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Genetic study of Sand gazelles (*Gazella subgutturosa marica*) from Saudi Arabia

Chromosomal and isozymic data

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Abstract

Presented the karyotype and electrophoretic variability at 20 loci of captive sand gazelles (*Gazella subgutturosa marica*) from Saudi Arabia. The most commonly encountered diploid numbers were 33 chromosomes for the males and 32 for the females, due to an X-to-autosome translocation commonly reported in the tribe Antilopini. Nevertheless, 4 females displayed diploid numbers of 31 chromosomes caused by a centric Robertsonian fusion in an heterozygous form. This probably results from previous hybridization with the subspecies *G. s. subgutturosa*. Percentage of polymorphic loci and mean heterozygosity were 15 % and 0.017, respectively. This latter quite low value, as well as the chromosomal polymorphism observed, may be due to previous lack of genetic management when this captive herd was founded. Nevertheless, the fact that some genetic variability remains in this endangered subspecies is encouraging in the perspective of its reintroduction in the wild, providing that the distribution of the chromosomal fusion and its possible consequences on reproduction and survival are checked.

Introduction

There is an urgent need for protection measures and establishment of captive-breeding programs for a number of species of gazelles (genus *Gazella*) that are seriously threatened today (Ryder 1987; Groves 1988). In this respect, one must know as precisely as possible the genetic status of the groups studied, both for breeding management purposes (Wayne et al. 1986; Templeton 1986) and for optimization of reintroduction plans (Allendorf 1986; Allendorf and Leary 1986).

In the present paper, we describe the genetic variability based on electrophoretic and karyologic results in a sample of *Gazella subgutturosa* from Saudi Arabia. The Goitred gazelle, or "rheem" *G. subgutturosa* is one of the three gazelle species native to Saudi Arabia (Thouless et al. 1991), where it is represented by the subspecies *G. subgutturosa marica,* the Sand gazelle (Harrison 1968). Although less threatened than the two other species (*G. gazella* and *G. [dorcas] saudiya*), the Sand gazelle has become rare in Saudi Arabia, and a captive-breeding program initiated by the National Commission for Wildlife Conservation and Development (NCWCD) has started in order to reintroduce the species into the wild (Thouless et al. 1991). Several hundreds of Sand gazelles are thus bred in Saudi Arabia, which are thought to represent a pure sample of the Arabian peninsula subspecies *G. subgutturosa marica,* according to external and skull morphology (Al Basri and Thouless, unpubl. data).

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Material and methods

Origin of the animals

The individuals studied originate from animals captured in the wild in different regions of Saudi Arabia (but precise locations are not known) between 1976 and 1982 and then bred near Riyadh in Prince Khaled farm, which became the King Khaled Wildlife Research Center (KKWRC) in 1986. Unfortunately, no details upon the numerical evolution of the herd during these first years are available, as no management of any kind was performed. At the KKWRC establishment, about 200 rheem were present, a number that has nearly doubled today. From here, a group of 24 animals has been brought into pre-release enclosures in the Mahazat as Saïd Reserve, the first site where reintroduction of the species is planned.

The isozyme survey was performed on 30 individuals, 19 of which belong to the group that is to be released in the Reserve. 23 animals from this latter group were karyotyped, as well as 7 additional individuals which will be reintroduced to the wild later on.

Karyotypes

The karyotypes were established from lymphocyte cell cultures. About 10 ml of peripheral blood were collected aseptically by jugular punction into heparinized sterile glass tubes. Ten drops of blood (0.5 ml) were distributed into vessels containing 9.5 ml of HAM'SF 12 nutritive mixture supplemented with 20 % fetal calf serum, antibiotics (100 UI) and concanavalin A (10 µg/ml). The culture was then incubated at 37 °C for 72 hours, and colcemid (final concentration 0.03 µg/ml) was added one hour before harvesting. The cells were then treated with a hypotonic solution of sodium citrate (0.85 %) for 20 min at 37 °C, fixed with Carnoy's solution, spread on previously cooled slides and stained with a 4 % Giemsa solution. The best metaphases were photographed and karyotypes were then prepared.

Protein electrophoresis

Blood samples were taken by jugular punction and stored in heparinized tubes at 4 °C until treatment. Saline solution was added before the first centrifugation, after which the plasma fraction was separated from the red cells. After several washes in saline solution, the red cell samples were prepared by a hypotonic shock in distilled water. The plasma and red cell samples were then kept at −30 °C until electrophoresis was performed.

Twenty loci were analysed using horizontal starch-gel electrophoresis according to Pasteur et al. (1987) with a starch concentration of 12 %. Staining procedures were according to Pasteur et al.

Table 1. **Enzymes studied, number of loci per enzymes, tissue (RBC = Red Blood Cells) and buffer system used**

(see text)

Enzyme	Loci	Tissu	Buffer
Aspartate-aminotransferase (AAT)	1	RBC	TME6.9/TME6.9
Acid-phosphatase (ACP)	1	RBC	TC6.4/TC6.0
Diaphorase (DIA)	1	RBC	TC6.4/TC6.0
Esterase (EST 10–14)	2	RBC	TME6.9/TME6.9
Glyoloxase (GLO)	1	RBC	TBE8.6/TBE8.6
Glucose 6-Phosphate dehydrogenase (G6PDH)	1	RBC	TME6.9/TME6.9
Glucose phosphate isomerase (GPI)	1	RBC	TC6.4/TC6.0
Lactate dehydrogenase (LDH)	1	RBC	TC6.4/TC6.0
Malate dehydrogenase (MDH)	1	RBC	TC6.4/TC6.0
Malic-enzyme (MOD)	1	RBC	TC6.4/TC6.0
Mannose phosphate isomerase (MPI)	1	RBC	TC6.4/TC6.0
Phosphogluconate dehydrogenase (PGD)	1	RBC	TC6.4/TC6.0
Purine nucleoside phosphorylase (NP)	1	RBC	TME6.9/TME6.9
Superoxyde dismutase (SOD)	1	RBC	TC6.4/TC6.0
Hemoglobin (Hb)	2	RBC	TBE8.6/TBE8.6
Albumin (ALB)	1	Plasma	LiOH8.3/LiOH8.1
Esterase (EST 1)	1	Plasma	LiOH8.3/LiOH8.1
Transferin (TRF)	1	Plasma	LiOH8.3/LiOH8.1

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(1987). Table 1 lists the loci and buffers used: Tris-Citrate (TC) pH 6.4 (gel) and 6.0 (electrode); Tris-Maleate-EDTA (TME) pH 6.9; Tris-Borate-EDTA (TBE) pH 8.6 and Tris-Lithium-Citrate-Borate (LiOH) pH 8.3 (gel) and 8.1 (electrode), as described by PASTEUR et al. (1987).

Results

Karyotypes

The diploid numbers in the 30 individuals studied were found to be as follows: 2n = 33 in the 11 males (Fig. 1), 2n = 32 in 15 females (Fig. 2) and 2n = 31 in 4 females (Fig. 3). The males have 26 meta-submetacentric, 6 acrocentric and 1 large submetacentric chromosomes. The 2n = 32 females have 26 meta-submetacentric, 4 acrocentric autosomes and 2 large submetacentric X chromosomes. In the 4 females whose diploid number equals 31, one more large metacentric chromosome is found, but there are only two acrocentric chromosomes. This pattern is probably reflecting the presence of a centric Robertsonian fusion in an heterozygous form in these 4 animals.

Protein electrophoresis

Three (Trf, Gpi and Np) of the twenty loci studied were polymorphic in the sample (Tab. 2). This yielded a percentage of polymorphic loci ($P_{99\%}$) of 15 %. Two alleles were found at each of the polymorphic loci, which resulted in a mean number of alleles per locus (A) of

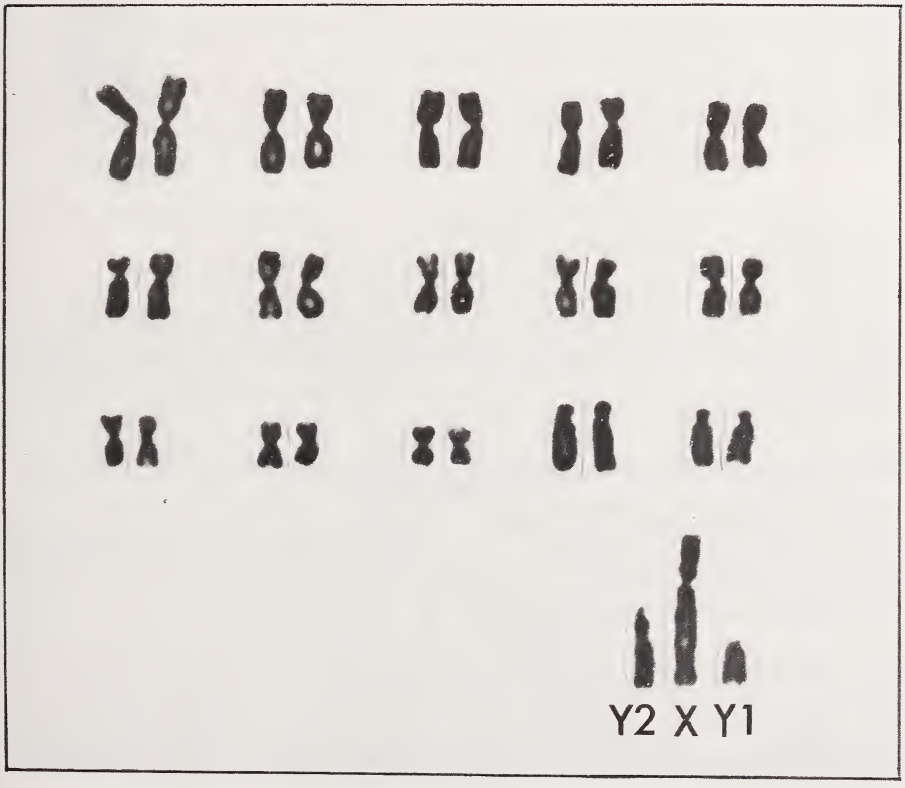

Fig. 1. Karyotype of a male *Gazella subgutturosa marica* with 2n = 33 chromosomes

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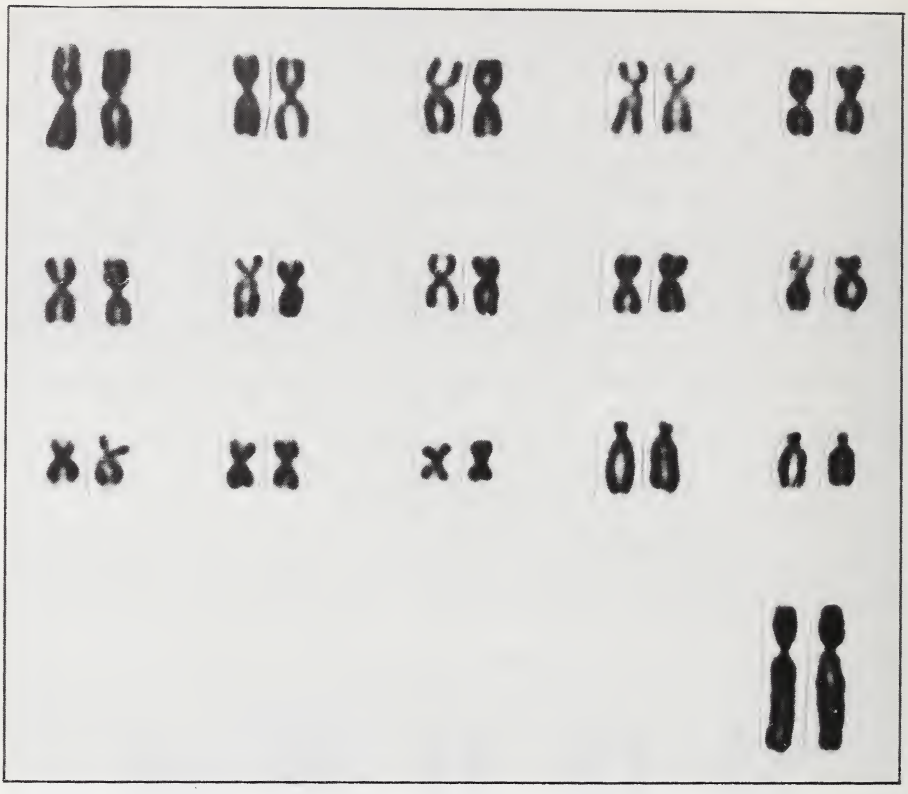

Fig. 2. Karyotype of a female *Gazella subgutturosa marica* with 2n = 32 chromosomes

1.17. From the allelic frequencies, heterozygosity was calculated at each of the three variable loci, and the mean heterozygosity (H) was 0.017 (Tab. 2).

It should be noted that the Trf and Np loci were polymorphic only within the 19 individuals from the Mahazat as Saïd Reserve. When calculated in this group of 19 individuals only, the value of H reaches 0.023. Nevertheless, the absence of the Np^{120} and Trf^{110} alleles in the 11 individuals from the KKWRC probably reflects a sampling effect as these two alleles are in low frequency and would likely be found in a larger group of Sand gazelles from KKWRC.

Table 2. **Allelic frequencies and heterozygosities for the polymorphic loci, and values of P, A and H for the whole sample**

Locus	Alleles	Allelic frequencies	Heterozygosity
Trf	100 110	0.97 0.03	0.064
Gpi	100 120	0.95 0.05	0.095
Np	100 120	0.90 0.10	0.180

Mean heterozygosity H = 0.017; Mean number of alleles per locus A = 1.17; Percentage of polymorphic loci P (99 %) = 15 %.

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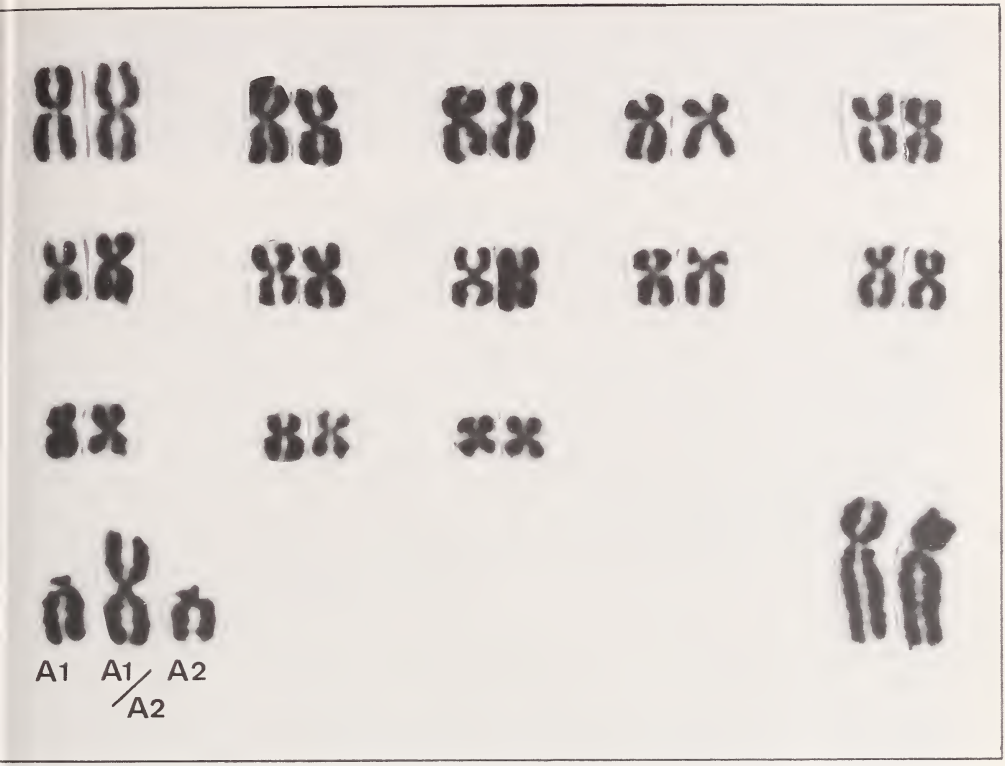

Fig. 3. Karyotype of a female *Gazella subgutturosa marica* with 2n = 31 chromosomes. The large A1/A2 metacentric autosome results from the fusion of two acrocentric ones (A1 and A2)

Discussion

Previously reported karyotypes of other members of the tribe Antilopini have been found to display peculiarities in the sex chromosome morphology. They have an unusually large X chromosome corresponding to an X-to-autosome translocation. Thus, males have one more chromosome (Y2) owing to the translocation of the acrocentric autosome onto the X chromosome (Wurster 1972). This is the case here.

Effron et al. (1976) found in a sample of *G. subgutturosa,* the origin and subspecific rank of which were not specified, a karyotype of 31 chromosomes in 2 males and of 30 in 1 female, with 28 metacentric autosomes. Wurster (1972) found the same result for 3 females studied. The subspecies name was not provided either, but the animals were called Persian gazelles, which is the usual name for *G. subgutturosa subgutturosa*. Diploid numbers of 30 and 31 are also reported for *G. subgutturosa* from China, where only *G. subutturosa subgutturosa* is met (Orlov, in Shi 1987).

In their sample of supposed Sand gazelle (*G. subgutturosa marica*), Kingswood and Kumamoto (1988) found chromosome numbers of 31 (N = 18 individuals), 32 (N = 19) and 33 (N = 1) in males, and 30 (N = 10), 31 (N = 12) and 32 (N = 11) in females. The Persian gazelles (*G. subgutturosa subgutturosa*) they studied have diploid numbers of 31 (N = 5) in males and 30 (N = 4) in females. Based on these data, as well as the fact that there was an exact homology of G-banding patterns between all chromosome pairs of a male

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Persian gazelle (2n = 31) and those of a male Sand gazelle (2n = 31), the authors argued the possibility that their Sand gazelle sample may in fact correspond to hybrids between the original stock of Sand gazelles and Persian gazelles, particularly since the origin of the animals sent to the USA was not well known. Moreover, breeding records were found to be different between Sand (or supposedly so) and Persian gazelles, Sand gazelles having less offspring. This fact could effectively result from on outbreeding depression following hybridization between two subspecies.

The results presented here confirm to a certain extent the hypothesis of Kingswood and Kumamoto (1988) concerning Goitred gazelles from the US zoos, as the true diploid numbers for *G. subgutturosa marica* seem to be 33 for males and 32 for females. Nevertheless, we also face the question of a probable introgression phenomenon with *G. subgutturosa subgutturosa* in the Saudi herd, as the 4 females with diploid numbers of 31 are likely to represent hybrids between the two subspecies. This hybridization seems, however, to be much less important than reported in gazelles from the US zoos, as the proportion of hybrids appears to be much lower. Anyway, in both cases morphological descriptions were useless to predict chromosomal findings, as the phenotype *"marica"* (smaller size, paler colour, better-developed horns in females, Harrison 1968) was observed in all specimens, even in those having a true *"subgutturosa"* caryotype (sample of Kingswood and Kumamoto 1988).

So far, we can't discuss the genetic variability of a pure sample of *G. subgutturosa marica*. Even without taking into account the two individuals (one of which has a Trf^{110} allele) with hybrid caryotypes that were included in our electrophoretic survey, we can't rule out the possible integration of Persian gazelles' genes into genomes of individuals chromosomally characterized as Sand gazelles, through recombination. Considering these restrictions, the percentage of polymorphic loci of 15 % observed in our sample is in the range of those found in a number of species of artiodactyles (review in Baccus et al. 1983, and in Vassart et al. in prep.). It is also close to the result of 14 % found by Templeton et al. (1987) in a captive herd of Speke's gazelle. Nevertheless, it appears to be somewhat lower than values obtained in samples of African gazelles *G. dorcas* and *G. thomsoni* (Vassart et al. in prep.). As far as the mean heterozygosity is concerned, the result found here (H = 0.017) is rather low when compared with data from the references cited above. This finding must be stressed, particularly in this case where a reintroduction program is going on, since sufficient heterozygosity is important in short-term success of a species in the wild (Allendorf 1986). This lower heterozygosity rate observed probably results in part from an absence of genetic management of this Sand gazelle herd, at least during a period of low effective size of the breeding group. At that time, genetic drift associated with group structure (see Lacy 1987) may have had important effects on genetic diversity.

Such failures in the management of the herd and in the checking of the animals' origin would also be responsible for the probable hybridization with *G. subgutturosa subgutturosa,* leading to the observed chromosomal polymorphism. The study of natural specimens of both subspecies in their particular range (and especially *G. subgutturosa marica*) is still needed to definitely clarify this situation. As far as the captive-breeding and reintroduction program is concerned, the distribution of the Robertsonian fusion must be precised by an extensive karyological study and its possible consequences on the adults breeding rate and juveniles survival and growth have to be documented (see Kingswood and Kumamoto 1988). Prior to that, it seems preferable to choose only those individuals displaying the 2n = 33 (males)/32 (females) karyotypes for reintroduction into the wild. This selection of individuals should also be achieved in such a way as to maintain as much genetic variability as possible. This last point could be achieved through a screening of the polymorphic loci described here on a larger sample of individuals, as well as through the finding of new variable loci.

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Zusammenfassung

Genetische Untersuchungen an Sandgazellen (Gazella subgutturosa marica) aus Saudi-Arabien. Chromosomale and elektrophoretische Daten

Bei in Gefangenschaft nachgezüchteten Sandgazellen (*Gazella subgutturosa marica*) aus Saudi-Arabien wurde der Karyotyp und die elektrophoretische Variabilität in 20 Loci untersucht. Männchen hatten 2n = 33 und Weibchen 2n = 32 Chromosomen infolge der bei den Antilopini häufig beobachteten Translokation des X-Autosoms. Indessen besaßen vier Weibchen 2n = 31 Chromosomen infolge zentrischer Robertsonischer Fusion in einer heterozygoten Form. Dies ist wahrscheinlich auf vorherige Hybridisierung mit der Unterart *G. subgutturosa subgutturosa* zurückzuführen. Der Prozentsatz polymorpher Loci sowie die mittlere Heterozygotierate beliefen sich auf 15 % beziehungsweise 0.017. Dieser letzte, ziemlich niedrige Wert sowie der beobachtete chromosomale Polymorphismus mögen auf vorherigen Mangel an genetischer Organisation zur Zeit der Gründung der Herde beruhen. Die Tatsache aber, daß eine gewisse genetische Variabilität in dieser gefährdeten Unterart verbleibt, ist ermutigend in Hinblick auf ihre Wiedereinbürgerung in die freie Wildbahn, vorausgesetzt, daß die Verteilung der chromosomalen Fusion und ihre möglichen Auswirkungen auf Reproduktion und Überleben überprüft werden.

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