Centromeric Heterochromatin and G-Banding of the Red Brocket Deer, *Mazama americana temama* (Cervoidea, Artiodactyla) with a Probable Non-Robertsonian Translocation

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Few studies have been published of the chromosome structure of animals in the tribe Odocoileini (American deer) despite the great number of species in this group. Of at least 12 species (Morris 1965), only *Odocoileus hemionus*, *O. virginianus* (Wurster and Benirschke 1967) and *Pudu pudu* (Koulischer et al. 1972) have been analyzed in detail. Taylor et al. (1969) mention the finding of 2n=68 in a single specimen of *Mazama americana* obtained from the Philadelphia Zoo and kindly made this material available to us for inspection.

We have recently had the opportunity to study a family of *Mazama americana temama* with very different results of those described by Taylor et al. (1969). Moreover, the new banding techniques now available allowed the identification of an unusual nonreciprocal translocation in one member of this family. This communication describes the findings of these studies.

Materials and methods

The San Diego Zoo had a breeding pair of red brocket deer obtained from Tamaulipas, Mexico, whose subspecies identification was established as *Mazama americana temama* by Mr. Clyde Hill, Curator of Mammals. The female had a singleton male newborn who died and, a week later, the doe died as well from peritonitis. Skin biopsies of all three animals were cultured according to the technique of Basrur et al. (1963). Fibroblasts from all were preserved with glycerol in liquid nitrogen. Lymphocyte cultures were successful by the whole blood technique from the offspring but pokeweed mitogen was substituted for phytohemagglutinin. The results were identical to the skin cultures. Giemsa banding was accomplished by two techniques: a) airdried slides were kept for one week at 37°C prior to treatment with 0.025% trypsin solution (90 seconds) in a Coplin jar, followed by two washes in water; b) flame-dried slides (1–2 weeks old) were incubated in 2× SSC at 60°C followed by a 30 second exposure to 0.5% trypsin solution, 95% alcohol wash and staining in Giemsa solution (3 ml Giemsa, 70 ml buffer [1 tablet Gurr buffer/1 liter water, pH 6.8]).

C-bands were obtained by placing 0.2 N HCl onto flame-dried slides for 30 minutes, rinse with water, followed by alkaline SSC for 2 minutes (1 part 0.7 N
NaOH, 6 parts $2 \times SSC$), one rinse with $2 \times SSC$, three rinses with 70% alcohol, 95% alcohol, air drying. Next, the slides were placed on filter paper wetted with $2 \times SSC$ in a Petri dish for 6 hours at $60^\circ C$, cooled to room temperature, rinsed with 70%, then 95% alcohol and then air dried. They were then stained in 4% Giemsa solution made up in phosphate buffer for 7 to 10 minutes.

R-bands were prepared according to the method of Verma and Lubs (1975) on airdried slides. Instead of color photographs we used Kodak Pan X film.

Metaphase plates were abundant and were photographed in a Zeiss photomicroscope with phase optics at $400 \times$ initial magnification, to be enlarged subsequently in the enlarger. One ideal metaphase (Fig. 1) was enlarged to a final magnification

Fig. 1. Karyotype of male *Mazama americana temama* offspring. The second element 22 is covered by a crystal.
of $\times 6500$, the chromosomes were cut out and weighed in order to get an estimate of the percentage of X and Y. It was calculated as described by Galton et al. (1965):

$$\frac{\text{both X (or Y)}}{\text{all autosomes}} \times 100$$

Results

The chromosome number of father and son was $2n=50$, that of the doe was $2n=49$ in all metaphases examined. In the male, there are 20 metacentric or sub-metacentric autosomes, one readily identified submetacentric X, 28 acrocentric autosomes and a metacentric Y. Two pairs of autosomes are significantly smaller than the remainder and of similar size as the Y chromosome (Fig. 1). In the best preparations the Y chromosome appears as a minute metacentric, more often, it is so small as to have an acrocentric configuration. The karyotypes are arranged with biarmed elements first and in order of descending size, sex chromosomes last.

In the female there were 20 pairs of metacentric or submetacentric autosomes, a pair of X-chromosomes, and 27 acrocentric autosomes. One of the smallest

Fig. 2. Giemsa banding of female Mazama with presumed tandem translocation of portion of one chromosome 24 onto end of long arm of first No. 4 (T). All other elements match well. X chromosome last, with characteristic bands.
acrocentric elements was consistently missing and is apparently translocated to the end of the long arm of one chromosome No. 4 (Fig. 2). Satellites were not observed on any element.

The X-autosome ratio was 5.82%, the Y: autosome + X ratio was 0.621%. Thus, the X falls into the general range of the "original-type" (Ohno et al. 1964).

Fig. 3. C-banding of male Mazama. Autosomes No. 10, No. 24 and X have only a small amount of heterochromatin. Note interstitial C-bands in No. 1 and No. 3.

C-bands

The amount of heterochromatin as exposed by the C-banding technique varies considerably among these chromosomes (Fig. 3). Two paracentromeric blocks are present in 1-5 and 7-9. In No. 6 and particularly in No. 10 only a small centromeric C-band exists, and those of No. 2 and X are also smaller than the C-bands of the other biarmed elements. An interstitial C-band is found in the long arms of 1 and 3 but there is no band at the site of the putative fusion of 4 and 24 (Fig. 4). All acrocentric autosomes have a paracentromeric C-band which varies greatly in width as seen in Fig. 4. In particular, that of the smallest 4 elements is diminutive and none is apparent in the Y chromosome.
G-bands

The banding pattern of the metacentric and acrocentric elements are distinctive and allow ready identification of all pairs (Figs. 2, 5). The X is also clearly identifiable and has a characteristic banding pattern, also seen in metacentric X chromosomes of other mammals.

Fig. 4. C-banding of female Mazama with presumed translocation t(4,24) at T. Note interstitial C-bands in No. 1 and No. 3.

One of the No. 4 elements of the doe has a longer long arm and is the presumed recipient of a portion of the missing acrocentric 24. The translocation is presumed to be tandem and, because no C-band is present at this site, it is assumed that the centromere of 24 was lost in the process of translocation (Figs. 2, 4). In all metaphases with elongated chromosomes the length of the long arm of one No. 4 element was distinctly longer and representative pairs from six additional metaphases are shown in Fig. 6. While it is true that occasionally other pairs had different lengths (e.g. No. 7 in Fig. 1 and No. 1 in Fig. 4), these were never consistent and are attributed to different contraction rates because of their position in the metaphases.

R-bands of the doe are displayed in Fig. 7. These were prepared in the hope
that the relatively heavy fluorescence of No. 24 might be displayed in the translocation element. This was not the case.

Fig. 5. Giemsa banding of adult male *Mazama*. The banding pattern is distinctive and permits identification of all pairs.

Discussion

Our finding of 2n=50 for *Mazama americana* differs markedly from the 2n=68 reported by Taylor *et al.* (1969) in a single specimen from the Philadelphia Zoo. The precise origin of the animal in Philadelphia cannot be ascertained and no frozen stock of cells is available for banding studies. In communications with the authors and curators at the Philadelphia Zoo there appears to be no doubt that the animal was correctly identified. The most likely explanation for this discrepancy would be that extensive polymorphism exists in the numerous subspecies of *Mazama*. This point would be interesting to verify on specimens with precise origin known as it
would be unusual amongst Cervidae to have such extensive variation. The finding of two distinctly smaller pairs of autosomes, both in the San Diego specimens and in that from Philadelphia, seems to be unique to Mazama and favors the above interpretation.

The findings here reported suggest a translocation between one of the smallest acrocentrics and metacentric autosome No. 4. Various techniques employed fail to delineate clearly what portion of No. 4 is present in the compound element although they suggest it is only a small piece. It does not possess the heavily stained C- or R-band and a translocation is only inferred by the con-

Fig. 6. Giemsa and R-banding of pair No. 4 of the female. The first element is consistently longer and appears to have an additional terminal band on the long arms.

Fig. 7. R-banding of female Mazama. Notice the strong fluorescence of the last three autosomes and its absence in the presumed translocation element.
sistently different length of the No. 4 chromosomes in the doe, a tiny additional G-band and the unlikelihood of monosomy 24 in a normal, reproducing animal. This latter possibility, however, cannot be ruled out entirely.

The Philadelphia specimen, a female, had 6 medium-sized elements and 62 acrocentrics, four of which are much smaller. The sex chromosomes are unknown. The Nombre fondamental (NF) would thus be 74, that of the San Diego specimens is 72, and of other Odocoileinae it would be 70–74 (Table 1). In general, the relationship may be explained then by a system of Robertsonian fusion as in other Artiodactyla and the presence of duplex centromeric C-bands further supports this assumption. In mice, evolution associated with fusion produces biarmed chromosomes with a similar structure (Gropp and Winking 1972). On the other hand, the reduction of NF from 74 to 72 must be accomplished by other mechanisms, such as tandem fusion. The finding of a probable fusion heterozygote in the doe here described supports that such fusion indeed may occur spontaneously and also that it is compatible with fertility. Pericentric inversion is another mechanism that could produce such a reduction but no direct support is yet available for this possibility.

Translocation polymorphism of the Robertsonian type has been described in a number of Artiodactyla. It has been seen in cattle (Gustavsson 1971, Darré et al. 1972, Bruère and Chapman 1973, Harvey 1971), in the goat (Padhe et al. 1971), in sheep (Bruère and Mills 1971, Nadler et al. 1973) and in pigs (McFee et al. 1966). It has been suggested that it may be a more common phenomenon associated with evolution in this order than in others (Wurster and Benirschke 1968). In general, no infertility or increased aneuploid offspring have been observed with this event (Bruère 1974), although the same author (1969) described an infertile ram possessing an autosomal translocation.

The range of chromosome numbers amongst the members of the Cervidae is particularly great when compared with other groups of artiodactyla (Taylor et al. 1969). Table 1 summarizes the findings to date. It will be seen that the chromosome number of most species is between 66 and 70 with a nombre fondamental around 70–74. The variation is usually due to a Robertsonian mechanism, except for the X in which inversion must have led from the more common acrocentric to a frequently submetacentric chromosome of the “original-type”, i.e. approximately 5% of the haploid genome when it has been calculated. The Muntiacinae clearly fall out of this group, both with their diploid number and NF. It may be considered that they should be removed from the subfamily on this ground.

Two other members fall remarkably out of line with their chromosome number: The Barasingha deer (C. duvauceli) and the presently reported Mazama americana temama, even though the NF is consistent with the other members of the Cervidae, excepting the muntjacs. Considerable Robertsonian evolution seems to have taken place in the Asiatic Cervinae to reduce the number to the 2n=56 of C. duvauceli, a now very rare species. Some of the related species are known to hybridize successfully in zoos, e.g. Cervus eldi and C. unicolor (marianus). Unfortunately, the karyotype of the former is still unknown. With respect to the South American species, too little is yet known of their karyotypic relationships to make any significant deduc-
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A = acrocentric; S = submetacentric; M = metacentric; NF = nombre fondamental.
tions. The reduction to 50 chromosomes in our specimens of *Mazama* is remarkable, considering the generally conservative nature of cervid chromosome evolution. It appears to have coincided with the radiation to South America of these ruminants and is similar to the evolution of carnivores, particularly the bears (Wurster 1969). It will be of great interest to ascertain the chromosome number and structure of the numerous other South American Odocoileinae which, unfortunately, are only so rarely accessible for study in zoological gardens.

**Summary**

The karyotype of the red brocket deer, *Mazama americana* is described. Skin biopsies of a pair and their young male offspring were cultured for the study of centromeric heterochromatin and G-bands. The chromosome number of father and son was 2n=50; that of the doe was 2n=49 in all metaphases examined. In the doe, one of smallest acrocentric elements was missing and is apparently translocated to the end of the long arm of one submetacentric chromosome No. 4. This translocation of the non-Robertsonian type has not been described in Artiodactyla before, moreover, the chromosome number differs from that reported to be as 68 in one single specimen reported previously.

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**References**


