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# Chromosome study of members of the subfamilies Caprinae and Bovinae, family Bovidae; the Musk Ox, Ibex, Aoudad, Congo buffalo and Gaur<sup>1</sup>

By H. HECK, D. WURSTER and K. BENIRSCHKE

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Improved methodology has enabled investigators in recent years to assess the chromosome number and structure of mammals more accurately than in the past. These techniques allow the examination of many animals previously unstudied and in many instances older findings, obtained by testicular squash methods or tissue sectioning, have to be corrected. More importantly perhaps, the recently developed possibility of immobilizing even valuable animals by Cap-chur gun and employing the safe and

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potent morphine-like compound M 99 (HARTHOORN and BLIGH, 1965) gives access to virtually any animal kept in the 200.

The findings of such studies open new fields of research and provide additional insight into possible reasons for hybrid sterility. They also add knowledge regarding taxonomic relationships between species and it is hoped that they will be employed in particular in those species which are threatened by extinction. They are easily undertaken when such animals are restrained for other reasons or when they die in zoological gardens.

The present report concerns principally the chromosome structure of the Musk ox (Ovibos moschatus moschatus [Zimmermann, 1780]) and Congo buffalo (Syncerus caffer nanus [Boddaert, 1785]). Two of these animals each had to be immobilized in order to move them into new quarters. Skin biopsies were taken at that time for tissue culture studies. Similarly, the Aoudad (Ammotragus lervia [Pallas, 1840]) and an Alpine Ibex (Capra ibex ibex Linné, 1758) were studied by this method. One male Gaur (Bos [Bibos] gaurus, H. Smith, 1827) was available from postmortem material.

# Immobilization

The necessity for handling animals arises quite frequently in any zoological garden. The use of tranquilizing and immobilizing drugs has made this task easier on the animals and the personnel involved. The Catskill Game Farm has used M-99 on a considerable number of hoofed animals. The M-99 is dissolved in acepromazine (10 mg/cc) in a concentration of one milligram of M-99 per cubic centimeter of acepromazine. Quite often more acepromazine is added to the required dose of M-99. An antidote to M-99 which has proven to be quite effective is Nalline. Most of the drugs are administered with the Cap-Chur Gun.

The following is a description of the transfer of a pair of musk oxen at the Catskill Game Farm. The female musk ox arrived from the Alberta Game Farm late in 1963, as a calf of the same year. The male arrived one year later, as a calf born 1964. As this pair matured, the quarters proved to be inadequate and the animals had to be moved in 1967 to new facilities erected especially for them. The now three year old bull was moved first. He was injected with 1.8 milligrams of M-99, plus 20 milligrams of acepromazine. A slight reaction was noticeable after  $3^{1/2}$  minutes and a good reaction after  $10^{3/4}$  minutes. A rope was put around his horns at  $14^{1/2}$  minutes. Two men in front and two men behind could lead this powerful bull about a quarter of a mile to the new stall, where he laid down. Twenty-eight minutes after the injection of M-99, he was given 30 milligrams of Nalline. At  $36^{1/4}$  minutes. For several hours after he was well-tranquilized.

The four year old cow was injected with 1.6 milligrams of M-99, plus 16 milligrams of acepromazine. There was a slight reaction after  $6^{1/2}$  minutes and very good reaction at 10 minutes and 10 seconds. Again, a rope was put around the horns after 10 minutes and she was led to the new quarters, where she laid down, like the bull.  $41^{1/2}$  minutes after the injection of M-99, she was given 30 milligrams of Nalline and was up after  $44^{1/2}$  minutes.

Full thickness skin biopsies of approximately 1 cm<sup>2</sup> were taken sterilely from the ear after shaving and alcohol washing. They were placed immediately into Eagle's medium to which 10% calf serum had been added and then shipped to the laboratory. Here, the skin was cut into small fragments, explanted into large Leighton tubes and cultured by a method similar to the procedur detailed by BASRUR et al. (1963). Following transfer to Carrell flasks and growth, these cultures were trypsinized after

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colchicine treatment and swelled with hypotonic Earle's solution. Then they were spread onto slides, stained with carbol fuchsin and the metaphases photographed. Certain cultures were treated with H<sub>3</sub>-thymidine to study the late replicatory behavior of the second X chromosome in females. These techniques have been adequately described elsewhere (SCHMID, 1963).

# Results

Musk ox: Both animals were found to have 48 chromosomes, which is in agreement with the recent findings by TIETZ and TEAL (1967). Six pairs of autosomes are submetacentric or metacentric in structure, the others being acrocentrics. The X chromosome is the largest acrocentric and similar in structure to the X of other members of this subfamily. The Y is the smallest element; it is metacentric and easily recognizable. In female cells one X is late replicating, as is true of most mammals; it is an "original"  $(5 \circ/6)$  type X (OHNO et al., 1964). The nombre fondamental (NF, MATTHEY, 1956) is 60 which is characteristic for all members of this group so far examined. Representative karyotypes are presented in Figures 1, 2.

*Ibex:* One female animal was available for study. Its karyotype is indistinguishable from that of the goat, reported by others (SOKOLOV, 1930; EVANS, 1965) and it is therefore not shown here. The diploid number is 2n=60; all chromosomes are acro-

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centric, except for the small Y.

Aoudad: One specimen of a male Barbary sheep was available. Its karyotype is shown in Figure 3. The chromosome number is 2n=58, the NF is again 60 with only one pair of metacentric autosomes present. It will be noted that this pair is relatively large compared with the metacentric elements of the other species here discussed. The X chromosome is an acrocentric element but it can not be precisely identified from the other acrocentric chromosomes. The Y is the smallest chromosome and usually has a submetacentric structure.

Congo buffalo: This species is included here because no previous cytogenetic obser-

Fig. 1 (above). Karyotype of male musk ox from skin biopsy. 2n=48. There are six pairs of autosomes with a submedian centromere, the remainder being acrocentrics. The sex chromosomes are last. The X chromosome is the largest acrocentric element, the Y is the smallest metacentric chromosome - Fig. 2 (middle). Karyotype of female musk ox from skin biopsy with corresponding autoradiographs shown below. One X chromosome is much more intensely labeled (shown last) which serves to identify the X of this species as the largest acrocentric - Fig. 3 (below). Karyotype of male aoudad. Only one pair of autosomes is metacentric, all others are acrocentric. The X is a larger acrocentric but not clearly identifiable, the Y is the smallest element with submetacentric or metacentric centromere. They are placed last.

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Fig. 4 (left). Karyotype of male Congo buffalo with three pairs of submetacentric or metacentric autosomes, the remainder is acrocentric. The X is an unusually large acrocentric, the Y a small submetacentric, shown last. — Fig. 5 (right). Karyotype of male gaur, possessing one pair of submetacentric autosomes (placed first), the remainder being acrocentriecs. The sex chromosomes (last) are similar to those of domestic cattle.

vations have been recorded on this animal and because its karyotype is useful in this context for the discussion of the Robertsonian mechanism of karyotype evolution. Both animals had similar karyotypes, a diploid value of 2n=54 with 3 pairs of metacentric autosomes, the remainder being acrocentrics. The X chromosome is a large acrocentric, in contrast to the metacentric of domestic cattle, and the Y chromosome is the smalllest element and probably submetacentric. The karyotype for the male is shown in Figure 4.

*Gaur:* This specimen showed 58 chromosomes and had a karyotype as shown in Figure 5. This differs from domestic cattle only by the presence of one submetacentric pair of autosomes, apparently a fusion product of two unequal-sized acrocentrics.

# Discussion

### Chromosome number

The chromosome complement of the musk ox has been ascertained recently by TIETZ and TEAL (1967). These investigators studied lymphocyte cultures of several musk oxen and show a metaphase plate which is in agreement with the findings described here. Although they examined several animals, they give no figures of how many were successfully studied. Nevertheless, the diploid value of 2n=48 can be considered accurate from these two studies.

The ibex with a complement 2n=60 is identical with the chromosome number of the goat which has been studied by many authors (SOKOLOV, 1930; GIMENEZ-MARTIN and LOPEZ-SAEZ, 1962; EVANS, 1965). The easy hybridization of ibex and goat (GRAY, 1954, 1966) and fertility of these hybrids testifies to the close relationship of these two species. Some consider the former among the wild ancestors of the domestic goat (HARRIS, 1962). The ibex has also been studied by HARD (1967), and his results are similar. Further, a recent report by HAUSCHLECK-JUNGEN and MEILI (1967) gives a quantitative comparison of ibex and goat chromosomes.

The Aoudad has a complement of 58 chromosomes and is between goat (2n=60) and sheep (2n=54 McFee et al., 1965; BUTTLE and HANCOCK, 1966). Its complement has not been published previously, but TAYLOR and HUNGERFORD (1967), who examined two specimens, finds a diploid value of 58 as well.

No previous reports are published on the cytology of the Congo buffalo. Its chromosome number of 54 differs considerably from that of domestic cattle with 2n = 60, as well as from other cattle studied by us, namely the banteng, and the yak (the gaur, however, was found to have 2n = 58 with two submetacentric autosomes) and by others (zebu: MAKINO, 1944; yak: ZUITIN, 1938, and the bison: MELANDER, 1959).

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# Hybridization

From various records summarized by GRAY (1954, 1966) and CHANG and HANCOCK (1967) the following hybrids of species with known chromosomes have been attempted in members of this group:

Ovibos moschatus x Bos taurus – alleged but unlikely Capra ibex x Capra hircus – both sexes fertile

x Hybrid Ammotragus lervia x Capra hircus – possible Ammotragus lervia x Capra hircus – recent success and fertility

x Ovis aries - apparently unsuccessful

Syncerus caffer x Bos taurus - no records of hybridization

Several attempts have been made to hybridize cattle x Indian water buffalo, none apparently with success. Only one older study indicates 2n=48 for the water buffalo (MAKINO, 1944) which should be repeated with modern techniques.

Numerous attempts of hybridization between goat and sheep have been undertaken. The subject has been discussed by CHANG and HANCOCK (1967), and GRAY (1966) gives many additional references. Undoubtedly some positive results have been achieved and, in early embryos of such crosses, the intermediate chromosome number of 57 has been verified. This methodology will play an important part in ascertaining the success of hybridization in adult animals of this cross, as well as of other hybrids, e. g. the mule (BENIRSCHKE et al., 1964). Moreover, from testicular structure and meiotic studies (BENIRSCHKE, 1967) an insight can be gained into the possibility of fertility by observing multivalent first meiotic stages once the chromosome structure of the parental species has been assessed accurately. The successful hybridization of goat and aoudad and the backcross of this hybrid to ibex have suggested to HALTENORTH (1961) that these species are more closely related than aoudad and sheep between which hybridization has failed. This has been disputed recently by SCHMITT (1963) on the basis of immunological studies of proteins. The present findings of 2n=58 for aoudad, however, tend to support the former author.

The nombre fondamental of all Bovidae reported here and most others studied in our laboratory is 60 and remarkably constant for this family. This is in striking contrast to its variability in Equidae (BENIRSCHKE, 1967) and suggests rearrangement of chromosome sets principally by Robertsonian mechanism, i. e. fusion of acrocentrics to produce metacentrics. Single steps in such a rearrangement might be *C. hircus* to *A. lervia*; further removed would be the sheep (2n=54), and musk ox with 2n=48, still further. These animals have similarly shaped X and Y chromosomes, but the X chromosome in particular, is different from that of *Bos* and *Syncerus*, suggesting a more distant relationship of these families. Similar differences were detected antigenically between proteins of *Bos* and *Ovibos* by MOODY (1958) and they were referred to by TIETZ and TEAL (1967). It is clear then that the "ox" of musk ox connotes no direct genetic relationship to Bovinae but that, at least Karyotypically, this species is more closely related to sheep, as has been suggested by many zoologists in the past.

Fusion of acrocentric elements is apparently a particularly common event in Bovidae. Not only does this process occur often in tissue culture but also, it is observed in lymphosarcoma (HARE et al., 1967) and spontaneously as a mutational event GUSTAVSSON, 1966; HERSCHLER and FECHHEIMER, 1966). The latter reports indicate considerable stability of such fusion products. Indeed, the progeny of some of the translocation carriers reported by GUSTAVSSON (1966) contains balanced translocation homozygotes with 58 chromosomes whose karyotype is indistinguishable from that of the wild gaur. Such translocations can occur between various elements of a more "primitive" (acrocentric-rich) karyotype and the fusion products would differ, consequently in size and structure. Fusion of two equal-sized elements would yield a Chromosome study of Caprinae and Bovinae

metacentric chromosome. that of unequal-sized elements results in a submetacentric new element. These may be large or and some such small translocational events have been well studied in human pedigrees. Barring additional rearrangements, such as pericentric inversions, it may be feasible, eventually, to trace evolutionary relationship in families such as reported here. Knowledge of such structural differences in closely related species will aid in explaining meiotic processes of hybrids, in particular hybrid sterility.

The possible evolutionary steps leading to the divergent species of Bovidae have been traced by PILGRIM (1947). He points out that many aspects are poorly understood and it is here, perhaps, where karyological findings may aid when all members of this large group have been studied and these findings can be fitted with the fossil record. A comparison of the findings here presented show that gaur and Congo buffalo are much more closely related to Bos than the other species described. The gaur has a karyotype most similar to cattle, having undergone one translocational step. Syncerus, on the other hand, seems further much removed with three submetacentric autosomal pairs, only that placed third in Figure 4 resembling the submeta-

Distribution of number of spreads examined with chromosome counts (Top axis) in the various animals. The modal number is the largest figure.

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centric of the gaur. Moreover, the similar sex chromosomes of cattle and gaur are much different from those of *Syncerus* which, in this respect, is much more like Caprini. This would correspond to the distant origin of these members as indicated by PILGRIM (1947).

The relationship of goat, Barbary sheep, sheep and musk ox is more complex when one compares their karvotypes. If one considers the acrocentric karvotype of goat and ibex (2n=60) as the more primitive, then by fusion of the two largest pairs the new metacentric autosome of the Barbary sheep (2n=58) could have arisen and their hybrid fertility attests to such a relatively simple rearrangement. In the sheep with 54 chromosomes, the largest metacentric may well correspond to that of Ammotragus, however, two additional pairs of central metacentric autosomes likely correspond to fusion products of the next largest acrocentric autosomes. None of these new metacentrics are found in the musk ox (2n=48). Here, the four largest autosomes are submetacentrics and can be construed to have arisen by fusion of much unequal acrocentrics, while pairs 5 and 6 (Fig. 1) may represent the metacentric fusion products of small acrocentrics. Clearly, this karvotype is very dissimilar from all other Boyidae so far considered. It will, therefore, be of great interest to ascertain whether the takin. presumably his closest relative, has a similar karvotype as well. Finally, it is pertinent to point to the karyotype of the Himalayan tahr (Hemitragus jemlahicus [H. Smith, 1826]) whose karvotype has been published by CHANDRA et al. (1967). This animal has also 48 chromosomes and, while autosomes 1-4 are structurally similar to those of Ovibos, the metacentric elements 5 and 6 are much larger and presumably of different origin. Such great divergence from the karyotype of the goat raises questions concerning their alleged closed relationship (PILGRIM, 1947; MORRIS, 1965). It is therefore hoped that a complete study of this family by a variety of techniques will shed additional light on the disputed taxonomic relationship of its members.

#### Addendum

Immediately after submitting this paper we received a reprint of the following publication: ULBRCH, F. & H. FISCHER. The Chromosomes of the Asiatic Buffalo (*Bubalis bubalis*) and the African Buffalo (*Syncerus caffer*). Zeitschr. Tierzüchtung u. Züchtungsbiol. 83/3: 219–223, 1967. These authors describe the chromosome complement of *Syncerus caffer* as 2n = 52 with 4 pairs of submetacentric and 21 pairs of acrocentric autosomes. Their karyotypes were obtained from blood samples taken from 3 animals of a population presumably in Kenya. The number of cells analyzed is not stated. The extra pair of submetacentrics may represent the spontaneous fusion of acrocentric elements commonly found in Bovidae as described above, and may be a stable feature in the population from which their specimens were obtained. Thus, this species may be either polymorphic or not a "good" species, and this can be clarified only through the study of more specimens.

#### Summary

Chromosome studies of five species of Bovidae are reported, the musk ox, ibex, aoudad, Congo buffalo and gaur. Their chromosome numbers are respectively 48, 60, 58, 54 and 58 and their karyotypes differ considerably. Simple Robertsonian mechanism of karyotype evolution may account for these changes since the nombre fondamental is 60 in all. These findings are discussed with respect to the possible interrelationship of these species.

#### Zusammenfassung

Der Bericht befaßt sich mit Chromosomen-Studien an Moschusochsen, Alpensteinbock, Mähnenschaf, Kongobüffel und Gaur. Die Anzahl der Chromosomen der genannten Arten ist in gleicher Reihenfolge 48, 60, 58, 54 und 58, wobei sich die Karyotypen beträchtlich unterscheiden. Einfacher Chromosomenumbau durch Fusionen vom Robertsonischen Typ kann für diese Unterschiede verantwortlich gemacht werden; denn die nombre fondamental ist bei allen 60. Weiterhin werden die Ergebnisse in Hinsicht auf die Verwandtschaft der untersuchten Arten diskutiert.

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