Islands to which not all specimens sampled on Farasan Islands were assigned (see results), we sought to infer the degree of historic inbreeding on the archipelago and thus, conducted another Friedman test using only animals with strong assignment ($Q > 0.9$) to this cluster and compared them to mainland animals.

For all calculations the level of missing data was set at 0.27, *i.e.*, data for at least eight of the 11 loci were available for all individuals. We tested for the presence of null alleles at each locus using Micro-checker v2.2.3 (Van Oosterhout *et al.* 2004).

Inference of population genetic structure and assignment of confiscated animals

STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was employed to identify the number of genetically distinct clusters (K) in the complete data set with the method presented by Evanno *et al.* (2005) using the web-based tool STRUCTURE HARVESTER v0.6.8 (Earl & VonHoldt 2011). STRUCTURE implements the Markov Chain Monte Carlo (MCMC) algorithm for the generalized Bayesian clustering method to classify individuals using genotypic data of unlinked markers. For each value of $K = 1$ through 14, ten iterations were run with a burn-in period of 10^6 generations, followed by a sampling phase of 10^6 iterations. Each simulation was performed using an ancestry model incorporating admixture, a model of correlated allele frequencies, but without any prior information corresponding to the origin of the samples. The same analysis was used to infer the origin of confiscated animals.

We detected a genetic cluster of gazelles endemic to the Farasan Islands ('Farasan cluster', see below). To infer if the assignment to this cluster resulted from statistic noise or true affiliation, we asked whether mean assignment of specimens from the Farasan Islands, mainland, and Jizan to the Farasan cluster would change with increasing K. Arcsine-transformed values for mean group assignment to the Farasan cluster (for the run with the highest $\ln \Pr(X|K)$ per K) were used as the dependent variable, 'origin' as a fixed factor and K as the covariate in an analysis of covariance (ANCOVA). F values were approximated using Wilks' lambda, and effect strengths were calculated as partial eta squared (η_p^2). In case of robust and replicable assignment in gazelles collected on Farasan Islands, but decreasing assignment with increasing K in others (reflecting statistic noise at lower values of K), we would expect slope heterogeneity between groups (*i.e.*, a significant interaction effect of 'origin × K').

As STRUCTURE results are sensitive to the violation of HWE, which was observed within our data set, results must be interpreted with caution. To secure that our conclusions were not affected by this, we also used the software GENETIX v4.05.2 (Belkhir *et al.* 2004) to calculate a multidimensional factorial correspondence analysis (FCA). The software groups individuals on multiple factorial axes based on shared alleles only and uses no *a priori* assumptions of group membership or a particular population genetic model (Belkhir *et al.* 2004).

Migration, time since island colonization and effective population size

We used an isolation-with-migration approach implemented in IMa2 (Hey 2010) to determine the direction and amount of gene flow between the Arabian mainland and Farasan Islands. One assumption of the model is that no other populations exist that are more closely related to the sampled populations than they are to one another. In order to analyze whether we could use all mainland individuals for the isolation-with-migration approach, we conducted several analyses of molecular variance (AMOVA) averaged over all loci using ARLEQUIN v3.5 to test for population structure among mainland animals. As we detected significant genetic structure for any grouping of mainland populations ($F_{ST} \geq 0.08$, $P < 0.001$), we decided to use only samples obtained from the south-western part of the Arabian Peninsula to compare with the island population as this population is the most likely one that colonized the Farasan Islands (white squares in Fig. 1).

In order to obtain a naïve estimate for mutation rates of each locus we considered the divergence time of *G. arabica* from its sister taxon *G. gazella*, calculated from an mtDNA-based phylogeny [95% Highest Posterior Density (HPD): $1.73–0.86 \times 10^6$ years], and correlated this information with the occurrence of unique alleles detected in a population genetic analysis of those two taxa using the same 11 microsatellite loci used here (Lerp *et al.* 2012). We counted the minimum number of mutational steps necessary to explain the observed unique alleles based on the assumptions of step-wise mutation and shared allele frequencies in both species representing the ancestral state. The number of steps was divided by the divergence time, and the specific mutation rate per locus was calculated as the mean of the rates inferred when using the upper and lower 95% HPD values. The mutation rate over all loci were calculated as the geometric mean of the per-locus rates (Won & Hey 2005).

We used ARLEQUIN v3.5 to calculate locus-wise θ under the stepwise mutation model to estimate priors for population size, splitting time, and migration rate as recommended in the IMa2 documentation. We ran one analysis to estimate the prior distribution and two analyses including our data with a total number of 15×10^6 steps, a burn-in phase of 150,000 steps and samples saved every 100 steps for each analysis, respectively. We analyzed both runs simultaneously to calculate the joint posterior density for the demographic parameters θ, *m* and *t* (Hey & Nielsen 2004) and to perform likelihood-ratio tests on nested models (Hey & Nielsen 2007).

Morphometric measurements of skulls

We performed a thorough morphometric analysis of potential differences in skull morphology between animals originating from the Farasan Islands and the mainland. In total, 45 skulls were measured for 32 linear measurements (Fig. S1). Horn measurements were taken from one side of the skull only to avoid overweighting these variables; usually this was the right side, except when the right horn was damaged or missing. Skulls from Farasan Islands (N = 14) were collected from dead animals found by rangers and later stored at KKWRC. Skulls of mainland gazelles came from the Natural History Museum, London (N = 8) and from the KKWRC breeding stock (N = 23) and were pooled because the KKWRC stock represents a mixed population founded with animals from geographically distant populations on the Arabian Peninsula.

Skull measurements were $\log_{10}$-transformed, and a principal components analysis (PCA) was conducted. The retained principle components with an eigenvalue > 1.0 were included as the dependent variables in a multivariate General Linear Model (GLM) with 'origin' (mainland vs. Farasan), 'sex', and the interaction of 'group × sex' as predictor variables. Box's test of equality of covariance matrices (a prerequisite of GLM) revealed a violation of this assumption (P = 0.002), so we conducted a series of non-parametric Kruskal-Wallis Rank Sum Tests for all comparisons between 'origin' and 'sex' using Bonferroni-corrected α-levels. The results (data not shown) confirmed those obtained by the GLM.

Results

Genetic variability and inbreeding coefficient

All microsatellite loci amplified reliably and all were polymorphic in each group (North, South-West, East, Farasan Islands, and Jizan; Fig.1) with numbers of alleles per locus ranging from two (BM302 and RM088) to 15 (MCM38; Table 1). We found 15 (North), 25 (South-West), 9 (East), 19 (Farasan) and 13 (Jizan) private alleles, respectively. Five (Jizan, East) to all loci (South-West) showed significant deviations from HWE (heterozygote deficiencies; Table 1).

The inbreeding coefficient F_{IS} did not significantly differ between groups (χ^2 = 1.91, df = 3, P = 0.59, N = 11); median F_{IS}-values (1st–3rd quartile) were 0.355 (0.172–0.544) for Farasan Islands, 0.137 (0.000–0.458) for the East group, 0.385 (0.277–0.507) for the South-West group and 0.333 (0.207–0.671) for the North group. When analyzing only those gazelles from Farasan Island assigned to the cluster endemic to the archipelago (*i.e.*, $Q > 0.9$, see below; median F_{IS} = 0.285, 1st–3rd quartile = 0.104–0.467), the Friedman test still remained non-significant (χ^2 = 4.44, df = 3, P = 0.22, N = 10).

Genetic structure between mainland and island populations

STRUCTURE HARVESTER (Earl & VonHoldt 2011) identified K = 2 as the uppermost hierarchical level of population differentiation following the procedure described in

Evanno *et al.* (2005) (Fig. 2a,b). All individuals from the mainland of the Arabian Peninsula were assigned to one group. The majority of animals from Farasan Islands were assigned to another, genetically distinct cluster, but several individuals were consistently assigned to the mainland cluster, with virtually all states of admixture in between (Fig. 2c). Assignment to the endemic 'Farasan cluster' did not correspond with particular mitochondrial lineages described in Wronski et al. (2010) (data not shown).

We tested the robustness of assignment to the 'Farasan cluster'. An ANCOVA using assignment scores as the dependent variable uncovered significant overall differences between the three groups (effect of 'origin' in Fig. 3). While assignment of Farasan and Jizan animals was virtually the same for values of $K = 2$ through 7, assignment of mainland gazelles to this particular cluster decreased with increasing K (see slope heterogeneity, expressed by the interaction term of 'origin × K' in Fig. 3). Overall assignment decreased slightly, but significantly, with increasing K (Fig. 3).

Biologically meaningful results (with at least some animals clearly assigned to one cluster) were obtained up to a number of genetically distinct clusters of $K = 7$ (Fig. S2). STRUCTURE HARVESTER uncovered the second highest value of ΔK for $K = 5$ and the third highest value for $K = 7$ (Fig. 2b). In these runs, mainland gazelles were assigned to different clusters without a clear geographical signal (Fig. S1).

The factorial correspondence analysis conducted with GENETIX retrieved three canonical axes explaining 80.76% of the total variance (Fig. 4). The first axis (explaining 36.14% of the variance) separated the majority of gazelles from Farasan Islands from mainland animals—corroborating the STRUCTURE analysis at $K = 2$ (Fig. 2c). The second and third axes separated groups of mainland gazelles (Fig. 4c). These results are in line with the pattern inferred from pairwise F_{ST}-values (Table 2), being highest for comparisons between Farasan Islands and each mainland group (0.184–

0.198) and considerably smaller, albeit still significant, for comparisons among mainland groups (0.087–0.049). The lowest value was found between North and South-West (0.049) and the highest between Farasan Islands and East (0.198; Table 2).

Population parameters of island and mainland populations

Maximum-likelihood estimates of the population mutation rate parameters (θ) inferred with the software IMa2 (Hey 2010) were 0.578 (90% HPD interval: 0.147 to 1.514) for Farasan Islands, 1.441 (90% HPD interval: 0.342 to 3.076) for South-West mainland and 76.38 (90% HPD interval: 55.12 to 97.60) for the ancestral population (Fig. 5a). This translates into a 2.5-fold and 132-fold higher effective population size of the South-West mainland and the ancestral population, respectively, compared to the Farasan Island population. The marginal posterior probability distribution of the divergence time parameter showed a peak close to zero (Fig. 5b) and corresponds with 1,843 years (90% HPD interval: 0 to 12,600 years) based on the geometric mean of locus-wise mutation rates per year (see IMa2 input file; DRYAD entry doi:). Migration parameters converged at the upper limits of the prior distribution (Fig. 5c), suggesting that migration may be much stronger than previously assumed.

Genetic assignment of confiscated animals

The analyses conducted with STRUCTURE and GENETIX revealed that gazelles confiscated at Akhoba Market largely originated from the Protected Area of the Farasan Islands (Fig. 2c). Other animals clustered more distantly from Farasan and mainland gazelles (Fig. 4). At $K \geq 4$, seven individuals were assigned to a separate cluster (*i.e.*, estimated group membership was $Q > 0.75$), with no equivalent found in the sampled mainland animals (Fig. S1). Furthermore, we found four specimens assigned to both the separate and the Farasan cluster suggesting hybrid origin ($Q =$

0.3–0.5). In STRUCTURE runs for $K = 7$ three individuals were assigned to a genetic cluster that occurs mainly in South-West Arabia (Fig. S1).

Skull morphology of island and mainland gazelles

The PCA of skull measurements retained five PC with eigenvalue > 1.0, with the first and second cumulatively explaining 68.2% of the total variance. Multivariate GLM uncovered significant differences between groups (*i.e.*, mainland *versus* Farasan Islands; $F_{5,37} = 95.87$, $P < 0.001$, $\eta^2 = 0.93$) and sexes ($F_{5,37} = 39.48$, $P < 0.001$, $\eta^2 = 0.84$). Finally, a significant effect of the interaction term of 'group × sex' ($F_{5,37} = 4.45$, $P = 0.003$, $\eta^2 = 0.38$) indicated a difference in sexual dimorphism between groups (Fig. 6).

Post hoc univariate GLMs uncovered significant effects only for the first two PC ($P \leq 0.036$); the first representing general size [as all except two variables had high (≥ 0.331) positive loadings], and the second representing horn size (Fig. 6). Qualitatively, Farasan gazelles were smaller and had shorter horns than mainland animals, and females were generally smaller and had shorter horns than males. Furthermore, the difference between sexes was more pronounced in the Farasan Island population, where females had disproportionately small horns (Fig. 6).

Discussion

Morphological and population genetic differentiation of Farasan gazelles

Using morphometric analyses of skulls we found Farasan gazelles to be smaller (following the 'Island Rule'; Van Valen 1973) and to exhibit stronger sexual dimorphism than mainland gazelles (Thouless & Al Bassri 1991; this study). Raia & Meiri (2006) suggested that island dwarfism in ungulates is driven by the release from interspecific competitors (and to a lesser extent from predators). The underlying idea is that interspecific competition and predation necessitate high investment into somatic maintenance and body growth, while release from those selective forces on islands allow for an increased investment into—and earlier onset of—reproduction, which brings about a decreased body size. In fact, gazelles living on the mainland of the Arabian Peninsula were competing for resources with Arabian oryx (*Oryx leucoryx*) and Saudi gazelles (*G. dorcas saudiya*), and more recently with feral camels (*Camelus dromedarius*) and domestic goats (*Capra hircus*; Habibi 1989; Dunham 1997), while no competitors and only few predators of juvenile gazelles (eagles) are present on Farasan Islands (Masseti 2010).

Hence, it seems tempting to simply argue that the conditions are met under which smaller body size in Farasan gazelles would be predicted to evolve. So, do Farasan gazelles show local adaptation in body size? Specifically, is the pattern of decreased body size and smaller horns (especially in females, which often do not develop any horns on the archipelago) caused by site-specific selection on loci involved in general body growth and size regulation, and horn development? A closer look at the population genetic pattern inferred from our microsatellite analyses suggests that Farasan gazelles were the most divergent group, and a genetic cluster being endemic to the archipelago was uncovered (Farasan cluster). However, about 30% of Farasan gazelles were not assigned to this cluster, but either to the mainland cluster or equally to both clusters. Furthermore, the results of the isolation-with-migration approach uncovered recent exchange of animals from the mainland onto Farasan Islands, likely owing to continued trading and translocation of gazelles (Groves 1997; see below). *E.g.*, it was reported that gazelles were moved onto the archipelago by traders and seamen as a form of game-ranching in historic times (Thouless & Al Bassri 1991). Assuming that the Farasan cluster represents locally adapted animals and the other cluster descendants of translocated mainland animals, and assuming that morphological differences have a heritable basis, one would expect to find at least a moderate number of animals (up to 30%) that exhibit morphological features typical of mainland gazelles, with distinctly larger body size, different fur coloration, and horned females. However, intense monitoring for several weeks per year over the past four years—including the photo-identification and camera trapping of hundreds of gazelles (Wronski *et al.* 2013)—revealed that virtually all gazelles on the archipelago uniformly show dwarfism (T. Wronski, *pers. obs.*).

We conclude that reduced body size in island *G. arabica* for the most part reflects phenotypic plasticity (Pigliucci 2001). The release from predation and interspecific competition on islands often leads to increased local population densities, translating into increased intraspecific competition. Limited resource availability is known to hamper body growth in ungulates (Skogland 1983; Choquenot 1991). Unlike pure desert dwelling gazelles (*e.g.*, *G. marica* and *G. dorcas saudiya*) inhabiting hyper-arid desert plains, Arabian gazelles typically live in upland areas of broken terrain and prefer to drink on a regular basis (Mendelssohn *et al.* 1995). Farasan Islands are certainly an atypical habitat for Arabian gazelles as the archipelago is devoid of any surface water (Flamand *et al.* 1988). We argue that the effects of increased intraspecific competition are even aggravated by those adverse ecological conditions. An alternative explanation would be that depressed growth is the result of inbreeding depression (Roldan *et al.* 1998), but our population genetic analyses found no signs of increased inbreeding on the archipelago.

In conclusion, our results support the view that Farasan gazelles represent no distinct, evolutionarily significant unit (see Thouless & Al Bassri 1991; Groves 1997) and thus, must not be treated as a distinct management unit (*sensu* Vogler & DeSalle 1994). Admittedly, separation of the 'Farasan cluster' from the mainland for a few thousand years has promoted some degree of population genetic differentiation, but any further considerations as to the distinctiveness of Farasan gazelles become obsolete in light of the observed repeated translocation of mainland animals onto the archipelago and thus, ongoing introgression of genetic material from the mainland cluster. Should a further decline of Arabian gazelles on the mainland necessitate action in this direction, and provided that hunting and poaching will be prevented at potential reintroduction sites, Farasan gazelles may become a valuable source of reintroduction onto the Arabian mainland. Still, as long as captive breeding programs for mainland *G. arabica* exist (Thouless 1991), animals from those initiatives should be given priority in reintroduction programs.

Gazella arabica on the Arabian Peninsula

Even though pairwise F_{ST}-values were highly significant for all comparisons of mainland groups, there was no deep genetic structuring between any groups of gazelles (*i.e.*, pairwise F_{ST}-values were ≤ 0.087). We conclude that the observed fragmentation of extant population (Mallon & Kingswood 2001) is a recent phenomenon, as isolation had only minor effects on allele distributions. Besides hunting, habitat loss through agricultural development, fencing of pasture, overgrazing by domestic livestock, and the construction of roads and settlements increased dramatically over the past 30 years and were the main reasons for the steep decline of Arabian gazelles on the mainland (IUCN/SSC Antelope Specialist Group 2008; Alwelaie 1989).

Results from our isolation-with-migration approach were consistent with a scenario of recent population fragmentation, since we inferred a more than 50-fold larger number of gazelles to have lived only a few centuries ago. However, results of historic abundance estimates, inferred from present genetic diversity, should be treated with caution, since they rely on a model with simplified assumptions (Palsbøll *et al.* 2012). Nevertheless, only a short time span was inferred for the estimations of θ

and μ, and it remains doubtless that gazelle populations (especially on the mainland of the Arabian Peninsula) underwent a severe decline during the past few centuries (IUCN/SSC Antelope Specialist Group 2008).

The inferred inbreeding coefficients were high, but comparable to other endangered ungulates, *e.g.*, Ethiopian walia ibex (*Capra walie*; Gebremedhin *et al.* 2009) or European bison (*Bison bonasus*; Daleszczyk & Bunevich 2009). Deviations from Hardy-Weinberg equilibrium were detected in all groups of mainland gazelles but were particular pronounced in the south-western group. Here, specimens were sampled from different, isolated subpopulations that sometimes harbor less than ten individuals (*e.g.*, Asir Mountains; Boug *et al.* 2012).

Gazella arabica from the Akhoba Market in Jizan

One aim of our study was to infer the origin of gazelles confiscated at the Akhoba pet market. Assignment of illegally traded animals to specific mainland population was not possible because little genetic structure was found among mainland groups (see above). Nevertheless, five animals confiscated at the Akhoba Market could be clearly assigned to the endemic Farasan cluster ($Q > 0.9$) and were most likely caught on the archipelago. This implies that illegal capture of live gazelles is existent, violating Saudi Arabian and international law (Child & Grainger 1990). Living gazelles are regularly confiscated by rangers on Farasan Islands, reflecting the strong demand for pet gazelles. Gazelles are chased until exhaustion using motorcycles and then captured alive (Supplementary Video 1). Local hunters, as well as tourists from as far away as Tabuk (northern Saudi Arabia), are said to hunt on Farasan Islands or purchase fresh game meat (T. Wronski, *pers. comm.* with rangers from Saudi Wildlife Authority). Hence, one of Saudi Arabia's most iconic species has been lost from most of its former range and now appears to be targeted at yet another site—the last remaining stronghold of the species in the Kingdom.

Other confiscated animals were assigned to a genetic cluster (at $K \geq 4$) that was not represented elsewhere in our data-set. Therefore, the origin of those animals could be only speculated upon. Since Jizan is close to the Yemen border, one likely explanation would be that animals were caught in Yemen (Fig. 1). The situation for gazelles in Yemen is unclear (Mallon & Al-Safadi 2001) and included samples obtained from Yemen were collected close to the Saudi border. Although gazelles in Yemen are thought to be rare, small populations may still exist (Mallon & Al-Safadi 2001) and live capture in those populations is also highly likely. Trading of wildlife is very common in Yemen since impoverished locals catch wildlife opportunistically (Stanton 2009). It is easily conceivable that a certain variety of wildlife (including gazelles) is regularly smuggled across the border into Saudi Arabia to achieve higher prices.

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Data Accessibility

Microsatellite data, IMa2 input file and skull measurements: DRYAD entry doi: XXX

Author contributions

H. L. conducted the molecular analyses (assisted by A. M. and R.-R. R.) under the supervision of M. Pl., M. Pf. and B. S. and wrote the first manuscript draft. T. W. collected samples; T. W. and E. V. B. performed skull measurements; H. L. conducted morphometric statistical analyses. All authors read and approved the final manuscript version.

Figures

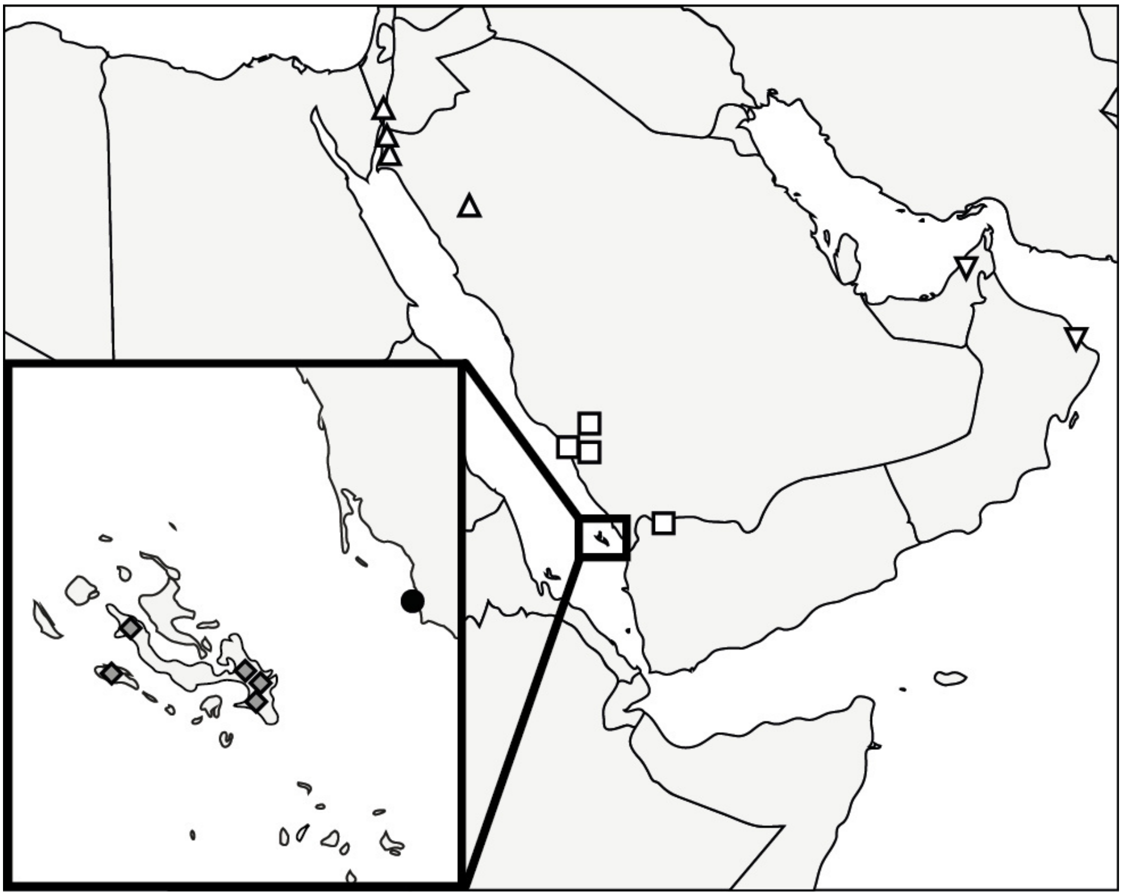


Figure 1 Sampling locations of Arabian gazelles (*G. arabica*) with known provenance. Individuals included in the North group (△), the South-West group (☐) and the East group (▽) are depicted as blank symbols in the main map, individuals from Farasan Islands (◆) and animals of unknown provenance confiscated at Akhoba Market in Jizan (●) are depicted on the inserted map.

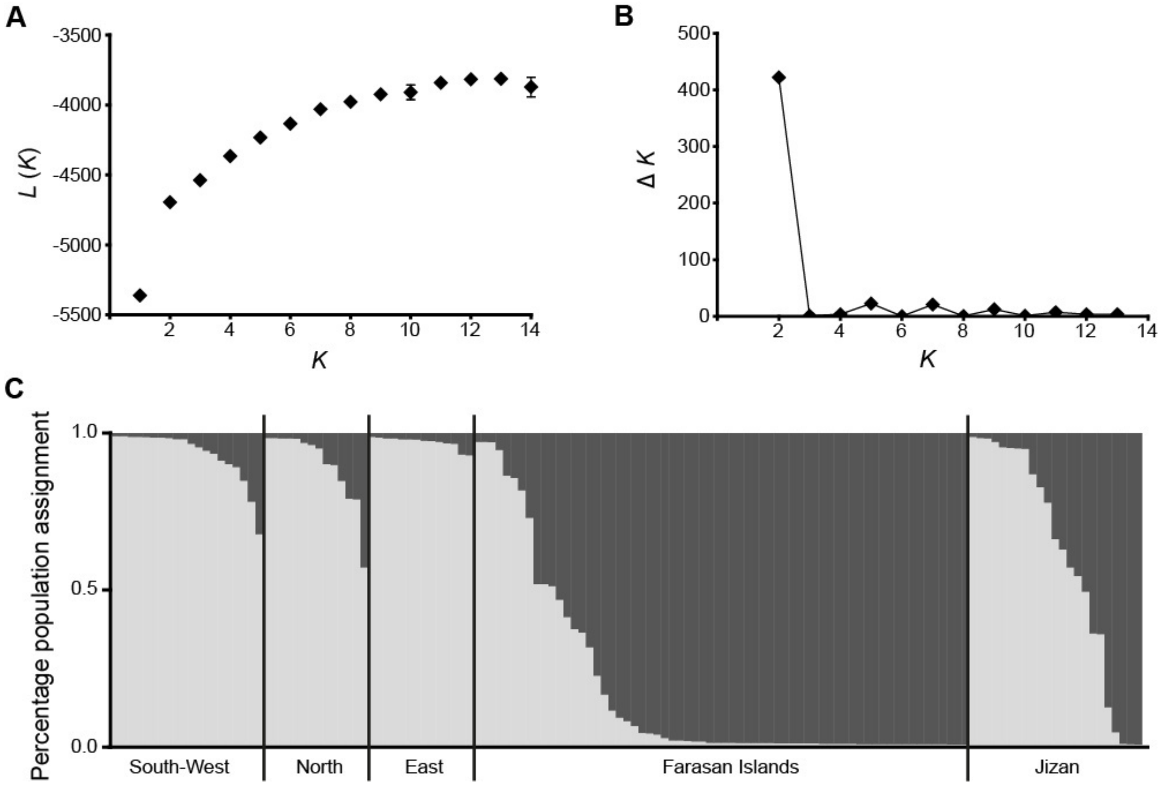


Figure 2 Detection of the most likely number of genetically distinct groups. (A) Mean $L(K)$ (± SD) over 10 runs per K as a function of K. (B) ΔK (Evanno *et al.* 2005) as a function of K. (C) Percentage population assignments to inferred genetic clusters at $K = 2$. Animals were sorted by Q values for each population separately.

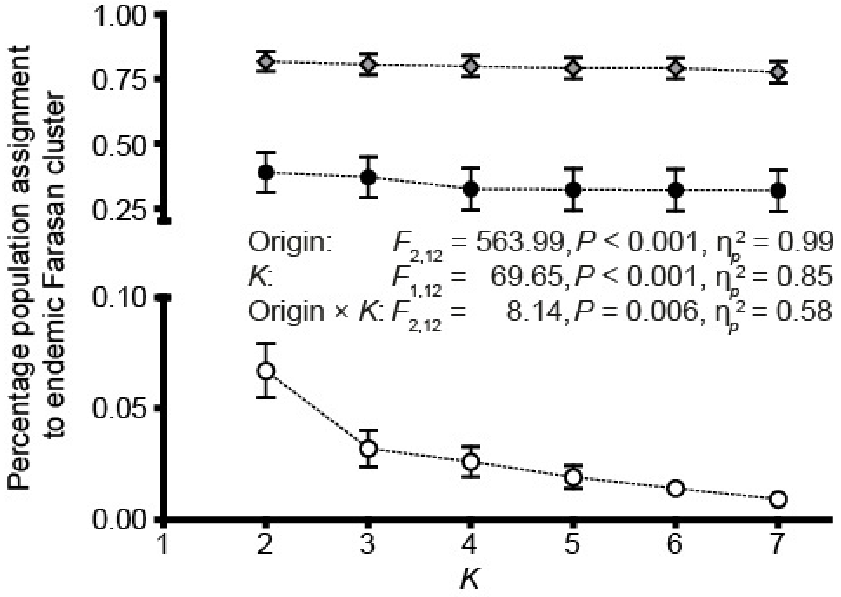


Figure 3 Mean (± SE) assignment of populations to the genetic cluster endemic to the Farasan Islands as a function of the number of genetic clusters (*K*) of mainland (○), Farasan (◆) and Jizan (●) populations. Results of ANCOVA for 'Origin' (fixed factor), *K* (covariate) and 'Origin × *K*' (representing the slope) are depicted.

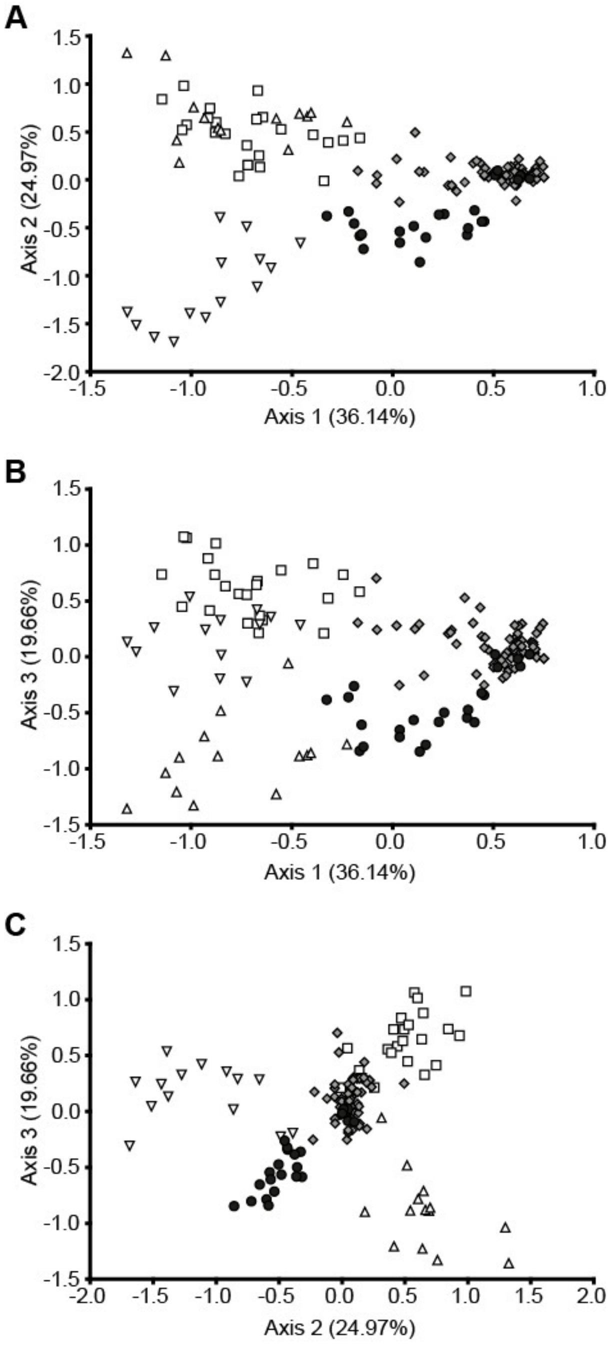


Figure 4 Factor correspondence analysis of allele frequencies. The first three axes are shown explaining 80.76% of variance within data. Symbols are equivalent to Fig. 1. (A) First two axes explaining 61.11% of the total variance; (B) first and third axes explaining 55.80% of variance, and (C) second and third axes explain 44.63% of variance.

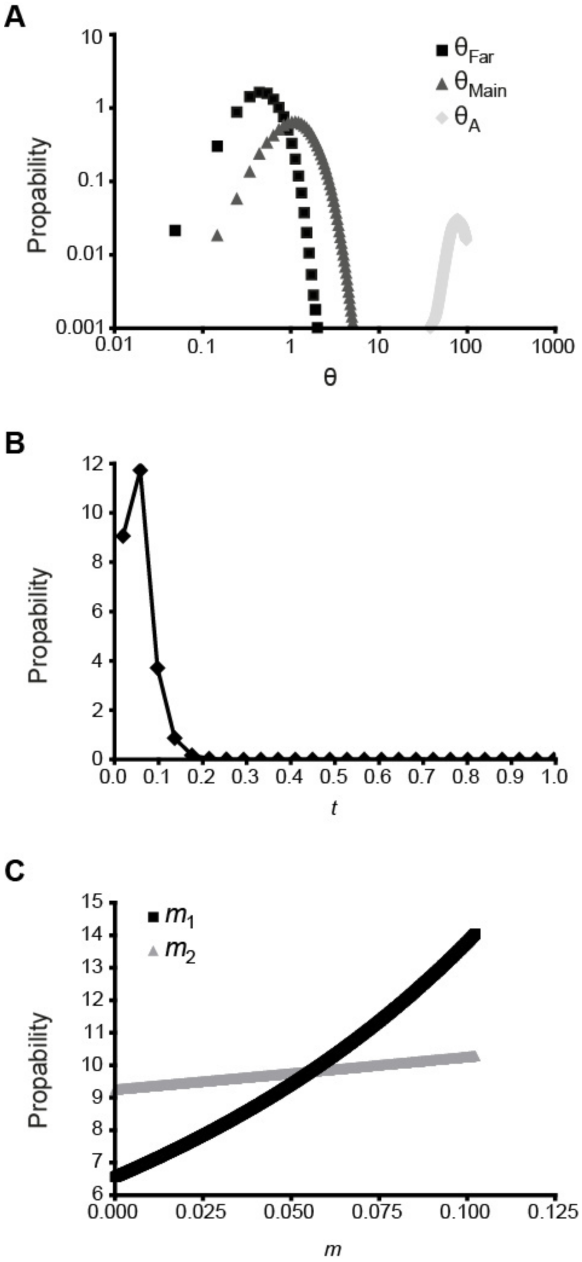


Figure 5 Marginal posterior probability distributions for (A) population size estimates for the Farasan (θ_{Far}), south-west (θ_{Main}) and ancestral populations (θ_A). (B) Splitting time (t), and (C) migration parameters for migration of gazelles onto (m_1) and from (m_2) the Farasan archipelago. Peak-values for θ and t can be converted into population size and splitting time in years, respectively, by using the geometric mean of locus-wise mutation rates per year (see main text).

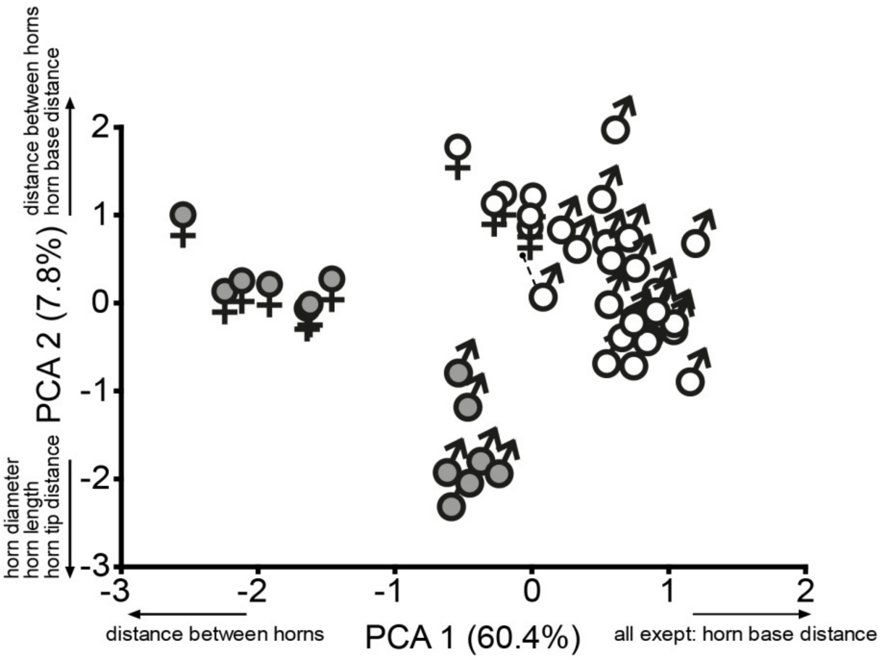


Figure 6 Principal component analysis (PCA) of skull measurements. The first two PC combined explain 68.2% of the total variance. Gazelles from Farasan Islands are presented in grey and mainland gazelles in white, sexes are indicated by symbols. Measurements with high positive or negative loadings are given on arrows besides the corresponding axis.

Tables

Table 1 Descriptive statistics of genetic variability for 11 microsatellite loci used in this study. For each locus, observed (H_O) and expected (H_E) heterozygosities as well as the number of observed alleles (N_A) and the range of allele sizes (S) are given. Significant deviations from HWE (*i.e.*, heterozygote deficiency) are indicated by asterisks, whereby *$P < 0.05$, **$P < 0.01$, and *** $P < 0.001$.

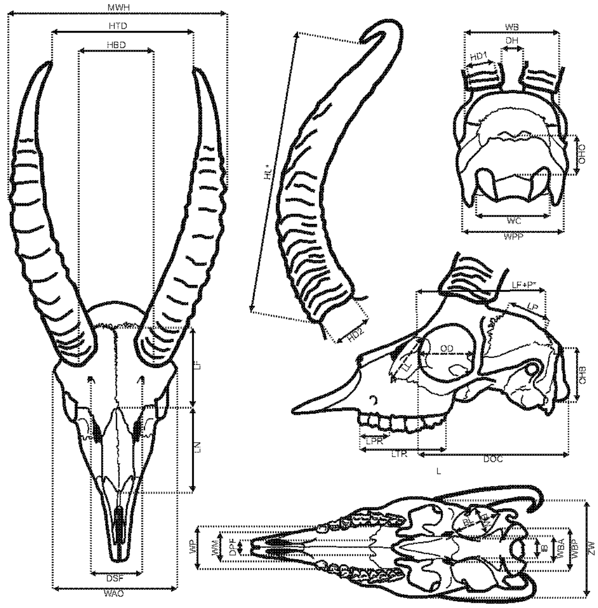
	North (N = 12)				**East (N = 14)**				**South-West (N = 22)**				**Farasan Islands (N = 66)**				**Akhoba Market Jizan (N = 23)**			
Locus	N_A	S	H_O	H_E	N_A	S	H_O	H_E	N_A	S	H_O	H_E	N_A	S	H_O	H_E	N_A	S	H_O	H_E
BM302	5	134-150	0.167	0.493***	3	138-146	0.286	0.265	5	134-148	0.091	0.482***	5	134-146	0.033	0.245***	2	128-138	0.043	0.043
BM415	6	126-146	0.750	0.696	4	138-144	0.385	0.443	8	124-146	0.45	0.764***	3	136-142	0.068	0.172***	3	138-142	0.391	0.428
CSSM043	7	243-263	0.800	0.837	9	255-279	0.429	0.865***	10	247-269	0.190	0.897***	11	237-271	0.391	0.797***	6	253-269	0.429	0.768***
TEXAN19	9	145-167	0.667	0.833*	6	143-157	0.857	0.791	9	147-169	0.591	0.873***	5	147-161	0.354	0.539**	7	143-157	0.957	0.839
BM4505	9	240-286	0.444	0.856***	10	238-300	0.714	0.894*	14	240-388	0.450	0.901***	12	228-370	0.218	0.473***	13	244-380	0.522	0.736***
SR-CRSP6	4	146-158	0.545	0.697*	5	154-162	0.692	0.791	8	140-160	0.611	0.848***	11	136-168	0.578	0.658**	5	146-158	0.609	0.705
MCM38	6	103-135	0.167	0.710***	8	111-165	0.857	0.836	15	113-165	0.700	0.914***	12	113-177	0.188	0.409***	13	111-185	0.739	0.813**
INRA40	7	186-246	0.556	0.784	6	200-220	0.375	0.742*	14	188-280	0.471	0.873***	7	188-302	0.480	0.617**	10	188-258	0.714	0.832*
OarFCB304	9	141-181	0.545	0.844***	11	139-171	0.571	0.886**	14	141-175	0.667	0.915***	10	143-169	0.683	0.824***	13	133-179	0.783	0.873
RM088	2	113-115	0.000	0.290**	2	113-115	0.429	0.423	3	113-121	0.318	0.513*	4	107-115	0.323	0.389	2	113-115	0.217	0.507**
TEXAN6	7	168-194	0.500	0.739**	7	168-190	0.429	0.778***	11	156-190	0.714	0.880*	10	154-182	0.364	0.562***	8	156-188	0.870	0.750

Table 2 Pairwise F_{ST} values between populations. All values were significant at $P < 0.001$.

	North	South-West	East	Farasan Islands
South-West	0.049			
East	0.087	0.054		
Farasan Islands	0.184	0.185	0.198	
Jizan	0.116	0.099	0.100	0.092

Supplementary Material

Supplementary Figures



Abbr. in Figure	Description
BL	bulla length
BW	bulla width
DH	distance between horns
DOC	distance orbit to condyle
DSF	distance of supra-orbital foramina
HBD	horn base distance (distance of the anteriormost parts of the pedicles)
HD1	horn diameter 1 (medio-lateral)
HD2	horn diameter 2 (antero-posterior)
HL	horn length 1, distance between the base of the horn sheath and the horn tip
HTD	horn tip distance
IB	inter-bulla distance
IPD	inter palatal-foramina distance
L F+P	length of frontal+parietal
LF	length of frontal
LL	length of lacrimal (maximum length of facial part)
LN	length of nasal
LP	length of parietal
LPR	length of premolar row
LTR	length of tooth row
MWH	maximum width of horns
OD	orbit diameter (horizontally)
OHB	occipital height, braincase only (dorsal of foramen magnum)
OHO	occipital height, occiput complete
WAO	width across orbits
WB	width of braincase
WBA	width of basioccipital anterior
WBP	width of basioccipital posterior
WC	width of codyle
WM	width of maxilla, measured at the midpoint of the diastema
WP	width of palate, measured at level of palatal foramina
WPP	width across paroccipital processes
ZW	zygomatic width (behind orbits)

*measurements marked with * in the Figure are measured with callipers and with a tape measure*

Figure S1 Skulls measurements taken for morphological analyses. Figure modified from Bärmann *et al.* (accepted).

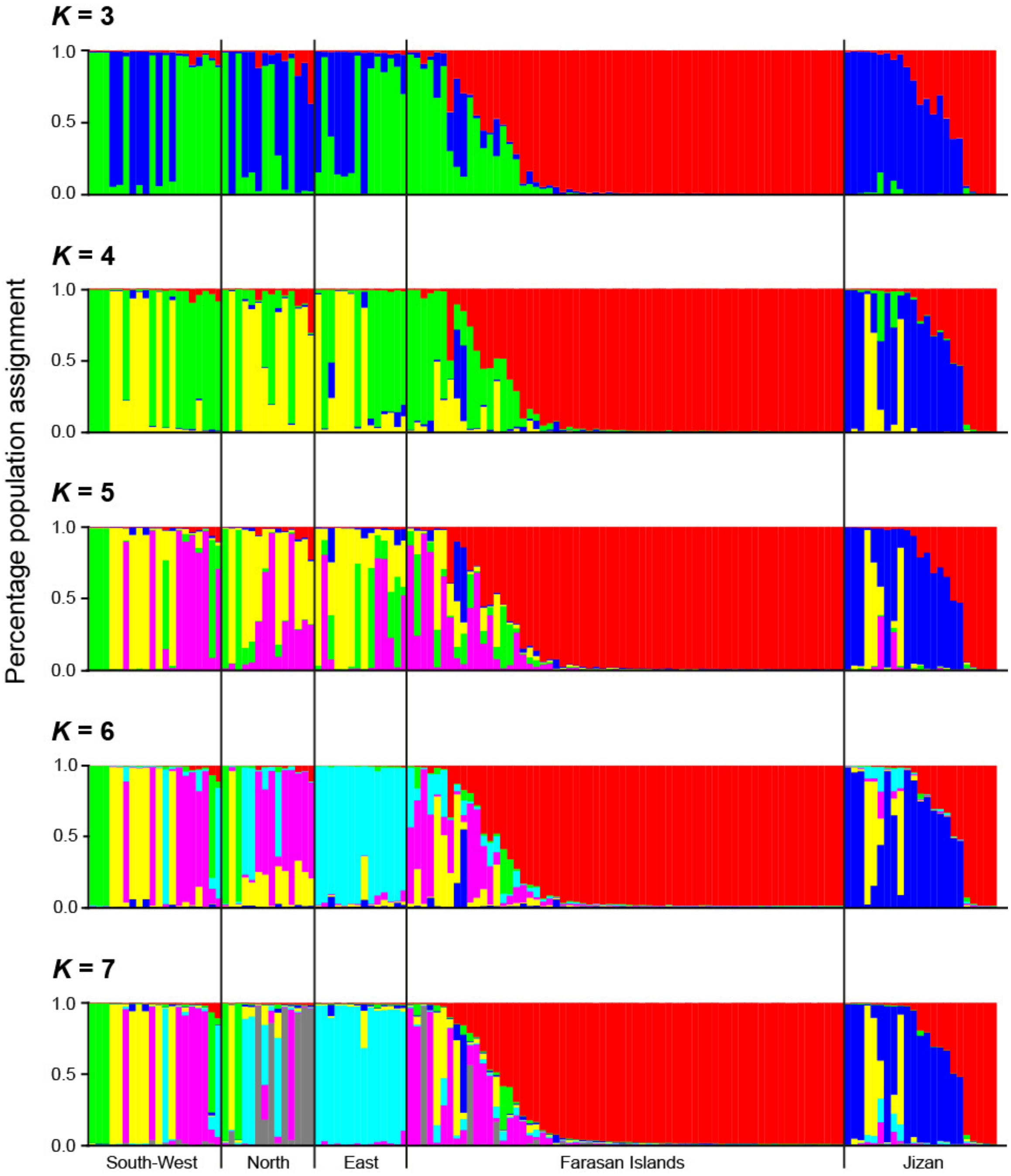


Figure S2 Percentage population assignments to inferred genetic clusters *K* ranging from 3 to 7. Animals were sorted by *Q* values of the genetic cluster endemic to Farasan (red) for each population separately.

Supplementary Tables

Table S1 List of specimens (wild, captive or confiscated) of *G.arabica* included in the population genetic analyses, origin, their collectors, sample types and group the specimens were designated.

Origin (coord.)	**Wild/ captive/ confiscated**	**Collector (number of samples)**	**Sample type**	**Group**
Southern Arava Valley	wild	R. King, R. Hammond, D. Blank, TAUM[a] (8)	tissue, DNA	North
Al Bad' (N 28°30', E 35°00')	wild	M. Sandouka (1)	tissue	North
BirMarshan (N 28°50' E 34°51')	wild	T. Wacher (1)	tissue	North
HarratUwayrid (N 26°50', E 37°45')	wild	K. Alageel (1)	tissue	North
Jordan	wild	O. Mohammed (1)	tissue	North
Wadi Al Safa, Dubai	wild	D. O'Donovan (2)	tissue	East
between Muscat and Sur, Oman	wild	M. Al Jahdhami (5)	tissue	East
Wadi Al Safa Wildlife Centre	captive	I. Nader, D. O'Donovan (7)	tissue, hairs	East
Wadi Tarj (N19°28', E42°21')	wild	T. Wronski (6)	tissue	South-West
Maqhshush (N18°38', E41°22')	wild	T. Wacher	feces	South-West
Al Hayla (N18°17', E41°49')	wild	T. Wacher (2)	hairs	South-West
Yemen (Amran)	wild	P. Vercamen (1)	hairs	South-West
National Wildlife Research Center	captive	P. Mésochina	tissue, blood	South-West
Tabalah (N20°05', E42°04')	wild	T. Wacher, R. Hammond (1)	hairs	South-West
Sharawrah	wild	T. Wacher	hairs	South-West
Al Wabra Wildlife Preservation	captive	D. Williamson (3)	blood, hairs	South-West
Farasan Islands (N16°40', E42°09')	wild	T.Wacher, O. Mohammed , M. Sandouka, H. Tatwany, S. Ostrowski,T. Wronski (66)	tissue, blood, hairs	Farasan Islands
Akhoba Market Jizan, unknown provenance	confiscated	S. Mubarak, S. Anajarriya (23)	tissue, blood	Jizan

[a] TAUM – Tel Aviv University Natural History Collection

Table S2 Microsatellite loci used in this study, original and antelope reference, used dye-label and number of multiplex reaction in which markers were amplified.

Locus	**Original reference**	**Antelope reference**	**Label**	**Multiplex No.**
BM302	Bishop *et al.* 1994	*Gazella dorcas* (Beja-Pereira *et al.* 2004)	CY5	1
BM415	Bishop *et al.* 1994	*G. dorcas* (Beja-Pereira *et al.* 2004)	DY-751	1
CSSM043	Moore *et al.* 1994	*G. cuvieri*, *G. dorcas* (Ruiz-Lópes *et al.* 2009)	CY5	1
TEXAN19	Burns *et al.* 1995a	*G. spekei* (Engel *et al.* 1996)	IRD700	1
BM4505	Bishop *et al.* 1994	*G. dorcas* (Beja-Pereira et al., 2004)	IRD700	2
SR-CRSP6	Bhebhe *et al.* 1994	*G. dorcas* (Beja-Pereira et al., 2004)	IRD700	2
MCM38	Hulme *et al.* 1994	*Aepyceros melampus* (Lorenzen & Siegismund 2004)	CY5	2
INRA40	Vaiman *et al.* 1994	*G. dorcas* (Beja-Pereira et al., 2004)	DY-751	2
OarFCB304	Buchanan & Crawford 1993	*G. cuvieri*, *G. dorcas* (Ruiz-Lópes *et al.* 2009)	CY5	3
RM088	Kossarek *et al.* 1995	this study	CY5	3
TEXAN6	Burns *et al.* 1995b	*G. spekei* (Engel *et al.* 1996)	IRD700	3

Supplementary References

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VI Eidesstattliche Erklärung

Ich erkläre hiermit an Eides Statt, dass ich die vorgelegte Dissertation über

The phylogeny of the genus *Gazella* and the phylogeography and population genetics of Arabian species

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