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Parallel Mosaicism of Supernumerary Chromosomes and Sex Chromosomes in *Echymipera kalabu* (*Marsupialia*)

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Abstract. *Echymipera kalabu* (*Peramelidae*: *Marsupialia*) does not have the full chromosome complement in all its adult somatic tissues. The chromosomes missing are the Y-chromosome in the male and an X-chromosome in the female. The full complement is present in the corneal epithelium and the reproductive tissue. A parallel mosaicism to this exists with respect to small supernumerary chromosomes which are found in certain animals of this species. These supernumeraries must be subject to the same control system as that which is responsible for the elimination of the sex chromosomes.

Introduction

We have described in previous papers an unusual and apparently regular form of sex chromosome mosaicism in two genera of the marsupial family *Peramelidae* (Hayman and Martin, 1965a; Hayman and Martin, 1969). The species in these genera do not have the complete diploid complement of chromosomes in all somatic tissues. Table 1 gives the present information about this situation and includes data on *Perory*ctes *longicauda*, a species whose chromosomes have not been studied previously. The data are accounted for by supposing that there is elimination of an X-chromosome from certain somatic tissue in the female and of the Y-chromosome from the same somatic tissue in the male.

This paper describes an extension of these observations to another species of the family *Peramelidae*, *Echymipera kalabu*, in which there is a similar elimination from certain somatic tissue of both the appropriate sex chromosome and also of supernumerary chromosomes which apparently occur frequently in this species. While certain aspects of this situation require additional study it is not likely that further collections of this species will become available in the foreseeable future. However, the importance of the observations which have been made would seem to justify publication at the present stage.

Table 1. *The chromosome numbers and sex chromosome constitutions in different tissues of adults of Isoodon spp., Perameles spp. and Peroryctes longicauda*

Species	Sex	Tissue	2n	Sex chromosome
Isoodon macrourus	♂ and ♀	thymus	13[a]	XO
		bone marrow	13	XO
		leucocytes	13	XO
		spleen	13	XO
		corneal epithelium	13	XO
Isoodon obesulus		intestinal epithelium	13[a]	XO
	♂	testes	14	XY
	♀	ovarian tissue	14	XX
Perameles nasuta	♂ and ♀	tissue culture of skin	14[c]	
		corneal epithelium	14	XX and XY
		intestinal epithelium	14[b]	XX and XY
		bone marrow	13	XO
		leucocytes	13	XO
Perameles gunni		spleen	13	XO
	♂	testes	14	XY
	♀	ovarian tissue	14	XX
Peroryctes longicauda	♀	corneal epithelium	14	XX
		spleen	13	XO

[a] Only *I. obesulus* examined.
[b] Only *P. nasuta* examined.
[c] Jackson and Ellem, 1968 (pouch young of *P. nasuta*).

Materials and Methods

Specimens of *Echymipera kalabu* were trapped in the Eastern Highlands and the Morabi district of New Guinea. The animals were injected intraperitoneally with 0.02% colchicine and killed after four hours. Slides showing chromosomes from the corneal epithelium (Fredga, 1964) and from the bone marrow and spleen were made in the field and subsequently sent to Adelaide for examination. The quality of the preparations varied, due to the difficulties under which they were made, but sufficient information has been obtained to offer an interpretation of the situation in this species.

Testicular material from one male was fixed in acetic-alcohol for one day, transferred to 70% alcohol and became available in the laboratory several weeks later.

Results

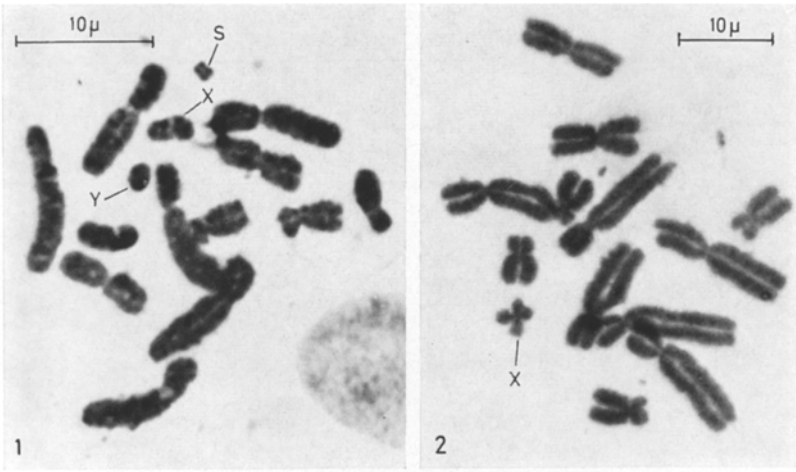
Table 2 lists the chromosome numbers found in tissues from a total of eight individuals (3 ♂ 5 ♀). The chromosome counts from the bone marrow and spleen, irrespective of sex, are always $2n = 13$ and the chromosome complement is always the same involving 6 pairs of homologues and an unpaired small metacentric chromosome (Fig. 2). There

Table 2. *The chromosome numbers and sex chromosome constitution of the specimens of Echymipera kalabu examined*

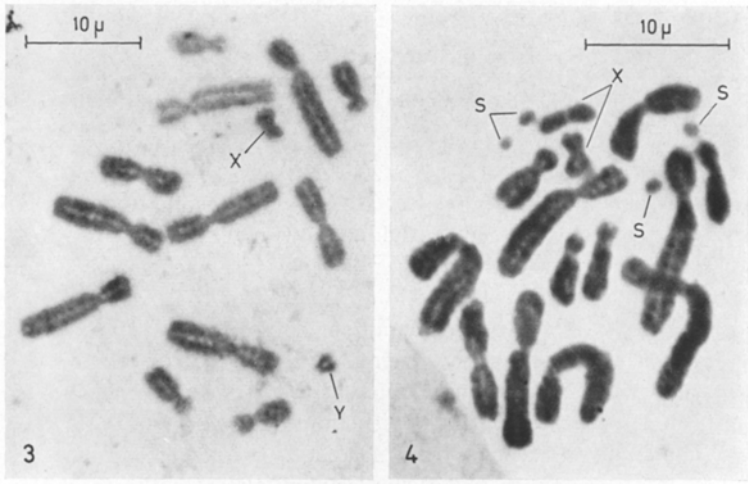
Individual	Sex	Tissue	2 [a]	Sex chromosome complement
A	♂	corneal epithelium	14 (+1)	XY
		marrow	13	XO
		testis	14 (+0, +1, +2)	XY
B	♀	corneal epithelium	14 (+2)	XX
		marrow	13	XO
C	♂	corneal epithelium	14	XY
		marrow	13	XO
D	♀	corneal epithelium	14 (+4)	XX
		marrow	13	XO
		spleen	13	XO
E	♀	corneal epithelium	14 (+3)	XX
		marrow	13	XO
F	♀	corneal epithelium	14 (+5)	XX
G	♂	corneal epithelium	14	XY
H	♀	corneal epithelium	14 (+2)	XX

[a] Numbers in brackets refer to the number of supernumerary chromosomes present (see text).

is no such constancy with respect to the chromosomal counts in the corneal epithelium between individuals, where counts from $2n = 14$ to $2n = 19$ have been obtained. Fig. 3 shows the chromosome complement from divisions in the corneal epithelium of the male G in which $2n = 14$. The autosomes of this complement are essentially the same as those found in the species of the genera *Isoodon*, *Perameles* and *Peroryctes*. The acrocentric element is interpreted as the Y-chromosome since it has never been found in the corneal epithelium of females, and occurs in all males. The small metacentric chromosome is the X-chromosome. These sex chromosomes appear to be smaller than those in the three genera named above. The additional chromosomes above $2n = 14$ found in divisions from the corneal epithelium of other individuals are always very small and metacentric (Figs. 1 and 4). The variation in the number of these chromosomes accounts for the variation in the total chromosome number. These small chromosomes have never been seen in marrow cells or spleen cells. These observations are based on a total of more than two hundred and fifty cells examined, and there is no evidence of within-tissue mosaicism.



Figs. 1 and 2. Chromosomes of individual A. Fig. 1. Corneal epithelium; one supernumerary chromosome is present. Fig. 2. Bone marrow; no supernumeraries present and the Y chromosome is missing



Figs. 3 and 4. Chromosomes in the corneal epithelium. Fig. 3. Individual G; no supernumeraries are present. Fig. 4. Individual D; four supernumerary chromosomes are present

We interpret these observations as follows. The basic chromosome number of *E. kalabu* is $2n = 14$ (XX/XY) and the chromosomes are very similar to those of species of *Isoodon* and *Perameles* we have studied. This chromosome complement is always present in the corneal epithelium. In the spleen and marrow only $2n = 13$ (XO) is found. Thus *E. kalabu*

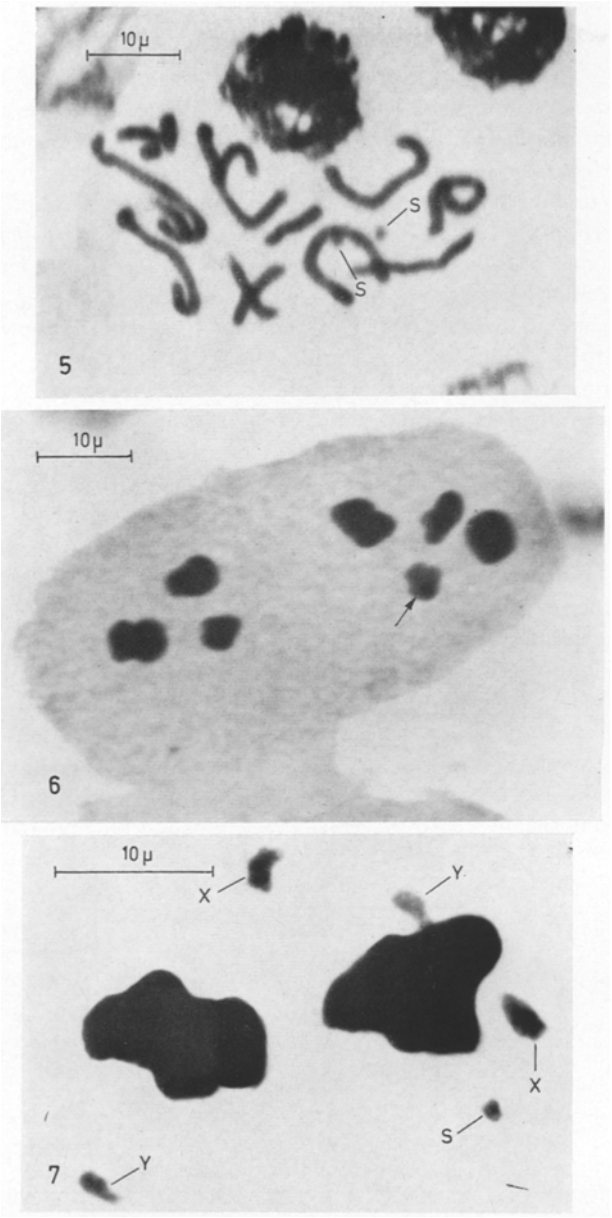


Fig. 5. Mitotic division in the testis of individual A; 2n = 14 and two supernumeraries are visible, one partly obscured by an autosome

Fig. 6. Diakinesis from meiotic divisions in individual A; no supernumeraries present. The sex bivalent is indicated

Fig. 7. Two separate cells at the "late" metaphase stage after the sex chromosomes have moved to the poles; in one of them a supernumerary is visible, in the other no supernumerary is visible

resembles *Perameles spp.* and *Peroryctes longicauda* (Table 1) in those tissues about which we have the necessary information. In addition to the basic complement of chromosomes some individuals of *E. kalabu* contain representatives of a class of chromosomes which are highly variable in number between individuals and which are not related to sex determination. These would fit the definition of supernumerary chromosomes or accessory chromosomes (Müntzing, 1958). The chromosomes of this class are confirmed to certain tissues and so far as our observations go are identical to the complete sex chromosome complement in their cycle of presence and absence.

The only testis material available was from individual A which had one supernumerary chromosome present in the corneal epithelium. Having been fixed under sub-optimal conditions, only a limited amount of analysis could be attempted. Cells were observed at spermatogonial mitosis, pachytene, diakinesis and metaphase I: some of these cells appeared to contain two supernumeraries, some one and some none (Figs. 5 —7). Where two supernumeraries were seen each was the same size as single supernumeraries and so were not the products of precocious divisions. Indubitably in some of these cells the small supernumerary chromosomes were hidden by autosomes. The question of how much of the observed variation was real and how much could be accounted for by chance invisibility will be discussed later.

At metaphase I in the material available the autosomal bivalents were clumped at the equator. The sex chromosomes, which are negatively heteropycnotic, move to the poles precociously and this separation was

Table 3. *Cells at metaphase I*

Number of supernumeraries visible	Early metaphase			Late metaphase		
	observed	expected[a]		observed	expected[a]	
		hypothesis A[b]	hypothesis B[b]		hypothesis A[b]	hypothesis B[c]
0	168	168.5	167.3	86	90.8	82.9
1	50	48.9	51.1	75	65.4	75.2
2	3	3.6	2.7	7	11.8	9.9

[a] The nature of hypotheses A and B is explained in the text.

[b] For hypothesis A the probability of observing a supernumerary chromosome at early metaphase is 0.127 and at late metaphase is 0.265. χ_2^2 for agreement (summed for early and late metaphase) is 3.71, $0.20 > P > 0.1$.

[c] For hypothesis B the frequencies of sperm mother cells with 0 and 2 supernumeraries are equal and estimated to be 0.185 each. The probability of observing a supernumerary at early metaphase is 0.255 and at late metaphase is 0.566. χ_1^2 for agreement is 0.986, $0.50 > P > 0.30$.

used to distinguish early from late metaphase. The supernumeraries which are also negatively heteropycnotic move to the poles at about the same time since their observable frequency at late metaphase is greater than that at early metaphase. The numbers of supernumeraries in early and late metaphase cells are shown in Table 3.

In 52 of the late metaphase cells with one supernumerary chromosome, the sex chromosomes could be distinguished by their size. The single supernumerary was with the X-chromosome in 30 cells and with the Y-chromosome in 22 cells. This does not differ significantly from equality, and suggests that sex and supernumerary chromosomes are inherited independently.

No pattern of association between the supernumerary chromosome and the sex vesicle was seen at pachytene.

Discussion

Variation in the Number of Supernumerary Chromosomes

Variation in the number of supernumerary chromosomes seen at metaphase I in individual A could result from two different situations. Firstly, if there were always two supernumeraries present in the germ line, and the variation in the number seen was due to chance hiding of one or both by other chromosomes, then the distribution of cells with 0, 1 and 2 supernumeraries should follow a binomial distribution. The expected numbers for the two classes of metaphase data are shown in Table 3 (hypothesis A). The differences between the expected and observed numbers are not significant. Alternatively, if the animal initially had one supernumerary chromosome, and during the divisions in the germ line preceding meiosis somatic non-disjunction occurred in some cells, then in addition to those with one, there would be an equal proportion of meiotic cells formed with no and two supernumerary chromosomes. A proportion of chance invisibility would be superimposed on this real variability. The expected frequencies have been calculated using the maximum likelihood method of Mather (1951) and are shown in Table 3 (hypothesis B). Again the differences between the expected and observed results are not significant. Consequently the variation can be accounted for by either situation and the matter cannot be resolved. However, both hypotheses suppose that supernumerary chromosomes in *E. kalabu* exhibit mitotic instability — as do supernumerary chromosomes in other species (Müntzing, 1958). In hypothesis A instability is postulated to occur in the corneal cell line of males in which supernumeraries are lost; in hypothesis B instability is postulated in the mitotic divisions during the formation of sperm mother cells.

As is shown in Table 2, sixteen supernumeraries were found in the corneal epithelial cells from five females while only one supernumerary was found in the same tissue from three males. This difference is significant ($P > 0.01$) i.e. there are fewer supernumerary chromosomes in the corneal cells of males than in females. If this difference represents the situation in the whole animal, then it cannot be explained by the preferential segregation of the supernumerary with the X-chromosome for which we found no evidence. If the difference is limited to certain tissues then there may be a different pattern between the sexes of the non-disjunction which is a necessary consequence of hypothesis A.

Until a considerable amount of additional information becomes available we can offer no further evidence for the basis of these variations in the number of supernumerary chromosomes.

The Elimination of Sex and Supernumerary Chromosomes

We have argued that the elimination of an X-chromosome from certain somatic tissues of these peramelids is an extreme form of the „inactive-X" hypothesis, dosage regulation being achieved by elimination rather than inactivation (Hayman and Martin, 1965a, 1969). We suggest that there is a characteristic feature of the inactive X-chromosome which is recognised by certain cell lines presumably early in development and this recognition results in the elimination of the X-chromosome. We have suggested that the Y-chromosome is also similarly characterised and it therefore is subject to the same control and suffers the same fate.

This same argument can be extended to the supernumerary chromosomes by supposing that they too share this common characteristic and are subject to the same control as are the sex chromosomes. While other models are possible we favour the view that the recognition and elimination system evolved initially as an extension of the dosage regulation system. On this view the supernumerary chromosomes are victims of a pre-adapted system which restricts them to certain tissues.

The observation that during stages of meiosis both the supernumeraries and the sex chromosomes show negative heteropycnosis suggests that their cycle of compaction may be associated with the recognition and elimination system. Where chromosome elimination has been studied in other organisms e.g. coccids, *Sciara* (Brown, 1966), it has been associated with the compaction of the eliminated elements. However, it is the condition of the eliminated chromosomes at the time of elimination that would be a critical test of this argument. It is possible that the supernumerary chromosomes are derived from sex chromosomal material. While such an origin would explain certain of their properties, there is no evidence for it.

Sex chromosome elimination has been reported in the mammalian species, *Microtus oregoni* (Ohno *et al.*, 1963, 1966) and inferred in *Akadon azarae* (Bianchi and Contreras, 1967) and *Choloepus hoffmanni* (Corin-Frederic, 1968). In each of these only the X-chromosome is eliminated. Consequently in these species the particular characterisation of the X-chromosome is not shared by the Y or the control system is limited to acting in the one sex.

Supernumerary chromosomes have been described in the marsupial *Schoinobates volans* (Hayman and Martin, 1965b) and there are probably two other occurrences. Gustavsson and Sundt (1965) report a variation between individuals in the chromosome number of the canine *Vulpes fulves* which can be interpreted as being due to the presence of supernumerary chromosomes and Shellhammer (personal communication) attributes a similar variation in *Reithrodontomys megalotis* (Muridae) to supernumerary chromosomes. The apparently infrequent occurrence of supernumerary chromosomes in mammals, compared to plants, may be a reflection of the degree of cytological knowledge in the two groups, particularly in the number of individuals of any one species studied. Certainly there are two features in which mammalian supernumeraries differ from plant supernumeraries. Firstly, they do not vary greatly in morphology — all reports describe them as small metacentric elements; secondly, they occur in greater frequency — individuals with 4 or 5 supernumeraries are common.

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