The Chromosomes of Four Species of Marsupials.

By

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With Plates 37 and 38.

THIS is a joint paper only in so far as the results of the two authors are combined. The first author is solely responsible for the first three species dealt with, and the second author for Potorous tridactylus. This work was carried out in the Zoological Laboratory of the University of Melbourne, the former author working under the terms of the Fred Knight Research Scholarship, and the latter under the Howitt Scholarship.

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Methods.—The material was fixed in either (1) Allen's modification of Bouin (in most cases omitting the urea), followed by aniline and bergamot oils; or (2) Flemming's fluid, followed by alcohol and xylol. Iron haematoxylin proved the most suitable stain. Fairly thick sections, about 15μ, were mostly used, mounted between two cover-slips, so as to allow a nucleus to be examined and drawn from both sides. This greatly facilitates the counting of the chromosomes.

1. TRICHOSURUS VULPECULA (figs. 1–9, Pl. 37; fig. 22, Pl. 38).

Material.—The material, which consisted of the testes from seven males and the ovary from one female, was obtained partly from specimens sent from Queensland by Dr. T. L. Bancroft, and partly from specimens caught in Melbourne.

Number of Chromosomes.—The diploid number of chromosomes as established by male and female counts is twenty, including the X and Y chromosomes in the male and the 2 X in the female (figs. 1 and 2, Pl. 37). The chromosome formula of the male is therefore 18 + XY, and of the female 18 + XX. In the male, X and Y are seen occupying approximately the centre of the plate.

The Meiotic Phase in the Male.—Synizesis proceeds in the usual way until the pachytene stage is reached (fig. 3, Pl. 37). It is at this stage that the sex-chromosomes first become identifiable, X as a fairly large element lying apparently always against or partly over the plasmosome. In fig. 3, Pl. 37, a much smaller rounded element is seen separate from X. This is probably the Y chromosome, but may possibly be a chromatoid body. The remaining chromosomes are still in the woolly stage. Fig. 4, Pl. 37, shows a metaphase plate of the first meiotic division, with nine bivalents and the XY pair.

The first meiotic division is reductional, X and Y travelling to opposite poles of the nucleus.

Figs. 5 and 6, Pl. 37, represent early and late anaphase respectively.

The telophase passes into a very characteristic resting stage, the secondary spermatocytes showing distinct dimorphism (fig. 7, Pl. 37), half the spermatocytes having a large chromatic body and the other half a small one, which are presumably X and Y respectively.

A second meiotic division showing the Y chromosome is shown in fig. 8, Pl. 37.

The expected two types of spermatids are found, one half containing X (female producing) and the other half Y (male producing) (fig. 9, Pl. 37).

2. Pseudochirus peregrinus (figs. 10–16, Pl. 37 ; fig. 24, Pl. 38).

Material.—The material consisted of the ovaries of one female, the testes from a half-grown male, and the testes of two full-grown males.

Number of Chromosomes.—The somatic number of chromosomes as established by both male and female counts is twenty (figs. 10, 11, Pl. 37). The chromosome formula of the male is therefore 18 + XY, and in the female 18 + XX. One of the ovaries was fixed in Bouin and gave no results as the chromosomes had clumped together. The other ovary was fixed in Flemming and gave good results, particularly in an unusually large prophase (fig. 10, Pl. 37).

The sex-chromosomes are distinguishable in the male mitosis, but it is difficult to determine which they are in the female, though there seems to be no doubt that they are two of the smallest chromosomes.

Sex-chromosomes.—The sex-chromosomes consist of a definite X and Y, as seen in figs. 12–16, Pl. 37. They are most clearly seen in the metaphase and early anaphase.

The first division of the nucleus is reductional for X and Y, and eventually gives rise to two types of spermatids—9 + X and 9 + Y.

The Meiotic Phase in the Male.—The meiotic phase in *Pseudochirus* is similar in the essential details to that of *Trichosurus vulpecula*, the metaphase stages (figs. 12, 13, Pl. 37) and the anaphases (figs. 14 and 16, Pl. 37) being particularly clear and definite.

Fig. 16 (*a* and *b*), Pl. 37, represents the two anaphase plates of the nucleus of a primary spermatocyte in which X and Y have not yet completely separated.

3. Phascolomys mitchelli (figs. 17–20, Pl. 37; figs. 21, 23, Pl. 38).

The material examined in this genus consisted of the testes from two wombats and the ovaries from a female.

Number of Chromosomes.—No mitoses could be found in the ovarian follicles, presumably owing to the fact that the animal had been in captivity for some years. It was therefore impossible to establish the female number directly. No spermatogonial mitoses were found that could be counted with any accuracy.

The diploid number of chromosomes, as deduced from the meiotic stages of the male, is fourteen including X and Y. The chromosome formula in the male is therefore 12+XY, and in the female is presumably 12+XX. The counts were taken in the metaphase and early anaphase of the first meiotic division.

The Meiotic Phase in the Male.—Meiosis takes the same course as in the other two forms dealt with. The sex-chromosomes are distinct as in the other forms, and follow the same course during meiosis.

The XY bivalent is clearly seen in figs. 17–20, Pl. 37. Fig. 21, Pl. 38, shows the differentiation between the two types of secondary spermatocytes.

Figs. 22, 23, and 24, Pl. 38, illustrate the relations of the XY bivalent in Trichosurus, Phascolomys, and Pseudochirus respectively.

The X chromosome in Pseudochirus differs from that of the other two forms in that it appears to be bipartite, consisting of a large element and a much smaller element attached to the larger. It is not unlike the X chromosome figured by Painter in the metaphase stages of the spermatogenesis of Didelphys virginiana (1922).

4. Potorous tridactylus (figs. 25–36, Pl. 38).

The living animals were obtained from Tasmania and were kept in captivity for about a year in Melbourne, during which time they failed to breed.

Number of Chromosomes.—The number of chromosomes in both male and female is twelve, including the sex-chromosomes which form the smallest pair. X and Y in the male are of much more nearly equal sizes than in the only other Macropod so far examined (M. ualabatus) (Agar, 1923). Moreover X is not attached to an autosome, either in the male or female, as it is in that species.

The Meiotic Phase in the Male.—The small number of chromosomes results in particularly clear figures of the meiotic divisions and of the relations of the XY pair, as illustrated in the figures. A noticeable characteristic of the Y chromosome (and to a less degree of X) is its frequently irregular shape, especially when drawn out on the anaphase spindle, giving the appearance of being composed of a number of elements (figs. 29, 31, 32, Pl. 38).

In a number of metaphases very small granules of chromatin were found on the spindle-fibres, quite isolated from the other chromosomes (fig. 28, Pl. 38). Their relation to the spindle-fibres distinguish them from the chromatoid bodies, of which several may be present, and which seem to be distributed quite capriciously at cell-division.

The expected two types of secondary spermatocytes and second meiotic divisions were easily found (figs. 33–6, Pl. 38).

Appended is a list of the Marsupials, with their respective chromosome numbers so far established, all except Didelphys having been worked out in the Melbourne University Zoological Laboratory :

	Number of Chromosomes including XY.	*Authority.*
A. Polyprotodontia		
1. Didelphyidae		
Didelphys virginiana	22	Painter, 'Journ. Exp. Zool.', vol. 35, 1922.
2. Dasyuridae		
Dasyurus maculatus	14	Greenwood, 'Quart. Journ. Micr. Sci.', vol. 67, 1923.
Sarcophilus ursinus	14	Greenwood, ibid.
B. Diprotodontia		
1. Macropodidae		
Macropus ualabatus	12	Agar, 'Quart. Journ. Micr. Sci.', vol. 67, 1923.
Potorous tridactylus	12	Ellery, this paper.
2. Phascolomyidae		
Phascolomys mitchelli	14	Altmann, this paper.
3. Phalangeridae		
Phascolarctus cinereus	16	Greenwood, 'Quart. Journ. Micr. Sci.', vol. 67, 1923.
Pseudochirus peregrinus	20	Altmann, this paper.
Trichosurus vulpecula	20	Altmann, this paper.
Petauroides volans	22	Agar, 'Quart. Journ. Micr. Sci.', vol. 67, 1923. In this species the XY pair was not identified.

EXPLANATION OF PLATES 37 AND 38.

All figures were drawn with the aid of a camera lucida, $\frac{1}{12}$ immersion lens and 20 Holos. ocular.

LETTERING.

Pl., plasmosome ; *chr.*, chromatoid body.

PLATE 37.

Trichosurus vulpecula.

Fig. 1.—Equatorial plate of a dividing follicle cell from the ovary.

Fig. 2.—Spermatogonial equatorial plate.

Fig. 3.—Pachytene stage. X, and probably Y, have condensed out.

Fig. 4.—Metaphase of the first meiotic division, showing nine autosome bivalents and the XY bivalent.

Fig. 5.—Anaphase of the first meiotic division.

Fig. 6.—Late anaphase ; autosomes not countable.

Fig. 7.—Two secondary spermatocytes, one containing X and the other Y.

Fig. 8.—Anaphase of a secondary spermatocyte containing the Y chromosome.

Fig. 9.—Showing the two types of spermatids.

Pseudochirus peregrinus.

Fig. 10.—Late prophase from an unusually large follicle cell from the ovary.

Fig. 11.—Spermatogonial equatorial plate.

Figs. 12, 13.—Early anaphases of the first meiotic division, showing nine autosome bivalents and the XY bivalent.

Fig. 14.—End view of a metaphase plate of the first meiotic division.

Fig. 15.—Late anaphase of the first meiotic division. All the chromosomes have completely separated.

Fig. 16 (*a*) Two anaphase plates of the nucleus of a primary spermatocyte. X and Y have not yet completely separated. (*b*) The same two plates with the slide moved slightly between the drawing of the upper and lower plates.

Phascolomys mitchelli.

Figs. 17, 18, 19.—Side view of three metaphases of the first meiotic division, showing six autosome bivalents and the XY bivalent. A chromatoid body is seen in fig. 17.

Fig. 20. Side view of an early anaphase. X and Y have separated.

PLATE 38.

Fig. 21.—Group of secondary spermatocytes showing dimorphism. Chromatoid bodies are also present. *Phascolomys mitchelli*.

Figs. 22, 23, 24.—Represent the XY bivalent taken from eight nuclei in *Trichosurus*, *Phascolomys*, and *Pseudochirus* respectively.

Potorous tridactylus.

Figs. 25, 26.—Metaphases from Graafian follicle cells. The two X chromosomes are in the centre of the ring. The chromosomes are numbered to indicate the homologous pairs.

Fig. 27.—Spermatogonial metaphase. The XY pair is in the centre.

Figs. 28, 29, 30, 31.—Side and end views of metaphases of the first meiotic division.

Fig. 32.—Anaphase of the first meiotic division.

Fig. 33.—Group of secondary spermatocyte nuclei to show dimorphism.

Figs. 34, 35.—Side and end views of a second meiotic division containing the Y chromosome.

Fig. 36.—Anaphase of a second division containing the X chromosome.

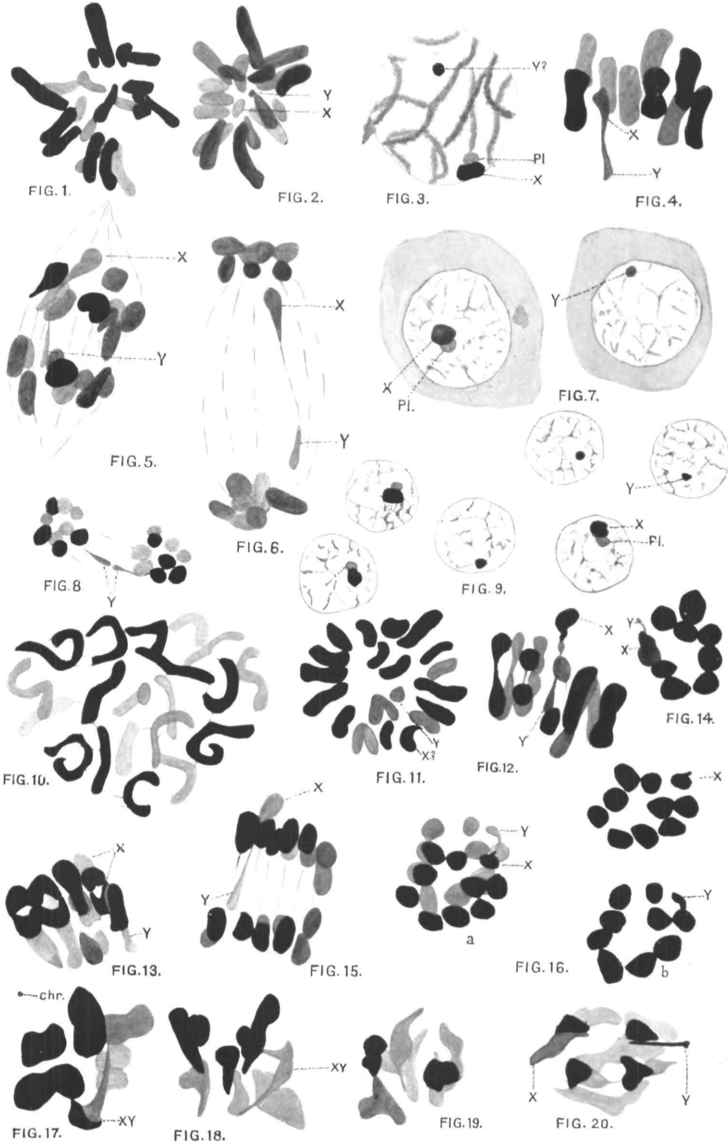
FIG. 1.
Y
X
FIG. 2.
Y?
Pl
X
FIG. 3.
X
Y
FIG. 4.
X
Y
FIG. 5.
X
Y
FIG. 6.
Y
X
Pl.
FIG. 7.
FIG. 8
Y
Y
X
Pl.
FIG. 9.
FIG. 10.
Y
X?
FIG. 11.
X
Y
FIG. 12.
Y
X
FIG. 14.
X
X
Y
FIG. 13.
X
Y
FIG. 15.
Y
X
a
Y
b
FIG. 16.
chr.
XY
FIG. 17.
XY
FIG. 18.
FIG. 19.
X
Y
FIG. 20.

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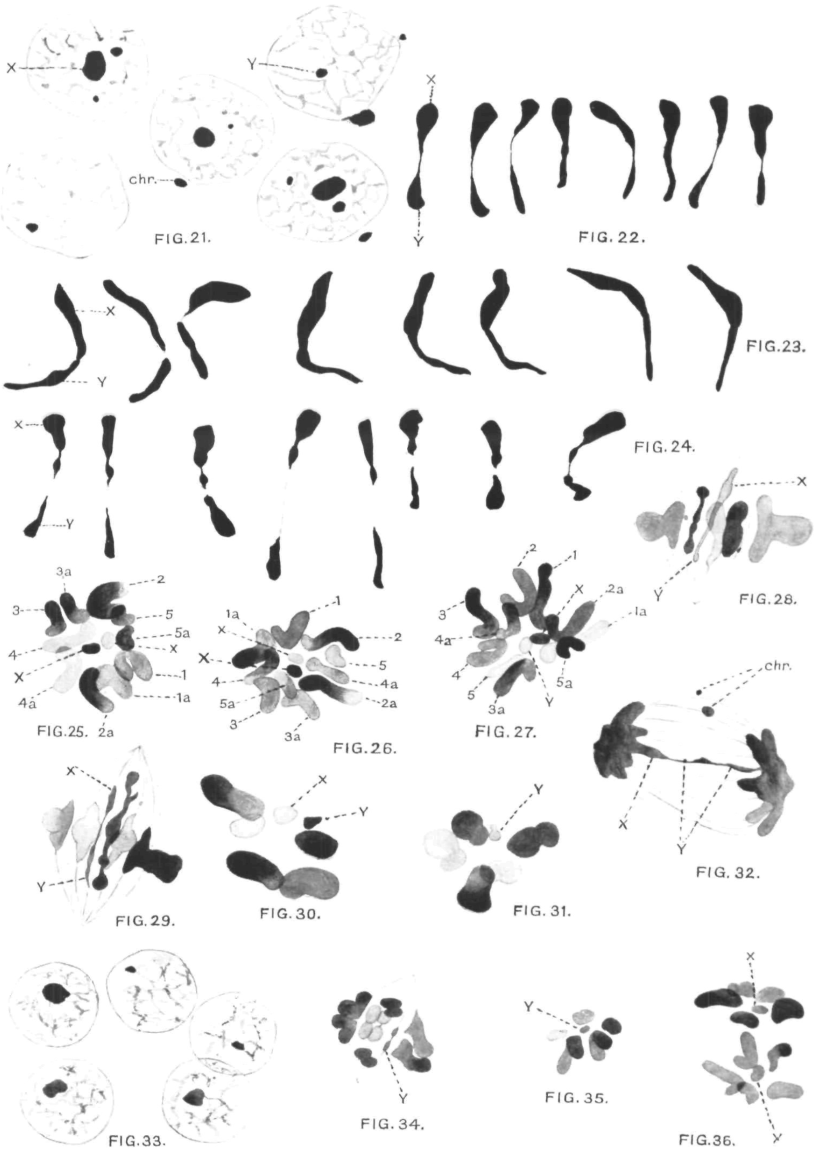

FIG. 21. FIG. 22. FIG. 23. FIG. 24. FIG. 25. FIG. 26. FIG. 27. FIG. 28. FIG. 29. FIG. 30. FIG. 31. FIG. 32. FIG. 33. FIG. 34. FIG. 35. FIG. 36.

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