

# SEX CHROMOSOME MOSAICISM IN THE MARSUPIAL GENERA ISOODON AND PERAMELES

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THE sex dependent variation in sex chromosomes of mammals with an XX/XY sex chromosome system requires a system of dosage compensation. Currently it is believed that such compensation is achieved by the random "inactivation" early in embryogenesis of all, or nearly all, of one of the two X chromosomes present in a normal female (LYON 1962; RUSSELL 1963). Associated with this "inactivation" of an X chromosome is a delay in its duplication of DNA (GRUMBACH, MORISHIMA, and TAYLOR 1962).

This paper presents evidence of the existence of a different cytological manifestation of sex chromosome dosage compensation in three species of marsupial bandicoots of the family Peramelidae.

## MATERIALS AND METHODS

Specimens of *Isoodon obesulus* (Shaw) were obtained from the vicinity of Perth, Western Australia; of *Isoodon macrourus* (Gould) from the vicinity of Townsville, Queensland, and of *Perameles nasuta* (Geoffrey) from the vicinity of Canberra, A.C.T. and Sydney, New South Wales. The leucocyte culture technique of MOORHEAD, NOWELL, MELLMAN and BATTIP (1960) was modified and applied to these species (HAYMAN and MARTIN 1965). Meiotic divisions were examined in the testes, and the somatic divisions in the liver and spleen were examined using the technique of FORD, HAMERTON and MOULE (1958).

## RESULTS

Table 1 records the chromosome numbers obtained from different tissues in each species. At least 20 divisions were counted from each of leucocyte, spleen and testicular tissue. Good quality divisions were harder to find in the liver and ovarian tissue and fewer cells were available for analysis in these tissues.

Characteristically, the germ line and somatic cells possessed different chromosomal complements. Somatic tissue always had 13 chromosomes and these are shown in Figure 1. Among all species there is great similarity, if not identity, between six pairs of chromosomes. There is always a single unpaired chromosome present, identical in appearance in both sexes of any species. This unpaired chromosome is similar in *Isoodon macrourus* and *Perameles nasuta* and different in *Isoodon obesulus*.

The meiotic stages in the testes always have  $2n = 14$  and a representative series of stages in meiosis from *Isoodon obesulus* is shown in Figures 2b, c and d. The sex

TABLE 1

*Chromosome numbers obtained from various tissues in the three species*

Species	Sex	Number examined	Germinal tissue	2n	Somatic tissue	2n
<i>Isoodon obesulus</i>	♂	3	testes	14	leucocytes	13
	♀	2	ovarian tissue	14	spleen	13
<i>Isoodon macrourus</i>	♂	3	testes	14	leucocytes	13
	♀	2			spleen	13
<i>Perameles nasuta</i>	♂	2	testes	14	leucocytes	13
	♀	2			liver	13
	♀	2	ovarian tissue	14	spleen	13
					leucocytes	13
					spleen	13

chromosomes can be recognized as being heteromorphic; one element is more metacentric and larger than the other. SHARMAN (1961) reports identical observations in the testes. The sex bivalent at metaphase I always shows reductional separation.

The ovarian tissue available for study was from adult or nearly adult individuals and the origin of the dividing cells is not known. In the two species examined (see Table 1) the chromosome number was  $2n = 14$  and it was possible to recognize the presence of two chromosomes similar to the single unpaired chromosome of the somatic complement. We assume that the same condition exists in the ovary of *Isoodon macrourus*.

These observations are interpreted as demonstrating that in the three species examined the female germ line is XX, the female soma X0, the male germ line XY, and the male soma X0.

#### DISCUSSION

There is complete chromosomal identity between the somatic tissue of both sexes, in each of the three species. We believe this to be an extreme manifestation of the dosage compensation mechanism generally found in mammals, elimination having replaced inactivation. In the species described in this paper the male embryo is presumably XY and during the development of the embryo the Y chromosome is lost from the somatic tissue; in the XX female embryo elimination of one of the two X chromosomes occurs from the soma. We are unable at present to demonstrate that there is random elimination of either the paternally derived X chromosome or the maternally derived X chromosome in the female soma. However, this is probably the case since the evolution of a system of elimination from one of inactivation can be envisaged. Such an evolutionary progression has been described in the coccids where heterochromatization of the paternal genome in the Lecanoid and Comstockiella systems is replaced by its elimination in the Diaspidid system (BROWN and MCKENZIE 1962).

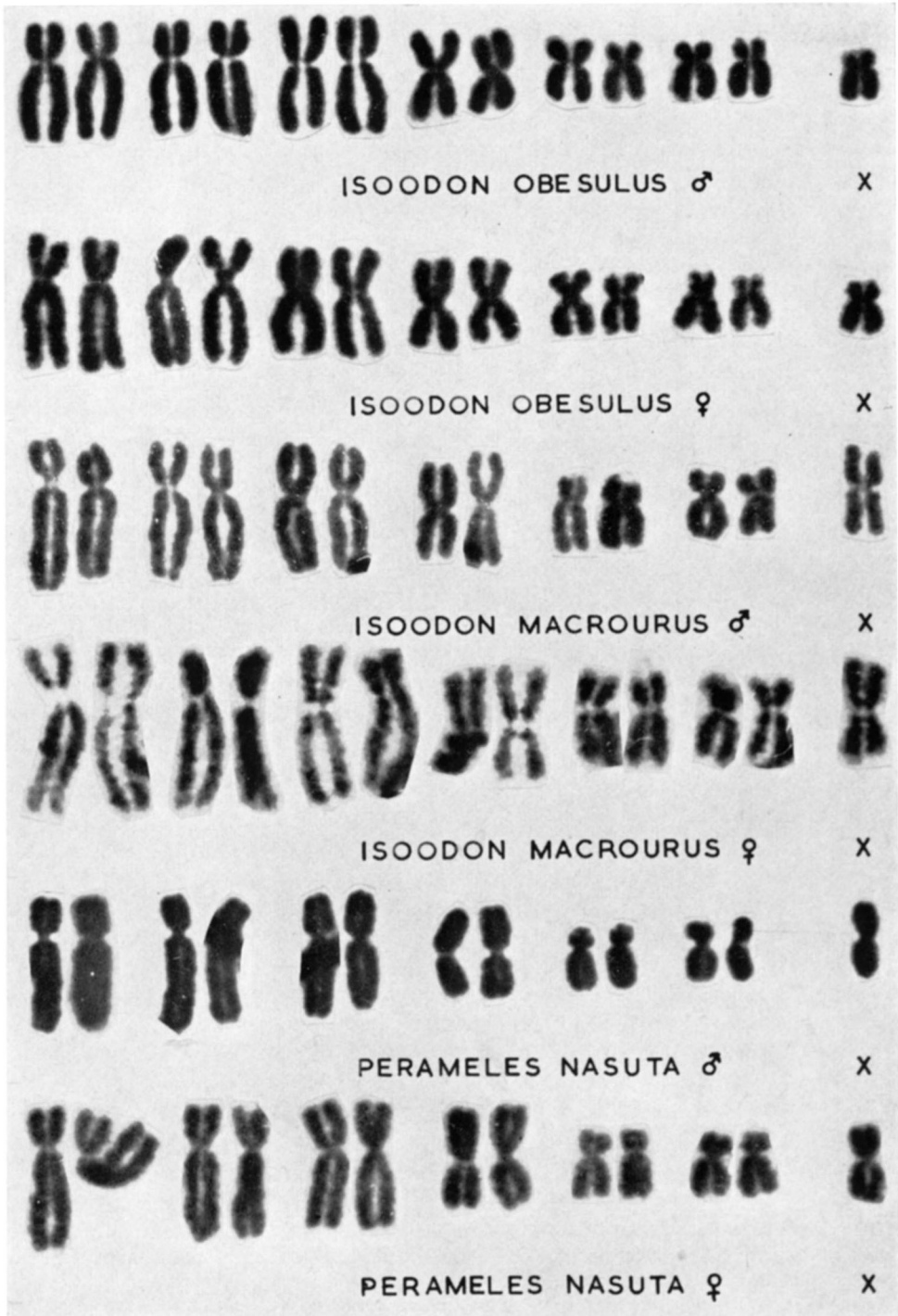


FIGURE 1.—Karyotype of the chromosomes from divisions in the leucocytes of *Isoodon obesulus*, *Isoodon macrourus*, and *Perameles nasuta*.

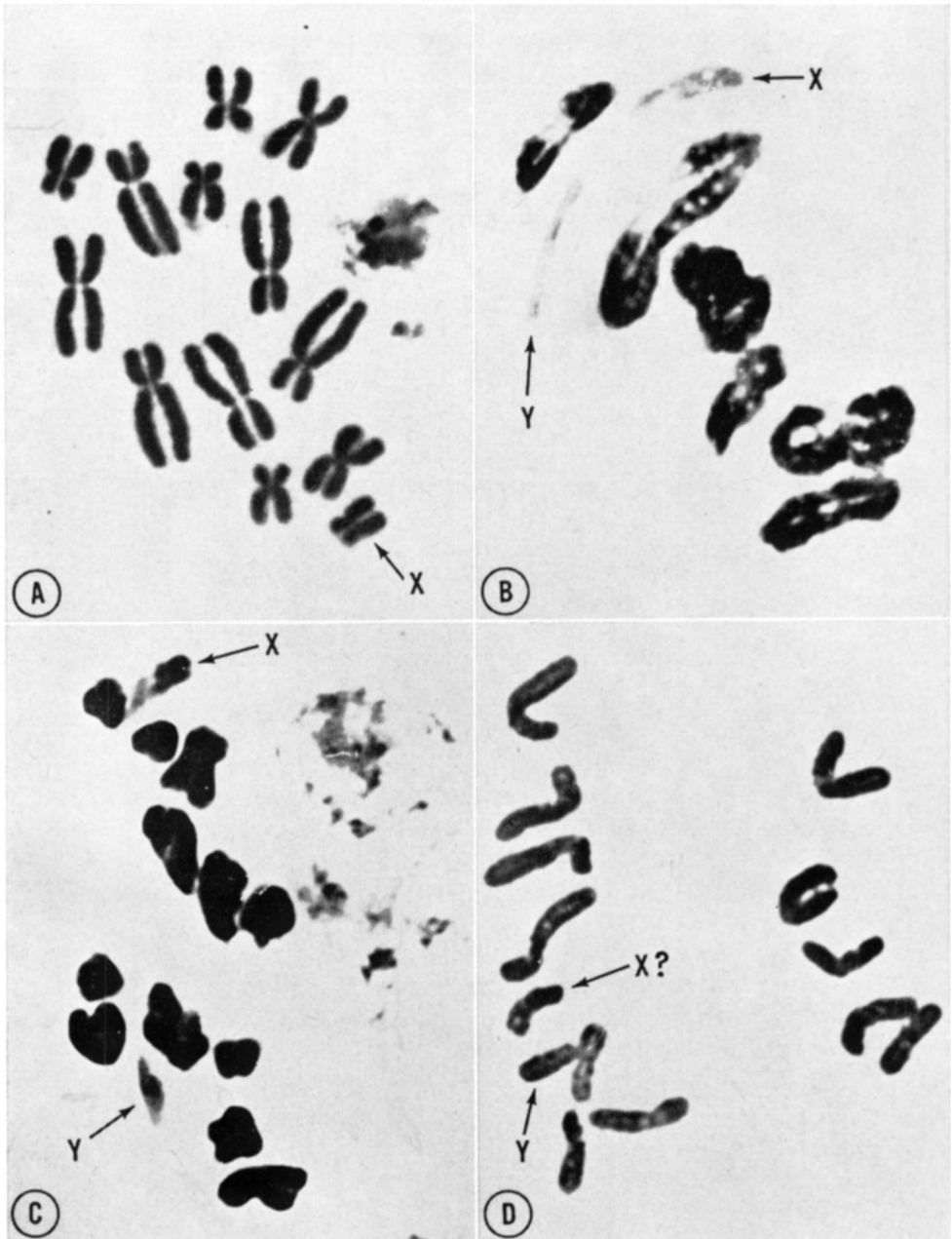


FIGURE 2.—A. Mitotic metaphase from dividing leucocyte of male *Isoodon obesulus* ( $\times 2300$ ). B. The chromosomes at metaphase I of meiosis in the male *Isoodon obesulus*. The faintly staining and heteromorphic XY bivalent is clearly shown ( $\times 2300$ ). C. Anaphase I of meiosis in the male *Isoodon obesulus*. The heteromorphic sex chromosomes are clearly shown and the larger element (the X chromosome) is faintly staining in only one arm (cf. metaphase I). Seven chromosomes are at each pole ( $\times 2700$ ). D. Spermatogonial division in the testes of *I. macrourus*  $2n = 14$ . The X chromosome + acrocentric Y element are indicated ( $\times 2700$ ).

The elimination of an X chromosome and the Y chromosome may have evolved from the delay in DNA synthesis which is present in these chromosomes in mammals. At the time in embryogenesis at which this difference between germ line and somatic cells occurs the delay may be exaggerated in the species of marsupials described in this paper so that the X and Y chromosomes may not complete their replication in time to take part in the normal cycle of cell division.

The delay in DNA synthesis of an X chromosome in somatic cells of the normal mammalian female is accounted for in terms of the "inactive X-hypothesis." However, considerable evidence exists that the Y chromosome is also late replicating in the somatic cells of man (SCHMID 1963), the Chinese hamster (TAYLOR 1960), the Syrian hamster (GALTON and HOLT 1964), cattle (GARTLER and BURT 1964), *Microtus oregonii* (HSU, SCHMID and STUBBLEFIELD 1964), the mouse (GALTON and HOLT 1965), the domestic cat (HSU and REARDEN 1965), the chinchilla (GALTON, BENIRSCHKE and OHNO 1965), and the marsupials *Potorous tridactylus* (WALEN personal communication) and *Macropus rufus* (HAYMAN and MARTIN unpublished observations). Indeed, in terms of the cycle of DNA replication, those species for which data are available show a striking parallel to the marsupial bandicoots if elimination is the equivalent of late replication. Concurrently with the random inactivation in the soma of one of the two X chromosomes present in the normal mammalian female may be the inactivation of all, or a major portion, of the Y chromosome in the soma of the normal mammalian male.

Not all tissues of the bandicoots have been examined, so it is an assumption to suppose that the soma is exclusively X0 in composition. This assumption implies that sex determination in both sexes must be initiated early in embryogenesis.

The mechanism described in these species affects X chromosomes which, while of presumably common evolutionary origin, are morphologically different and indicate the capacity of this mechanism to persist while evolutionary modification of chromosome morphology occurs. The only other species of the Peramelidae available to us was *Thylacomys lagotis* (Reid) in which we found  $2n = 18$  (XX) in the female soma and  $2n = 19$  ( $XY_1Y_2$ ) in the male soma (unpublished data).

The pattern of chromosome distribution between germ line and soma reported in *Perameles* and *Isodon* is similar to, but not identical with, the situation reported in *Microtus oregonii* (OHNO, JAINCHILL and STENIUS 1963). In this species the male germ line is 0Y, the male soma XY, the female soma X0, and the eggs are presumed to always have one X chromosome. While the number of X chromosomes present in the soma of both sexes is identical, the evolution of this system of dosage compensation has resulted in a situation different to that operating in *Perameles* or *Isodon*.

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ADDENDUM: Since this paper was accepted for publication we have been informed that Drs. L. JACKSON and K. ELLEM of the Jefferson Medical College, Philadelphia (personal communication) have examined the chromosomes of *Perameles nasuta* in cells derived from skin tissue culture. In both sexes they find 14 chromosomes including both sex chromosomes. Thus unless the species is polymorphic, it appears probable that the mosaicism is not confined to the germ line and soma, but is of a more complex nature.

## SUMMARY

The somatic chromosomes of males and females of *Perameles nasuta*, *Isodon obesulus* and *Isodon macrourus* have been counted in liver and spleen cells and in leucocytes. The chromosome number is  $2n = 13$  and the sex chromosome constitution X0. The chromosome number in the male germ line is  $2n = 14$  (XY). The chromosome number in ovarian tissue of females of the first two species (and, by extrapolation, of the third species) is  $2n = 14$  (XX).

Such mosaicism in X-chromosome constitution between the germ line and the soma of the females is explicable in terms of a mechanism for dosage compensation. A parallel exists between elimination of an X and a Y chromosome from the soma in these species and their delayed duplication of DNA during mitosis in other mammals. It is suggested that an "inactive" Y chromosome in the male soma may occur as commonly in mammals as the "inactive" X in the female soma.

## LITERATURE CITED

- BROWN, S. W., and H. L. MCKENZIE, 1962 Evolutionary patterns in the armoured scale insects and their allies. *Hilgardia* **33**: 141-171.
- FORD, C. E., J. L. HAMERTON, and R. H. MOULE, 1958 Chromosome changes in primary and transplanted reticular neoplasms of the mouse. *J. Cell. Comp. Physiol.* **52** (suppl. 1.): 235-270.
- GALTON, M., and S. F. HOLT, 1964 DNA replication patterns of the sex chromosomes in somatic cells of the Syrian hamster. *Cytogenetics* **3**: 97-111. — 1965 Asynchronous replication of the mouse sex chromosomes. *Exptl. Cell Res.* **37**: 111-116.
- GALTON, M., K. BENIRSCHKE, and S. OHNO, 1965 Sex chromosomes of the chinchilla. Allocyclic and duplication sequence in the somatic cells and behaviour in meiosis. *Chromosoma* **16**: 668-680.
- GARTLER, S. M., and B. BURT, 1964 Replication patterns of bovine sex chromosomes in cell culture. *Cytogenetics* **3**: 135-142.
- GRUMBACH, M., S. MORISHIMA, and J. H. TAYLOR, 1962 Human sex chromosome abnormalities in relation to DNA synthesis and heterochromatinisation. *Proc. Natl. Acad. Sci. U.S.A.* **49**: 581-589.
- HAYMAN, D. L., and P. G. MARTIN, 1965 An autoradiographic study of DNA synthesis in the sex chromosomes of two marsupials with an XX/XY<sub>1</sub>Y<sub>2</sub> sex chromosome mechanism. *Cytogenetics* (In press).
- HSU, T. C., W. SCHMID, and E. STUBBLEFIELD, 1964 DNA replication sequences in higher animals. *Symp. Soc. Study Develop. Growth* **23**: 83-112.
- HSU, T. C., and H. H. REARDEN, 1965 Further karyological studies on Felidae. *Chromosoma* **16**: 365-371.
- LYON, M. E., 1962 Sex chromatin and gene action in mammalian X-chromosome. *Am. J. Human Genet.* **14**: 135-148.
- MOORHEAD, P. S., P. C. NOWELL, W. J. MELLMAN, D. M. BATTIP, and D. A. HUNGERFORD, 1960 Chromosome preparations in leucocytes cultured from human peripheral blood. *Exptl. Cell Res.* **20**: 613-616.
- OHNO, S., J. JAINCHILL, and C. STENIUS, 1963 The creeping vole (*Microtus oregonii*) as a gonosomal mosaic. 1. The OY/XY constitution of the male. *Cytogenetics* **2**: 232-239.
- RUSSELL, L. B., 1963 Mammalian X-chromosome action: Inactivation limited in spread and in region of origin. *Science* **140**: 976-978.
- SCHMID, W., 1963 DNA replication patterns of human chromosomes. *Cytogenetics* **2**: 175-193.
- SHARMAN, G. B., 1961 The mitotic chromosomes of marsupials and their bearing on taxonomy and phylogeny. *Australian J. Zool.* **9**: 38-60.
- TAYLOR, J. H., 1960 Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. Biophys. Biochem. Cytol.* **7**: 455-464.