Studies on a male eland × kudu hybrid

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Summary. An accidental mating between a male eland and a female kudu produced an animal with primarily eland phenotypic characteristics. Despite pronounced male behaviour the animal was azoospermic. Histological examination of the testis showed complete lack of germ cells. Chromosome studies with analysis of Giemsa bands showed that the parental karyotypes differed by two reciprocal translocations and one pericentric inversion, involving chromosomes 1 and 3, 5 and 11, and 9 respectively. All other chromosomes had identical banding patterns.

Introduction

Interspecific hybrids are of scientific interest and the reasons for their sterility in particular have been repeatedly investigated. We have had the opportunity recently to study in some detail the hybrid between a male eland (*Taurotragus oryx pattersonianus*) and a female kudu (*Tragelaphus strepsiceros bea*). Reference to such a cross is only once made in the literature (Boulineau, 1933). This author describes very briefly one male and one female hybrid of this type, and depicts their adult phenotypes, but gives no details of their fertility or other studies.

The chromosomes of these bovids of the Subfamily Tragelaphinae are of interest. Both species possess 32 elements in the female but only 31 in the male, with the Y-chromosome attached to autosome 14 (Wallace & Fairall, 1967; Hsu & Benirschke, 1971). Despite the similarity of chromosome number, the arm lengths of several chromosomes differ in these species, most notably those of chromosomes 1. No detailed banding studies have been published of the karyotypes.

This paper describes a study over a 3-year period of the phenotypic changes of a male eland × kudu hybrid, its electroejaculation and testis morphology, and the details of chromosome structure of the hybrid and kudu.

Materials and Methods

The hybrid is the offspring of an East African male eland (*Taurotragus oryx pattersonianus*) and a female East African kudu (*Tragelaphus strepsiceros bea*). The cross occurred accidentally at the San Diego Wild Animal Park and is assured because of the absence of a male kudu from the group. At 3 years of age the animal was immobilized by intramuscular injection of 12 mg etorphine hydrochloride (M99: D-M Pharmaceuticals, Rockville, Maryland) and 10 mg acepromazine (Ayerst Laboratories, New York) and skin biopsies were taken for tissue culture. These were grown according to the method described by Basur *et al.* (1963), and chromosome preparations were made in the usual manner. They were stained with Giemsa solution after pretreatment with trypsin: the flamed slides were treated for 2 hr in two changes of SSC at 60°C; washed in 0.5% trypsin solution for 30 sec; washed briefly in 95% alcohol; stained for 5 min in Giemsa solution (3 ml Giemsa/70 ml water); and finally washed again.

While the animal was immobilized, semen was collected after stimulation with a rectal probe in a manner similar to that employed for collection of bovine semen (Hill *et al.*, 1956). The semen was examined fresh and after fixation and staining with fast green. One scrotal testis was removed at the same time, cut into two parts, and fixed in Bouin's solution. Sections were cut after embedding in paraffin wax and were stained with haematoxylin and eosin.

Results

Phenotype

Mating of the parent animals occurred sometime in January or February 1971, and parturition on 26 September 1971 was uneventful. One month after birth, the offspring appeared similar to a young kudu and distinctive differences appeared only subsequently. At 2 months, the legs had become heavier and were more like those of a young eland. The coat colour was brown, intermediate between that of eland and kudu, the ears were rounded as in kudu but not so large, and the face had the markings of kudu but was not as narrow. At 5 months, the body structure and coat colour were those of a young eland except for the lateral stripes and facial markings of kudu. Ears and tail were intermediate. At 10 months he had most of the characteristics of an eland, i.e. body structure, coat coloration, black patches on front legs, horns growing straight and backwards, but still possessed the lateral stripes and facial markings of a kudu and held his head upwards. After 10 months, the body structure of eland predominated and the horns changed from that of a young eland phenotype to an intermediate form. At present (Pl. 1, Fig. 1) his body is heavy, oxen in appearance, and a dewlap is developing. The heavy legs have black patches in the front, but the facial markings, lateral white stripes and a fringe that extends from neck to tail, are like those of a kudu. Some mixed phenotypic characteristics are pointed (eland) but flared (kudu) ears, intermediate horn structure resembling more the giant eland, and a tail half the length of that of an eland but with a terminal hair tuft (kudu). The hybrid has male behaviour and strong male scent.

Testis

No spermatozoa were recognized by direct microscopic examination of the 5 ml ejaculated semen. Only detritus and some epithelial cells were present in stained smears. The size of the testis was adjudged to be normal and both testes were descended. No spermatozoa were present in the epididymis.

The histological appearance of the testis supported the findings at electroejaculation. All seminiferous tubules lacked germinal cells and only Sertoli cells were present. No mitotic or meiotic activity was found; the Leydig tissue was abundant (Pl. 1, Figs 2 and 3).

Chromosomes

The Giemsa banding of a male kudu karyotype was available for comparison with the chromosomes of the hybrid. It had 31 chromosomes, in accordance with reports from the literature (Hsu & Benirschke, 1971), and possessed the Y/14 translocation characteristic for these bovids. All elements could be specifically identified by this banding technique and pairing was unequivocal (Pl. 2, Fig. 4). The metacentric chromosome shown last is composed of one autosome 14, the bottom portion, and the Y, represented here as the top half of this element.

No Giemsa-banded preparation from an eland was available to us but previous studies had shown its chromosome number to be the same as those of kudu, although the arm lengths of several chromosomes differed (Hsu & Benirschke, 1971). The Giemsa-banded karyotype of the hybrid is shown in Pl. 2, Fig. 5. The arrangement follows that of the kudu karyotype (Pl. 2, Fig. 4) and, since the kudu elements can be identified by banding structure, they are always placed on the right

EXPLANATION OF PLATE 1

Fig. 1. Eland × kudu hybrid at 3 years of age. Note the intermediate horn shape, dewlap, stripes, dorsal fringe, black patches on front legs and tail.

Fig. 2. The appearance of the testis of the hybrid. Note the abundance of interstitial cells and absence of germ cells. The tubules are lined with Sertoli cells only. H & E, ×134.

Fig. 3. Higher magnification of the testis. An occasional tubule (centre) possesses larger cells, but these cannot be classified as germ cells with certainty. H & E, ×207.

(Facing p. 14)

PLATE 2

side of the pairs. The translocated Y-chromosome must have come from the eland sire, and the solitary chromosome 14 from the kudu dam. No difference in banding pattern could be ascertained in many chromosomes and hence they were paired with the kudu elements. They were thought to be homologous and are here labelled chromosomes 2, 4, 6, 7, 8, 10, 12, 13, 14, 15, Y. The X-chromosome was identical to that of the kudu.

Chromosome pairs 1, 3, 5, 9 and 11 are believed to be different in these two species and, by comparing the banding patterns, the rearrangements identified in the hybrid were as follows: Chromosome 1: $E_{1p} = K_{3q}$; $E_{1q} = K_{1q}$; Chromosome 3: $E_{3p} = K_{3p}$; $E_{3q} = K_{1p}$; Chromosome 5: $E_{5p} = K_{5p}$; $E_{5q} = K_{5q}$; Chromosome 9: pericentric inversion; Chromosome 11: $E_{11p} = K_{11p} + K_{5q}$; $E_{11q} = K_{11q}$.

It is suggested that reciprocal translocations occurred during the speciation process among the arms of chromosomes 1 and 3, and between 5 and 11; that a pericentric inversion took place in chromosome 9; and that the exchange between the arms of chromosomes 1 and 3 involved whole arms. When elongated chromosomes of the respective types are compared one with another then the banding pattern of these arms is identical. Conversely, the exchange between chromosomes 5 and 11 appears to involve only a portion of the long arm of what is here designated Kudu 5 (K_{5q}). Because of the difference in centromere position in the chromosomes of pair 9, but their mostly identical banding pattern, it is assumed that a simple pericentric inversion with breaking points near the centromere occurred. These rearrangements are depicted schematically in Text-fig. 1.

Text-fig. 1. Schematic diagram of the rearrangements that distinguish the eland from the kudu karyotypes. E = eland, K = kudu.

Discussion

This study confirms the chromosome number of eland and kudu and the peculiarity of their attached Y-chromosomes. Moreover, analysis of the banding pattern in the kudu allows for the first time unequivocal identification of all elements. The karyotype displayed in Pl. 2, Fig. 4 is arbitrarily

EXPLANATION OF PLATE 2

Fig. 4. Karyotype of a male kudu, Giemsa banding technique. All chromosomes can be paired easily. The Y is attached to one autosome, chromosome 14.

Fig. 5. Karyotype of the hybrid. Homology is apparent in all pairs except 1, 3, 5, 9 and 11. So far as the chromosomes are identifiable individually, the eland element is placed left in the pairs, the kudu to the right. Giemsa banding.

arranged according to the length of chromosomes and it follows those shown by previous investigators.

The karyotype analysis of the hybrid allows, by inference, the elucidation of the eland karyotype. Ten autosomal pairs and the sex chromosomes are believed to be identical in structure because pairs with the same banding patterns were identified. In pairs 1, 3, 5, 9 and 11, banding patterns and centromere positions differ and, from the banding analysis, reciprocal translocations are assumed to have occurred between whole arms of 1 and 3, and between portions of one arm of 5 and 11. The difference in banding patterns of pair 9 and the difference in arm lengths suggest a pericentric inversion as the simplest explanation for chromosome change.

Thus, eland and kudu differ in the morphological arrangement of five chromosomes. When this change has occurred, of course, is unknown, as is the direction of the change. A comparison with karyotypes of related species (nyala, sitatunga) is not helpful because of their considerably different chromosome complements.

The sterility of the hybrid was unexpected before this study because of the generally similar karyotypic structure of eland and kudu. Animals with more divergent chromosome sets produce male hybrids with a complete set of germ cells and sterility can be inferred to be secondary to failure of meiotic pairing. For example, meiotic activity is frequently seen in the testes of mules and hinnies, but spermatozoa are not usually formed because first meiosis cannot be completed. When the parental chromosome sets are less divergent, fertility may be feasible, as in horse $\times$ Przewalski horse hybrids (Koulischer & Frechkop, 1966).

There are many causes of hybrid sterility (Basrur, 1969) and only some are the result of chromosomal incompatibility at first meiosis. The reason for the absence of germ cells in the eland $\times$ kudu hybrid is unknown. A parallel situation is found in female mules. In this cross, normal germ cells are present in the fetus but disappear during later development (Benirschke & Sullivan, 1966; Taylor & Short, 1973). This change may also be the result of meiotic incompatibility, but cannot be held responsible for the deficiency in the present male hybrid. It will be interesting to learn from other eland $\times$ kudu crosses whether this deficiency of germ cells is a constant event and whether it also extends to females.

No other hybrids are known to occur of either kudu or eland with other species. Gray (1971) reviews the literature and reports that repeated matings of male elands with domestic and zebu cows proved infertile. Similarly, artificial insemination has been unsuccessful.

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