Phylogenetic Reanalysis of the Saudi Gazelle and Its Implications for Conservation

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Abstract: The identification of taxonomically appropriate populations of endangered species for captive breeding and reintroduction programs is fundamental to the success of those programs. The Saudi gazelle (Gazella saudiya) was endemic to the Arabian peninsula but is now considered extinct in the wild and is potentially a candidate for captive breeding and reintroduction. Using 375 base pairs of mitochondrial DNA (mtDNA) cytochrome b gene derived from museum samples collected from the wild prior to the presumed extinction of this species, we show that G. saudiya is the sister taxon of the African dorcas gazelle (G. dorcas). Reciprocal monophyly of G. saudiya mtDNA haplotypes with G. dorcas, coupled with morphological distinctiveness, suggests that it is an evolutionarily significant unit. These data indicate that captive populations identified previously as potential sources of G. saudiya for captive breeding appear incorrectly designated and are irrelevant to the conservation of G. saudiya. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of several private collections of living gazelles in Saudi Arabia provides no evidence for the survival of G. saudiya. We recommend that field surveys be undertaken to establish whether G. saudiya is indeed extinct in the wild and that other private collections within the Arabian peninsula be screened genetically. We urge caution when captive animals of unknown provenance are used to investigate the phylogenetics of cryptic species groups.

Reanálisis Filogenético de la Gacela Saudi y las Implicaciones para Su Conservación

Resumen: La identificación de poblaciones taxonómicamente apropiadas de especies en peligro para programas de reproducción en cautiverio y de reintroducción es fundamental para su éxito. La Gacela Saudi (Gazella saudiya) fue endémica a la península de Arabia pero ahora está considerada como extinta en su medio y es un candidato potencial para reproducción en cautiverio y reintroducción. Utilizando 375 pares de bases de ADN mitocondrial (ADNmt) del gene citocromo b derivados de muestras de museos colectadas en el medio silvestre antes de la extinción de la especie, mostramos que G. saudiya es el taxón hermano de la gacela dorcas africana (G. dorcas). La monofilia recíproca de haplotipos de ADNmt de G. saudiya con G. dorcas, unido a diferencias morfológicas, sugiere que es una unidad evolutiva significativa. Estos datos indican que las poblaciones cautivas identificadas previamente como fuente potencial de G. saudiya para reproducción en cautiverio están incorrectamente identificadas y son irrelevantes para la conservación de G. saudiya. El análisis PCR-RFLP de varias colecciones privadas de gacelas vivas en Arabia Saudita no proporcionan evidencia para la supervivencia de G. saudiya. Recomendamos que se realicen muestreos en el campo para establecer si en efecto G. saudiya está extinta en su hábitat y que se examinen genéticamente las otras colecciones privadas en la península Arábiga. Recomendamos precaución cuando animales cautivos de origen desconocido son utilizados para investigar la filogenia de grupos de especies críticas.
Introduction

The utility of molecular genetic data in the study of phylogeny and systematics is uncontroversial, but the use of these data to identify taxonomic units worthy of conservation remains contentious (Barrowclough & Flesness 1996). Recently, this debate has focused on the use of evolutionarily significant units (ESUs) and management units (MUs) in conservation (Moritz 1994; Vogler & De-Salle 1994; Cracraft 1997; Pennock & Dimmick 1997; Waples 1998). The ESU is an attempt to provide an operational definition of a conservation unit based largely on phylogenetic criteria (Ryder 1986; Moritz 1994). Failure to identify such units can be expensive, potentially leading to species extinction (Daugherty et al. 1990) and the misdirection of resources to the management of less fundamental taxonomic units (Avise & Nelson 1989).

Captive breeding and reintroduction programs are a high-profile and expensive approach to species conservation, so the decision to initiate such measures must be based on sound information (Snyder et al. 1996). In particular, knowledge of systematics of the taxon is crucial. Most taxonomy is based on morphological characters, but the existence of morphologically cryptic species, which are genetically divergent, can make identification of conservation units difficult (Baker et al. 1995; Barratt et al. 1997). In the worse-case scenario, cryptic variation and confused taxonomy could lead to the wrong species being captively bred and reintroduced.

Many gazelle species (Gazella) are endangered because of over-hunting and habitat destruction (Ryder 1987; Saleh 1987; Thouless et al. 1991; Baillie & Groombridge 1996). Therefore, conservation agencies have initiated measures to halt population declines and reestablish populations by reintroducing captively bred animals (Dunham et al. 1993; Haque & Smith 1996; Wacher & Kichenside 1998). Gazelles are good candidates for reintroduction because the main cause of decline, over-hunting, is easier to remedy than other factors such as habitat destruction (Dunham 1997). This has been borne out by the success with which reintroduced populations of gazelles have been established in the wild, particularly in Saudi Arabia (Haque & Smith 1996; Dunham 1997).

Gazelles are recognized as being one of the most taxonomically complex groups within the bovids (Groves & Harrison 1967; Groves 1997), a fact that has hindered conservation efforts (Ryder 1987). This problem is caused largely by considerable intraspecific variation being confused with interspecific similarity, particularly in characters such as horn-shape, body size, and pelage coloration—characters favored by morphological taxonomists to distinguish taxa (Groves 1996). The Saudi gazelle (Gazella saudiya) is an example of how taxonomic confusion can cause problems in conservation and how molecular genetics can potentially help solve these problems.

Case History of G. saudiya

G. saudiya, a species considered endemic to the Arabian peninsula, was once common on the gravel plains to the east of the Hejaz and Asir mountains in western Arabia (Vesey-Fitzgerald 1952). Over-hunting decimated wild populations, and the taxon is now considered extinct in the wild (Thouless et al. 1991; Baillie & Groombridge 1996). It is possible that G. saudiya survives in captive collections in Arabia; if so, it would be a prime candidate for captive breeding and reintroduction (Sausman & Correll 1994).

The search for extant populations of G. saudiya has been hampered by confusion over the species’ taxonomic position. Initially described as a distinct subspecies of the mountain gazelle, Gazella gazella (Carruthers & Schwarz 1935), its classification has oscillated from a distinct species, G. saudiya (Morrison-Scott 1939), to a subspecies of the dorcas gazelle, G. dorcas saudiya (Ellerman & Morrison-Scott 1951), and back to G. saudiya (Groves 1988). This confusion has been exacerbated by the identification of three captive populations of putative Saudi gazelles at Al Areen Wildlife Park (AAWP), Bahrain, at Al Ain Zoo (AAZ), United Arab Emirates, and in the private collection of Sheikh Al Thani, in Al Wabra (AW), Qatar (Williamson & Tatwany 1996).

Visual appearance and karyotype data have shown that the AW population is distinct from both AAWP and AAZ populations. Al Wabra animals have both typical G. dorcas appearance and karyotype (2n = 30/31), whereas the AAWP and AAZ animals are more similar in both respects to the Indian gazelle, G. bennetti (2n = 49–52) (Kingswood et al. 1997). Rebholz et al. (1996) interpreted these findings as suggesting that an Arabian form of G. dorcas (of which AW animals were held to be examples) and G. saudiya (of which AAWP and AAZ animals were held to be examples) were once sympatric in Arabia.

More detailed studies of the AAWP population, using karyotypic and mitochondrial DNA (mtDNA) sequence variation, indicated that this population may contain hybrid animals (Kumamoto et al. 1995; Rebholz & Harley 1997). Rebholz and Harley (1997) found two divergent cytochrome b (cyt b) haplotypes, one of which matched those found in captive populations of G. bennetti, whereas the other was a novel haplotype interpreted as being genuine G. saudiya mtDNA. Rebholz and Harley (1997) suggested that the AAWP G. saudiya were a hybrid mixture of G. saudiya and G. bennetti and that urgent measures were required to identify pure G. saudiya within the population. They also suggested that, once identified, large-scale captive breeding of G. saudiya for reintroduction should take place as a conservation measure.

The relative importance of the three captive populations of putative G. saudiya (AAWP, AAZ, and AW) is
not clear, however. None of the populations has a documented history, and there is no evidence to connect these captive animals to the wild-collected specimens of *G. saudiya* first described by Carruthers and Schwarz (1935). Historically, gazelles have been traded within Arabia and often imported from both Africa and Asia (Newby 1990). Also, gazelles in Arabia are often kept in multispecies groups in which there is an increased risk of hybridization (Habibi 1989; R.L.H. & T.J.W., personal observation).

To address these problems, we present partial DNA sequences of the 5′ region of the cytochrome b gene of mtDNA, obtained from skins of *G. saudiya* currently held at the Natural History Museum, London. These skins were collected before 1940, before the species was extirpated from the wild, and are, to our knowledge, the only specimens of wild *G. saudiya*. We use these sequences, and orthologous sequences from other gazelle species, to investigate the phylogenetic position of *G. saudiya* within the genus *Gazella* and to assess the relevance of captive collections for the future conservation of *G. saudiya* (Rebholz & Harley 1997). Furthermore, we discuss the status of the taxon *G. saudiya* in relation to the concept of evolutionarily significant units (Ryder 1986; Moritz 1994).

**Methods**

**DNA Samples**

The DNA samples were obtained from museum skins collected prior to presumed extirpation in the wild. As a conservative starting point, we considered *G. saudiya* to include all gazelle specimens with the *saudiya* epithet: namely, *G. saudiya, G. dorcas saudiya, G. gazella saudiya,* and *G. arabica saudiya*. Skin samples suitable for the extraction of DNA are known from the Natural History Museum, London (NHM) (18 specimens), and the Harrison Zoological Museum, Sevenoaks, Kent (HZM) (1 specimen). Samples from all but one of these skins (*G. saudiya* NHM40.314) were used for DNA extraction (Table 1; Fig. 1). In addition, a sample from the skin of a late-term calf (NHM40.299), identified as *Gazella arabica*, was included within the *G. saudiya* group (Table 1).

**Table 1.** *Gazella* samples used in DNA sequencing and phylogenetic reconstruction.

<table>
<thead>
<tr>
<th>Taxon name(^a)</th>
<th>Identification no.</th>
<th>Date collected</th>
<th>Origin</th>
<th>Location(^b)</th>
<th>Approximate lat./long.</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. arabica</em> (<em>G. saudiya</em>)</td>
<td>NHM40.299</td>
<td>1936</td>
<td>wild</td>
<td>Sirr Al Yamani (1)</td>
<td>N16.20 E46.50</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. arabica saudiya</em></td>
<td>NHM35.2.2.1</td>
<td>1934</td>
<td>wild</td>
<td>Kuwait (2)</td>
<td>N29.40 E48.00</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.301</td>
<td>1936</td>
<td>wild</td>
<td>Sirr Al Yamani (1)</td>
<td>N16.20 E46.50</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.302</td>
<td>1936</td>
<td>wild</td>
<td>Taraf Al Ain (3)</td>
<td>N15.50 E47.40</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.303</td>
<td>1936</td>
<td>wild</td>
<td>Wadi Markha (4)</td>
<td>N14.57 E46.35</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.304</td>
<td>1936</td>
<td>wild</td>
<td>Wadi Naq'a (5)</td>
<td>N15.30 E47.15</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.305</td>
<td>1936</td>
<td>wild</td>
<td>Arq Abu Da’ir (6)</td>
<td>N16.40 E45.15</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.306</td>
<td>1936</td>
<td>wild</td>
<td>Alamy Abayadh (7)</td>
<td>N16.00 E45.42</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.307</td>
<td>1936</td>
<td>wild</td>
<td>Ruwaik Tract (8)</td>
<td>N15.55 E46.10</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.308</td>
<td>1936</td>
<td>wild</td>
<td>Jau Al Khudaif (9)</td>
<td>N15.50 E46.35</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. saudiya</em></td>
<td>NHM40.309-313; NHM40.315-316</td>
<td>1936</td>
<td>wild</td>
<td>Dhalim (10)</td>
<td>N22.50 E41.40</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. dorcas saudiya</em></td>
<td>NHM48.384</td>
<td>1946</td>
<td>wild</td>
<td>Sahafa Plain (11)</td>
<td>N26.00 E39.00</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. dorcas saudiya</em></td>
<td>HZM1.4060</td>
<td>1953</td>
<td>wild</td>
<td>Abu Al Jir, Iraq (12)</td>
<td>N32.50 E40.00</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. bennetti</em></td>
<td>NHM35.12.21.8</td>
<td>1934</td>
<td>wild</td>
<td>Turbat, Pakistan (13)</td>
<td>N26.00 E39.00</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. dorcas</em></td>
<td>KKWRC026</td>
<td>1996</td>
<td>captive</td>
<td>Sudan?</td>
<td>unknown</td>
<td>blood</td>
</tr>
<tr>
<td><em>G. dorcas</em></td>
<td>KKWRC777</td>
<td>1997</td>
<td>wild</td>
<td>Wadi Rayan, Egypt (14)</td>
<td>N29.00 E30.17</td>
<td>shed hair</td>
</tr>
<tr>
<td><em>G. dorcas</em></td>
<td>KKWRC639</td>
<td>1997</td>
<td>wild</td>
<td>Sinai, Egypt (15)</td>
<td>N28.05 E34.20</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. pelzelni</em></td>
<td>NHM36.5.20.18</td>
<td>1934</td>
<td>wild</td>
<td>Danakil, Ethiopia (16)</td>
<td>N14.00 E40.50</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. pelzelni</em></td>
<td>NHM36.5.20.20</td>
<td>1934</td>
<td>wild</td>
<td>Danakil, Ethiopia? (16)</td>
<td>unknown</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. gazella sp.</em></td>
<td>KKWRC254</td>
<td>1994</td>
<td>wild</td>
<td>Makshush, Saudi Arabia (17)</td>
<td>N18.35 E41.30</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. gazella muscatensis</em></td>
<td>HZM26.4534</td>
<td>1967</td>
<td>wild</td>
<td>Wadi Umma, Oman (18)</td>
<td>N24.00 E57.00</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. gazella gazella</em></td>
<td>KKWRC-GH1</td>
<td>1995</td>
<td>wild</td>
<td>Golan Heights, Israel (19)</td>
<td>N33.00 E35.00</td>
<td>tissue</td>
</tr>
<tr>
<td><em>G. leptoceros</em></td>
<td>KKWRC770</td>
<td>1997</td>
<td>wild</td>
<td>Hatiyat Umm Ghuzlan, Egypt (20)</td>
<td>N29.17 E25.14</td>
<td>blood spot</td>
</tr>
<tr>
<td><em>G. subgutturosa subgutturosa</em></td>
<td>NHM70.2087</td>
<td>1913</td>
<td>wild</td>
<td>Samarra, Iraq (21)</td>
<td>N34.14 E43.56</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. subgutturosa yarkandensis</em></td>
<td>NHM35.8.26.498</td>
<td>1934</td>
<td>wild</td>
<td>unknown</td>
<td>unknown</td>
<td>dried skin</td>
</tr>
<tr>
<td><em>G. subgutturosa marica</em></td>
<td>KKWRC618</td>
<td>1995</td>
<td>wild</td>
<td>Al Khunfah (22)</td>
<td>N28.20 E38.38</td>
<td>blood</td>
</tr>
<tr>
<td><em>G. subgutturosa marica</em></td>
<td>HZM1.5725</td>
<td>1969</td>
<td>wild</td>
<td>Ramlat Fasd, Arabia (23)</td>
<td>N19.20 E53.30</td>
<td>dried skin</td>
</tr>
</tbody>
</table>

\(^a\)Not all *Gazella saudiya* samples yielded DNA in sufficient quality or quantity to allow PCR amplification.

\(^b\)Numbers in parentheses refer to geographical location in Fig. 1.
because the collector’s notes (H. St. John Philby) unequivocally indicate that this was the unborn offspring of a female *G. saudiya* (NHM40.301).

Blood or tissue samples were obtained from the three captive populations of putative *G. saudiya*. These included four samples from AAWP: KKWRC153, KKWRC705, KKWRC707, KKWRC708; three samples from AAZ: KKWRC154, KKWRC155, KKWRC216; and three samples from AW: KKWRC001, KKWRC002, KKWRC003 (Fig. 1). Alphanumeric designations refer to samples held in the gazelle DNA database of the King Khalid Wildlife Research Centre (KKWRC). Other than *G. saudiya*, we included samples from five species, *G. bennetti*, *G. dorcas*, *G. gazella*, *G. leptoceros*, and *G. subgutturosa*. Whenever possible, we used samples from across each species’ range to maximize intraspecific variation. All samples, except one from a captive *G. dorcas* KKWRC026, were collected from the wild (Table 1; Fig. 1). KKWRC026 originated from a captive group of *G. dorcas*, the founders of which are thought to have originated from west of the Nile in Sudan (S. Mubarak, personal communication). Wild samples were collected from known locations, except those from *G. subgutturosa yarkandensis* (NHM55.8.26.498) and *G. pelzelni* (NHM55.5.20.20), for which precise locations are unavailable. *G. s. yarkandensis* is associated with specimens from the eastern part of the range of *G. subgutturosa*, whereas *G. pelzelni* was collected by W. Thesiger in Ethiopia, probably near the Danakil.

**DNA Extraction**

We extracted DNA through a variety of protocols, depending on sample type (Table 1). Single hairs, or sand particles coated in dried blood, were placed in 250 μL of a 5% suspension of Chelex (Bio-Rad) in 10 mM Tris-HCl (pH 8.0) and digested overnight with proteinase K (20 μL of 20 mg/mL) at 55°C. After digestion the suspension was heated to 99°C for 5 minutes to denature proteinase K. Five microlitres were used in PCR reactions.

Small pieces of skin (about 2 × 2 mm) were chopped finely and digested with a conventional Tris-HCl, SDS, proteinase K protocol but with the addition of dithiothreitol (DTT) to a final concentration of 0.1M. Extractions were performed with a conventional phenol/chloroform protocol (Ausebel et al. 1996). The DNA in aqueous solution was purified by centrifugation through a Prospin-Ultra microconcentrator following the manufacturer’s instructions (Life Sciences International).

Using conventional protocols (Ausebel et al. 1996), we extracted DNA from whole blood and tissue. Blood samples collected in sodium or lithium heparin often failed to give PCR products even though DNA concentration and quality was high, suggesting that heparin was inhibiting *Taq* polymerase activity (Beutler et al. 1990). Washing blood samples (fresh or previously frozen) with 0.9% saline prior to digestion increased the likelihood of PCR amplification. For all methods, we monitored potential contamination using blank extractions containing no DNA.

**DNA Amplification and Sequencing**

The 486 base pairs (bp) of the 5’ region of the cytochrome *b* gene of mtDNA were PCR-amplified with the versatile primers L14724 and H15149 (Kocher et al. 1989; Irwin et al. 1991). Museum samples of *G. saudiya* that failed to amplify for this entire region could in some cases be amplified with primer pairs L14724/H14927 (product size: 250 bp) and L14979/H15149 (product...
size: 223 bp) (Irwin et al. 1991). All reactions were performed in a 25-μL reaction volume with 0.5 units of Taq polymerase (Gibco), 1x reaction buffer (Gibco), 1.5 mM MgCl₂, 40 μM of each dNTP, and 200 nM of each primer. Amplifications for all primer pairs were performed with the following cycle profile: 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds. We monitored contamination using blank PCR reactions containing no template DNA.

Double-stranded PCR products were sequenced with a Thermostsequenase-based cycle-sequencing kit using an end-labeled primer protocol (Amersham-Pharmacia Biotech). Both L14724 and H15149 were used as sequencing primers.

Sequence reactions were run on 6% acrylamide denaturing sequencing gels and visualized by autoradiography. For museum samples, we amplified and sequenced each sample from at least two separate DNA extractions. Autoradiographs were scored by eye, with each individual sequence read at least twice. Nucleotides were numbered with reference to the complete mtDNA genome of Bos taurus (Anderson et al. 1982).

We aligned sequences using Sequencher 3.0 (Gene Codes Corporation) and reconstructed phylogenetic relationships by cladistic and phenetic methods using PAUP* (version 4.0b2; Swofford 1998). Maximum parsimony (MP) trees were constructed with the branch and bound algorithm. Corrected nucleotide distances were estimated with the Hasegawa-Kishino-Yano model (HKY85; Hasegawa et al. 1985), and trees were reconstructed with the neighbor-joining (NJ) algorithm (Saitou & Nei 1987). We assessed the robustness of the NJ tree topology by bootstrap resampling the observed data 1000 times and reconstructing trees using each resampled data set (Felsenstein 1985). We used published cytochrome b sequence from Grant’s gazelle (Gazella granti; Genbank accession number AF028820; Arctander et al. 1996) to root MP and NJ trees by outgroup comparison.

Results

PCR Amplification and Sequencing from G. saudiya Museum Skins

Seven of the 19 G. saudiya museum skin samples (NHM40.2.21, NHM40.299, NHM40.303, NHM40.310, NHM40.313, NHM40.316, and HZM1.4060) yielded DNA of sufficient quality and quantity to allow PCR amplification of the 487 bp fragment of cytochrome b (L14724-H15149). A 250 bp region (L14724-H14927) was also successfully amplified for an eighth skin (NHM40.312).

Four sequence haplotypes were found, with the majority of sequences possessing one of two haplotypes (haplotype A, NHM35.2.2.1, NHM40.303; haplotype B, NHM40.299, NHM40.310, NHM40.313) that differed by a single transition (A→G) at position 14780 (Table 2). The sequence from NHM40.312 terminated at nucleotide 14710, so it was not possible to determine whether it belonged to haplotype A or B, but it clearly belonged to the A/B haplotype group. The remaining two sequences, from NHM40.316 and HZM1.4060, were divergent from haplotypes A and B, with uncorrected distances (calculated from 375 bp between nucleotides 14514-14888) of 0.04 (NHM40.316 - haplotype A); 0.043 (NHM40.316 - haplotype B); 0.061 (HZM1.4060 - haplotype A); and 0.064 (HZM1.4060 - haplotype B).

Cytochrome b Sequence from Putative G. saudiya from Captive Collections

Analysis of 10 putative G. saudiya revealed five cytochrome b haplotypes that could be arranged into three divergent haplotype groups (Table 2). The four putative G. saudiya from AAWP possessed one of two divergent haplotypes (uncorrected distance 0.029). Of the four, three (KKWRC153, KKWRC705, KKWRC708) possessed a haplotype described as G. saudiya by Rebholz and Harley (1997). The fourth animal (KKWRC707) matched the G. bennetti haplotype described by Rebholz and Harley (1997), as did all three animals sampled from AAZ (KKWRC154, KKWRC155, KKWRC216). From the AW collection, two haplotypes were found (KKWRC001 and KKWRC003) that differed by a single transition. These haplotypes were divergent from the haplotypes found in animals at both AAWP and AAZ. Uncorrected distances varied from 0.061 to 0.064 for comparisons with the G. saudiya haplotype (sensu Rebholz & Harley 1997) and from 0.050 to 0.056 with G. bennetti haplotypes.

Phylogenetic Analyses

To make phylogenetic sense of the diversity of mtDNA haplotypes attributed to G. saudiya, we aligned representative sequences of the major haplotype groups to cytochrome b sequences (375 bp) of 15 individuals belonging to six species within the genus Gazella (GENBANK accession numbers AF187692-AF187722; Table 1). Of the 375 base pairs of cytochrome b, 16.8% of characters were variable. Most variation was at third positions of codons (88.9%), with 7.9% at first positions and 3.2% at second positions.

Two most-parsimonious trees of length 88 were found that differ in the position of the G. gazella clade (Fig. 2).
Both haplotypes A and B, derived from *G. saudiya* museum skins, formed a monophyletic clade with *G. dorcas*. The two divergent haplotypes within the museum samples of *G. saudiya* (NHM40.316 and HZM1.4060) grouped with other taxa: NHM40.316 with *G. gazella* and HZM1.4060 with *G. subgutturosa marica* (Figs. 2 & 3). This suggests that these skins were incorrectly identified as *G. saudiya* or, less likely, were wild hybrids. Misidentification is likely because HZM1.4060 was immature (horns not fully developed), whereas NHM40.316 had a dark flank stripe, a character that is absent in *G. gazella*. Therefore, we hereafter consider NHM40.316 and HZM1.4060 to be misidentified.

The cytochrome b haplotype of captive *G. saudiya* (sensu Rebholz & Harley 1997) was divergent from the NHM *G. saudiya* (uncorrected distances 0.051–0.054) but identical to the haplotype found in *G. subgutturosa varkandensis* (NHM35.8.26.498) and *G. subgutturosa subgutturosa* (NHM70.2087). These findings are inconsistent with previous conclusions that AAWP represents one of only two known populations of *G. saudiya* (Rebholz et al. 1991; 1996; Kumamoto et al. 1995; Rebholz & Harley 1997). Unexpectedly, *G. subgutturosa* was paraphyletic, with the Arabian subspecies, *G. s. marica*, recovered as the sister taxon of North African, *G. leptoceros* (Figs. 2 & 3). This arrangement has historical precedent because, based on morphological data, Ellerman and Morrison-Scott (1951) considered the Arabian taxon *marica* a subspecies of *G. leptoceros* rather than *G. subgutturosa*.

As Rebholz and Harley (1997) reported, the other haplotype group found within the AAWP and the AAZ populations (Table 2) was derived from *G. bennetti*. This suggests that the AAWP population may be of mixed ancestry, but more likely between *G. subgutturosa* and *G. bennetti* rather than between *G. saudiya* and *G. bennetti*, as has been suggested previously (Kumamoto et al. 1995; Rebholz & Harley 1997).

The two closely related haplotypes found in the AW collection (KKWR001 and KKWRC003) grouped within the *G. dorcas* clade and the KKWRC003 haplotype were identical to that sequenced from a putative Sudanese *G. dorcas* (KKWR002). Thus, out of the three populations of putative *G. saudiya*, it was the AW haplotypes, rather than the AAWP or AAZ haplotypes, that were most similar to the museum *G. saudiya* haplotypes.

The NJ tree yielded a similar topology to the MP tree with the *G. saudiya/G. dorcas* monophyletic group supported strongly with a bootstrap value of 100% (Fig. 3). Likewise, *G. s. marica* was recovered (96% bootstrap

<table>
<thead>
<tr>
<th>Source</th>
<th>NHM40.312 museum</th>
<th>NHM40.316 museum</th>
<th>HZM1.4060 museum</th>
<th>KKWRC027</th>
<th>KKWRC154 putative <em>G. saudiya</em></th>
<th>KKWRC216 putative <em>G. saudiya</em></th>
<th>KKWRC001 putative <em>G. saudiya</em></th>
<th>KKWRC002 putative <em>G. saudiya</em></th>
<th>KKWRC003 putative <em>G. saudiya</em></th>
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</thead>
<tbody>
<tr>
<td>AA</td>
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<td>T C</td>
<td>T C T</td>
<td>T C C</td>
<td>T C G</td>
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<td>A T T</td>
</tr>
<tr>
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<td>T T A</td>
<td>A A A</td>
<td>T C T</td>
<td>T C C</td>
<td>T C A</td>
<td>C T T</td>
<td>C T T</td>
<td>A T T</td>
<td>A T T</td>
</tr>
</tbody>
</table>

* Dots (.) represent identical nucleotides and asterisks (*) unsequenced nucleotides. Variable nucleotides are numbered with reference to the complete mtDNA sequence of *Bos taurus* (Anderson et al. 1982).

Table 2. Variable nucleotides from a 375 bp region of cytochrome b derived from museum samples of known provenance and captive populations of putative *G. saudiya* including those described by Rebholz and Harley (1997).*
support) as the sister group to *G. leptoceros*. Branch-lengths indicated that the overall divergence within the *G. saudiya*/*G. dorcas* clade was low, raising the question as to whether *G. saudiya* is a species distinct from *G. dorcas*.

**Genetic Distinctiveness of *G. saudiya* from *G. dorcas***

The proportion of nucleotide differences between *G. saudiya* and *G. dorcas* haplotypes was small, ranging from 0.005 (pairwise comparison: NHM40.310 and NHM36.5.20.20) to 0.019 (NHM35.2.2.1 and KKWRC001/KKWRC777). Nonetheless, there were two C→T transitions (positions 14648 and 14658; Table 2) that distinguished all *G. saudiya* from all *G. dorcas* that we sequenced. These two diagnostic substitutions were shared by all six *G. saudiya* (NHM35.2.2.1, NHM40.299, NHM40.303, NHM40.310, NHM40.312, NHM40.313), a significant finding because the haplotypes were derived from animals collected from geographically distant locations within Arabia (Fig. 1).

Both diagnostic transitions fell within four base-cutting restriction-enzyme recognition sites. The transition at position 14648 caused the loss in *G. saudiya* of a Tsp509I site (AATT→AATC), whereas that at 14658 caused the gain of a HaeIII site (GGCT→GGCC). The RFLP analyses using these two enzymes provided a simple and quick method to survey captive animals for the presence of *G. saudiya* mtDNA.

Sixty-eight animals, considered on morphological grounds to be *G. dorcas*, were surveyed. The majority (51 individuals) were animals held at the King Khalid Wildlife Research Centre (KKWRC) and were believed to have originated from west of the Nile in Sudan; thus, there were a priori reasons to expect these animals to have African *G. dorcas* cytochrome *b* haplotypes. The remaining 17 originated from seven private collections within Saudi Arabia, and no details of their history or provenance are known. The RFLP results showed that for positions 14648 and 14658 all samples had mtDNA haplotypes consistent with the origin of the matriline from African *G. dorcas*, rather than the Arabian *G. saudiya*.

**Discussion***

Our results are relevant to two issues. The first is the phylogenetic position of *G. saudiya* and its taxonomic
distinctiveness from *G. dorcas*; *G. saudiya*’s distinction would require separate conservation measures. The second is the relevance of captive populations of putative *G. saudiya* (sensu Rebholz & Harley 1997) at AAWP, AAZ, and AW to the conservation of *G. saudiya*.

**Taxonomic Distinctiveness of *G. saudiya* from *G. dorcas***

Low levels of differentiation between *G. saudiya* and *G. dorcas* raise the questions of whether *G. saudiya* is genetically distinct from *G. dorcas* and whether it should be considered a separate ESU (Ryder 1986; Moritz 1994). Although the ESU concept has gained considerable acceptance within conservation biology (Waits et al. 1998; Manceau et al. 1999), its precise definition is still being debated (Moritz 1994; Vogler & DeSalle 1994; Cracraft 1997). Its definitions vary from the identification of minimum diagnosable units, even when those units are based on single nucleotide differences (Vogler & DeSalle 1994), to concordant patterns of divergence for molecular, morphological, and ecological characters (Barrowclough & Flesness 1996). Moritz (1994) defines ESUs as “reciprocally monophyletic for mtDNA alleles and showing significant divergence of allele frequencies at nuclear loci.”

*G. saudiya* and *G. dorcas* are closely related, and genetic distances between them are small. But despite the relatively short sequences analyzed, *G. saudiya* and *G. dorcas* mtDNA haplotypes are reciprocally monophyletic. We have no data from nuclear loci; but some morphological characters are congruent with both mtDNA sequence data and geographical separation. Therefore, we believe that *G. saudiya* is distinct from *G. dorcas* and thus requires conservation as a separate ESU.

**Captive Populations: the Relevance of Putative *G. saudiya* to Conservation***

Our findings suggest that the identification of animals at AAWP and AAZ as *G. saudiya* (sensu Rebholz et al. 1991; Kumamoto et al. 1995; Rebholz & Harley 1997) is incorrect. First, *G. saudiya* (as defined by the NHM samples) is the sister taxon to *G. dorcas*, not *G. bennettii*. Second, the novel haplotype from AAWP, described by Rebholz and Harley (1997), is derived from *G. s. subgutturosa*, not *G. saudiya*.

Nevertheless, because mtDNA is inherited maternally there are several scenarios in which the population of putative *G. saudiya* (sensu Rebholz & Harley 1997) at AAWP may have true *G. saudiya* ancestry yet still be compatible with the current mtDNA results: (1) insufficient sampling of living animals in our study and previous studies (Rebholz & Harley 1997) has left true *G. saudiya* haplotypes unsampled; (2) only male *G. saudiya* contributed to the founding of the population; and (3) female *G. saudiya* founders contributed to the gene pool but their mtDNA has been lost due to mtDNA lineage extinction.

The NHM skins and skulls of *G. saudiya* are characterized by the absence of a dark flank stripe, the presence of dark carpal and tarsal tufts, and straight horns in both males and females. The AAWP and AAZ animals share these characters, and their designation as *G. saudiya* in 1987 was based on their presence (C. Groves, personal communication). Prior to this, the gazelles at AAWP had been designated as Qatari subgutturosa (Groves 1996; Rebholz & Harley 1997), a name that, interestingly, predicts our finding that *G. s. subgutturosa* at least partially founded the population. Since Groves’ initial identification, both karyotypes and DNA sequences have been used to provide scientific support for the view that AAWP and AAZ gazelles were *G. saudiya* and that *G. saudiya* is only distantly related to *G. dorcas* (Rebholz et al. 1991; Kumamoto et al. 1995; Rebholz & Harley 1997).

The conclusions of these studies, however, relied on the initial assumption that gazelles at AAWP and AAZ were identified correctly as *G. saudiya*, an assumption that our data do not support. The only direct evidence, other than pelage coloration, to connect the captive populations of putative *G. saudiya* to the museum skins of *G. saudiya* was the association of a single female skull from AAWP to the NHM *G. saudiya* skulls when a number of craniometric and horn measurements were analyzed by discriminant functions (Groves 1996). The skull was larger than all NHM *G. saudiya* skulls, but this was attributed to captive rearing (Groves 1996). Besides the limitations of sample size, we suggest that these morphological analyses cannot be considered strong evidence for the relationship of AAWP putative *G. saudiya* with NHM *G. saudiya* because genetic evidence strongly suggests that the population is composed, at least partially, of hybrids (Rebholz & Harley 1997; this study). We believe that the unknown consequences of hybridization and captive rearing on these morphometric characters in this individual casts doubt on this evidence.

Of all three captive populations of putative *G. saudiya*, the animals at AW are most closely related to the museum specimens of *G. saudiya*. The founders of the AW population are reported to have originated from Tareeq Afif (Greth & Williamson 1996), which is <150 km from Dham and is the origin of three *G. saudiya* NHM museum skins (NHM40.310, NHM40.312, NHM40.313) we sampled (Table 1; Fig. 1). All AW animals (KKWRC001, KKWRC002, KKWRC003) had haplotypes consistent with African rather than Arabian origin. Given the proximity of Tareeq Afif to Dham and the lack of physical barriers between the two locations that might have impeded gene flow, it is unlikely that the AW gazelles originated from a genetically separate population of *G. sau-
diya. Furthermore, the physical appearance of the AW gazelles differs from that of NM skins because AW gazelles possess a dark flank stripe and lack dark carpal and tarsal tufts. We suggest that the G. dorcas gazelles at AW are of African origin and unsuitable as a source of G. saudiya for captive breeding and reintroduction.

Importance of Museum Samples

In addition to the incorrect designation of AAWP animals as G. saudiya, confusion over the phylogenetic relationship between G. s. subgutturosa and G. s. marica has arisen because of the use of captive animals to reconstruct phylogenetic relationships. Rehholz and Harley (1999), using data from partial cytochrome b and complete cytochrome c oxidase III mtDNA gene sequences, found that G. s. subgutturosa and G. s. marica shared the same haplotype, which is in contrast to our findings that sequence divergence is >5% (0.054–0.058). The animal sampled by Rehholz and Harley (1999) originated from the AW collection (Rehholz 1996) and may therefore be of uncertain provenance. We suggest that either G. s. subgutturosa has been misidentified or G. s. marica and G. s. subgutturosa have been mixed in captivity so that the mtDNA sequence originates from G. s. marica rather than from G. s. subgutturosa.

Endangered species are by definition rare, and obtaining samples of known provenance is difficult. Our study shows how DNA sequence data derived from museum skins collected from known locations can be important in constructing phylogenies. Furthermore, the case study of G. saudiya highlights the dangers of presuming the origin of captive animals without sound documentation.

Conservation Recommendations

We show that the three captive populations of putative G. saudiya (AW, AAWP, and AAZ) are of little relevance to the future conservation of this taxon. Moreover, RFLP surveys of privately owned gazelle collections within Saudi Arabia have failed to provide evidence of surviving G. saudiya. Although the current status of G. saudiya is uncertain, we demonstrated a rapid method (PCR-RFLP) for the identification of this taxon. We suggest the following conservation measures.

We recommend that field surveys be conducted in areas where G. saudiya has been recorded previously. These surveys should focus on (1) southwestern Saudi Arabia, where the disputed border between Yemen and Saudi Arabia may have discouraged hunting in the recent past, and (2) northwestern Saudi Arabia, where there are reports of as-yet unidentified gazelles (O. Llewellyn, personal communication). When gazelles, or signs of gazelles, are found, shed hairs, suitable for DNA extraction, should be collected to aid identification.

We suggest that further PCR-RFLP surveys of private collections of gazelles be initiated in an attempt to identify potential sources of G. saudiya for captive breeding and reintroduction. If both prove fruitless, then G. saudiya, as we identified it, must be considered extinct.

A third option is the introduction of a closely related taxon into the former range of G. saudiya. The phylogenetic affinity of G. saudiya to G. dorcas suggests that the introduction of G. dorcas would be most suitable. A factor that, in our view, precludes the introduction of G. dorcas is the unknown interaction between G. dorcas and the mountain gazelle G. gazella. The location of museums skins indicates that G. saudiya and G. gazella existed sympatrically in Saudi Arabia, so it is possible that interspecific competition between G. saudiya and G. gazella may have led to behavioral changes that minimized competition. In contrast, G. dorcas is sympatric with G. gazella only in the Arava Valley, where there is a population of fewer than 20 individuals of G. gazella acacia (Mendelsssohn et al. 1995; D. Blank, personal communication). Based on paleogeographic data, Tchernov et al. (1986) suggest that the range of G. dorcas has expanded from northeastern Africa and has replaced G. gazella in Sinai and southern Israel by competitive exclusion, with the Arava Valley population of G. gazella a relict of a more widespread distribution. If this is so, then the release of G. dorcas into the former range of G. saudiya might be detrimental to small, highly fragmented populations of G. gazella that are under continued threat from hunting and habitat destruction (Magin & Greth 1994). Resources would be better directed toward protecting existing wild populations of G. gazella than introducing a non-native, although closely related, taxon.

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Literature Cited


