

Sex-chromosome Mosaicism in the Lemur-like Possum *Hemibelideus lemuroides* (Marsupialia: Petauridae)

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Abstract

A regular system of sex chromosome mosaicism in a somatic tissue is reported in *H. lemuroides*. Spermatogonial mitosis and cultured fibroblast cells are $2n = 20$, while most bone marrow cells from both males and females are $2n = 19$. In males the Y chromosome is lost and in females one of the X chromosomes.

Introduction

The regular elimination of a sex chromosome from somatic tissues is known only in a few mammalian species. In the eutherian species *Acomys selousi* (Matthey 1968) and *Choloepus hoffmani* (Corin-Frederic 1969) an X chromosome is eliminated from some somatic tissue in the female, while in *Microtus oregoni* an X chromosome is lost from male germ cells (Ohno *et al.* 1963). Polymorphism for X chromosome loss in bone marrow and spleen cells, with XX, Xx (x is a partially deleted X chromosome), Xx/XO mosaics and XO individuals, has been reported in the South American field mouse *Akodon azarae* (Bianchi and Contreras 1967). Y-chromosome elimination appears not to occur in male soma in these species.

The elimination of an X chromosome in females and the Y chromosome in males in the marsupial bandicoot genera *Perameles* and *Isoodon* has been the subject of several investigations (Hayman and Martin 1965, 1974; Walton 1971; Close 1979). The X or Y chromosome is lost from some or all cells of the bone marrow, liver, spleen, corneal and intestinal epithelium (*Isoodon* only), thymus, lung and kidney, while both sex chromosomes are retained in cultured skin fibroblasts, ovarian and testicular tissues. In *Isoodon* only, corneal and intestinal epithelia are mosaic for sex-chromosome loss (Close, unpublished data). The bandicoot genera *Echymipera* and *Peroryctes* also eliminate one sex chromosome from some somatic tissues, but they are not as well studied as *Perameles* and *Isoodon* (Hayman *et al.* 1969; Hayman and Martin 1974; Sharman 1974).

The marsupial greater glider (*Petauroides volans*) has a system of Y-chromosome elimination from all or some cells in the bone marrow, liver and spleen, but not cultured fibroblasts or testicular tissue in males (Murray *et al.* 1979; Murray and McKay 1979). An X chromosome is not lost from these tissues in females. We report here that the lemur-like possum, *Hemibelideus lemuroides*, a form closely related to *P. volans*, has a system of sex-chromosome elimination from some somatic tissues in both males and females.

Materials and Methods

Animals were collected at Mt Haig, Qld, 30 km south-west of Cairns. Material for cytological analysis was collected immediately after death from animals shot in the field. Accession numbers of voucher specimens of skins and skulls will be supplied on request to G. M. McKay.

Bone marrow was flushed from ends of long bones using Bacto haemo-agglutination buffer (Difco Laboratories) containing 2.0 µg colchicine per millilitre, the cell suspension being kept at body temperature for 1½ h to increase the number of metaphase stages of mitosis. After centrifugation at approximately 800 rev/min the supernatant was discarded and the pellet resuspended in 0.53% (w/v) KCl and kept at body temperature for 15 min. After further centrifugation the pellet was fixed in methanol-acetic acid (3:1 v/v).

Testis tubules were fixed using the technique of Murray *et al.* (1979).

Fibroblast cultures were initiated following the technique of Murtagh (1977).

Slides from fixed bone marrow and cultured fibroblasts were prepared by dropping a suspension in methanol-acetic acid (3:1 v/v) onto cold slides wet with 60% (v/v) acetic acid which were air-dried beneath a bench lamp. Testis tubules fixed in methanol-acetic acid (3:1 v/v) were centrifuged to pack about 0.15 ml of tubules into the bottom of a 10-ml centrifuge tube. 1.0–1.5 ml of 60% (v/v) acetic acid was added and the opaque suspension which appeared at the top of the acetic acid was drawn into a 2.5-ml disposable syringe fitted with a No. 20 or 21 gauge needle. The material was expressed onto a dry slide on a hot plate at 80°C to form a spreading drop which was immediately drawn back into the syringe so as to leave a film of spread cells on the slide. Slides were stained with 1% lacto-aceto-orcein stain.

Table 1. Chromosome counts for the *Hemibelideus lemuroides* examined cytologically

Animal No.	Sex ^A	Tissue ^B	No. of chromosomes	No. of cells scored
1	M	BM	19	9
		Testis	20	1
2	F	BM	19	6
3	F	BM	19	6
4	M	BM	19	18
5	F	BM	19	4
6	F, py	Liver	20	2
7	F	BM	19	18
			20	6
8	F	BM	19	21
9	M	BM	19	23
10	M	Testis	20	1
		BM	19	10
11	M	BM	19	8
12	M	F	20	15
13	F	BM	19	6

^A py, pouch young from animal No. 5.

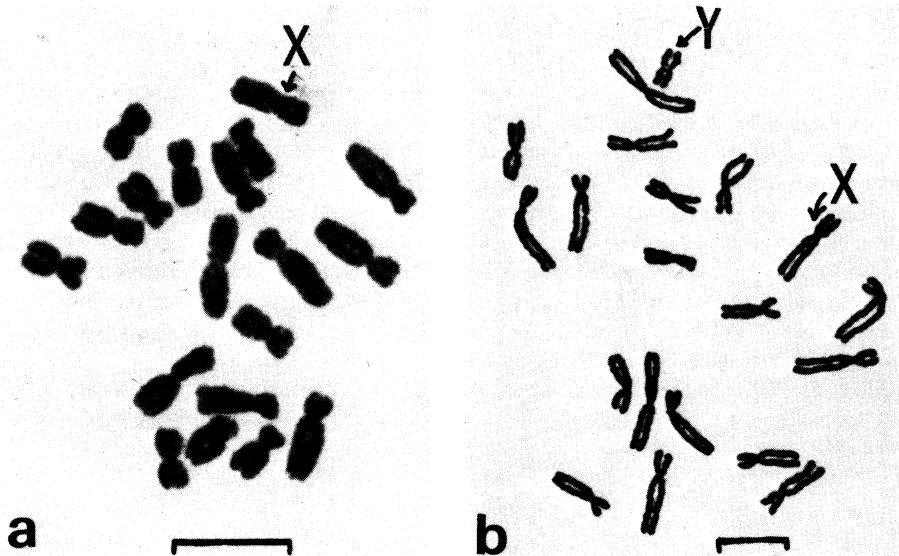
^B BM, Bone marrow; F, fibroblasts.

Results

The chromosome number was examined in 13 *H. lemuroides* (Table 1). Nineteen chromosomes were found in all bone marrow cells from 11 individuals (6 males, 5 females) while another animal (female) was mosaic, having 18 cells with a $2n = 19$ and a further six cells with $2n = 20$. Cultured fibroblast cells and two liver cells from a pouch young female were $2n = 20$. Spermatogonial mitoses and cultured fibroblasts in males were $2n = 20$. Representative $2n = 19$ and $2n = 20$ cells are presented in Fig. 1.

The morphology of the chromosomes in the $2n = 19$ cells were identical in both males and females (Fig. 1a). In spermatogonial cells and male fibroblasts ($2n = 20$) an additional, small metacentric chromosome was present (Fig. 1b), which was not observed in $2n = 20$ fibroblast culture cells from females. This male-specific chromosome is the Y chromosome. Two copies of a submetacentric chromosome were observed in $2n = 20$ female cells, while only one such chromosome was observed in $2n = 19$ cells from both males and females.

This is presumed to be the X chromosome. Thus females eliminate an X chromosome and males eliminate the Y chromosome from bone marrow cells. Karyotypes and data derived from selective chromosome staining techniques are presented by McQuade (1984).



Figs 1a and 1b. Bone marrow cell from female No. 13 illustrating $2n = 19$, XO condition (a) and a cultured fibroblast cell from male No. 12 illustrating the $2n = 20$, XY condition (b). Arrows indicate sex chromosomes. Bars equal $10 \mu\text{m}$.

Discussion

Data are presented which show there is a system for the selective elimination of one sex chromosome from bone marrow cells of both male and female *H. lemuroides*. The Y chromosome is lost from cells in the male while one of the two X chromosomes is eliminated from bone marrow cells in females. This system of sex-chromosome mosaicism appears similar to that found in the marsupial bandicoots (Hayman and Martin 1965, 1974). Hayman and Martin (1969) reported a $2n = 20$ from a female lemur-like possum, with the chromosome counts being obtained from corneal epithelium cells (D. L. Hayman, personal communication). In the bandicoot, *Perameles nasuta*, sex-chromosome elimination occurs in bone marrow but not in cells of the cornea, while in *Isoodon* a sex chromosome is eliminated from both bone marrow cells and corneal epithelium (Hayman and Martin 1969). The pattern of tissues in which elimination occurs in *H. lemuroides* appears close to that found in *P. nasuta*. However, considerably more study will be needed on the pattern of sex-chromosome loss from various tissues over a range of developmental times in *H. lemuroides*, particularly in view of the occurrence of 6 out of 24 $2n = 20$ cells in one adult, before a more careful comparison between the systems of sex-chromosome mosaicism in bandicoots and lemur-like possum can be made. Further studies on these phenomena in the marsupial bandicoots are included in Close (1984).

H. lemuroides and the closely related *P. volans* (the greater glider) both have systems of sex-chromosome mosaicism. In *P. volans*, the loss of a sex chromosome is restricted to the Y chromosome in males (Murray *et al.* 1979; Murray and McKay 1979), while one sex chromosome is lost from some somatic tissues in both sexes in the lemur-like possum.

The phenomenon of sex-chromosome loss in bandicoots (Hayman and Martin 1965, 1969, 1974; Walton 1971) and X-chromosome loss in eutherian mammals (Ohno *et al.* 1963; Bianchi and Contreras 1967; Corin-Frederic 1969) has been attributed to an extreme form, or end result of, X-chromosome inactivation in mammals. This explanation, although unproven, could also apply to the sex-chromosome mosaicism system reported here for *H. lemuroides*, but as we pointed out earlier (Murray *et al.* 1979; Murray and McKay 1979) this explanation does not necessarily fit the Y-chromosome elimination system observed in *P. volans*. However, the finding of X- and Y-chromosome loss from somatic tissues of *H. lemuroides* suggests an alternative explanation for the absence of X-chromosome elimination from somatic tissues of female *P. volans*.

As the occurrence of sex-chromosome elimination from somatic tissues is a rare phenomenon, it is reasonable to assume that the mechanism leading to elimination arose in the common ancestor to both the lemur-like possum and greater glider, rather than having arisen independently in these closely related species. During their continued evolution, after the lines leading to *H. lemuroides* and *P. volans* diverged from their common ancestor, chromosomal rearrangements became fixed, resulting in the present karyotypes of $2n = 20$ for *H. lemuroides* and $2n = 22$ for *P. volans* (Murray *et al.* 1979; McKay *et al.*, unpublished data).

If an autosomal segment was translocated to the X chromosome and became fixed in the line leading to *P. volans* there would have been selection pressure against the elimination of the rearranged X chromosome to prevent the loss of the autosomal material. There is no evidence at present to indicate that an X-autosome rearrangement has occurred, although a G-banding study of the chromosomes from both species could possibly resolve this question.

Alternative possibilities are (1) the X-chromosome region needed for elimination has been lost in *P. volans* through some rearrangement; (2) the common ancestor had only Y-chromosome elimination and a subsequent X-Y rearrangement in *H. lemuroides* has led to elimination of recognition sites on both the X and Y chromosome; or (3) the two systems are not related, having arisen independently. As pointed out above, we favour the hypothesis that the mechanism for sex-chromosome loss from somatic tissue arose in the common ancestor to *Hemibelideus* and *Petauroides*, with subsequent modification to produce the different systems observed in the two genera.

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References

- Bianchi, N. V., and Contreras, J. R. (1967). The chromosomes of the field mouse *Akodon azarae* (Cricetidae, Rodentia) with special reference to sex chromosome anomalies. *Cytogenetics* **6**, 306–13.
- Close, R. L. (1979). Sex chromosome mosaicism in liver, thymus, spleen and regenerating liver of *Perameles nasuta* and *Isoodon macrourus*. *Aust. J. Biol. Sci.* **32**, 615–24.
- Close, R. L. (1984). Rates of sex chromosome loss during development in different tissues of the bandicoots *Perameles nasuta* and *Isoodon macrourus* (Marsupialia: Peramelidae). *Aust. J. Biol. Sci.* **37**, 53–6.
- Corin-Frederic, J. (1969). Les formules gonosomiques dites aberrantes chez les Mammifères Eutherians. Exemple particulier du Paresseux *Choloepus hoffmani* Peters (Edente, Xenarthre, famille des Bradypodidae). *Chromosoma (Berl.)* **27**, 268–87.
- Hayman, D. L., and Martin, P. G. (1965). Sex chromosome mosaicism in the marsupial genera *Isoodon* and *Perameles*. *Genetics* **52**, 1201–6.

- Hayman, D. L., and Martin, P. G. (1969). Cytogenetics of marsupials. In 'Comparative Mammalian Cytogenetics'. (Ed. K. Benirschke.) pp. 121-217. (Springer-Verlag: New York.)
- Hayman, D. L., and Martin, P. G. (1974). Mammalia I: Monotremata and Marsupialia. In 'Animal Cytogenetics'. Vol. 4. Chordata. (Ed. B. John.) (Gebrüder Borntraeger: Stuttgart, Berlin.)
- Hayman, D. L., Martin, P. G., and Waller, P. F. (1969). Parallel mosaicism of supernumerary chromosomes and sex chromosomes in *Echymipera kalubu* (Marsupialia). *Chromosoma (Berl.)* **27**, 371-80.
- Matthey, R. (1968). Cytogenétique et taxonomie du genre *Acomys*, *A. percivali* Dollman et *A. wilsoni* Thomas, espèces d'Abyssinie. *Mammalia (Paris)* **32**, 621-7.
- McQuade, L. R. (1984). Taxonomic relationship of the greater glider, *Petauroides volans*, and the lemur-like possum, *Hemibelideus lemuroides*. In 'Possums and Gliders'. (Eds A. P. Smith and I. D. Hume.) (Aust. Mamm. Soc.: Sydney.) In press.
- Murray, J. D., and McKay, G. M. (1979). Y chromosome mosaicism in pouch young of the marsupial greater glider (Marsupialia: Petauridae). *Chromosoma (Berl.)* **72**, 329-37.
- Murray, J. D., McKay, G. M., and Sharman, G. B. (1979). Studies on metatherian sex chromosomes. IX. Sex chromosomes of the greater glider (Marsupialia: Petauridae). *Aust. J. Biol. Sci.* **32**, 375-86.
- Murtagh, C. E. (1977). A unique cytogenetic system in monotremes. *Chromosoma (Berl.)* **65**, 37-57.
- Ohno, S., Jainchill, J., and Stenins, C. (1963). The creeping vole (*Microtus oregoni*) as a gonosomic mosaic I. The OY/XY constitution of the male. *Cytogenetics* **2**, 232-9.
- Sharman, G. B. (1974). Marsupial taxonomy and phylogeny. *J. Aust. Mammal. Soc.* **1**, 137-54.
- Walton, S. M. (1971). Sex chromosome mosaicism in pouch young of marsupials *Perameles* and *Isodon*. *Cytogenetics* **10**, 115-20.

