G-BANDING AND CHROMOSOMAL EVOLUTION

IN AUSTRALIAN MARSUPIALS

by

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SUMMARY

This G-banding study was designed primarily to evaluate the disputed hypothesis of a 2n=14 complement as ancestral, and thus of increases in chromosome number as a major mode of karyotypic evolution for Australian marsupials.

The 2n=14 chromosome complements of fourteen species of marsupials from four different taxonomic families, each representing one of the major superfamilies of Australian marsupials were G-banded. The close similarity and particular small differences in G-banding pattern between these complements could be simply explained only if 2n=14 had been commonly ancestral for all these species. As 2n=22 had also been proposed as the ancestral chromosome number, the 2n=22 complements of three marsupial species from two families, and that of a species with 2n=20 chromosomes from a third family, were also G-banded and compared. The G-banding patterns of the 2n=22 complements of different families were quite distinct and could most simply be related to each other, to the 2n=20 complement of the third family and to the G-banded complements of all other Australian marsupials by a common 2n=20 intermediate. This complement could be considered derived from the 2n=14 G-banded complement that was proposed as ancestral for the four major superfamilies of Australian marsupials by the Robertsonian fission of three pairs of autosomes. Thus comparison of these G-banded chromosomes has implicated increases in chromosome number, and in particular Robertsonian fission, in the evolution of Australian marsupials.

In addition pathways of chromosomal change were examined by a G-banding study of a further eight species of Macropodidae, which is karyotypically the most diverse family of Australian marsupials. The

G-banding pattern homologies were consistent with the hypothesis of a 2n=22 complement as ancestral for these species, with the decreases in chromosome number being largely attributable to centric events. For *Macropus* species, the 2n=16 chromosome complements that appear similar after general chromosome staining were shown to represent three distinctively G-banded complements, with the differences between these being changes in the arm components of three pairs of chromosomes.

Thus the application of G-banding techniques has allowed a re-assessment of the relationships of general stained chromosome complements of similar format, and therefore of the processes of chromosomal change in Australian marsupials.

DECLARATION

This thesis contains no material which has been accepted for the award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material published or written by another person, except where due reference is given.

R. H. Rofe

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CHAPTER 1

CHROMOSOME BANDING AND KARYOTYPIC EVOLUTION

Any reproducible increase in the linear differentiation of the chromosome is potentially useful for better defining karyotypic differences and consequently contributing to our understanding of chromosomal evolution.

1.1 Polytene chromosomes

The impact of such increased resolution is well illustrated by the classical studies of Dipteran chromosomes, and the genus *Drosophila* (Patterson and Stone, 1952; and for a review, White, 1973a) is used here as an example.

Species of *Drosophila* show very little variation in the number and gross morphology of their mitotic chromosomes. However, in a number of tissues of these flies there are very long multi-banded polytene chromosomes; and when the banding patterns of these have been compared there are very few species that are karyotypically indistinguishable. Analysis of these polytene chromosomes has shown that the banding pattern (as well as the genic content) of a particular segment is conserved during karyotypic repatterning in *Drosophila*. The observable chromosome changes can be accounted for by intra-chromosomal rearrangements and whole arm-translocations, with the most common type of change being pericentric inversion. Many species have chromosomes showing overlapping inversion differences, and, therefore, if it assumed that each inversion occurred only once, the phylogenetic relationships of these species may be deduced from the sequence of such events (Sturtevant and Dobzhansky, 1936).

The polytene chromosomes of *Drosophila* have also been used by Muller to study radiation induced chromosome breakage. The mechanisms

of chromosomal rearrangement that were formulated largely from his observations (Muller, 1940) have been widely accepted and applied to all eukaryotes.

1.2 Metaphase chromosome banding techniques

Most animals and plants do not have chromosomes with linear differentiation equivalent to that of the Dipteran polytene chromosome and therefore the facets of chromosomal evolution that may be deduced from the study of these particular chromosomes have been much more limited. However, in recent years a number of "chromosome banding" techniques have been developed. These increase linear resolution by selectively staining regions of metaphase chromosomes. Some of these chromosome banding techniques, those that are particularly relevant to the subject of this thesis, are now reviewed.

G-banding

Reproducible patterns consisting of many transverse bands can be produced along the length of animal chromosomes after specific treatments and staining with Giemsa stain - thus G-bands (Dutrillaux *et al.*, 1971; Drets and Shaw, 1971; Seabright, 1971; Sumner *et al.*, 1971). Such bands may be produced by many different reagents; for example, salts, bases chelating agents, detergents, proteolytic enzymes and oxidizing agents (Kato and Yosida, 1972; Kato and Moriwaki, 1972; Shiraishi and Yosida, 1972; Lee *et al.*, 1973; Utakoji, 1972). Electron microscopy studies (Burkholder, 1975) indicate that chromatin is rearranged during G-banding. However, G-bands can be observed in very gently prepared chromosomes (Bahr *et al.*, 1973; McKay, 1973; Yunis and Sanchez, 1973) and mitotic G-bands correspond well with chromomere patterns at pachytene of meiosis (Hungerford *et al.*, 1971; Okada and Comings, 1974). Thus G-banding techniques are considered to accentuate an existing pattern of chromatin

packaging that is usually obscured by the condensation of metaphase chromosomes. The appearance of clear G-bands after treatment with specific protein denaturants and protease under mild non DNA-denaturing conditions indicates the role of chromosomal proteins in band production. The stain itself is thought to play some role, since Giemsa significantly enhances G-banding pattern relative to Feulgen staining (Comings *et al.*, 1973) and dyes showing pronounced metachromasia and side-stacking show good banding, whereas those lacking such properties do not (Comings and Avelino, 1975).

The observations that G-bands replicate late in the DNA synthetic period (Ganner and Evans, 1971; Dutrillaux, 1975), and that there is a preferential distribution of intermediate repetitive DNA in G-bands (Sanchez and Yunis, 1974) and of mRNA in non G-bands (Yunis *et al.*, 1977) have been interpreted as indicating G-bands to be regional concentrations of non-coding chromatin.

C-banding

Using a modification of a technique for the *in situ* hybridization of radioactive nucleic acid, Arrighi and Hsu (1971) showed that the centromeric regions of many animal chromosomes could be differentially stained. These dark bands, which may also be induced in plant chromosomes, were called C-bands. A good correlation exists between C-bands and constitutive heterochromatin (as defined by Brown, 1966) and sites of satellite or highly repeated DNA. However, these terms should not be used synonomously as the correlations are not absolute (Hennig and Walker, 1970; Arrighi *et al.*, 1974; John and King, 1977). Also different C-banding patterns may be revealed for the same complement by applying alternative C-banding methods (Voiculescu *et al.*, 1972) and the possibility of such variation must be recognized when comparisons are made.

C-bands appear to be chromosome regions that are particularly resistant to dispersal by the band-inducing agents used (Comings *et al.*, 1973; Merrick *et al.*, 1973). However, C-bands are demonstrably heterogeneous by other banding methods (for example, fluorochrome staining and *in situ* hybridization of satellite DNAs) and even display such heterogeneity within a complement (Jalal *et al.*, 1974). Thus C-bands appear to be chromosome regions of often quite different chemical composition that nevertheless show a common structural response to C-banding agents. It is therefore not surprising that no simple and specific common function of C-bands has been accepted.

N-banding

Matsui and Sasaki (1973) and Funaki *et al.* (1975) treated the chromosomes from a number of animal and plant species with hot acidic solution. In all cases the bands produced marked the site of the secondary constriction or presumptive nucleolar-organizing region. Silver staining techniques also reveal such N-bands (Howell *et al.*, 1975). In the mitotic chromosomes of animals the location of N-bands correlates well with sites (as demonstrated by *in situ* hybridization) of ribosomal RNA genes (Matsui, 1974; Goodpasture and Bloom, 1975). Secondary constrictions generally correspond well with the latter (Hsu *et al.*, 1975).

N-bands appear to result from the selective staining of acidic protein (Matsui and Sasaki, 1973; Schwarzacher *et al.*, 1978) associated with functional nucleolar organizer regions (Miller *et al.*, 1976; Schwarzacher *et al.*, 1978).

Other-banding techniques

In addition to G-, C- and N- there are many other chromosome banding methods. Some of these produce (like C-banding) localized bands (for example the G11- and Cd-bands; Bobrow *et al.*, 1972; Eiberg, 1974).

Others reveal (as does G-banding) periodic bands along the length of animal chromosomes (for example Q- and R-bands; Caspersson *et al.*, 1971 and Dutrillaux and Lejeune, 1971). Many periodic band-inducing agents have different target sites in chromatin and therefore show slightly different banding patterns. However, while the differential action of these reagents manifests the chemical heterogeneity of chromosomal regions, these banding patterns reflect essentially the same repeating structural organization of chromatin along the length of the metaphase chromosome. The individual mechanisms of such periodic band induction and the general functional significance of these relatively large chromosomal domains remain to be elucidated.

Plant chromosomes respond to only some of these band-inducing treatments for metaphase chromosomes (for example, C-, N- and Q-, but not G-banding) and only localized bands are produced. Nevertheless such bands have facilitated chromosome identification and as in animals, have revealed extensive and unsuspected chromosomal polymorphism.

However, the periodic bands that can be induced in animal chromosomes have much greater resolving power than the more localized bands and thus have allowed the unequivocal identification of most chromosome pairs of animal complements. Also, as these striated chromosomes give much better definition of karyotypic differences, pathways of chromosomal evolution in different animal groups can be both determined and examined in much greater detail than before. In order to provide some frame of reference for an assessment of the contribution of studies of banded metaphase chromosomes in general, and also the research of this thesis, to knowledge of the modes and mechanisms of karyotypic evolution in animals, the history of the present concepts of chromosomal change is considered.

1.3 Concepts of chromosomal change

Before the advent of metaphase chromosome banding methods, the most readily observable karyotypic differences for most eukaryotes were changes in the number and gross morphology of chromosomes. Thus concepts of chromosomal evolution were dominated by mechanisms of such changes; and in particular Robertsonian fusion, the related principle of a "nombre fundamental", and pericentric inversion.

Robertsonian or centric fusion is presently conceived as the apparent replacement of two acrocentric chromosomes in a complement by a metacentric. Robertson (1916), to whom "Robertsonian" refers, regarded this process as completely reversible; that is, that a metacentric may be replaced in a complement by two acrocentric chromosomes.

The concept of a "nombre fundamental", NF, (Matthey, 1945) follows directly from Robertson's proposals, as it assumes that the units of karyotypic evolution are chromosome arms, the number of which (NF), remains constant for closely related animals. Matthey has argued that a metacentric chromosome produced by Robertsonian fusion has a double centromere, and that the splitting of a metacentric chromosome constitutes Robertsonian fission. He points out that, unlike reciprocal translocations, these mechanisms can directly account for the units of change being chromosome arms, because they are by nature centric phenomena. Although Matthey regards fission as a possibility, he believes that the fusion process has predominated in the evolution of vertebrate genomes; as in some groups, animals he considers to be morphologically primitive have the highest chromosome numbers, and for most eutherian groups there are less species with chromosome numbers above the overall modal number, than there are below (Matthey, 1973). This latter argument depends to some extent on the choice of a convenient modal number (in the range 2n=40 to 56) for any group and even then there are some groups (for example, Artiodactyla and Primates) in which fission

appears to have predominated.

Mechanisms of Robertsonian change

There are several mechanisms of so-called Robertsonian change and the elements of a number of these are shown in Figure 1.1.

The principles of chromosomal rearrangement that Muller (1940) proposed on the basis of chromosome breakage experiments in *Drosophila* have been invoked for Robertsonian changes. These were that each functional rearranged chromosome must have received a single centromere and two telomeres from a pre-existing chromosome. Therefore on Mullerian arguments, (a) of Figure 1.1, Robertsonian changes are visualized as very unequal reciprocal translocations (Darlington, 1937).

In Mullerian fusion a small dispensable chromosome or unstable fragments are formed from parts of the acrocentric chromosomes. The first evidence of phylogenetic reduction in chromosome number by such a mechanism was presented by Tobgy (1943) in a study of the chromosomes of *Crepis* species.

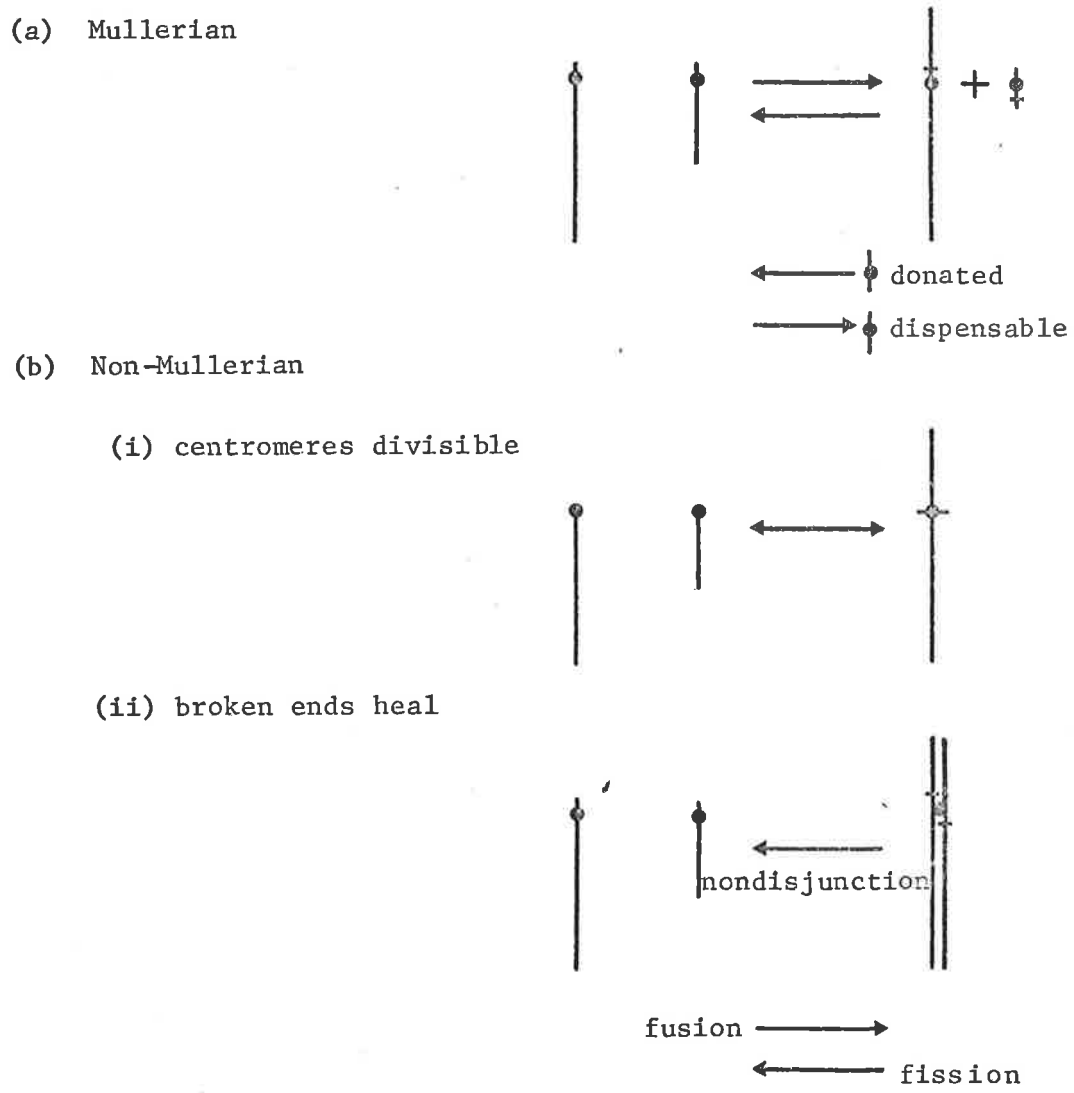
Mullerian fission, by the reverse reciprocal translocation process of (a) of Figure 1.1, is still hypothetical; it requires a dispensable or transitory chromosome to donate the intact centromere and telomeres for the acrocentric products.

Given this requirement it is interesting to recall that Robertson himself conceived fission as a direct process; the replacement of a single metacentric by two acrocentric chromosomes.

> "That Vs may be formed by the fusion of non-homologous rods by their proximal ends and also that rods may be formed by the breaking of a V at its proximal end, the apex, is to be inferred from the presence of a V and its rod-mates in one and the same individual."
>
> Robertson, 1916.

Figure 1.1

Mechanisms of Robertsonian change.



-chromosome breaks, which are indicated for the fission direction only.

The elements of different mechanisms of the gain and loss of chromosomes without detailed reference to theories of centromere structure (for example, Lima de Faria (1956), Marks (1957) and John and Freeman (1975)).

If, in contrast to Muller's belief, the centromere is regarded as divisible, Robertsonian changes may also be achieved following breakage within the centromeres of chromosomes, (b) (i) of Figure 1.1. It has been suggested that Robertsonian fission by such a process contributes significantly to karyotypic evolution (John and Hewitt, 1968; Todd, 1970). This mechanism assumes that there are functional telocentric chromosomes and there is some support but no direct evidence for this view.

Lima de Faria (1956) interpreted the observable structure of the kinetochore as a tandem reversed repeat and suggested that functional telocentrics can be produced by misdivision of this region. John and Hewitt (1968) considered, on the basis of the appearance of chromosomes at meiosis, that telocentric chromosomes do exist in animals. In plants there have been a small number of observations of what appear to be telocentrics produced from a single metacentric chromosome (Marks, 1957). In animals observations like those of Southern (1969) are the most direct evidence for such a process of Robertsonian fission. He found one male grasshopper, *Myrmeleotettix maculatus*, heterozygous for a metacentric and two acrocentric chromosomes (individuals of this species are usually homozygous metacentric for this chromosome pair). The 'telocentrics' were stable and 95% of the gametes produced had balanced chromosome complements. However, it is not known whether such "telocentrics" can be transmitted and produce viable offspring. More direct evidence of Robertsonian fission by a mechanism of centromere splitting would be provided by a mosaic heterozygote for such fission chromosomes, especially such an animal that produced viable offspring with both derived acrocentric chromosomes.

On the argument that broken chromosome ends may heal (McClintock, 1941), Robertsonian fission may be achieved by another non-Mullerian mechanism, (b) (ii) of Figure 1.1, which does not require division of

the centromere. In particular, two acrocentric chromosomes may be produced from a single metacentric by breakage and healing in different arms of a metacentric chromosome at G2, followed by non-disjunction (Hayman and Martin, 1974).

Thus, as shown in Figure 1.1, there are several conceivable mechanisms of Robertsonian change. However, as Mullerian principles of chromosome rearrangement have been most widely accepted, such changes have been predominately interpreted as fusions. As White (1973b) who uses "dissociation" for "Mullerian fission" explains:

> "... the mechanism of fusion seems an easier one than of dissociation (at least as conceived of by the present author) ... thus most students of vertebrate and, especially, mammalian karyotypes have interpreted differences in chromosome numbers in terms of evolutionary fusions rather than dissociation."

and as he believes "probably rightly".

Therefore, when accounting for changes in chromosome number, Robertsonian fusion and the related principle of a "nombre fundamental" have dominated the thinking of cytogeneticists in the past. Likewise, for explaining changes in chromosome morphology that are unaccompanied by changes in chromosome number, pericentric inversions have been invoked.

The pervasive influence of the latter concept is well illustrated by the hailing of entirely C-banding short arms (*Peromyscus*) as a "new mechanism" of chromosome evolution (Duffey, 1972); although as John and King (1977) point out, the presence of heterochromatic supernumerary arms on the chromosomes of grasshoppers had been known for decades.

The application of metaphase chromosome banding methods has also stimulated a recent revival of interest in non-Mullerian mechanisms of Robertsonian fusion. C-, Cd- and Q-banding studies (for example, Niebuhr, 1972; Lau and Hsu, 1977) have supported previous suggestions

that some biarmed chromosomes may actually be dicentrics. In addition these indications of non-Mullerian mechanisms of chromosomal change have generated a small amount of renewed interest in the possibility of non-Mullerian fission and therefore the feasibility of Robertsonian fission as a means of changing chromosome number (John and Freeman, 1975).

1.4 The periodic-banding of metaphase chromosomes and its implications for karyotypic evolution

Of the bands that may be induced on metaphase chromosomes, the periodic-bands give the greatest resolution of karyotypic differences and therefore have contributed most substantially to a better understanding of chromosomal evolution. An extremely large number of papers have been published in this area and therefore only a few examples that best illustrate the main impact of such studies will be reviewed. These examples are from mammals.

The outstanding demonstration from comparative periodic- (G-, Q- or R-) banding studies has been the extent of conservation of chromosomal segments. For example, in Primates, not only are almost all of the periodic bands of chromosomes of the gorilla, chimpanzee and orangutan completely homologous to those of each other species and to those of Man (see Miller, 1977, for review), but also to those of the more distantly related baboon (Dutrillaux *et al.*, 1978). These bands thus appear to have remained unaltered since the divergence of the lineages of the baboon and Man, which is approximately 30 million years B.P.

There are some whole chromosomes that are homologously banded in a number of primate species (for example, six such chromosomes are shared by the baboon and Man). However, homologously banded segments are often found in morphologically distinct chromosomes in different primate species. Thus banding pattern is conserved during rearrangement and this enables the nature of past chromosome changes in primates to be ascertained, in

a similar way to that in which polytene chromosomes are used to define karyotypic differences in *Drosophila*. For example, there are two pairs of banded chromosomes in the gorilla, chimpanzee and orangutan (all possessing 2n=48 chromosomes) that correspond in a Robertsonian manner to the arms of one banded chromosome pair in Man (2n=46) and the chromosomes of these species are also differentiated by several pericentric inversions, a small number of paracentric inversions and more complex rearrangements. The baboon, with 2n=42 chromosomes, has a banded karyotype closest to that of the orangutan, differing by three fusions, a number of pericentric inversions and three more complex rearrangements.

As with the polytene chromosomes of *Drosophila*, segments with homologous banding patterns in primates also show conservation of genetic loci (for example Finaz *et al.*, 1977).

The conservation of periodic banding pattern and also the identifiable karyotypic differences have allowed the construction of pathways of chromosomal evolution and thus phylogenies for some groups of species. For example, Dutrillaux *et al.* (1975) using the relationships between the periodic-banding patterns of chromosomes of the chimpanzee, gorilla and orangutan that are homologous to pairs 2 and 7 in Man, have suggested that the orangutan lineage diverged first from an ancestor common to all these primates, then the lineage of the gorilla, and finally the lineages of the chimpanzee and Man diverged.

Periodic-banding studies have confirmed the major roles of both Robertsonian change and pericentric inversion in many mammals as well as the Primates. For some groups there are many Robertsonian differences and comparatively few others. For example, the main karyotypic differences between twelve species of Bovidae with chromosome numbers ranging from 2n=31♂ to 2n=60 can be accounted for by Robertsonian changes, with other rearrangements appearing to have been fixed quite infrequently. This

G-banding pattern homology of chromosome arms also extends into the related superfamilies, Giraffoidea and Cervoidea (Buckland and Evans, 1978a). As an extreme example of Robertsonian differences, the giraffe with 2n=30 chromosomes, has eleven G-banded chromosome pairs that correspond to Robertsonian combinations of twenty two acrocentric pairs of the goat complement (2n=60).

Such a large number of karyotypic differences is perhaps to be expected between animals from different taxonomic superfamilies. However, G-banding has also substantiated the occurrence of many Robertsonian differences between the karyotypes of closely related species. For example, comparison of the G-banded chromosomes of two species of woodrats, *Neotoma*, with quite different chromosome numbers (2n=38 and 2n=52) have confirmed that these karyotypes differ by at least seven such changes (Mascarello *et al.*, 1974).

Periodic banding studies have also revealed some less expected karyotypic relationships and thus shown that the simplest or most generally accepted explanation of cytological differences may be in error. Metacentric chromosomes of similar morphology may be seen to possess quite different arm components after G-banding (Pathak *et al.*, 1973a; Bianchi *et al.*, 1976) and even whole karyotypes that appeared to be similar on morphological grounds may be seen to be quite different after G-banding (Stock, 1975). As an example involving karyotypic differences, there are two bat *Rhogeessa* species with 2n=30 and 2n=34 chromosomes. After general chromosome staining these complements appeared to differ by two Robertsonian changes. However, G-banding shows that the 2n=30 species has metacentrics 2/15, 4/10, 13/8, 7/11 and 1/5 whereas three metacentrics of the other species have quite different arm components, 4/15, 10/13, 1/11 (Bickham and Baker, 1977).

The possible variety of chromosomal rearrangements has also been emphasized by periodic banding studies, with the result that cytogeneticists

are now more than ever aware of the diversity of chromosomal evolution. As well as Robertsonian changes and pericentric inversions, tandem fusions, paracentric inversions, and many other rearrangements appear to have been significant modes of karyotypic evolution for some mammalian groups. For example, the G-banded chromosome complement of the Rhesus macaque, *Macaca mulatta*, with 2n=42 chromosomes, and that of the African green monkey, *Cercopithecus aethiops*, with 2=60, can be simply related by centromere-telomere translocations, as well as Robertsonian changes, when the C-banding short arms of the African green monkey G-banded chromosomes are deleted (Stock and Hsu, 1973). Also, although the chromosome evolution of horses (Equidae) has clearly involved some Robertsonian changes, as had been earlier suggested on the basis of general stained chromosomes, G-banding studies (Ryder *et al.*, 1978) have shown that simple changes such as single Robertsonian fusions or fissions and pericentric inversions are insufficient to account for the karyotypic differences between species; and many highly complex rearrangements are believed to have occurred. Nevertheless it is possible that these complex differences may be the products of many simple changes.

1.5 The impact of metaphase chromosome banding

In summary, the immediate impact of metaphase chromosome banding on the cytogenetics of eukaryotes has been the facilitation of chromosome identification and the demonstration of outstanding variation. In animals this variation, which has been revealed by the application of localized banding methods, is in marked contrast to the conservative properties of chromosomes that are demonstrable with periodic-banding techniques. For example, it is now clear that although each human being may show extremely close G-banding homology with a chimpanzee, that each person has their own personal and characteristic suite of C-, Gll-, N- and fluorescent-banding variants, all of which appear to be inherited

(Craig-Holmes *et al.*, 1973; Magenis *et al.*, 1978; Varley, 1977; Verma and Lubs, 1975).

Studies of banded metaphase chromosomes have also provided better definition of karyotypic differences and thus of pathways of chromosomal evolution. Metaphase chromosome banding has emphasized that although Robertsonian changes and pericentric inversion are clearly important types of chromosomal change, changes in the amount of C-banding material and other types of rearrangements have contributed significantly to karyotypic evolution.

However, while the banding of metaphase chromosomes has provided much information on the sorts of karyotypic differences, it has given little clarification of the detailed mechanisms of chromosome change. In particular, although centromeric banding has indicated that Robertsonian fusions may be produced by non-Mullerian mechanisms, metaphase chromosome banding has otherwise contributed very little to our understanding of the mechanisms and directionality of Robertsonian changes. However, evidence for the directionality of Robertsonian change may be indirectly available from periodic-banding studies of particular animal groups where the general stained complements suggest that either fission or fusion is a much more probable explanation of the karyotypic relationships. One such group is the Australian marsupials.

CHAPTER 2

THE MARSUPIALS AND THEIR CHROMOSOMES

2.1 The marsupials

Simpson (1945) classified existing mammals in two subclasses, Prototheria (consisting of the single order Monotremata) and Theria (infraclasses Metatheria and Eutheria). The marsupials comprise his single order Marsupialia of the Metatheria. The date of eutherian-marsupial divergence, as estimated by amino acid sequence analysis of myoglobin and haemoglobin is 130 million years B.P. (Air *et al.*, 1971), and this is in general accord with the fossil record.

Marsupials are presently found in America and the Australian region and there is little evidence that they have been abundant elsewhere. There are approximately eighty extant species in both South and Central America and one hundred and seventy in Australasia, but only one, *Didelphis virginiana*, in North America; although this region has an extensive fossil history of marsupials (Clemens, 1971; Fox, 1971).

The most commonly accepted explanation for the disjunct distribution of present day marsupials between America and Australasia is that they originated in America and migrated to Australia via Antarctica, after separation of New Zealand from the southern land mass, approximately 80 million years B.P., but before Australia drifted northwards to its present position (Clemens, 1977; Keast, 1977).

Studies of the serological affinities of marsupial species indicate that animals of the two extant American superfamilies are as distinct from each other as either are from Australasian marsupials (Hayman *et al.*, 1971; Kirsch, 1977a).

The oldest certain marsupial fossils have been found in North America and date from the late Cretaceous, approximately 80 million years B.P. (Fox, 1971). At present the earliest marsupial fossil finds in Australia have been early Miocene, approximately 22 million years B.P. (Tedford *et al.*, 1975), although most living Australasian families of marsupials are believed to have been present before this time (Archer and Bartholomai, 1978), having been formed about 60 million years B.P. (Stonehouse, 1977).

On the basis of morphology, anatomy and serology, marsupials have been variously classified in family and higher category groups (Simpson, 1945; Ride, 1964; Kirsch, 1968, 1977b). According to Kirsch (1977b) there are two main groups of living marsupials in America and four in Australia. The families of existing marsupials are listed in super-families, following Kirsch and Calaby, 1977, in Table 2.1. In this thesis specific names are also those of Kirsch and Calaby (1977).

2.2 Their chromosomes

The DNA content of marsupial nuclei shows a similar range to that of eutherian mammals (Bachmann, 1972; Hayman and Martin, 1974). However, chromosome numbers are much lower in marsupials, ranging from 2n=10 to 2n=32. Approximately fifty per cent of living species have been examined cytologically and as can be seen in Figure 2.1 2n=14 and 2n=22 are the most frequent chromosome numbers (Sharman, 1973; Hayman and Martin, 1974; Reig *et al.*, 1977) and both are found in Australian and American marsupials (see also Table 2.1). As the best criterion for an ancestral-like property is generally its wider taxonomic distribution in the group of animals being considered, the simplest interpretation of the distribution of chromosome numbers is that either 2n=14 or 2n=22 may have been ancestral for all marsupials. If either

Table 2.1

Living marsupials and their known chromosome numbers.

SUPERFAMILY	FAMILY	NUMBER OF GENERA	NUMBER OF SPECIES	2n
Didelphoidea	Didelphidae	11-3	70	$\overset{*}{14}$,18,22
	Microbiotheriidae	1	1	$\overset{*}{14}$
≠	Thylacinidae	1	1	-
Caenolestoidea	Caenolestidae	3	7	$\overset{*}{14}$
≠ Dasyuroidea	Dasyuridae	14	49	$\overset{*}{14}$
	Myrmecobiidae	1	1	$\overset{*}{14}$
≠ Perameloidea	Peramelidae	7	16	$\overset{*}{14}$
	Thylacomyidae	1	2	18♀,19♂
≠ Phalangeroidea	Phalangeridae	3	11	14,20
	Burramyidae	4	7	$\overset{*}{14}$
	Petauridae	5-5	22	10,16,18, 20,22
	Macropodidae	17	56	(10♀,11♂),12, (12♀,13♂),14, (16♀,15♂),16, 18,20,22,24,32
≠ Vombatoidea	Vombatidae	2	3	$\overset{*}{14}$
	Phascolarctidae	1	1	16
≠ Tarsipedoidea	Tarsipedidae	1	1	24
≠ Notoryctoidea	Notoryctidae	1	1	20

≠ Australian marsupials

-x x is the number of subgenera

$\overset{*}{14}$ 2n=14 basic complement

\- cytologically unknown family

The number of genera and species are taken from Kirsch and Calaby, 1977, and chromosome numbers from Hayman, 1977.

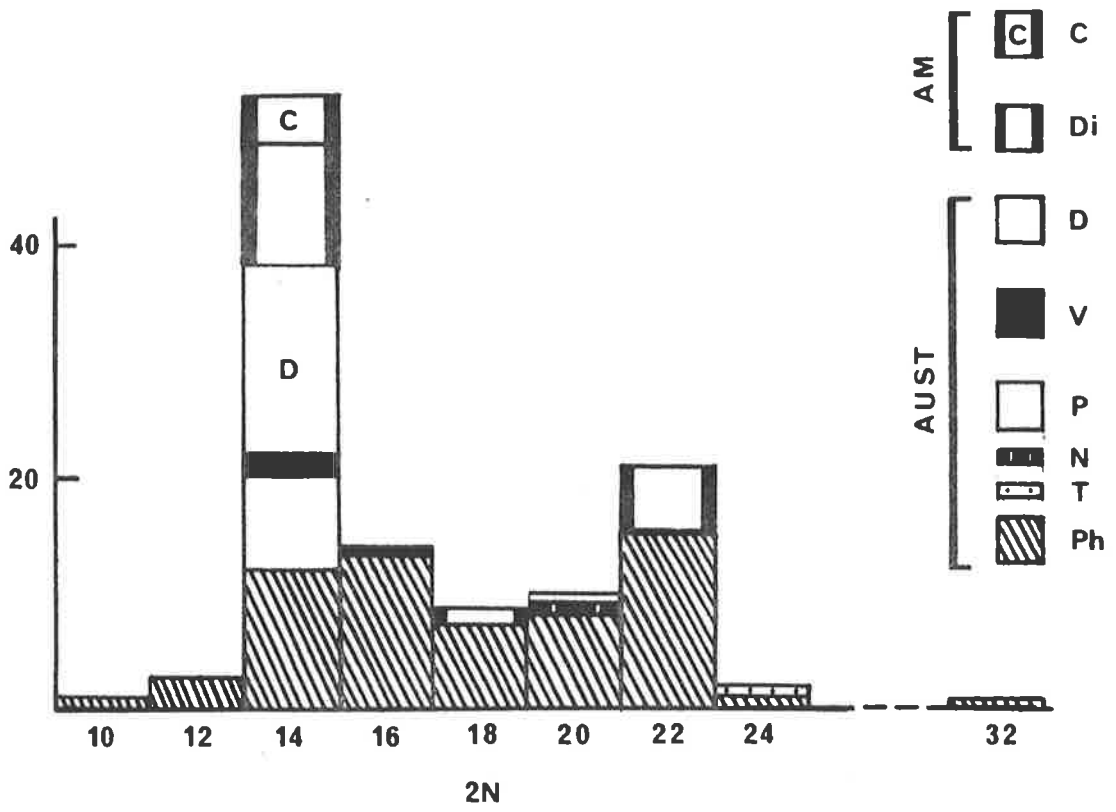
Figure 2.1

The distribution, by superfamily, of diploid chromosome number in marsupials.

Marsupials with multiple sex chromosome systems have been allotted the chromosome number of the XX♀, XY♂ complement from which these can be regarded as derived.

X-axis diploid chromosome number
Y-axis number of species

AM	American superfamilies
C	Caenolestoidea
Di	Didelphoidea
AUST	Australian superfamilies
D	Dasyuroidea
V	Vombatoidea
P	Perameloidea
N	Notoryctoidea
T	Tarsipedoidea
Ph	Phalangeroidea


AM
C
C
Di
AUST
D
V
P
N
T
Ph
40
20
C
D
10
12
14
16
18
20
22
24
32
2N

2n=14 or 2n=22 is assumed ancestral, it is necessary to postulate independent evolution of the alternative number in several American and Australian lineages, and as the relationship between ancestral complements with the two modal numbers has been regarded as essentially Robertsonian (Biggers *et al.*, 1965; Martin and Hayman, 1967; Hayman and Martin, 1974; Sharman, 1973)either centric fission or fusion is implicated in the evolution of many marsupial complements. Both 2n=22 and 2n=14 have been favoured as possible ancestral chromosome numbers for all marsupials (Sharman, 1961; Matthey, 1973; Martin and Hayman, 1967; Hayman and Martin, 1974). The differences of opinion mainly arise in the considered directionality of the Robertsonian process.

2n=22 as ancestral

Sharman (1973, 1974) adopted the view that 2n=22 was the more likely ancestral number for marsupials, arguing that chromosome fusion is a demonstrable mechanism of karyotype evolution in marsupials, and that there are very few unequivocal reports of chromosome fission in animals. The multiple sex chromosome systems of a number of marsupials were advances as unequivocal examples of chromosome fusion in marsupials.

He also considered that the presence of 2n=22 in several living didelphids (some of the oldest fossils are didelphid), and the observation that very few marsupials have 2n greater than 22, suggested that 2n=22 was a more likely ancestral chromosome number than 2n=14. No particular format was proposed for the 2n=22 ancestral complement. The very similar 2n=14 complements (Sharman, 1961; Martin and Hayman, 1967) were necessarily explained as convergent karyotype forms produced by independent chromosome number reduction in each major lineage of marsupials. Sharman indicated that periodic-banding techniques when applied to the similar 2n=14 complements may provide some indication of their origin, and it is clear that he believed that with 2n=22 as ancestral at least the arm

components of the chromosomes of some of these complements would be different.

Matthey (1973) has documented "strong arguments in support" of 2n=22 as the ancestral chromosome number for all marsupials. These were -

(1) The oldest fossil remains do not differ from living Didelphidae. Animals of the genus *Didelphis* have 2n=22 chromosomes.

(2) The uniformity of the 2n=14 karyotypes is an indication of "frozen formulae" produced by centric fusion rather than direct similarity of ancestral types.

(3) In eutherians, multiple sex chromosome systems are most common in animals with low diploid chromosome numbers. Four marsupials with 18, 16, 12 and 10 chromosomes in the female have multiple sex chromosome systems.

(4) The nuclear DNA difference between *Didelphis* (2n=22) and *Potorous tridactylus* (2n=12♀, 13♂) is due to fragment loss during chromosome fusion.

However, Hayman (1977) points out that (4) is untenable when all nuclear DNA data are considered, (3) is not discordant with Hayman and Martin's interpretation (1974) of karyotype evolution in marsupials, and (2) is based on the assumption that 2n=22 or a higher number was ancestral for marsupials. Further, a morphologically primitive marsupial (*Didelphis*) need not have a "primitive" karyotype, (1).

In addition it is noteworthy that the karyotypes of living Didelphidae are not all 2n=22; 2n=14 and 2n=18 also occur in this American family of marsupials (Biggers *et al.*, 1965; Reig and Bianchi, 1969).

2n=14 as ancestral and the "2n=14 basic karyotype" concept

Hayman and Martin preferred 2n=14 as the ancestral chromosome number for all marsupials (Martin and Hayman, 1967; Hayman and Martin, 1969, 1974). They argued that this was the simplest interpretation of the cytogenetic data, as many of the 2n=14 complements in all superfamilies are very similar after general chromosome staining, whereas the 2n=22 complements show a diversity of karyotypic format. Also, although both 2n=14 and 2n=22 occur in both American and Australian marsupials, similar 2n=14 complements occur in all major superfamilies, but 2n=22 is present in only two (see Table 2.1). At the family level 2n=14 also predominates. In addition they argued that increase in chromosome number (which given 2n=14 as ancestral, is required in all but two superfamilies of marsupials) is a demonstrable karyotypic change in marsupials, as *Aepyprymnus rufescens* has many more chromosomes (2n=32) than either modal number.

Thus the main force of their argument was the close similarity of the 2n=14 complements of many diverse marsupials (see 2n=14* in Table 2.1). These 2n=14 complements were proposed as retaining essentially ancestral-karyotype format, consisting of three large metacentric or submetacentric chromosomes, one medium sized metacentric and two small autosomes, one of which may have a satellite, and a sex chromosome pair. This karyotypic form was called the "2n=14 basic complement".

Initially Martin and Hayman (1967) assessed the similarity of the 2n=14 basic complements of animals from the four main superfamilies of Australian marsupials using chromosome and chromosome arm length measurements corrected for nuclear DNA values. If two or more chromosomes were "shared" (for each chromosome the length of both arms not statistically different in the two species) by any two karyotypes, this was regarded as indicative of relationship. *Cercartetus* (superfamily Phalangeroidea) shared two chromosomes with *Vombatus*

(Vombatoidea), which in turn shared two with *Perameles* (Perameloidea). The similar nuclear DNA values and total lengths for four chromosomes of *Cercartetus* and a number of dasyurids (Dasyuroidea) were submitted as evidence of relationship.

Subsequent to this first formal proposal of 2n=14-basic as a possible ancestral complement for all marsupials, this karyotype form was described in the until then, cytologically unknown, American superfamily, the Caenolestoidea (Hayman *et al.*, 1971).

In 1974, Hayman and Martin published a much more extensive comparison of 2n=14 basic complements. For each Australian and American family of marsupials in which 2n=14 basic complements occur, an average 2n=14 complement was defined by mean per cent arm-length measurements for each chromosome, taken over several species. Whenever possible, length measurements were corrected for nuclear DNA values. In a similar way, average 2n=14 karyotypes for each superfamily of marsupials were constructed and then these averagedto give an overall "average basic karyotype" for all five superfamilies of Australian and American marsupials. (The Phalangeroidea of Hayman and Martin (1974) is equivalent to both Phalangeroidea and Vombatoidea of Table 2.1. The latter classification means that the 2n=14 basic karyotype is referable to six major superfamilies of marsupials.)

The concept of such a 2n=14 "average basic karyotype" for all marsupials was founded on the following assumptions:

(1) DNA is distributed proportionally along the length of all chromosome arms and therefore chromosome lengths are a valid way to compare complements;

(2) the deviations of the superfamily averages of chromosome lengths from the total are not large;

(3) the species studied are representative of the taxonomic diversity of marsupials.

The first assumption appeared to be justified, as the 2n=14 karyotypic format remained essentially similar even with variation in nuclear DNA content.

The second point could not be proven, but changes in arm ratio could account for most of the deviations and therefore pericentric inversions were proposed for chromosome 6 of Didelphoidea, chromosomes 2, 4 and 6 of Dasyuroidea and chromosome 1 of Phalangeroidea (Phalangeroidea plus Vombatoidea of Table 2.1).

For the third assumption to be acceptable, the exclusion of three non-basic 2n=14 complements from the calculation of superfamily averages required justification. These included the complements of some of the tree-kangaroos, *Dendrolagus* species and of the cuscuses, *Phalanger* species. The complement of the potoroo *Potorous tridactylus* with 2n=12♀, 13♂ (XX♀, XY_1Y_2♂) is also most simply derived from a non-basic 2n=14 (XX♀, XY♂) complement. The format of these non-basic 2n=14 complements is quite different for each of these three genera, and is also quite different from the form of the 2n=14 basic complement. Taxonomically the *Dendrolagus* species and *Potorous tridactylus* are macropods and therefore their exclusion could be justified, as their 2n=14 non-basic complements were explicable as fusion derivatives of the 2n=22 complement proposed (Martin and Hayman, 1966) as ancestral for all kangaroos and wallabies (family Macropodidae). The 2n=14 karyotypes of the cuscuses were also postulated as having arisen from a complement with higher chromosome number.

By the slightly different classification adopted in this thesis one other taxon known to possess 2n=14 chromosomes was not included in the calculations of Hayman and Martin. This is the monotypic Myrmecobiidae. The 2n=14 complement of this animal is of "basic form" and very similar to those of Dasyuridae.

A necessary corollary of a 2n=14 basic chromosome complement for all marsupials was the independent occurrence of increases in chromosome number in many superfamilies of living marsupials. Moreover, since the relationship between the higher and lower chromosome numbers was regarded as essentially Robertsonian, Robertsonian fission was implicated. In most cases, not only was independent fissioning required, but fission of several chromosomes of the basic-14 complement. Hayman and Martin nevertheless considered that:

> "The hypothesis of the conservation of this basic complement of 2n=14 seems more likely given our present knowledge than the hypothesis that in two highly disjunct populations containing diverse superfamilies chromosome fusion has arrived at a numerically and morphologically similar solution to the problems of chromosome number."
>
> Hayman, 1977.

The major objection to the concept of a 2n=14 basic complement for marsupials has been this necessary proposal of widespread chromosome fissioning. As discussed previously, Mullerian mechanisms of chromosomal rearrangement have been widely accepted and therefore fission has been regarded as unlikely, because it requires a dispensable or transitory donor chromosome. Mullerian fission is in fact a possibility in marsupials as potential donors in the guise of supernumerary chromosomes do occur in some marsupial complements (Hayman and Martin, 1965a; Hayman *et al.*, 1969). If non-Mullerian fission is allowed, the 2n=14-basic complement is obviously the best candidate for the putative form of the ancestral karyotype.

The X and Y chromosomes

Sex determination in marsupials is of the XX♀, XY♂; where the Y chromosome is believed to be male determining (Sharman *et al.*, 1970). One X chromosome in female marsupials is genetically inactive and late replicating and in macropods at least, this is the paternally derived

X chromosome (Sharman, 1971; Richardson *et al.*, 1971; Cooper *et al.*, 1971).

Ohno (1969) has indicated extensive homology of X chromosomes between mammals and in accordance with his view there are a number of X-linked genes in marsupials (Cooper *et al.*, 1977; Donald and Hope, personal communication) and these are known to be X-linked in eutherians. However, when the mean per cent lengths (corrected for nuclear DNA values) of the smallest X chromosomes (presumably Ohno's basic unit) of Australian marsupials and that of the X chromosomes of Man were compared, that of marsupials appeared smaller (Hayman and Martin, 1974).

X and Y chromosomes also show considerable size variation within Marsupialia. This may be largely due to changes in the amount of C-banding material (Hayman and Martin, 1974; Hayman and Rofe, 1977). When the G-banding pattern of the non-C-banding, asynchronously DNA-replicating region of different sized X chromosomes was compared, it was similar in several kangaroos and wallabies (Macropodidae). This pattern was also similar to that of the brush-tailed possum X chromosome (Phalangeridae), but different to that of Dasyuridae (Hayman and Rofe, 1977).

Pathways of chromosomal evolution

With the 2n=14 basic complement as the starting point, Hayman and Martin (1974) have proposed pathways of chromosomal evolution for all known marsupial complements. Where these pathways are specifically relevant to the research of this thesis they are described in detail in later chapters. Following fission of the 2n=14 basic complement, the main evolutionary changes proposed were centric fusion, pericentric inversion and changes in DNA content. The distribution of chromosome numbers in marsupials was accounted for by proposing that acrocentricity of pairs 5 and 6 of the 2n=14 basic complement prevented the fission of

these chromosomes in most instances.

The pathways of chromosomal evolution that Hayman and Martin have proposed on the basis of the 2n=14 basic complement and their other specific hypotheses of karyotypic evolution in marsupials, are, according to Kirsch (1977a), compatible with phylogenies inferred from the entirely different type of information provided by serology and dentition. He believes this correspondence to be

> "the most convincing argument that 2n=14 is primitive"

and believes that

> "if 2n=14 is *not* the ancestral state it is then impossible to construct any sort of reasonable phylogeny."

Chromosome banding studies

Apart from the C-, N- and G-banding studies (Hayman and Martin, 1974; Hayman and Rofe, 1977) referred to in previous sections, there have also been a number of banding studies of the chromosomes of isolated species of marsupials (Pearson *et al.*, 1971; Grewal *et al.*, 1971; Sinha *et al.*, 1972; Sinha and Kakati, 1976; Brown and Cohen, 1973; Yunis *et al.*, 1973; Curcuru-Giordano *et al.*, 1974; Dunsmuir, 1976; Murray, 1977; Venolia, 1977). Where these reports are relevant to the work of this thesis they are discussed in later chapters.

CHAPTER 3

THE SCOPE OF THE THESIS

The chromosomes of Australian marsupials are ideal for a G-banding study of karyotypic evolution; diploid chromosome number is low, the chromosomes are large and there is considerable karyotypic diversity. Most importantly there is the specific and controversial hypothesis of a "2n=14 basic complement" and its implications for the directionality of Robertsonian change in these marsupials, to be evaluated with G-banding.

The research of this thesis was initially undertaken to see if G-banding homology could be used to define karyotypic differences in Australian marsupials. If so, the primary aim was to examine the "2n=14 basic complement" proposal by comparing the G-banding patterns of such complements possessed by diverse species of marsupials. If these complements are similar because they have retained essentially ancestral format, it is possible that this will be reflected by their G-banding patterns.

As will be evident in the following presentation of results, G-banding can be used to trace pathways of chromosome evolution in Australian marsupials. The species studied in this thesis are listed in Table 3.1.

The chromosomes of fourteen species of marsupials with 2n=14 basic complements were G-banded and compared. These marsupials are from at least two genera of each of four taxonomic families; Dasyuridae (marsupial cats and mice), Peramelidae (bandicoots), Vombatidae (wombats), and Burramyidae (pygmy phalangers). Each of these families represents one of the four major superfamilies of Australian marsupials, Dasyuroidea,

Table 3.1

The species studied.

SUPERFAMILY	FAMILY	SPECIES	2n
Dasyuroidea			
	Dasyuridae		
		Ningaui species	14
		Sminthopsis crassicaudata (Gould, 1844)	14
		Antechinomys laniger (Gould, 1856)	14
		Dasyuroides burnei Spencer, 1896	14
		Dasyurus viverrinus (Shaw, 1800)	14
		Dasyurus hallucatus Gould, 1842	14
		Antechinus flavipes (Waterhouse, 1838)	14
		Planigale maculatus (Gould, 1851)	14
Perameloidea			
	Peramelidae		
		Isoodon obesulus (Shaw, 1797)	14
		Perameles nasuta Geoffroy, 1804	14
Phalangeroidea			
	Phalangeridae		
		Trichosurus vulpecula (Kerr, 1792)	20
	Burramyidae		
		Acrobates pygmaeus (Shaw, 1793)	14
		Cercartetus concinnus (Gould, 1845)	14
	Petauridae		
		Petaurus norfolcensis (Kerr, 1792)	22
	Macropodidae		
		Petrogale penicillata (Griffith, 1827)	22
		Thylogale billardierii (Desmarest, 1822)	22
		Macropus rufus (Desmarest, 1822)	20
		Macropus robustus Gould, 1841	16
		Macropus fuliginosus (Desmarest, 1817)	16
		Macropus giganteus Shaw, 1790	16
		Macropus eugenii (Desmarest, 1817)	16
		Macropus parryi (Bennett, 1835)	16
		Macropus rufogriseus (Desmarest, 1817)	16
		Wallabia bicolor (Desmarest, 1804)	10♀, 11♂
Vombatoidea			
	Vombatidae		
		Lasiorhinus latifrons (Owen, 1845)	14
		Vombatus ursinus (Shaw, 1800)	14

Perameloidea, Vombatoidea and Phalangeroidea, respectively. The two remaining superfamilies each consist of one living species (Table 2.1) and may be monotypic. These animals do not have 2n=14 chromosomes.

With the exception of another monotypic family, the Myrmecobiidae, or numbat (Table 2.1), every Australian family known to possess a 2n=14 basic chromosome complement was sampled in the G-banding study. Thus the species studied were representative of the taxonomic diversity of Australian marsupials with such complements.

One of the four major superfamilies of Australian marsupials, the Dasyuroidea, consists of animals known only to possess 2n=14 basic complements. In two other superfamilies, Perameloidea and Vombatoidea, 2n=14 basic complements predominate, with only one species of each superfamily being known to have alternative chromosome number. Thus most of karyotypic diversity of Australian marsupials is displayed by the Phalangeroidea (Table 2.1 and Figure 3.1).

The Phalangeroidea is also the only Australian superfamily in which 2n=22 complements (a favoured ancestral chromosome number) are presently known, and this number is found only in the families Macropodidae (kangaroos and wallabies) and Petauridae (ringtails and gliding phalangers) (see Figure 3.1). The 2n=22 chromosome complements of two macropods and one petaurid, and the 2n=20 complement of the brush-tailed possum, *Trichosurus vulpecula*, Phalangeridae, were examined in an attempt to assess the proposal of 2n=22 as the ancestral chromosome number for Australian marsupials.

As can also be seen from Figure 3.1, the complements of the family Macropodidae constitute most of the range in chromosome number of the Phalangeroidea and thus of all Australian marsupials. Therefore in addition to the two species with 2n=22 chromosomes, several macropods with other chromosome numbers were studied (Table 3.1). Specific

Figure 3.1

The distribution, by superfamily, of diploid chromosome number in Australian marsupials.

D	Dasyuroidea
V	Vombatoidea
P	Perameloidea
N	Notoryctoidea
T	Tarsipedoidea
Ph	Phalangeroidea

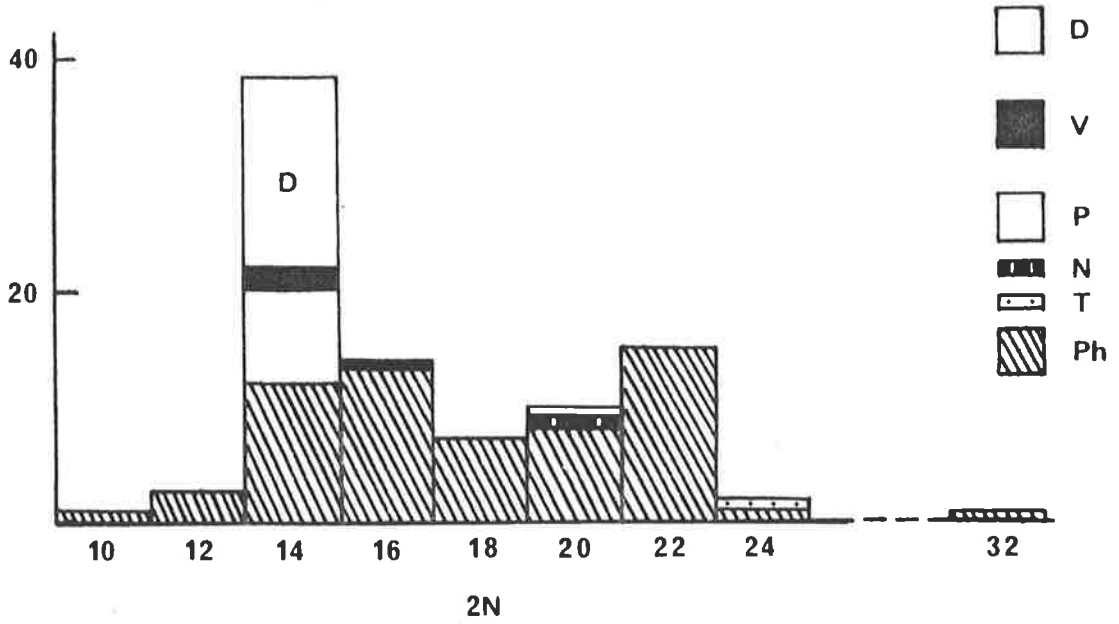
The distribution, by family, of diploid chromosome number in the superfamily Phalangeroidea.

B	Burramyidae
Pha	Phalangeridae
Pe	Petauridae
M	Macropodidae

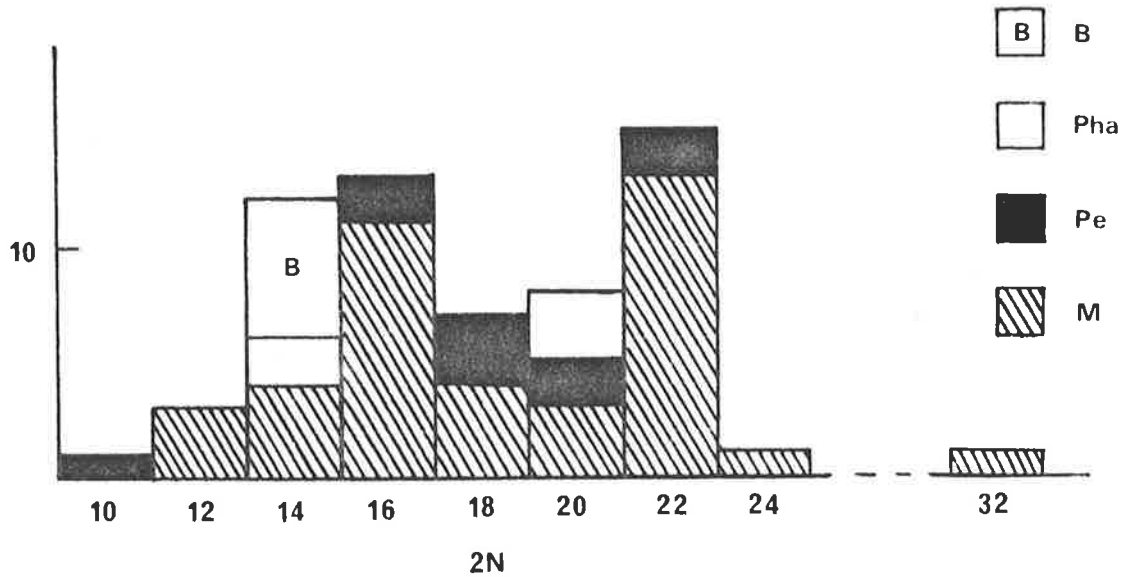
Marsupials with multiple sex chromosome systems have been allotted the chromosome number of the XX♀, XY♂ complement from which these can be regarded as derived.

X-axes diploid chromosome number; Y-axes number of species.

Aust sfs



Ph fs



relationships had been proposed for these macropodid complements (particularly Robertsonian differences attributable to fusions) and these were to be evaluated with G-banding. C- and N-banding patterns were also studied in this family, as nuclear DNA content, and the size and morphology of the sex chromosomes show quite substantial variation, even between species of kangaroos and wallabies that produce viable hybrid offspring.

The research outlined includes several comparisons of the G-banding patterns of complements with the same number of morphologically similar chromosomes. However, these are comparisons of chromosome homology at different taxonomic levels. Apart from the inter-superfamilial comparison of the 2n=14 basic complements, there is an intra-familial comparison of the 2n=14 basic complement of Dasyuridae (eight species) and an intra-generic comparison of the similar 2n=16 complements of *Macropus* species, Macropodidae (six species). Of the several families with 2n=14 basic complements, the family Dasyuridae was chosen for intensive study as animals were available from many of the several living genera of this family (Tables 2.1 and 3.1), and almost all dasyurid complements show extremely similar morphology after general chromosome staining. One of the objects of the G-banding study of the 2n=16 complements of *Macropus* species was to better define the minor differences between these complements that are observable after general chromosome staining.

The latter two studies of morphologically similar chromosome complements were also undertaken partly to serve as reference studies for the inter-superfamilial comparison of the G-banded 2n=14 basic complements.

CHAPTER 4

MATERIALS AND METHODS

4.1 Cell culture

Chromosomes for banding analysis were obtained from *in vitro* culture of marsupial cells. Unless otherwise indicated, cultures were initiated and chromosome preparations made by the author.

Fibroblast cultures

Primary cultures were routinely initiated using skin biopsies from the ear or groin of the animal. The cells of the resulting cultures were fibroblastic in general appearance and will be simply referred to as "fibroblasts". The following procedure is that adopted for culture initiation, and is chiefly that of Commonwealth Serum Laboratories (C.S.L.).

(a) If necessary, remove hair from the sample area. Swab with 70% alcohol. Allow to dry.

(b) Use sterile scissors or an ear punch, excise the tissue and collect into Eagles Basal Medium (BME) supplemented with

  (i) anti-fungal, mycostatin (Squibb Nystatin B.P.) 500 units per ml,

  (ii) anti-bacterial, Benzyl penicillin (C.S.L.) 100 units per ml, Gentamicin sulphate (Roussel Pharmaceuticals), 16 units per ml.

(c) Within two hours transfer the tissue to BME with a ten-fold concentration of antifungal and antibacterial agents. Mince the tissue coarsely with scissors, and wash the pieces through approximately six fresh BME solutions. Finally, mince to

pieces small enough to pass up the bore of a thirty drop per ml Pasteur pipette.

(d) Remove the medium and add 0.5 ml of chicken embryo extract, CEE (lyophilized CEE (C.S.L.) dissolved in 5 ml of BME) to the pieces of tissue. Then spread the pieces over the surface of a previously scratched glass culture bottle. Remove the excess fluid.

(e) Add 0.25 ml of chicken plasma (lyophilized chicken plasma (C.S.L.) plus 2.5 ml of BME) dropwise to the pieces. Rewash the pieces drop by drop with this plasma two or three times.

(f) Remove the excess fluid, incubate at 37°C for 30 minutes.

(g) Remove the excess fluid, being careful not to disturb the clot. Add complete growth medium (BME plus 15% foetal calf serum (FCS)). Return to the 37°C incubator.

Cells were usually grown in 10 ml of medium (BME plus 15% FCS) in Faulding 'Falco' glass baby bottles placed in an incubator at 37°C. When necessary the pH of the medium was adjusted to near neutral with 5% CO_2 in medical air. Trypsin versene (C.S.L.) was used to disperse cell sheets before subculture or chromosome preparation. Cells were frozen in 2 ml of a solution comprising 70% BME, 20% FCS and 10% DMSO (dimethylsulphoxide) and stored in liquid nitrogen.

A cell line of *Macropus parryi*, female, was obtained from the Commonwealth Serum Laboratories. Professor D.W. Cooper supplied the cell culture of *Macropus parryi*, male, and Dr. J.A. Marshall Graves that of the female *Macropus giganteus*.

Short-term lymphocyte cultures

Lymphocytes of a number of macropodid and other marsupials were cultured by a method essentially that of Moorhead *et al.* (1960). Blood was obtained either by venous-caudal or cardiac puncture. The plasma and lymphocyte layer were collected after either

(a) allowing the heparinized blood to settle naturally

or (b) adding dextran (5% v/v) to facilitate separation

or (c) spinning at low speed in a bench centrifuge.

In some cases lymphocytes were collected after density gradient separation of diluted blood in a Ficoll/Hypaque mixture (Coghlan and Hope, unpublished method). The resultant lymphocyte band was washed well with phosphate buffered saline (PBS) and added to culture bottles.

Five millilitres of medium 199, 1640 or F10 each supplemented with 20% FCS, 0.05 ml of PHA-M (Difco-Phytohaemagglutinin form M) and 0.02 ml of preservative free heparin (C.S.L. Heparin-Injection 1000 units/ml) was used as culture medium. One half to one millilitre of cell-containing plasma or PBS was added to each culture bottle. Cells were grown at 37°C for approximately 72 hours.

4.2 Chromosome preparation

For fibroblast cultures, the preparation of fixed mitotic cells was attempted as soon as a sample of the culture had been frozen in liquid nitrogen and the cells were known to be viable when recovered. Culture samples were frozen as early in the history of the culture as possible.

Depending on culture growth rate, colchicine was added for one half to two hours prior to harvest. Amounts of colchicine varied from 0.02 ml of 0.002% weight of colchicine per volume of water (w/v) to 0.05 ml of 0.02% w/v per 10 ml of culture medium. Some cell cultures (particularly the fibroblasts of the bandicoot species) were markedly colchicine sensitive,

and only very limited exposure avoided extensive endoreduplication of metaphase chromosomes.

Cell suspensions (lymphocyte cultures or dispersed fibroblast monolayers) were centrifuged. The resultant cell pellet was resuspended in 0.075M KCL at 37°C. Fibroblasts required 30 minutes in hypertonic KCL, lymphocytes 10 to 15 minutes. Cells were fixed in freshly prepared 3:1 methanol:acetic acid. Slide preparations were made for silver N-banding after 15 minutes, and for other purposes after three changes of fixative and 4 to 30 hours fixation. Cells suspended in fresh fixative were added dropwise to very clean, dry slides. Slides were stored at room temperature until required.

Dr. D.L. Hayman provided chromosome preparations from the lymphocytes of many macropodid marsupials and Dr. P.R. Baverstock and M. Gelder, Dr. R.M. Hope and Professor G.B. Sharman fixed cells of *Petaurus norfolcensis*, *Macropus rufus* and *Petrogale penicillata* respectively.

4.3 Chromosome banding techniques

Good banding (clear, high contrast) was dependent on the quality (high mitotic index and good fixation particularly) and age of the chromosome preparation.

G-banding

For detailed patterns, extended chromosomes were necessary. Cell cultures therefore received limited exposure to colchicine.

The trypsin-banding method of Seabright (1971), for human chromosomes, was used to produce G-bands on marsupial chromosomes. A minor modification of the method was a further ten-fold dilution of the trypsin stock solution with Sorenson's phosphate buffer, pH 7.0.

The trypsin treatment time was 10 to 120 seconds at room temperature and chromosomes were stained with 2 to 10% v/v Gurr's R66 Giemsa stock

solution in Sorenson's phosphate buffer (pH 7.0) for 3 to 20 minutes.

Most chromosomes remained very sensitive to trypsin treatment for several weeks after slide preparation. Treating immature preparations, even for short periods, resulted in fuzzy or bloated, poorly banded, chromosomes. The age of a lymphocyte preparation for optimal G-banding was variable; however most fibroblast preparations banded best after two to six months. Chromosomes from fibroblast cultures could always be satisfactorily G-banded, but good banding of lymphocyte chromosomes could be achieved only intermittently.

C-banding

The method (RNA-ase step omitted) of Arrighi and Hsu (1971) with sodium hydroxide, or that of Sumner (1972) using barium hydroxide, was used to C-band marsupial chromosomes. In general, to achieve optimal C-banding, chromosome preparations from fibroblast cultures required less ageing than lymphocytes. However, each preparation required independent evaluation as the best results were sometimes obtained within the first three days, and other times only after several months.

N-banding

Marsupial chromosomes were N-banded by three methods. Two of these involved hot acid treatment; hot aqueous trichloro-acetic acid (5% by weight of TCA) followed by dilute HCl (Matsui and Sasaki, 1973), or hot acidic phosphate solution (Funaki *et al.*, 1975). Chromosomes were then stained with 10% v/v Gurr's R66 Giemsa in pH 7.0 Sorenson's phosphate buffer.

The third N-banding method was the "Ag-I method" of Bloom and Goodpasture (1976), which is simply the treatment of slide preparations with 50% w/v of silver nitrate ($AgNO_3$) in water. N-banding using $AgNO_3$ required 12 to 72 hours at 37°C and chromosomes were often counter-stained for 10

to 60 seconds with 10% R66 (v/v) in Sorenson's phosphate buffer. Cells fixed only briefly before slide preparation showed a greater proportion of N-banded chromosome complements. This treatment with $AgNO_3$ solution was preferable to the hot acidic recipes for inducing N-bands, as the chromosomes retained good morphology.

4.4 The analysis and presentation of the results

At least 15 cells in which each chromosome was clearly visible were routinely recorded per banding technique per animal. Well banded cells were photographed with Agfa-Gaevert Copex Pan 35 mm high contrast film using a 100X oil immersion objective. The film was developed with a fine grain developer, Kodak D11, and prints were made at a standard magnification on Ilfospeed photographic paper.

G-banding analysis

For each species a large number (usually more than thirty) of cells with G-banded chromosomes were photographed and printed and the G-banding patterns analysed as follows:

Several cells each with a complete set of non-overlapping G-banded chromosomes were karyotyped. In every well-banded complement each autosomal pair and each type of sex chromosome could be distinguished by G-banding pattern alone or by a combination of this and size.

For each of these chromosome pairs or chromosomes, well banded and more extended chromosomes with that particular pattern were then cut from prints of 10 to 30 cells. These chromosomes were aligned to provide a measure of the inherent variation (presumably the result of differential contraction and staining) of the pattern of that pair (or chromosome).

A chart was then constructed in which each autosomal pair and each type of sex chromosome was represented by such a line of G-banded chromosomes.

As is obvious from the karyotypes and charts presented in the following chapters, the G-banding pattern of each particular pair or chromosome is reproducible and characteristic and such charts are a much better representation of the G-banded chromosome complement of a species than one or several individual karyotypes.

They are also a much better basis for the comparison of the G-banding patterns of two species than one or several karyotypes of each, as chromosome preparations may differ in pattern display due to chromosome contraction and staining response. Allowance can very easily be made for such variation using the charts (for example, the longer chromosomes of one chart may be compared with the shorter chromosomes of the other).

Thus the resolution of karyotypic differences is much greater when such charts are used instead of a small number of G-banding karyotypes.

For the G-banding patterns of chromosome regions of two different species to be considered homologous, the pattern differences between one such region in one species and any analogous segment in the other, were of the same order of magnitude as the intraspecific variation within either row of these two chromosome segments. This method of karyotype analysis clearly and consistently revealed G-banding pattern homologies.

The presentation of the results

The results of the chromosome banding studies are presented by taxonomic families and, where appropriate, intra-familial chromosome evolution is discussed in the same section. The G-banded 2n=14 basic complements are shown first, followed by their comparison (Chapter 5). Chromosome evolution in Macropodidae is then considered (Chapter 6) followed by the presentation of G-banding karyotypes from two further phalangeroid families (Chapter 7). Chromosome evolution in the super-family Phalangeroidea is then examined (Chapter 8, comparing results from Chapters 5.4, 6 and 7), and finally the implications of the present

G-banding data for chromosome evolution in Australian marsupials as a whole (Chapter 9).

Each section of results is introduced by a brief description of the taxonomic composition and cytogenetics of the particular group of marsupials to be considered. In these introductions the numbers of taxa are taken from Kirsch and Calaby, 1977; the cytological details are to be found in Sharman, 1973; Hayman and Martin, 1974; and the chromosome and nuclear DNA measurements are those of Hayman and Martin (1974).

Generally one G-banding karyotype and chart is presented per species and these are of the chromosomes of one individual. Chromosomes have been numbered according to the measurements of Hayman and Martin (1974). Any departures from this are indicated in the text.

For the preparation of figures in which inter-specific G-banding pattern homologies are illustrated only chromosomes of average contraction were used, as many of the 'land-mark' features of contracted chromosomes are no longer conspicuous in very extended chromosomes.

CHAPTER 5

THE G-BANDED "2n=14 BASIC" CHROMOSOME COMPLEMENTS

The marsupial species with 2n=14 basic complements that were studied with G-banding are listed together with the sex of the animals and their source localities in Table 5.1. All chromosome preparations were made from fibroblast cultures.

5.1 Chromosome evolution in Dasyuridae, marsupial cats and mice

Of the forty-nine listed species (involving fourteen genera) of dasyurids, fifteen (from nine genera) have been studied cytologically with a general chromosome stain. All (but the *Ningaui* sp. for which only chromosome number has been published) have morphologically similar 2n=14 chromosome complements. This high degree of karyotypic homogeneity has been emphasized by the measurements of the general stained chromosomes of eleven dasyurid species (seven genera). For seven of these eleven (six genera) nuclear DNA content was also measured and the values were very similar. Only slight deviations of four chromosomes of these seven species from a postulated "basic dasyurid karyotype" (defined by the chromosome measurements) were observed and these were proposed to be due to intra-chromosomal rearrangements (Hayman and Martin, 1974).

To see whether this extreme karyotypic constancy extended to G-banding pattern homologies, the chromosomes of eight dasyurid species (seven genera) were G-banded. The species studied (see Table 5.1) are representative of the taxonomic diversity of the group.

Table 5.1

The species possessing "2n=14 basic" complements that were studied with G-banding.

FAMILY	SPECIES	COMMON NAMES	SEX	SOURCE
Dasyuridae		marsupial cats and mice		
	Ningaui sp.	a marsupial mouse	♂	Billiatt Conservation Park, South Australia.
	Sminthopsis crassicaudata	the fat-tailed marsupial mouse	♂	Taplan, South Australia, stock.
	Antechinomys laniger	the jerboa marsupial mouse	♂	Beetoota, Queensland.
	Dasyuroides burnei	Byrne's pouched mouse	♂	Coorabulka, Queensland, stock.
	Dasyurus viverrinus	the eastern-native cat	♂	Lake Leake, Tasmania.
	Dasyurus hallucatus	the little northern native cat	♀	Groote Eylandt, Northern Territory.
	Antechinus flavipes	the yellow-footed marsupial mouse	♀	Norton Summit, South Australia.
	Planigale maculatus	the pygmy marsupial mouse	♂	Humpty Doo, Northern Territory, stock.
Peramelidae		bandicoots		
	Isoodon obesulus	the short-nosed bandicoot	♀	Mt. Gambier, South Australia.
	Perameles nasuta	the long-nosed bandicoot	♂	Sydney environs, New South Wales.
Vombatidae		wombats		
	Lasiorhinus latifrons	the hairy-nosed wombat	♀	Blanchetown region, South Australia
	Vombatus ursinus	the common wombat	♀	South-eastern Tasmania.
Burramyidae		pygmy phalangers		
	Acrobates pygmaeus	the pygmy glider	♀	Langkoop, Victoria.
	Cercartetus concinnus	the pygmy possum	♂	West-central Eyre Peninsula, South Australia.

The G-banded chromosomes

Figure 5.1 *N-Pm* are the G-banding karyotypes, and Figures 5.2 to 5.9 the G-banding charts, for each dasyurid species studied.

The general stained chromosomes of six of these species have previously been measured and pairs 2 and 6 were exactly metacentric. In all G-banded complements the two arms of these chromosomes could be easily distinguished and one arm with a particular banding pattern is consistently shown as the short arm in karyotypes and charts.

The karyotypes of *Planagale maculatus*, Figure 5.1 *Pm*, and the as yet unnamed *Ningaui* species, Figure 5.1 *N*, are newly described. That of the *Ningaui* sp. is a novel dasyurid karyotype, as of the karyotypically known dasyurids only this species possesses a submetacentric chromosome pair 6, all others have a metacentric pair 6.

As can be seen from the karyotypes and charts, for each species every chromosome pair has a different and distinctive G-banding pattern. There is a secondary constriction near the end of the short arm in general stained pair 5 chromosomes of all these dasyurids, and this region is visible as an attenuation in some G-banded chromosomes. The Y chromosomes are extremely small. They were missing from some cells karyotyped and have been omitted from the charts, but are shown in Figure 5.10.

The G-banding patterns of the chromosomes of these dasyurid species were compared and the results are illustrated in Figure 5.10. For each species each G-banded autosomal pair and the sex chromosomes are represented in this figure. These chromosomes are aligned with those of the same pair number from the other seven species. Each chromosome of any one species has a single, similarly sized chromosome with a corresponding G-banding pattern in all other species and there is detailed and exact pattern homology of almost all chromosome regions between all species. Only the chromosome pairs 6 of the *Ningaui* sp. and *Antechinomys laniger* (marked with *a* and *n* respectively in Figure 5.10) differ from each

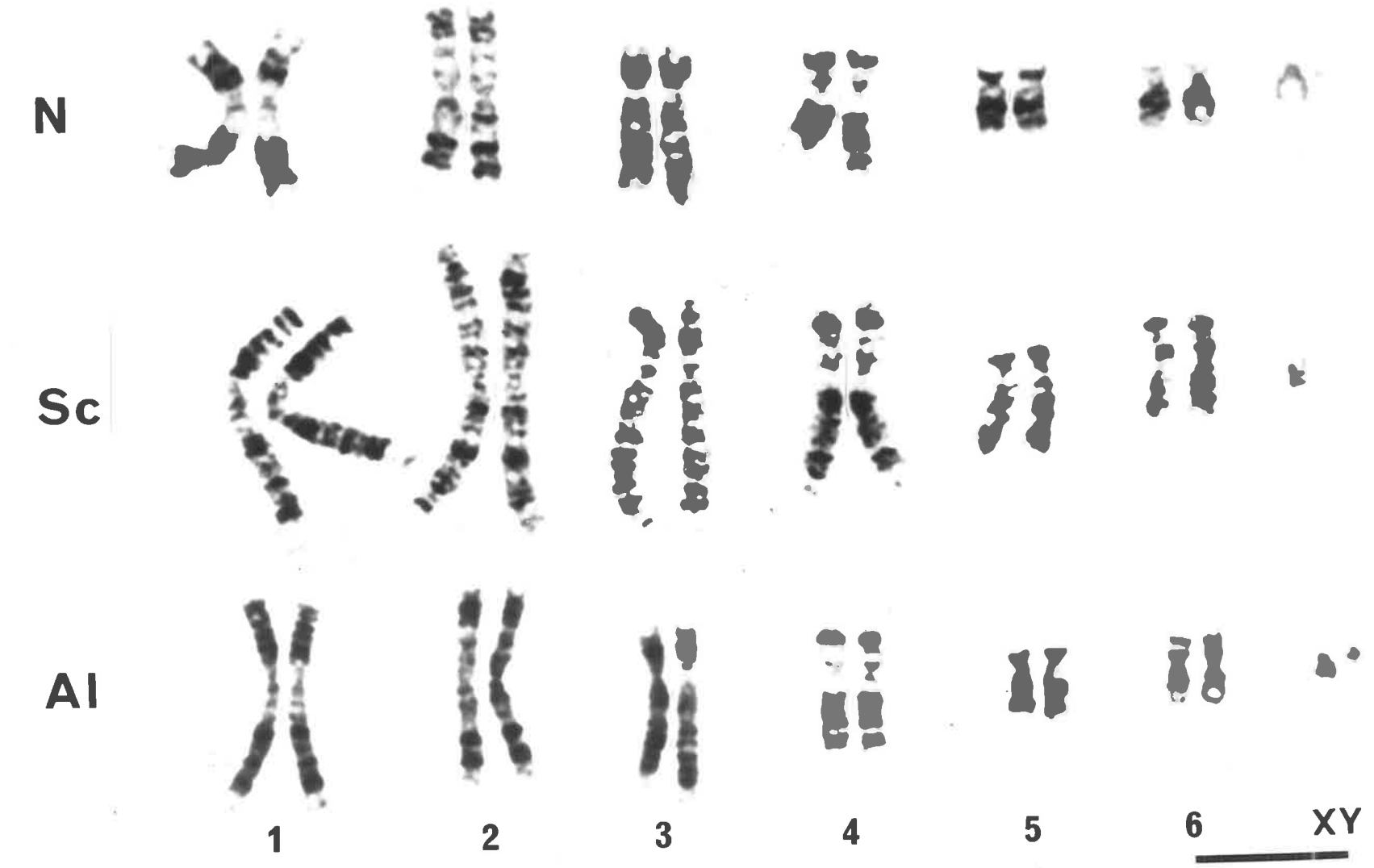
Figure 5.1

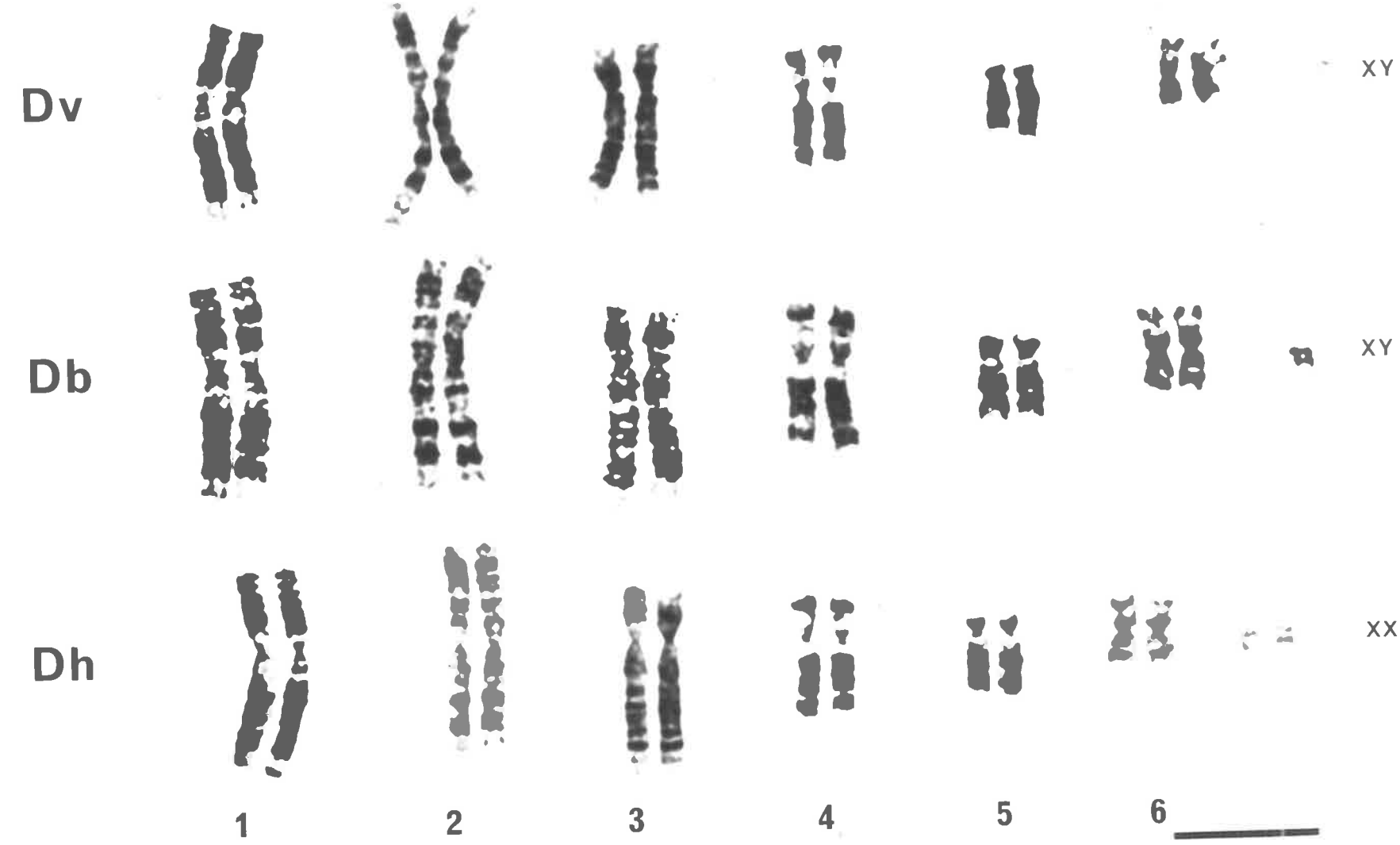
The G-banding karyotypes of dasyurids.

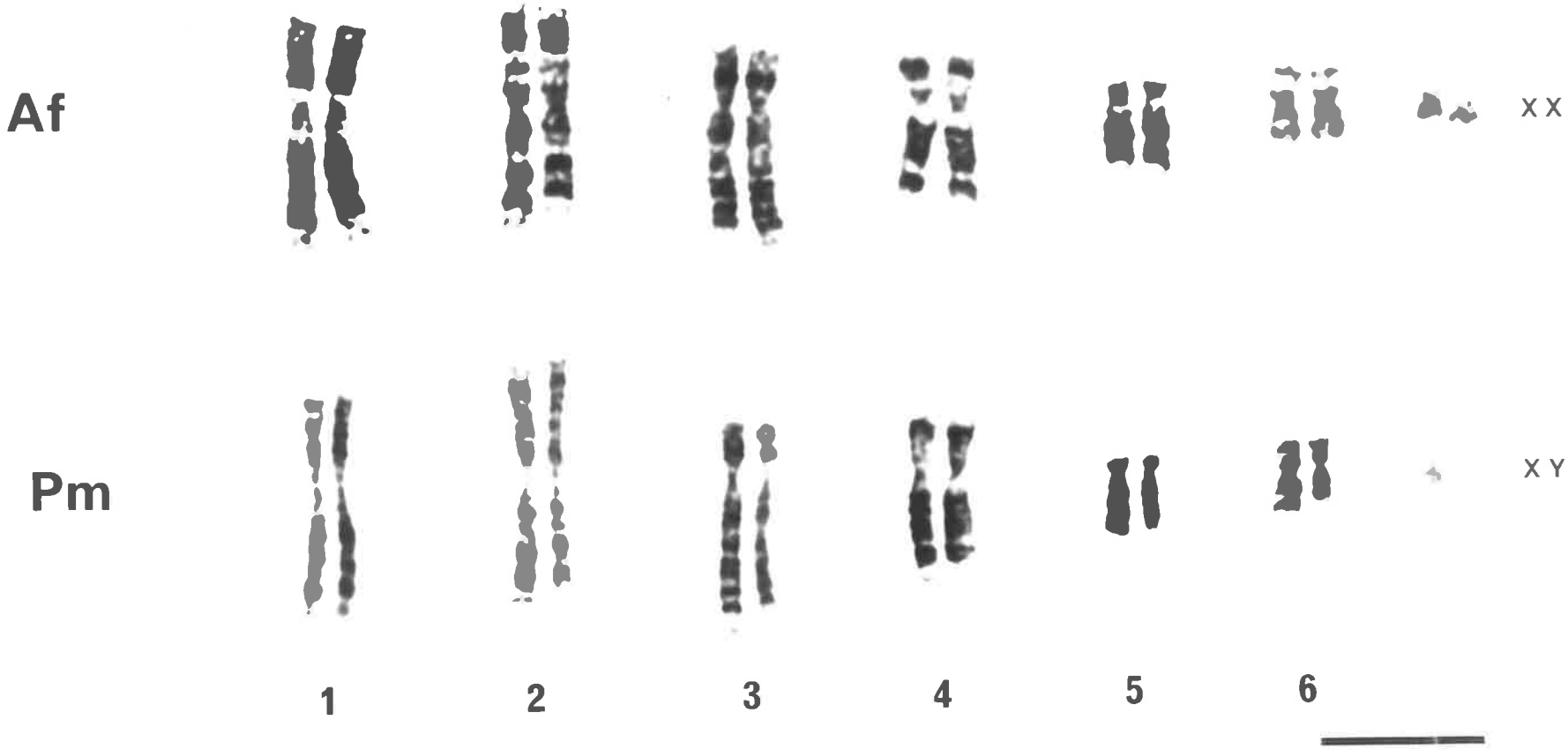
N	*Ningaui* sp.
Sc	*Sminthopsis crassicaudata*
Al	*Antechinomys laniger*
Dv	*Dasyurus viverrinus*
Db	*Dasyuroides burnei*
Dh	*Dasyurus hallucatus*
Af	*Antechinus flavipes*
Pm	*Planagale maculatus*

Where Y chromosomes are missing from the karyotypes of male dasyurids, G-banded representatives are shown in Figure 5.10.

In this and all following figures the bar line indicates ten microns (10×10^{-6} metres).


N
Sc
Al
1
2
3
4
5
6
XY


Dv
Db
Dh
XY
XY
XX
1
2
3
4
5
6


Af
Pm
XX
XY
1
2
3
4
5
6

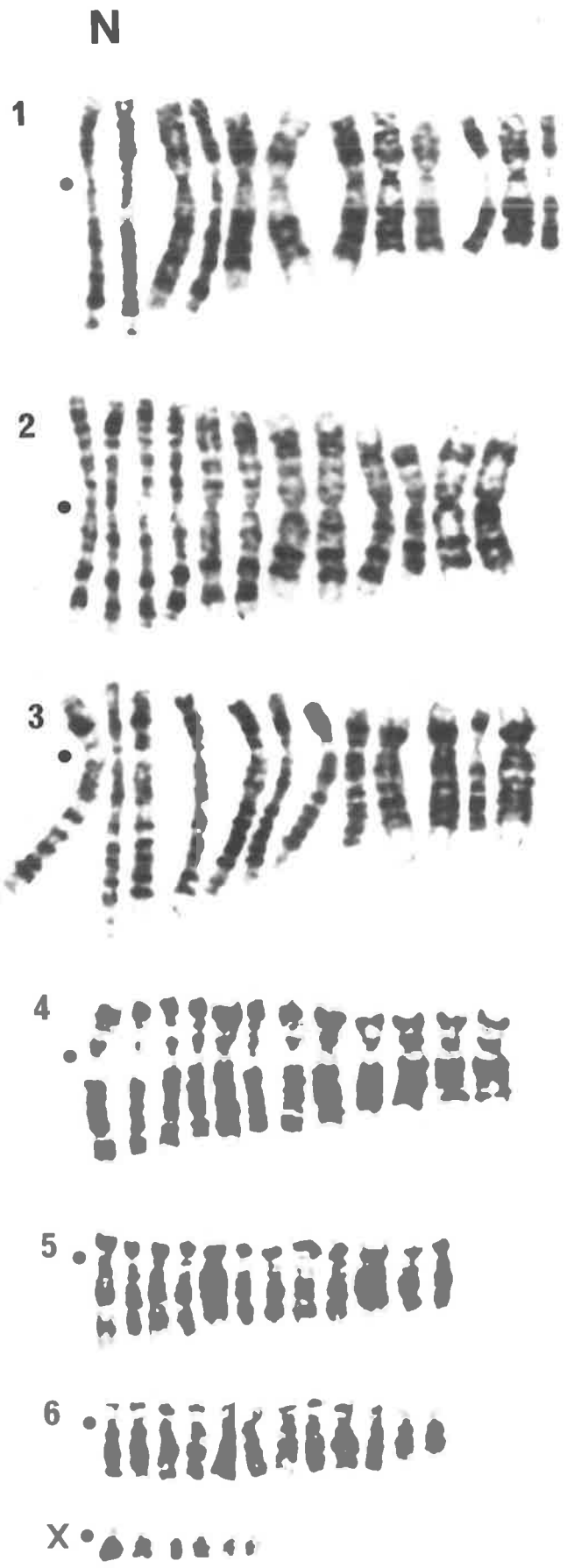
Figures 5.2 to 5.9

The G-banding charts for dasyurids.

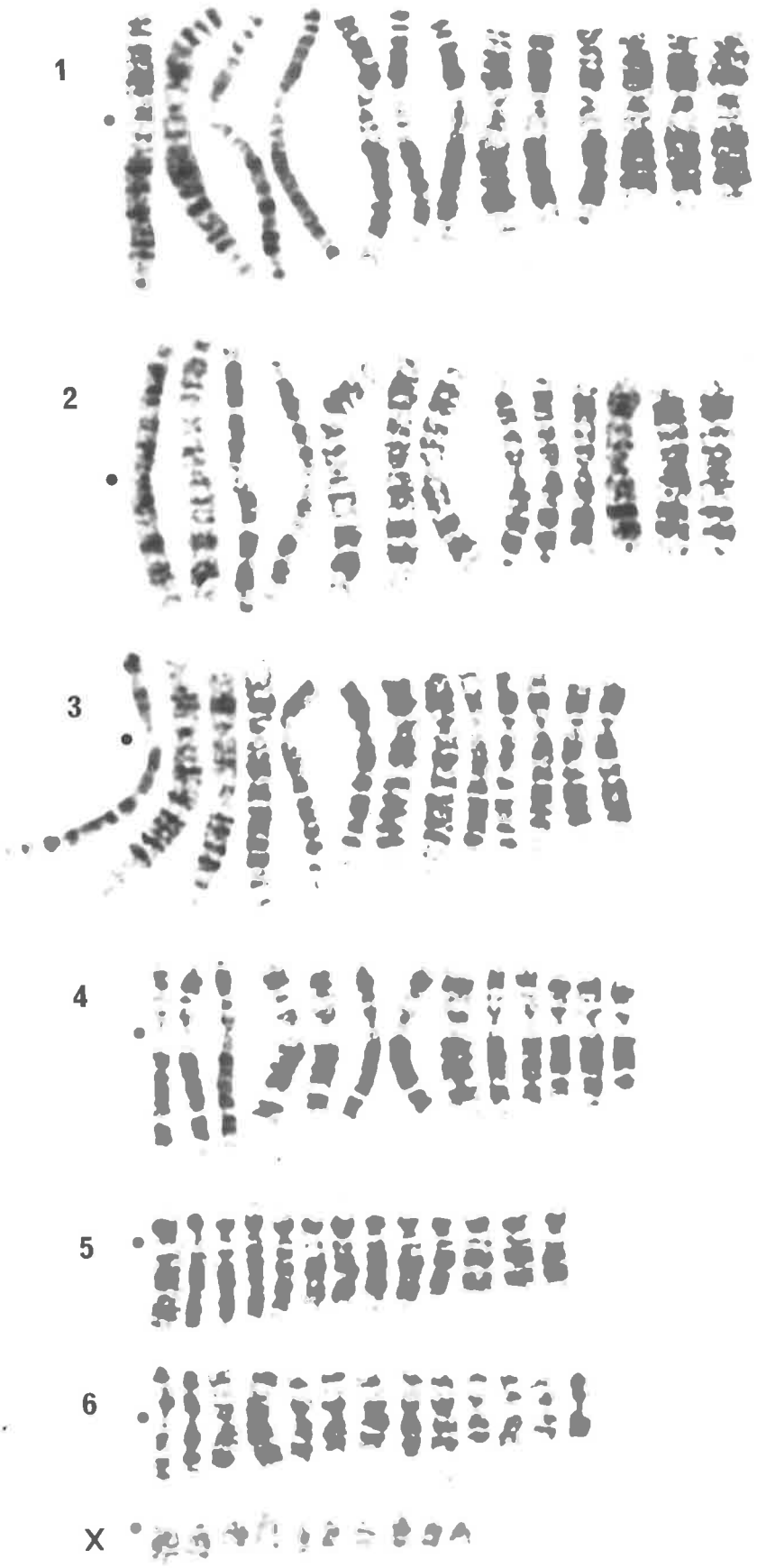
5.2	*N*	*Ningaui* sp.
5.3	*Sc*	*Sminthopsis crassicaudata*
5.4	*Al*	*Antechinomys laniger*
5.5	*Db*	*Dasyuroides burnei*
5.6	*Dv*	*Dasyurus viverrinus*
5.7	*Dh*	*Dasyurus hallucatus*
5.8	*Af*	*Antechinus flavipes*
5.9	*Pm*	*Planagale maculatus*

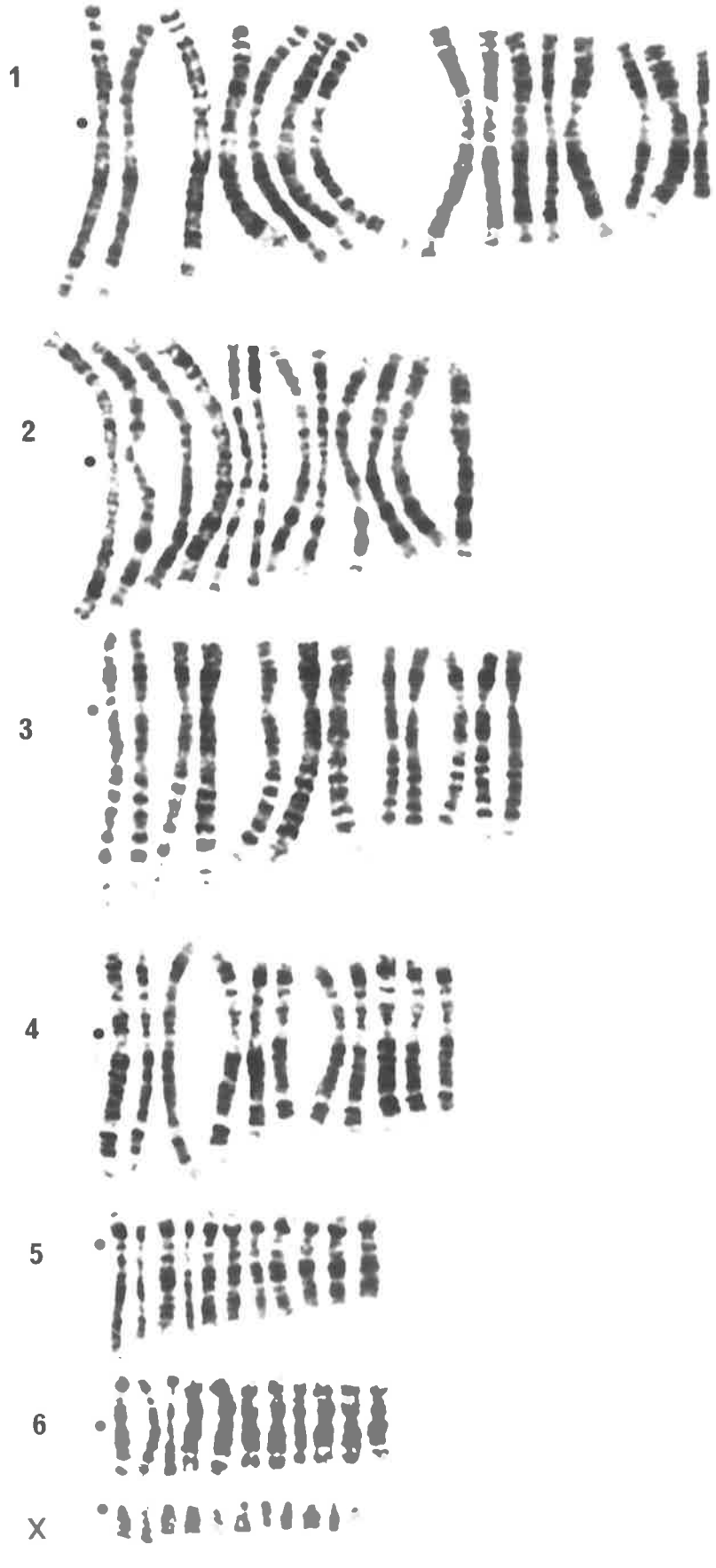
Where Y chromosomes are missing from the charts of male dasyurids, G-banded representatives are shown in Figure 5.10.

In this and all following G-banding charts centromere positions are indicated by dots.

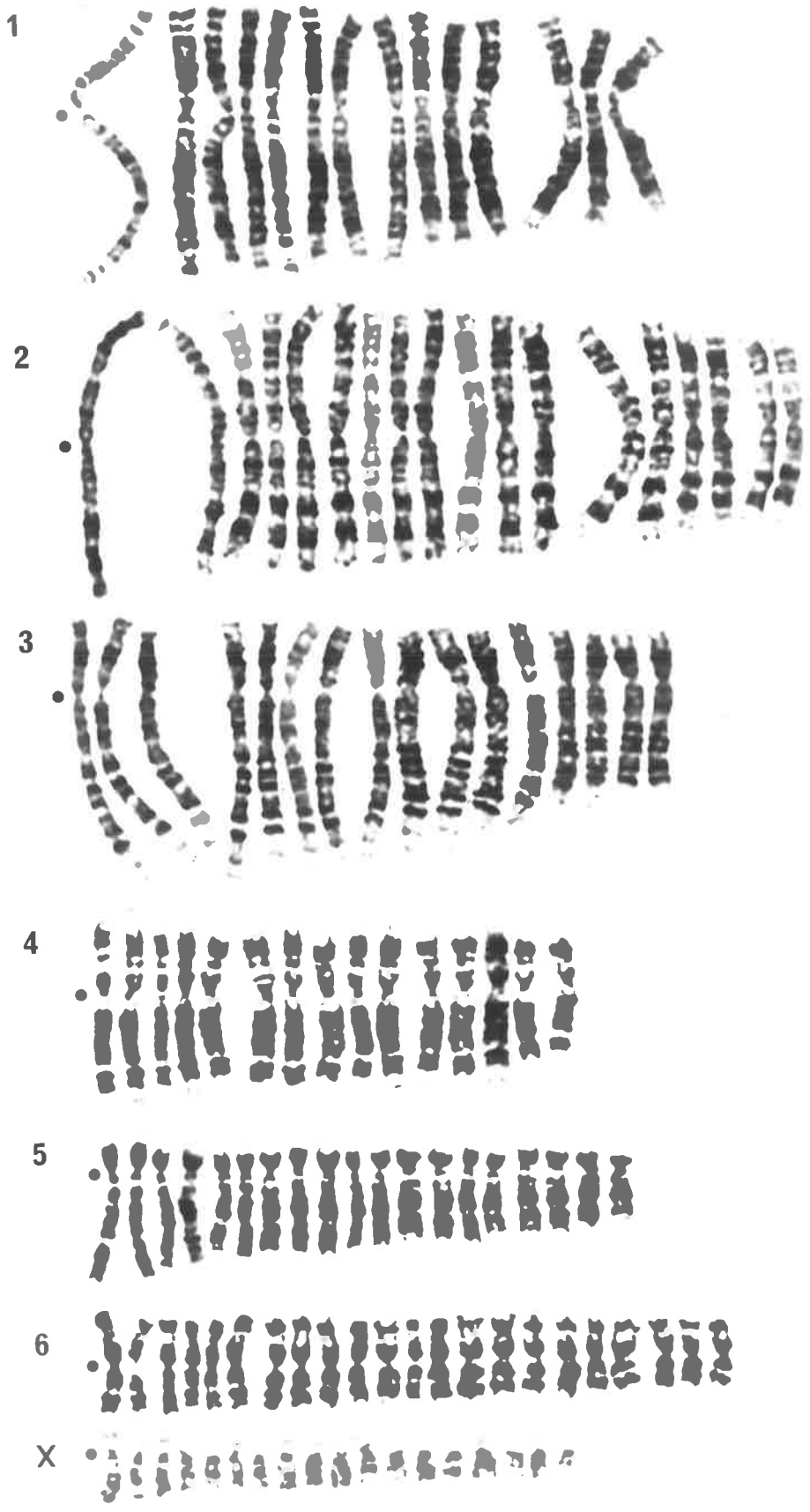

N
1
2
3
4
5
6
X

Sc



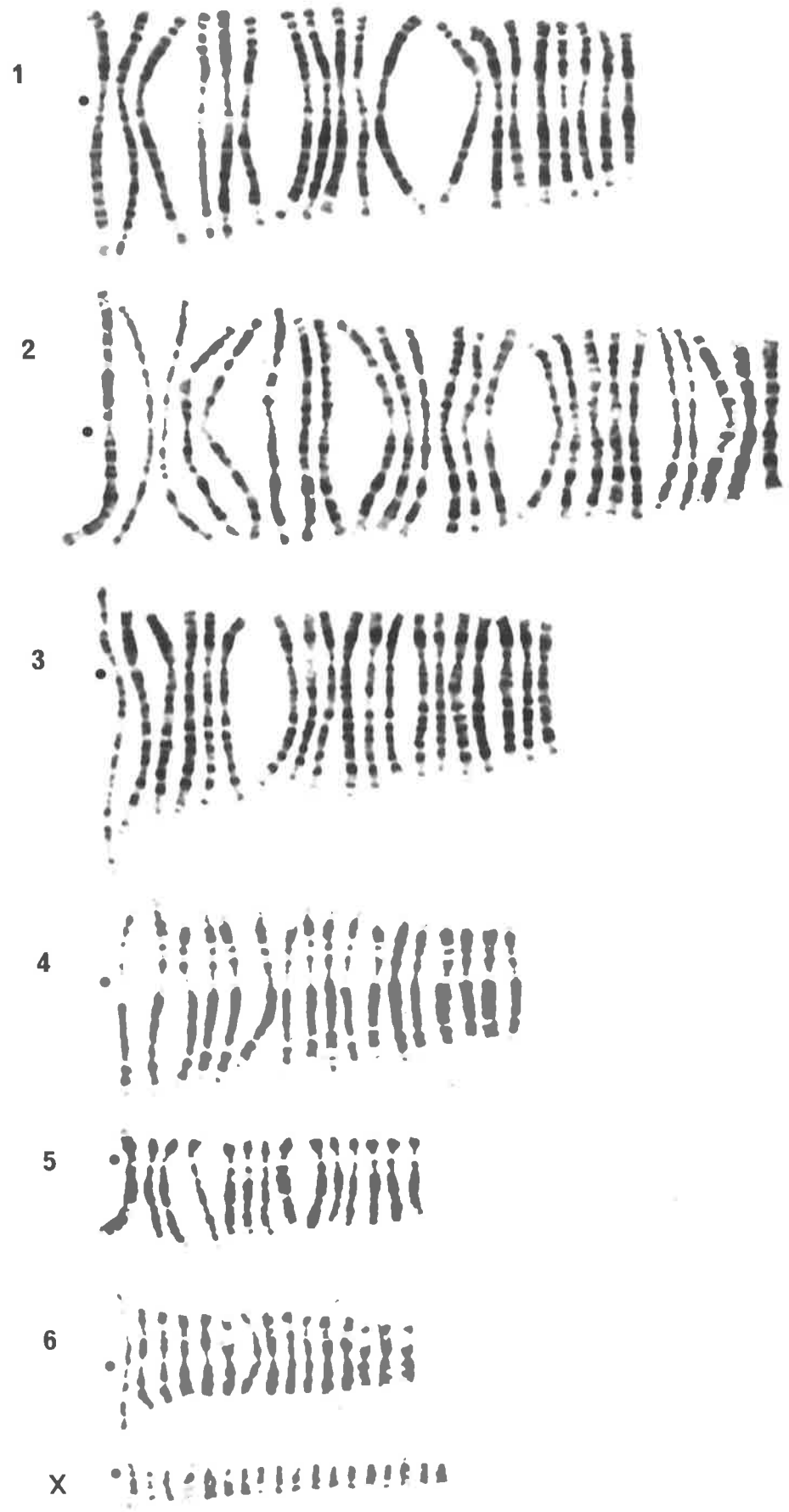

AI
1
2
3
4
5
6
X

Db

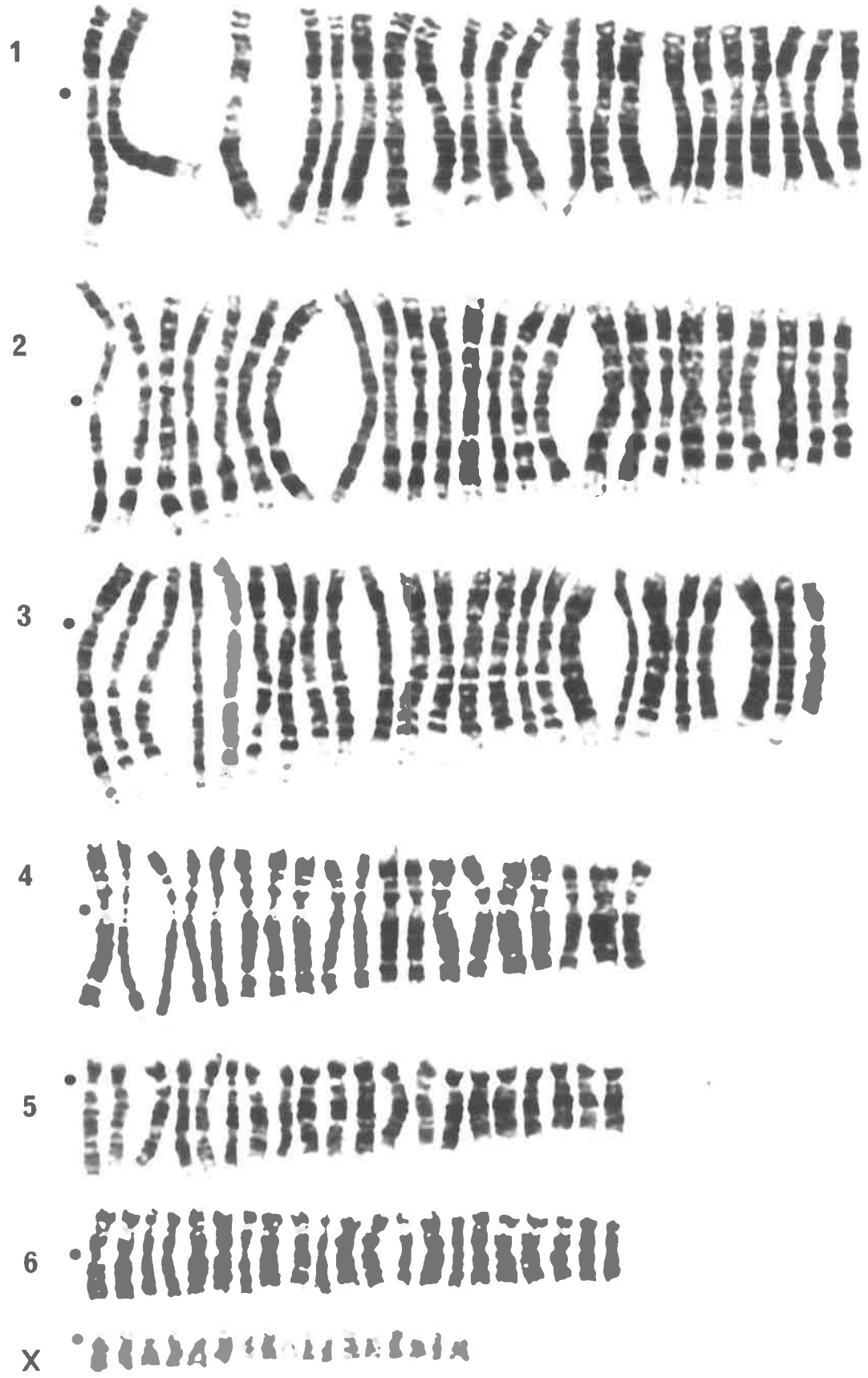


Dv

Dh



Af



Pm

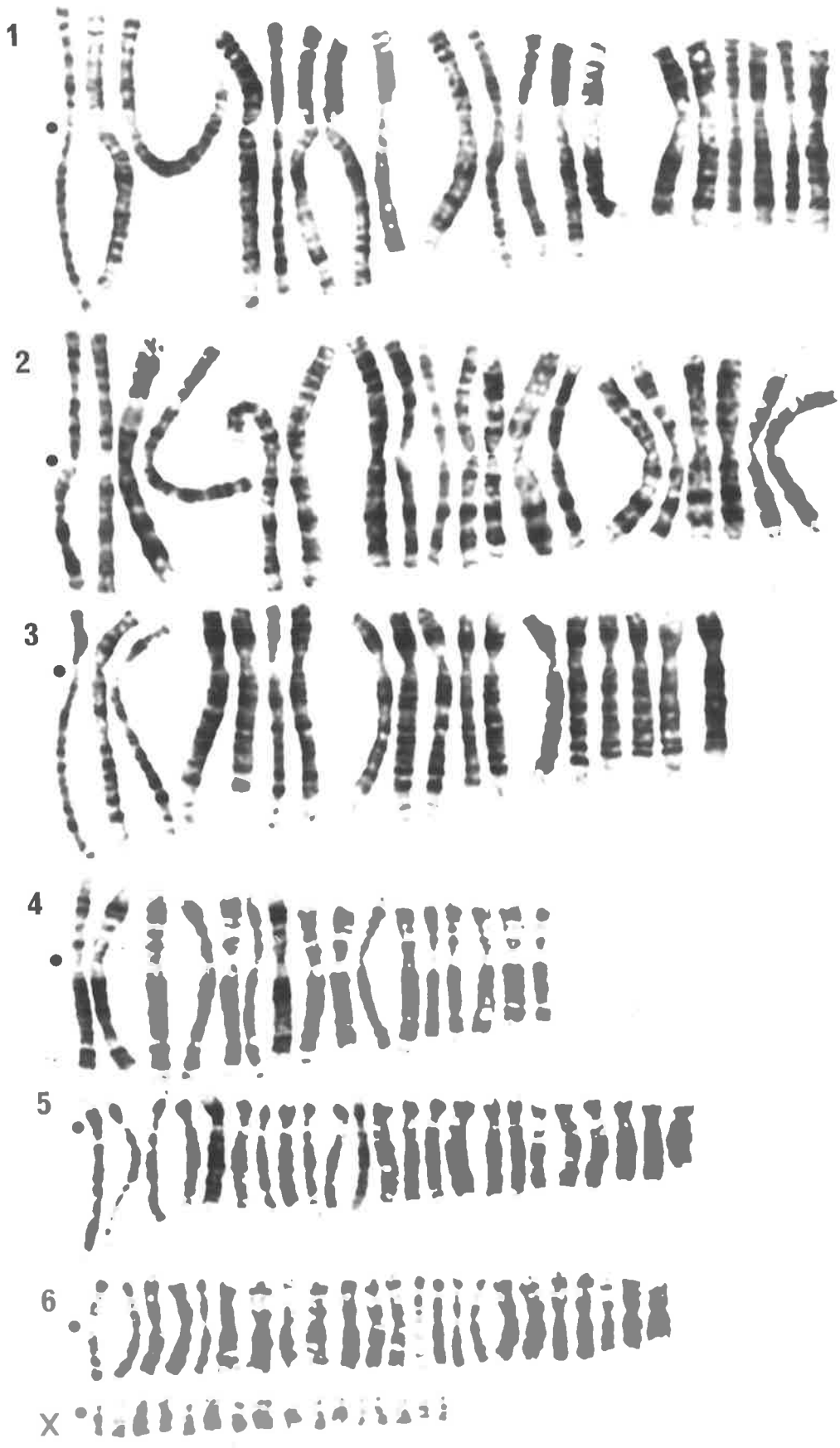


Figure 5.10

The G-banded dasyurid chromosomes.

For each species a single G-banded representative of each autosomal pair and of the X and Y (if the specimen is male) chromosome aligned with the chromosomes of corresponding size and arm ratio in the other seven species. Chromosomes marked with *n* and *a* deviate from the most common G-banding pattern.

Centromere positions are marked with dots.

N	*Ningaui* sp.
Sc	*Sminthopsis crassicaudata*
Al	*Antechinomys laniger*
Db	*Dasyuroides burnei*
Dv	*Dasyurus viverrinus*
Dh	*Dasyurus hallucatus*
Af	*Antechinus flavipes*
Pm	*Planagale maculatus*


1
2
3
4
5
6
n
a
X
Y
N Sc Al Db Dv Dh Af Pm
N Sc Al Db Dv Dh Af Pm

other and the sixth chromosomes of all other species in G-banding pattern.

Thus all dasyurid species studied possess a basic G-banded karyotype (with the exception of chromosomes 6 of the *Ningaui* sp. and *A. laniger*) as defined by Figure 5.10 and the concept of a "basic dasyurid karyotype" based on measurements of general stained chromosomes can be extended to the proposal of "a basic G-banded dasyurid karyotype" of the type in Figure 5.10. The G-banding pattern of each chromosome of this basic G-banded karyotype is now described, with the 'land-mark' features of each presented first, and then the overall pattern.

Pair 1

The most distinctive G-banding features of this chromosome are the striped appearance of the distal quarter of the short arm (three clear dark bands with white interband areas), and a dark region in the long arm. In this latter 'land-mark' region there are four dark bands which are spaced approximately one-third, three-fifths and five-sixths of the arm's length from the centromere, with two dark bands marking the most distal position.

In the short arm a grey region occurs proximal to the striped 'land-mark' region, then a dark region with several bands, the most distal of these often being the darkest. A pale area follows, then a small band flanking the centromere.

The long arm of this chromosome, apart from the dark 'land-mark' region, is pale. The small band flanking the centromere is followed by a discrete band and an indistinct band in the pale area proximal to the 'land-mark' region. In extended chromosomes bands are often visible between the first and second, and between the second and third most proximal 'land-mark' bands. The distal end of the long arm is pale with two grey bands, the most distal being darker.

The extreme ends of both chromosome arms are unstained.

Pair 2

Overall, this chromosome appears paler than all other G-banded chromosomes. The major G-banding pattern features are the two broad dark bands in the distal half of the long arm. Both these bands are composites of several thin bands (for example, see Figure 5.1 *Sc*).

There are at least seven main bands in the short arm and the centre is marked by a very characteristic pale interband. From the distal end of this arm the first band is pale, the next two are broader and grey, the fourth is narrow and the fifth often visible as a grey doublet. The sixth band is thin and dark and the seventh is a small band flanking the centromere. Major bands are often visibly composite, and a fine band is frequently seen between the sixth and near-centromeric seventh band.

In the long arm there are two major bands (which may appear complex) between the centromere and the two dark 'land-mark' bands. A small band flanks the centromere and bands may be visible between this band and the first, and between the first and the second most proximal major bands. The end of the arm is marked by a grey band.

Pair 3

The 'land-mark' features of this G-banded chromosome are the striped region of the distal half of the long arm (five dark bands, alternating with some white interbands), and the long pale distal end of this arm. The short arm has a broad dark central band that often appears double.

The short arm is lightly banded on both sides of the median dark band, with a small band flanking the centromere.

In the long arm, there is a grey area close to the centromere, in which four or five bands are often visible. This region is followed by a characteristic pale area about one-third of the length of the chromosome arm from the centromere and then by the striped 'land-mark' region in which the first, second and fourth most proximal interbands are white, and the

third is grey with a minor band often visible. There are two faint bands in the long pale distal region of this chromosome arm.

Pair 4

This is overall a dark chromosome, especially the long arm with its large block of dark bands.

The short arm has a dark doublet band in the distal half, then two discrete bands and a small near-centric band.

The long arm has a small band flanking the centromere, then a pale region in which there are several light bands. A large dark-staining block then extends over half of the arm. At least six bands are regularly seen in this block. Separated from this area by a small clear interband are two very dark broad bands.

One or two faint bands are regularly seen in the pale distal regions of both arms.

Pair 5

The salient features of this G-banded chromosome are the broad black band extending from near the centromere to the middle of the short arm and two concentrations of dark bands in the long arm.

A faint band is often associated with the secondary constriction of the distal half of the short arm.

In the long arm the centromere is flanked by a small band, which is followed by a white region, another band and then the bands of the most proximal dark 'land-mark' region. The pale area between the two dark 'land-mark' regions often displays a discrete band. The most distal dark region is followed by a white area marked by a faint band at the end of the arm. In elongated chromosomes each of the two dark 'land-mark' regions of the long arm are visibly composed of at least three dark bands.

Pair 6

All dasyurids studied, except the *Ningaui* sp. and *Antechinomys laniger* possess chromosomes with the "basic" G-banding pattern. This consists of a dark band (doublet) at the distal end of the short arm, followed proximally by a large pale region bisected by a faint band. A large dark band flanks the centromere. The most proximal region of the long arm is grey, with a dark band which may appear double. This is flanked by a pale interband area in which there are two dark bands (the most proximal often the darkest of the chromosome). The extreme ends of both arms are unstained.

The X chromosome

The short arm shows one faint band and the centromere is often marked by a small band. The long arm is pale grey with a maximum of three greyish bands.

[The X chromosomes of some species showed more G-banding detail than others. As the best banded X chromosomes were always chosen for each species, these differences may simply reflect the proportion of cells with elongated chromosomes in different cultures, or be a chance effect. For example, the detailed G-banding pattern of the *Dasyuroides burnei* X chromosome (see Figure 5.5) may have been observed for the *Ningaui* sp. if the X chromosomes had been of comparable length and staining quality. When X chromosomes of comparable quality are compared (Figure 5.10) there are no outstanding pattern differences.]

The Y chromosome

An extremely small chromosome with one or two G-bands.

[Because of the small size of these chromosomes it is not known whether the dark and light bands may sometimes show represent differentiation of non-centromeric material or of the centromere and surrounding Y chromosome material.]

If the basic G-banded karyotype of Figure 5.10 is assumed commonly ancestral for the dasyurids studied, the deviant banding patterns of the sixth chromosomes, *n* and *a* in Figure 5.10, may be accounted for by simple and independent rearrangements of chromosomes with the ancestral-type of banding pattern.

It will be general practice in this thesis to interpret differences in G-banding pattern as single paracentric, pericentric inversions or Robertsonian changes, unless this is inconsistent with the G-band sequences. Although this conservative approach is adopted it is recognized that more complex changes may account for the pattern differences. For example, the *A. laniger* pair 6 (*a* in Figure 5.10), as shown below, is assumed to have been formed from the basic type by a paracentric inversion (or in this special case, an addition of chromosome material). However, this chromosome may also have been formed from the basic type by a translation event (either the light or the dark band of the differential segment of the *Al* and *Sc* chromosomes, Figure 5.11, being broken out of the *Sc* chromosome and being inserted with a third break at the appropriate position).

It is also acknowledged that the interpretation of all karyotypic differences in this thesis is limited by the resolution of the present G-banding patterns, and that some of the proposed relationships may subsequently be revised by other studies.

The deviation of pair 6 of *A. laniger* from that of all other dasyurids studied would probably pass unnoticed with general chromosome staining. The G-banding pattern of this pair is similar to the basic dasyurid pattern, but whereas the first major band of the long arm is flanked distally by a white region then two dark bands in the basic pattern, in *A. laniger* it appears that the second dark band precedes the white area which is then followed by only one dark band. This deviation may be explained by a paracentric inversion of a chromosome with the basic type of pattern; see Figure 5.11 *Al(i)*. However, the *A. laniger* sixth chromosome appears to have a longer long arm than the other metacentric dasyurid sixth

Figure 5.11

The relationships of the deviant dasyurid pair 6 chromosomes, *n* and *a*, to that of *Sminthopsis crassicaudata*, *Sc*, with the more common G-banding pattern.

— the breakpoints of inversions.

■ material additional to that of *Sc*.

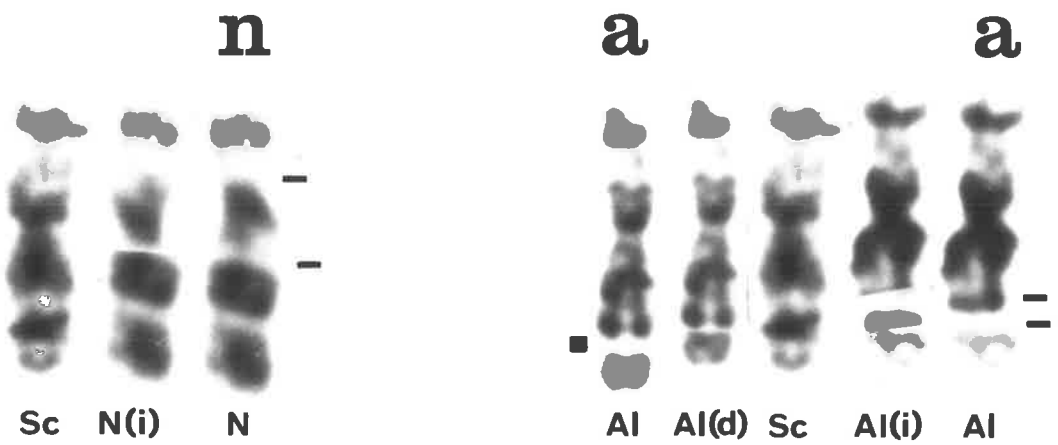
N *Ningaui* sp. chromosome.

N(i) *Ningaui* sp. chromosome with a segment, including the centromere, inverted to show the resultant pattern homology with *Sc*.

Al *Antechinomys laniger* chromosome.

Al(i) *Antechinomys laniger* chromosome with a segment inverted to show the resultant pattern homology with *Sc*.

Al(d) *Antechinomys laniger* chromosome with a segment deleted to show the resultant pattern homology with *Sc*.


n
a
a
Sc
N(i)
N
Al
Al(d)
Sc
Al(i)
Al

chromosomes. Therefore it is also possible that the white area in this chromosome may be chromosomal material additional to that of the basic pair 6; see Figure 5.11 *Al(d)*.

The G-banding pattern of the *Ningaui* sp. chromosome 6 can be accounted for by a pericentric inversion of the basic G-banded sixth pair; Figure 5.11 *N(i)*. The large dark band flanking the centromere and that part of the pale area containing the faint median band of the short arm of the G-banded basic pair 6, have been relocated in the long arm of the *Ningaui* sp. chromosome.

In contrast to Hayman and Martin (1974) who measured the chromosomes of an *Antechinus flavipes* and found that pair 3 was less metacentric than the third chromosome of other dasyurids, the G-banded third autosome of the individual of this species studied here does not appear to deviate from the basic G-banded chromosome 6. Both these *A. flavipes* were collected from the Adelaide Hills. The general stained sixth chromosomes of the animal studied in this thesis were not measured, but they appeared to have similar arm ratio to those of most other dasyurids studied.

The basic G-banded dasyurid karyotype and the intra-familial conservation of G-banded complements in animals

The seven genera of dasyurids represented by the species studied with G-banding have probably been independent evolutionary lineages since late Miocene; approximately ten million years B.P. (Archer and Kirsch, 1977). Therefore there have presumably been substantial opportunities for repatterning the ancestral G-banding karyotype. That this has not occurred suggests that this particular configuration of 2n=14 chromosomes has been selectively favoured in dasyurids.

The only karyotypic differences observed, while appearing to be independently derived, are both chromosome 6 rearrangements. This may be

a chance effect. These variants occur in two genera (*Ningaui* and *Antechinomys*) that are considered, on the basis of dentition and cranial morphology, to be quite closely related to *Sminthopsis* (*Antechinomys laniger* specifically to *S. crassicaudata*) and to each other (Archer, 1975, 1977). *Antechinomys* and *Sminthopsis* also show close serum protein affinity (Kirsch, 1977a). It is therefore possible that further G-banding studies will, on the basis of shared derived chromosomes, provide insights into the phylogenetic relationships and taxonomy of species of these two genera and *A. laniger* where other methods cannot.

As explained in Chapter 3, the Dasyuridae were chosen for a detailed G-banding study of the intra-familial conservation of the 2n=14 basic complement as there are more genera of living Dasyuridae than of other families of Australian marsupials with such complements, and animals were available from several genera that are representative of the taxonomic diversity of this family.

There have been relatively few, comparable G-banding studies of the intra-familial conservation of whole chromosome complements in animals. In the cat family Felidae, the morphologically similar complements of fourteen species have been G-banded (Wurster-Hill and Gray, 1973; Roubin *et al.*, 1973). However, eleven of these were *Felis* species. The G-banding karyotypes of all species were very similar, but a small number of previously undetected differences were revealed. These were mostly differences in the extent of negatively stained areas in particular G-banded chromosomes. A previously cryptic pericentric inversion difference characterized a G-banded chromosome pair of the two *Panthera* species studied but not of other species. Seven species of *Felis* with two pairs of similarly sized presumably homologous acrocentrics were shown with G-banding to possess only one pair in common and a proposed centric fusion difference was considered after G-banding to result from the tandem fusion

of two acrocentric chromosomes.

G-banding studies that have involved sampling a number of genera of other families showing conservation of karyotypic form include those of Emydidae (turtles), ten species, six genera (Bickham and Baker, 1976), and Phocidae (seals), seven species, six genera (Arnason, 1974, 1977). No G-banding pattern differences, other than those predicted from general stained chromosomes, were detected. However, apparently no detailed comparison of extended G-banded chromosomes was attempted and thus small but significant differences such as those detected between the jerboa marsupial mouse (*Antechinomys laniger*) and most other dasyurids of this thesis, and between several cat species (Wurster-Hill and Gray, 1973), may well exist.

5.2 The G-banded chromosomes of two species of Peramelidae, bandicoots

The sixteen species of living peramelid bandicoots are classified in seven genera; three of these each contain only one living species and have not been studied cytologically. The eight species (four genera) whose chromosomes have been examined, have 2n=14 chromosomes of "2n=14 basic" morphology. The length measurements for each chromosome pair of five species (four genera) do not differ markedly from the average of these measurements.

The chromosomes of *Isoodon obesulus*, the short-nosed bandicoot, and *Perameles nasuta*, the long-nosed bandicoot (see Table 5.1) were G-banded. At the time of sampling most fibroblast cells of the male long-nosed bandicoot were diploid, but most mitotic cells of the female short-nosed bandicoot had 2n=15 chromosomes. It is not known whether the latter cells were derived from an original population with this or a lower chromosome number.

Figures 5.12 *Io*, 5.13, 5.12 *Pn*, 5.14 are the G-banding karyotypes and charts for the short-nosed bandicoot and long-nosed bandicoot respectively. Although the chromosomes of the long-nosed bandicoot are numbered according to the measurements of Hayman and Martin (1974) those of the short-nosed bandicoot are not. Pairs 2 and 3 of the *I. obesulus* complements in this thesis correspond respectively to the third and second largest chromosomes as measured by Hayman and Martin. Their measurements also show pairs 4 of both these species to be exactly metacentric chromosomes. The chromosome arm of pair 4 that has a G-banding pattern most similar to that of the short arm of pair 4 of the basic G-banded dasyurid complement was chosen as the short arm for these two peramelids.

Three of the fifteen chromosomes in all the 2n=15 complements of the short-nosed bandicoot are apparently X chromosomes as the complements otherwise possess very similar G-banded chromosomes to those of the long-nosed bandicoot. This suggestion is supported by the C-banding patterns of these complements (Rofe, unpublished) as in each complement of the short-nosed bandicoot, three chromosomes of the appropriate size show characteristic (see Figure 5.24) C-banding patterns.

The large secondary constriction of the general stained pair 6 of the long-nosed bandicoot remains quite pronounced after G-banding, but no G-banded chromosome of the short-nosed bandicoot is so clearly marked.

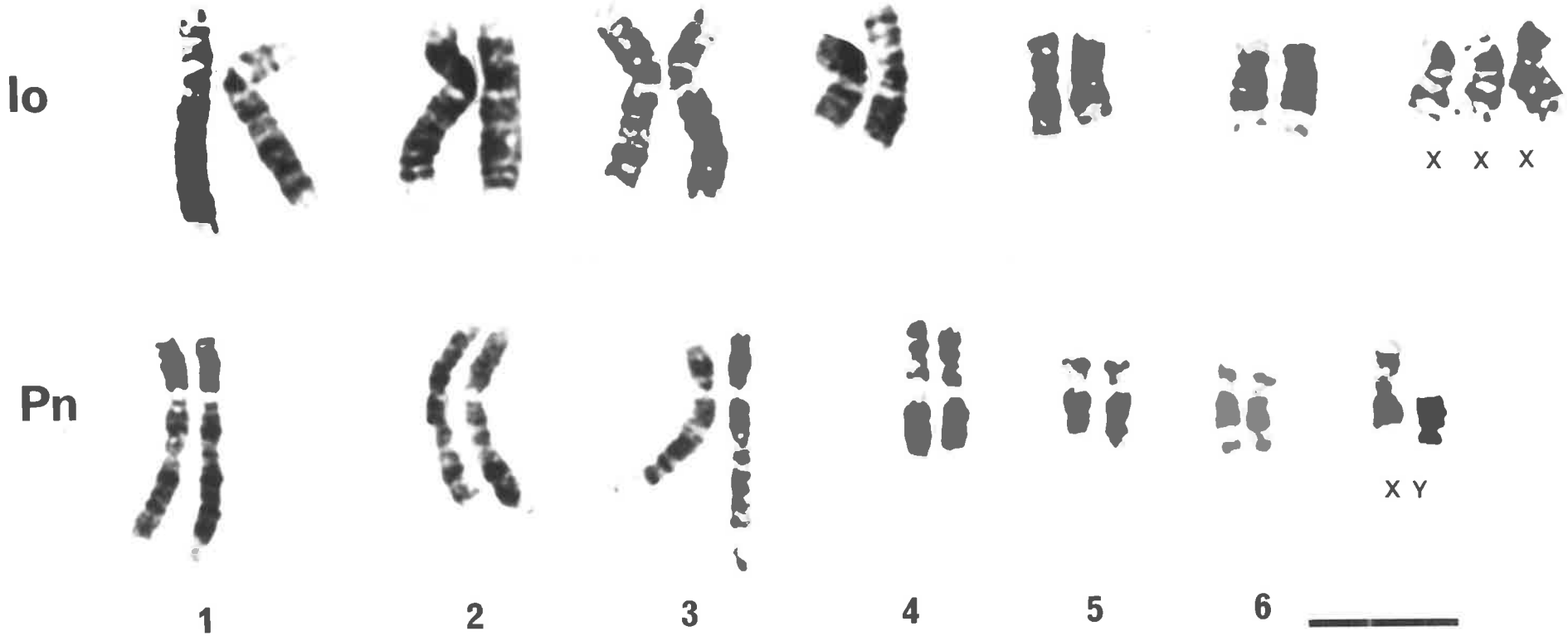
The G-banded complements of these two species are obviously very similar and the G-banding patterns of the autosomes can be described as essentially those of the basic dasyurid autosomes of the corresponding number. The peramelid pairs 1, 2, 4 and 6 do show deviations from dasyurid patterns and these differences are considered in section 5.5.

The X chromosomes of these two peramelids are clearly different. That of the long-nosed bandicoot is much larger than that of the short-nosed bandicoot and shows a quite different G-banding pattern (Figures 5.15 (a) and 5.24). Brief descriptions of the patterns of the X

Figure 5.12

The G-banding karyotypes of peramelids.

Io *Isoodon obesulus*
Pn *Perameles nasuta*


Io
X X X
Pn
X Y
1
2
3
4
5
6

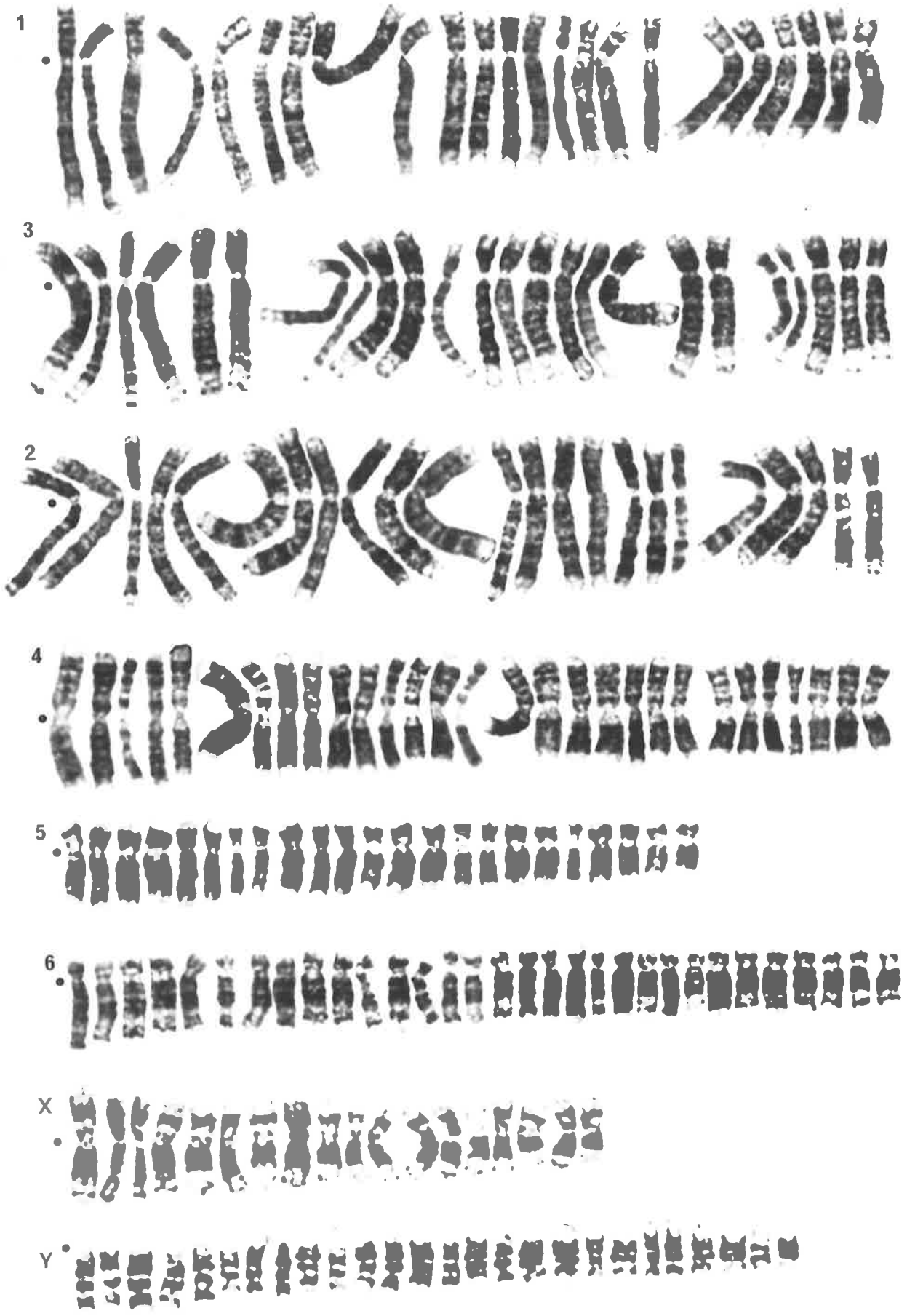
Figures 5.13 and 5.14

The G-banding charts for peramelids.

5.13	*Io*	*Isoodon obesulus*
5.14	*Pn*	*Perameles nasuta*

Io

Pn



chromosome of the short-nosed bandicoot and of the X and Y chromosomes of the long-nosed bandicoot are now given.

The X chromosome of *I. obesulus*, the short-nosed bandicoot, has a strikingly contrasting G-banding pattern. In the short arm there is a dark band in the distal third and also near the centromere. In the long arm there is a dark band adjacent to the centromere, followed by three equally spaced bands, the most distal being near terminal. The first of these three bands is light and the second is darkest and broadest. The interbands closest to the centromere are whitest.

In the short arm of the X-chromosome of *P. nasuta*, the long-nosed bandicoot, there is a pale distal region, a central dark doublet, then a pale area becoming darker most proximally. The long arm is lightly G-banded near the centromere, followed by a wide dark area composed of several discrete dark bands, the most distal being the darkest. There is a large white distal region with a band of medium stain intensity near the end.

The Y chromosome shows the most contrasting G-banding pattern of the *P. nasuta* complement. The short arm is pale. The long arm is pale near the centromere, followed by a grey region, a definite dark band, a whitish interband area, another clear dark band and a second pale region. The distal third of the long arm is grey, and whitish terminally.

In addition to the X chromosomes there are some small differences between the G-banding patterns of the autosomes of these two bandicoot species (see Figure 5.15 (a) and the karyotypes and charts). The autosomes show differing G-banding patterns in the centromeric regions, which are areas that stain darkly after C-banding (Rofe, unpublished). In the short-nosed bandicoot some of these regions show dark G-bands (for example, pairs 4 and 5), some are grey (pair 6), and some show a pattern of dark and light bands (pairs 1, 2 and 3). In contrast, the centromeric C-banding regions of all chromosomes of the long-nosed

Figure 5.15

Composite G-banding karyotypes for -

(a) peramelids

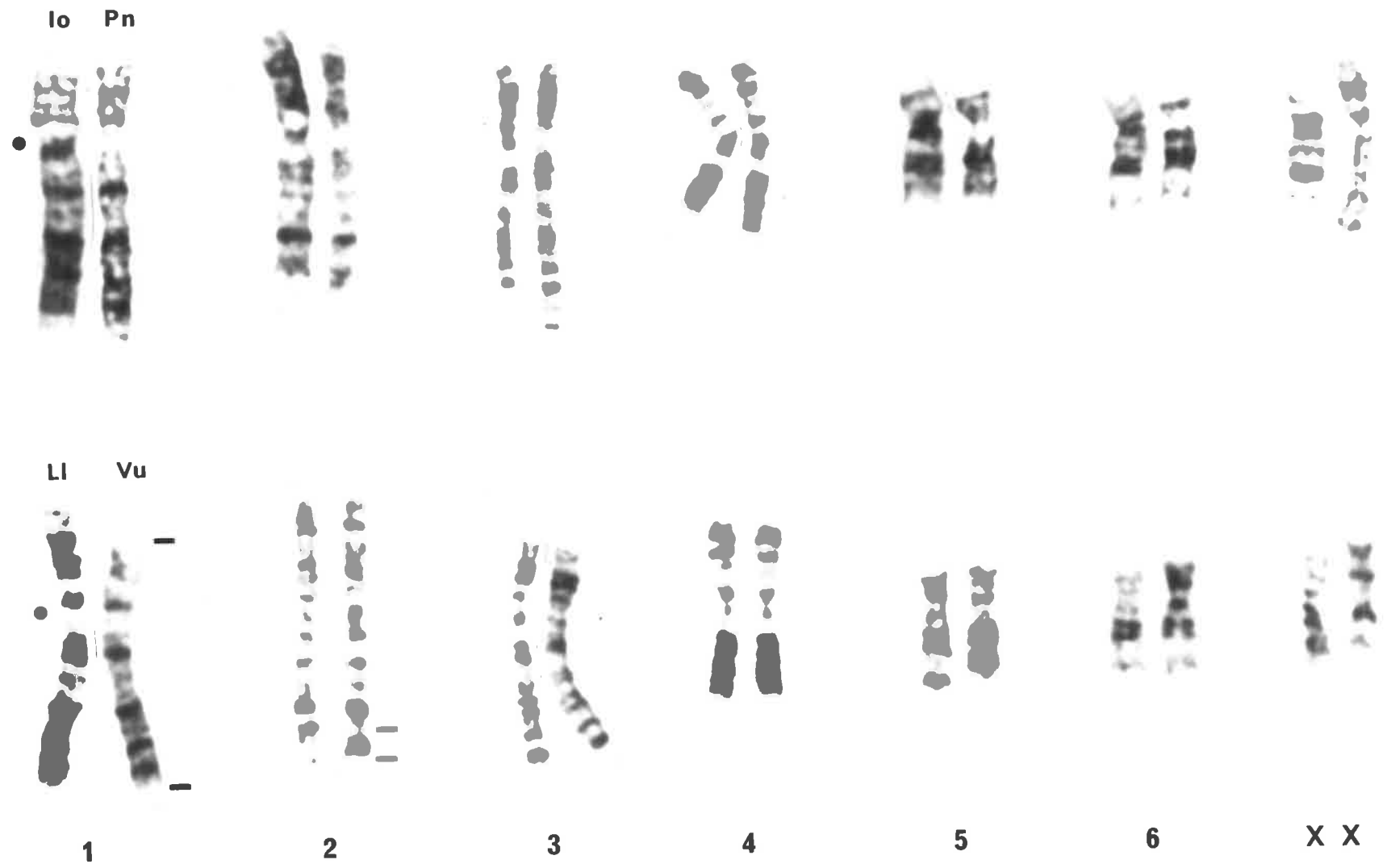
The chromosome on the left of each pair is from *Isoodon obesulus*, *Io*, and that on the right from *Perameles nasuta*, *Pn*.

(b) vombatids

The chromosome on the left of each pair is from *Lasiorhinus latifrons*, *Ll*, and that on the right from *Vombatus ursinus*, *Vu*.

— breakpoints of inversion differences between *Ll* and *Vu* chromosomes

Centromere positions are indicated by dots.


Io
Pn
Ll
Vu
1
2
3
4
5
6
X X

bandicoot are white and flanked on both sides by grey areas after G-banding.

As well as these near-centromeric differences, pair 3 of the short-nosed bandicoot shows a pronounced white area in the proximal one-sixth of the long arm. There is no equivalent G-banding feature in the third chromosome pair of the long-nosed bandicoot studied.

5.3 The G-banded chromosomes of two species of Vombatidae, wombats

There are only three species (two genera) of living wombats and the general stained chromosomes of two of these, *Lasiorhinus latifrons* and *Vombatus ursinus* have been studied. Both have 2n=14 basic chromosome complements, but according to chromosome measurements only one chromosome pair (pair 4) is of very similar size and arm ratio in the two species. A pericentric inversion has been proposed to account for the differences between the largest chromosome pairs of these species (Martin and Hayman, 1967).

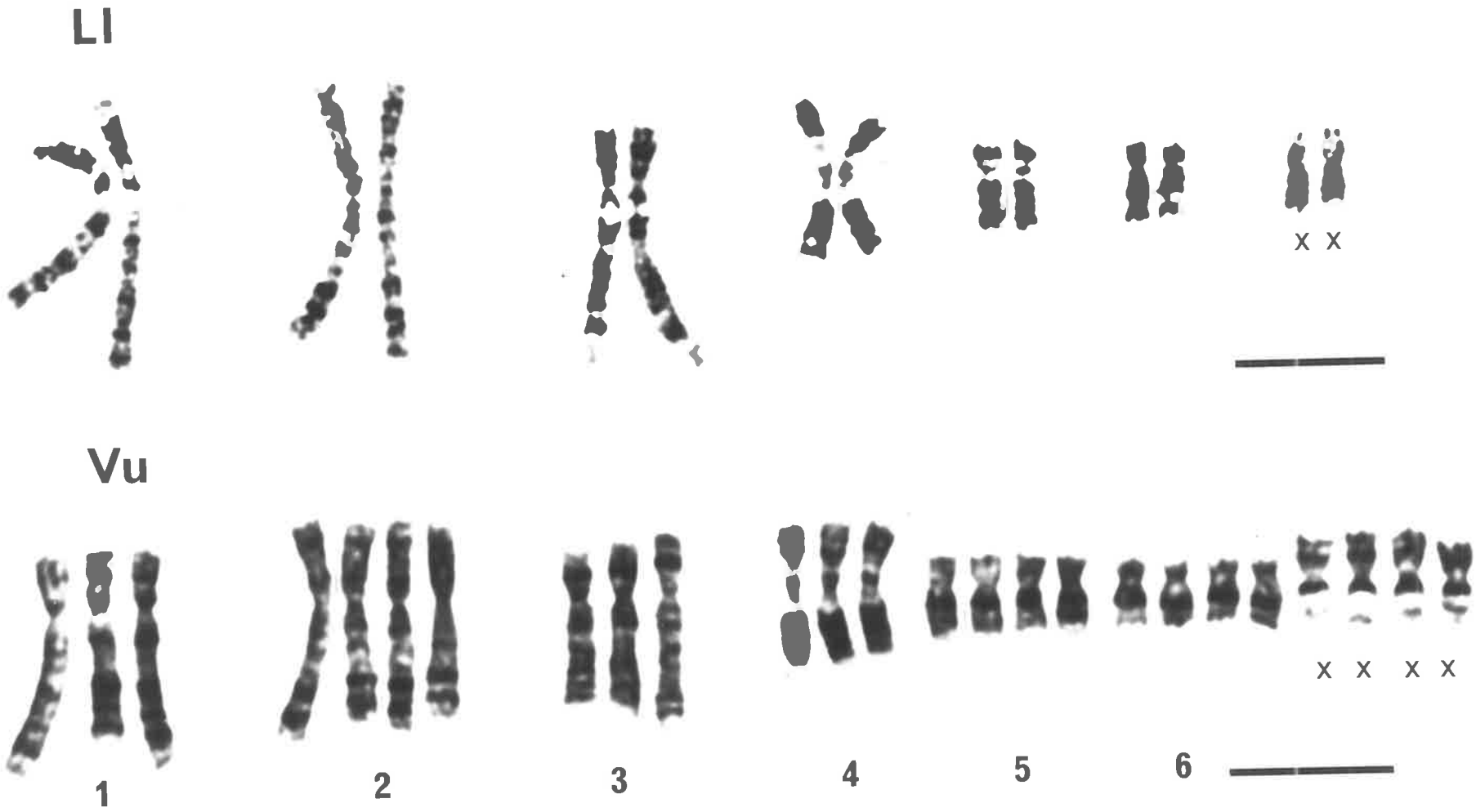
The chromosome complements of the wombat species referred to above *L. latifrons*, the hairy-nosed wombat and *V. ursinus*, the common wombat (see Table 5.1) were G-banded. Figures 5.16 *Ll*, 5.17, 5.16 *Vu* and 5.18 are the G-banding karyotypes and charts for the hairy-nosed wombat and the common wombat respectively.

At the time chromosome preparations were made for G-banding, most cells of the female hairy-nosed wombat culture were diploid, but those of the female common wombat culture usually possessed 23, 24, 25 or 26 chromosomes. G-banding showed that these cells were essentially tetraploid, but missing three or four chromosomes, usually one of each of pairs 1, 3 and 4. Some of these cells also possessed a chromosome (*X-T* in Figure 5.18) formed by a translocation between one chromosome 1 and an X chromosome. The breakpoints of this translocation are shown on chromosomes 1 and X in Figure 5.18. As the G-banded complement of

Figure 5.16

The G-banding karyotypes of vombatids.

Ll *Lasiorhinus latifrons*
Vu *Vombatus ursinus*


LI
x x
Vu
x x x x
1
2
3
4
5
6

Figures 5.17 and 5.18

The G-banding charts for vombatids.

5.17 *Ll* *Lasiorhinus latifrons*
5.18 *Vu* *Vombatus ursinus*

for 5.18 — breakpoints of the X-autosome (*X-T*) translocation

the smaller dots indicate G-banded *Vu* chromosomes that are mismatched, those in pair 1 are pair 3 chromosomes, and vice versa.

LI

the common wombat appeared to be otherwise unaltered, comparison of these G-banded chromosomes with those of the hairy-nosed wombat was considered legitimate.

Measurements show that chromosome pairs 4 of the complements of both wombats are exactly metacentric. The G-banded short arm of these chromosomes was chosen to correspond in G-banding pattern to that of the short arm of pair 4 of the peramelids studied.

The G-banded chromosome complements of these two wombats are very similar and the patterns of the autosomes may be described as essentially those of peramelid chromosomes of corresponding number. The X chromosomes, although of similar size and arm ratio in the two species, possess quite different G-banding patterns (Figures 5.15 (b) and 5.24). Also, while the X chromosome of the hairy-nosed wombat shows no marked secondary constriction, that of the common wombat possesses a clear attenuation in the distal half of the long arm. The G-banding patterns of the X chromosomes may be described as follows:

The short arm of the X chromosome of the hairy-nosed wombat, *L. latifrons*, is pale grey with two thin grey bands. There is a small band flanking the centromere and the distal end is grey. The whole of the long arm distal to a small dark centric band appears dark, with generally indistinct G bands. The distal end of the arm is white.

In the short arm of the X-chromosome of the common wombat, *V. ursinus*, there are two large dark bands on a grey background. There is a pale region near the centromere and the distal end is white. In the long arm distal to a thin grey centric band, there is a small pale region followed by a characteristic broad black band flanking the white secondary constriction region. The trabant is variably G-banded, often showing a dark band proximally and a fainter distal band.

Although the G-banding patterns of the autosomes of these two species are similar there are three striking differences (see Figure 5.15 (b)).

Firstly, the largest chromosomes of the two species differ markedly in morphology and G-banding pattern. This difference may be accounted for (as proposed by Martin and Hayman, 1967) by a pericentric inversion, with the breakpoints of such an event as indicated on chromosome 1 of the common wombat in Figure 5.15 (b). Secondly, the two dark 'land-mark' bands in chromosome pair 2 of the hairy-nosed wombat are much closer than in pair 2 of the common wombat. This difference may be accounted for by a paracentric inversion, the breakpoints being shown on chromosome 2 of the common wombat in Figure 5.15 (b). There may also be other differences between the G-banding pattern of these pair 2 chromosomes, namely in the short arms near the centromeres, but these are difficult to define. Thirdly, pair 3 of the hairy-nosed wombat shows a very distinctive G-banding feature that is not seen in the homologously banded chromosomes of the common wombat. In the proximal one-sixth of the long arm of pair 3 of the hairy-nosed wombat there is a white bulbous region which often extends past the boundary of the remainder of the G-banded chromosome.

5.4 The G-banded chromosomes of two species of Burramyidae, pygmy phalangers

There are four genera of living burramyids and of these only *Cercartetus* is known to include more than one living species. Species from three of the four genera have been examined with a general chromosome stain and all have 2n=14 chromosome complements of the "basic" format. The main karyotypic differences are those of chromosome pairs 5 and 6 which are acrocentric in *Acrobates pygmaeus* and *Burramys parvus* and submetacentric in *Cercartetus* species. Pair 2 of *Burramys parvus* is acrocentric whereas the second chromosomes of other burramyids are submetacentric (Gunson *et al.*, 1968). The chromosome measurements of three

species of *Cercartetus* and of *Acrobates pygmaeus* indicate a number of further minor karyotypic differences for most chromosomes of these species.

In terms of the "2n=14 basic complement" proposal for Australian marsupials the burramyid karyotypes are a focal point as they are the only postulated "2n=14 basic" complements in the karyotypically diverse superfamily, the Phalangeroidea (see Figure 3.1).

The chromosome complements of two burramyids, *Acrobates pygmaeus*, the pygmy glider, and *Cercartetus concinnus*, the pygmy possum, were G-banded.

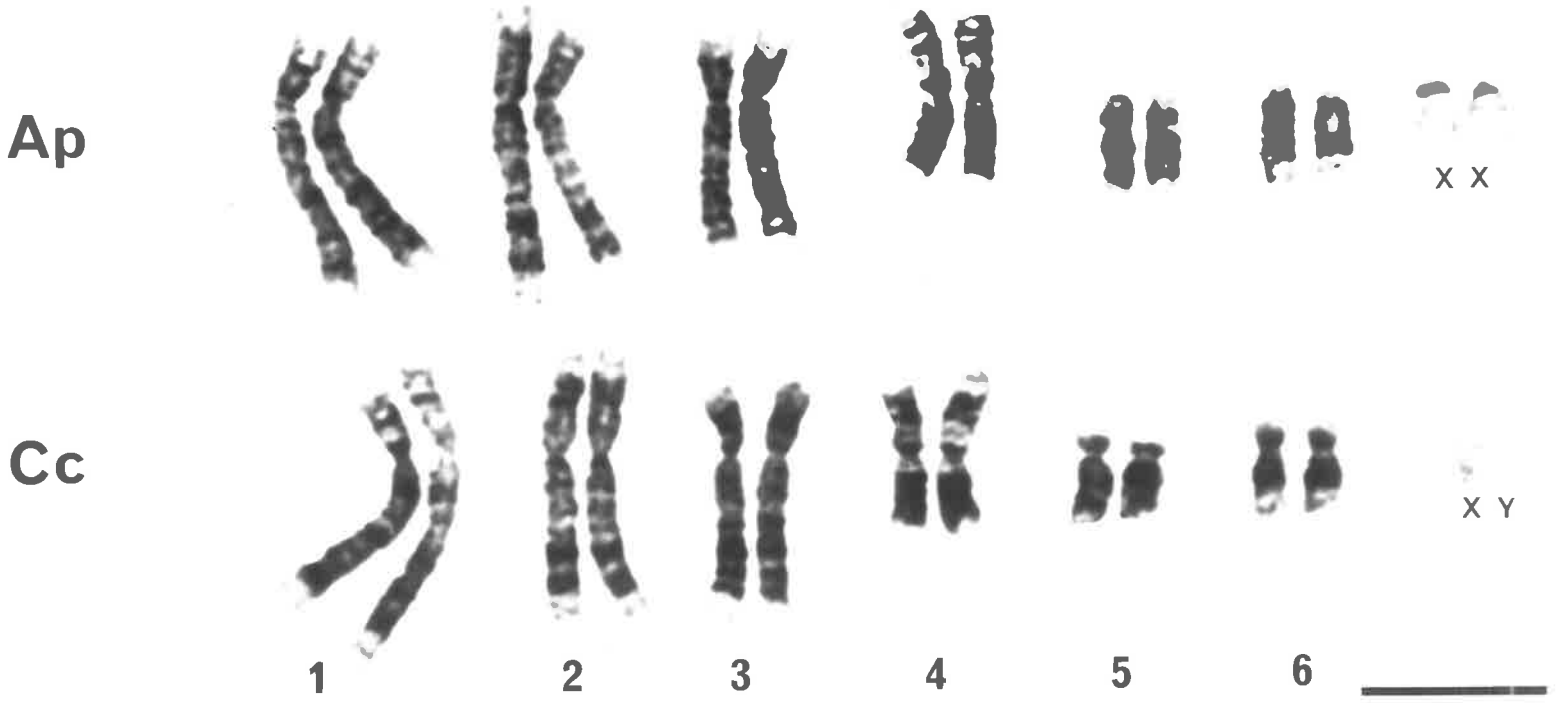
The G-banding karyotypes and charts for these two species are shown in Figures 5.19, 5.20 and 5.21. Measurements of the general stained chromosomes of the pygmy glider have given equivalent lengths and arm ratios for chromosome pairs 2 and 3 and also for pairs 5 and 6. Pair 4 is exactly metacentric. Chromosomes with G-banding patterns corresponding to those of pairs 2, 3, 5 and 6 in the wombats were chosen as pairs 2, 3, 5 and 6 respectively in the pygmy glider and as for the wombat species, the more darkly banded arm of pair 4 chromosomes was chosen as the long arm for the pygmy glider. In the general stained chromosome complements of the pygmy possum that have been measured pairs 2 and 3 are of similar length. The more metacentric G-banded pair was chosen as pair 2 in G-banding karyotypes and charts. The Y chromosome of the pygmy possum is very small and has been omitted from the chart, Figure 5.21.

The chromosomal G-banding patterns of both pygmy phalangers are very similar and the patterns of the autosomes may be described as chiefly those of the autosomes of the two wombat species studied. However, the X-chromosomes of the two burramyids differ in G-banding pattern (see Figure 5.22 and 5.24). The pygmy glider X chromosome appears longer than that of pygmy possum, with a dark procentric band, a white area and perhaps the next more distal G-band of the long arm of the glider X missing from the X chromosome of the possum. The G-band pattern of the possum X

Figure 5.19

The G-banding karyotypes of burramyids.

Ap *Acrobates pygmaeus*
Cc *Cercartetus concinnus*

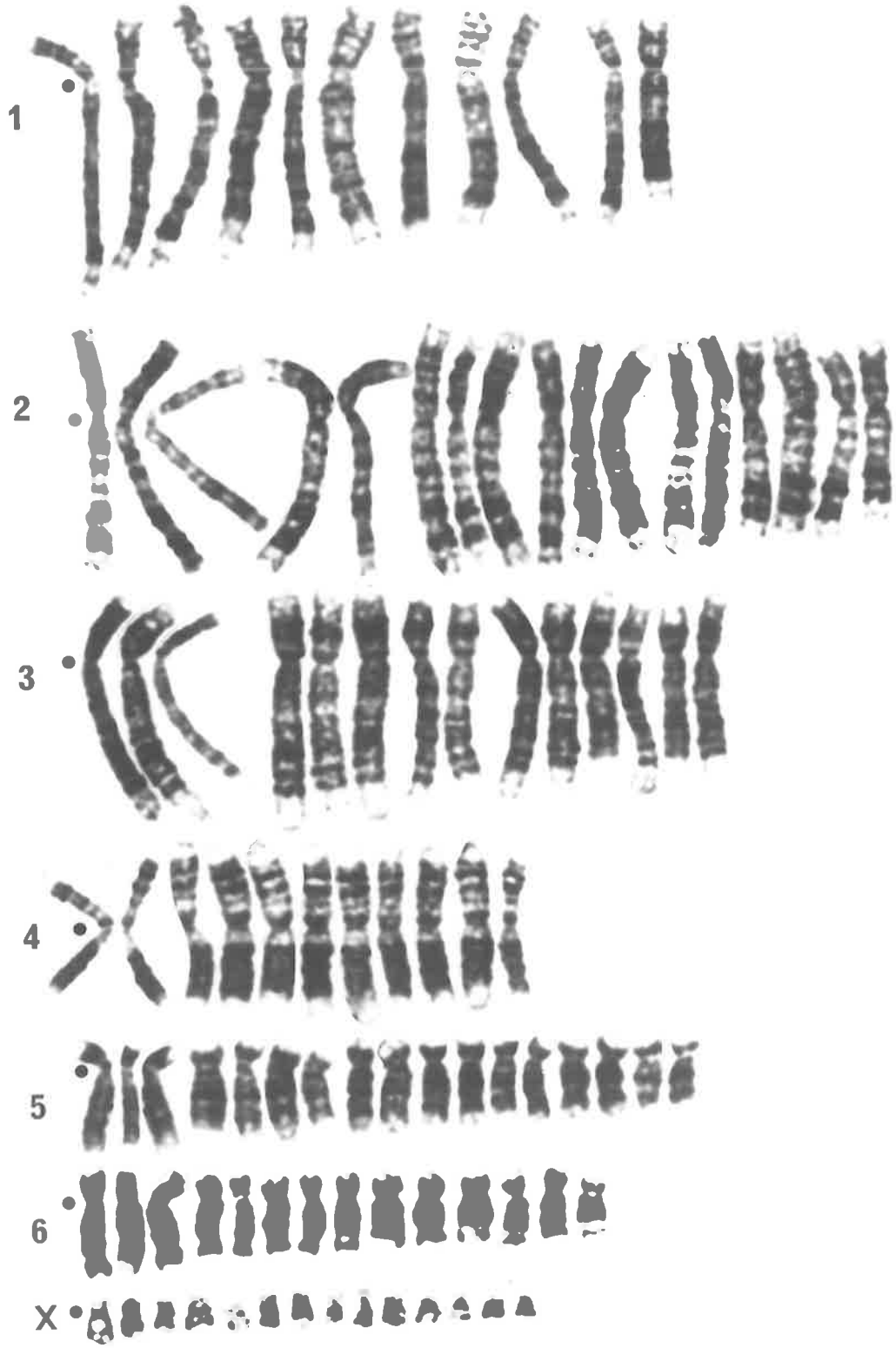

Ap
Cc
X X
X Y
1
2
3
4
5
6

Figures 5.20 and 5.21

The G-banding charts for burramyids.

5.20	*Ap*	*Acrobates pygmaeus*
5.21	*Cc*	*Cercartetus concinnus*

Ap


Cc
1
2
3
4
5
6
X

(pale with approximately three grey bands) corresponds to the distal two-thirds of the long arm pattern of the glider G-banded X chromosome. Chromosome material appears to have been either added to the glider X chromosome or deleted from that of the possum.

Although each G-banded autosomal pair of the pygmy glider corresponds in G-banding pattern to a chromosome of similar size in the pygmy possum there are a number of small differences (Figure 5.22). Pericentric inversions (the breakpoints of which are indicated on the possum chromosomes in Figure 5.22) may account for the differences in centromere position in pairs 5 and 6 of these two species. The G-banding patterns of these chromosomes have remained substantially unchanged. The second chromosomes of these pygmy phalangers also appear to have slightly different arm ratios, the possum chromosome being more metacentric than that of the glider. G banding shows that the difference may be attributed to a small pericentric inversion, the small G-band flanking the centromere in the long arm of *A. pygmaeus* appearing in the short arm of the *C. concinnus* second chromosome (see Figure 5.22). Such near centromeric differences in G-banding pattern must, however, be interpreted with caution, as they are within the bounds of the C-bands displayed by these chromosomes (Rofe, unpublished). Gunson *et al.* (1968) have previously reported a centromere position difference for chromosome 2 of *A. pygmaeus* and *Cercartetus* species, but it is not clear which chromosome was regarded as more metacentric.

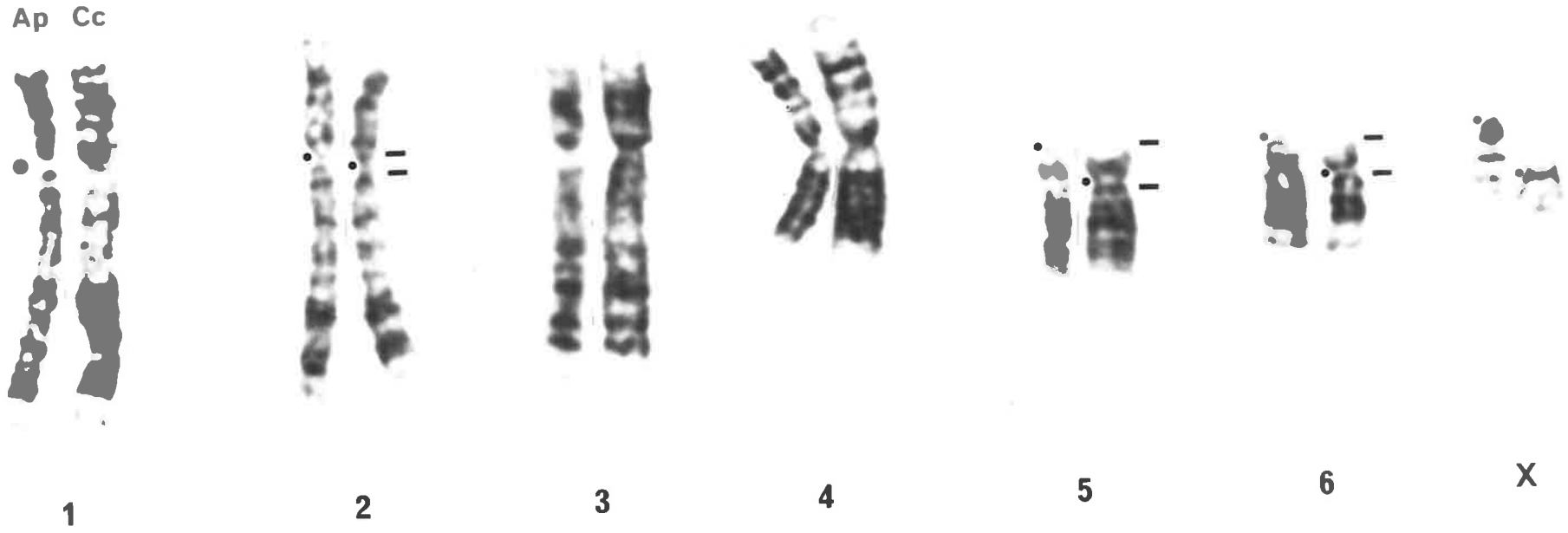
Figure 5.22

Composite G-banding karyotypes for burramyids.

The chromosome on the left of each pair is from *Acrobates pygmaeus*, *Ap*, and that on the right from *Cercartetus concinnus*, *Cc*.

— breakpoints of pericentric inversion differences between *Ap* and *Cc* chromosomes.

Where centromere positions differ in the two species they are indicated by smaller dots.


Ap Cc
1
2
3
4
5
6
X

5.5 A comparison of the G-banded 2n=14 basic complements

The marsupials with 2n=14 basic complements that were studied with G-banding earlier in this chapter are from four different families, each representing one of the four major superfamilies of Australian marsupials (Tables 2.1 and 3.1). Their G-banded chromosome complements are compared in Figures 5.23 and 5.24. As all dasyurid species studied had virtually identical G-banding patterns, chromosomes from only three of the eight species are shown in these figures.

The autosomes

Figure 5.23 contains representative G-banded autosomes for each of these species and the gross G-banding patterns of the autosomal complements of all these marsupials are clearly similar. For each complement every G-banded autosome shows almost band-for-band pattern homology with an autosome of corresponding size in all other species. The major deviations from the most common patterns are marked with black dots in Figure 5.23. Five of these, *Ll 1*, *Ll 2*, *Ap 2*, *Ap 5* and *Ap 6* are intra-familial G-banding pattern differences that have been interpreted in earlier sections of this chapter as inversion differences (Figures 5.15 (b) and 5.22). The remainder are discussed later in this section.

An interesting deviation from the most usual pattern for chromosomes 3 is a feature which is common to the G-banded *Ll 3* and *Io 3* (see also Figures 5.13 and 5.17), a clear white area near the centromere in the long arm of these chromosomes. The possible significance of this observation is unknown.

There are also many small interfamilial differences in centromere position and G-banding sequence between some of these chromosomes (for example, between chromosomes 5). However, only differences in G-banding pattern that involve large segments and thus that may be readily defined, have been noted.

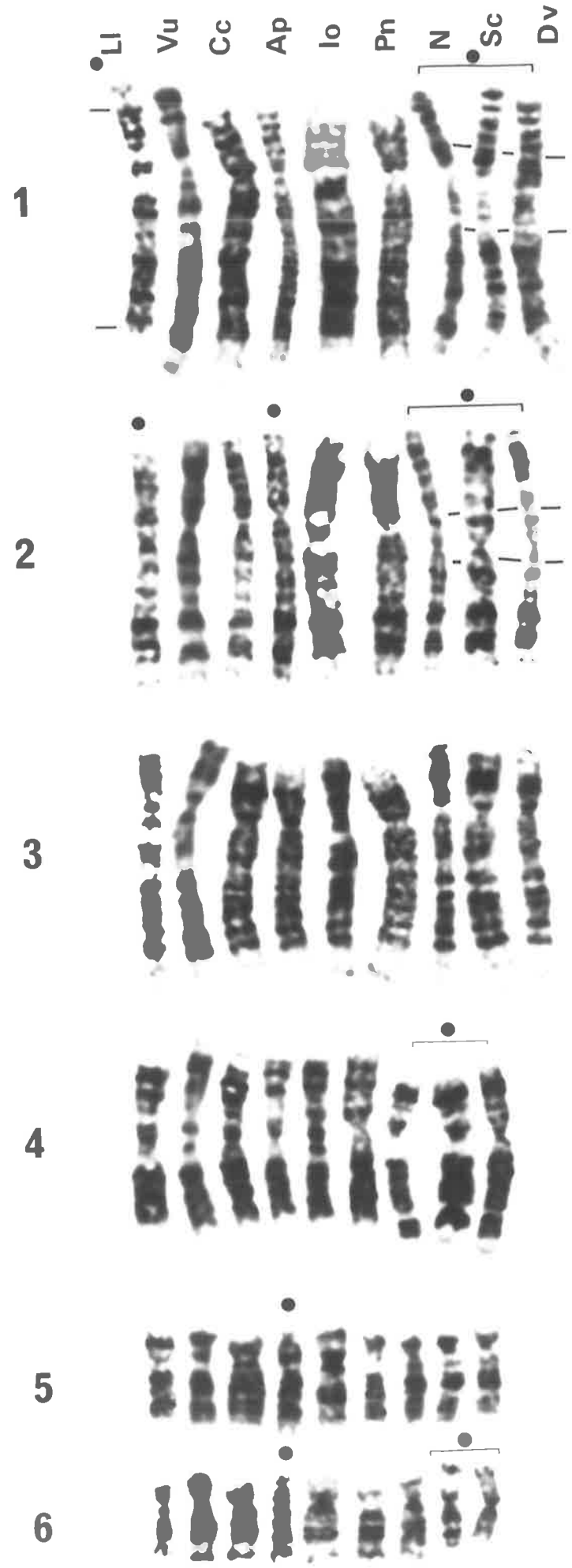
Figure 5.23

A comparison of the G-banded autosomes of the "2n=14 basic" complements.

Vombatoidea	Vombatidae	*Lasiorhinus latifrons*	*Ll*
		Vombatus ursinus	*Vu*
Phalangeroidea	Burramyidae	*Cercartetus concinnus*	*Cc*
		Acrobates pygmaeus	*Ap*
Perameloidea	Peramelidae	*Isoodon obesulus*	*Io*
		Perameles nasuta	*Pn*
Dasyuroidea	Dasyuridae	*Ningaui* sp.	*N*
		Sminthopsis crassicaudata	*Sc*
		Dasyurus viverrinus	*Dv*

The dots indicate chromosomes that deviate from the most common G-banding patterns.

= indicate the breakpoints of some pericentric inversions.


Ll
Vu
Cc
Ap
Io
Pn
N
Sc
Dv
1
2
3
4
5
6

The X and Y chromosomes

The general morphology and G-banding patterns of the X chromosomes, Figure 5.24, are very variable. The variation in size can be partly attributed to changes in the amount and distribution of C-banding material; with the notable exception of the X chromosomes of the female *Lasiorhinus latifrons*. However, the long armsof the X chromosomes of this animal consistently G-banded more diffusely than the short armsand in a male of the same species, using a different C-banding method (barium hydroxide instead of sodium hydroxide) the long arm often appeared darker than the short arm of the X chromosome (Rofe, unpublished).

Thus it is conceivable that the non C-banding portions of the X chromosomes and the short arm of *Ll X* in Figure 5.24, represent a basic X chromosomal region common to all these marsupials. However, these regions, as well as being quite small, do not show a particularly distinctive G-banding pattern even when undivided by C-banding material. Therefore their G-banding pattern homologies may be more apparent than real.

The Y chromosomes of these species also show considerable variation in size and morphology, with many being minute. They are variably C-banded and no comparisons of their G-banding patterns were attempted.

The following consideration of the results is confined to the autosomes, which, unlike the X and Y chromosomes, do show demonstrable G-banding pattern homologies in all species.

The interpretation of the data

The simplest interpretation of the present data alone is to assume that all species of Figure 5.23 had a common ancestor with 2n=14 chromosomes. As these species with 2n=14 basic complements were otherwise selected solely on the criteria of availability and are from families of different taxonomic

Figure 5.24

A comparison of the G- and C-banded X chromosomes

of the "2n=14" basic complements.

Ll *Lasiorhinus latifrons*
Vu *Vombatus ursinus*

Cc *Cercartetus concinnus*
Ap *Acrobates pygmaeus*

Io *Isoodon obesulus*
Pn *Perameles nasuta*

N *Ningaui*
Sc *Sminthopsis crassicaudata*
Dv *Dasyurus viverrinus*

The first line is the G-banded chromosomes for which centromere position is indicated by —. The second line shows a representation of the C-banding pattern for each of these X chromosomes and the band-inducing method used is indicated below each chromosome, n for C-banding with Na(OH); b for $Ba(OH)_2$.

Figure 5.25

The relationship of the G-banded dasyurid pair 4 chromosomes

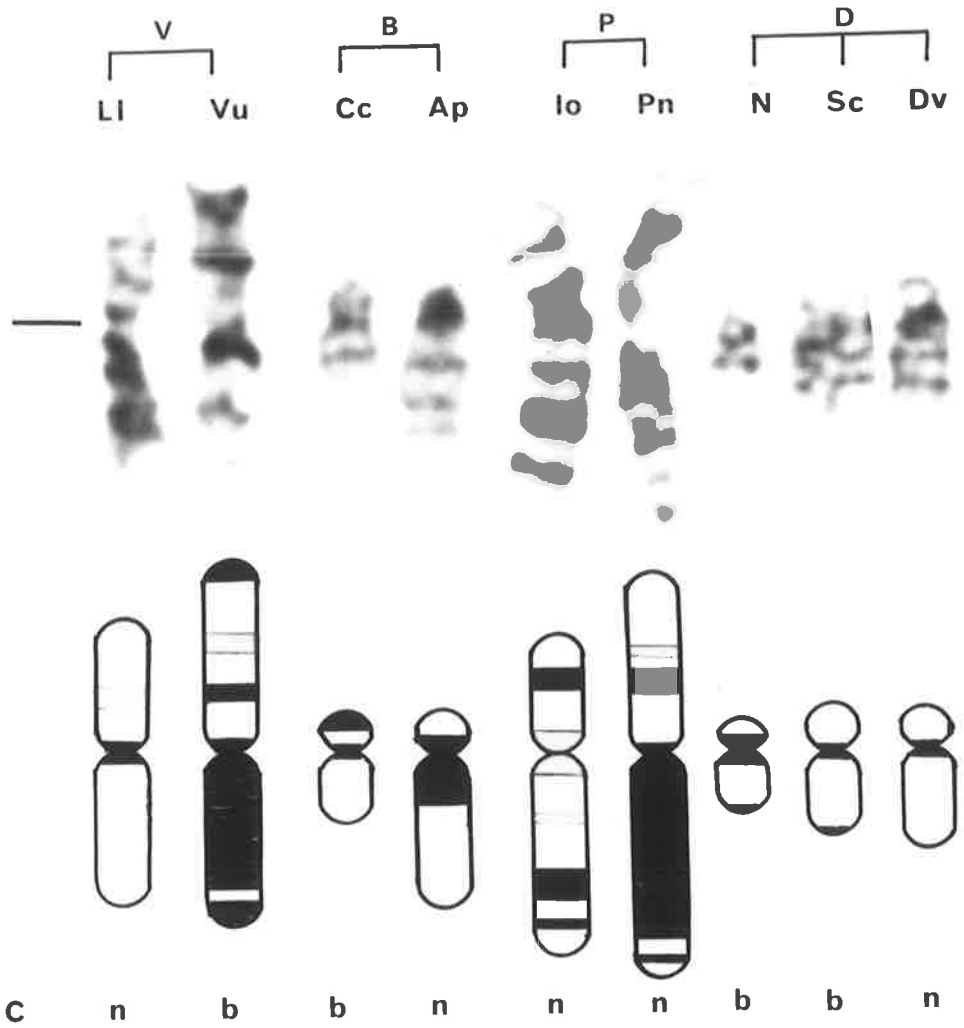
to those with the more common G-banding pattern.

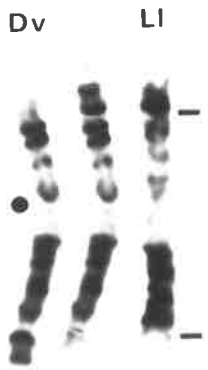
Dv *Dasyurus viverrinus*
Ll *Lasiorhinus latifrons*

the dot indicates centromere positions.

the dashes indicate the breakpoint of the pericentric inversion producing the *Dv* chromosome from an *Ll*-like chromosome.

both chromosomes on the left are from *Dv*, the centre one has been cut and the ends inverted to show the resultant pattern homology with the *Ll* chromosome 4.


V
B
P
D
Ll
Vu
Cc
Ap
Io
Pn
N
Sc
Dv
C
n
b
b
n
n
n
b
b
n


Dv
Ll

superfamilies it is reasonable to further assume that all these superfamilies, that is, all the major superfamilies of Australian marsupials (see Table 2.1) had a common ancestor with 2n=14 chromosomes.

The best representation of the G-banding pattern of each ancestral autosome is the type occurring in the greatest number and variety of taxa. These are the unmarked chromosomes in Figure 5.23. The marked chromosomes are thus considered derived. Five of these, *Ll 1*, *Ll 2*, *Ap 2*, *Ap 5* and *Ap 6*, can each be related by inversion to a chromosome with the ancestral type of G-banding pattern (to *Vu 1*, *Vu 2*, *Cc 2*, *Cc 5* and *Cc 6* respectively) possessed by a marsupial species in the same taxonomic family.

All four other derived types of G-banded chromosomes are common to all dasyurids (with the exception of chromosome 6 of the *Ningaui* species). Thus the unity of Dasyuridae with respect to G-banding pattern is shown. The sixth chromosome of the *Ningaui* species appears to have a G-banding pattern much like the ancestral type for this chromosome. However, as *Ningaui* sp. is characteristically dasyurid in other respects, and all dasyurids studied have a metacentric sixth chromosome pair, the submetacentric chromosome 6 of the *Ningaui* sp. is interpreted as having arisen from one with the typical dasyurid pattern by pericentric inversion (see Figure 5.11). Therefore the similarity of the G-banding pattern of the *Ningaui* sp. chromosome 6 to that of an ancestral type is clearly a convergence, the breakpoints of the *Ningaui* sp. inversion being similar to those of the initial ancestral dasyurid pair 6 inversion.

The G-banding patterns of dasyurid chromosomes 1, 2, 4 and 6 can be simply explained as the products of pericentric inversions of chromosomes with ancestral-type G-banding patterns. This is shown for chromosome 4 in Figure 5.25 and can be visualized for pair 6 in Figure 5.11 if the *Ningaui* sp. chromosome is labelled as an ancestral type. The breakpoints of the pericentric inversions producing chromosomes 1 and 2 of dasyurids from ancestral types are marked on the dasyurid chromosomes in Figure 5.23.

The comparison of the autosomal G-banding patterns of these species also shows that although chromosomes 1 of *Lasiorhinus latifrons*, and those characteristic of the dasyurid species are of similar length and arm ratio, they possess quite different G-banding patterns. These differences can be simply accounted for by independent and different pericentric inversions of pair 1 chromosomes with ancestral-type G-banding patterns.

The 2n=14 basic karyotype concept

On the basis of the 2n=14 basic complement proposal (Hayman and Martin, 1974) the G-banded chromosome complements of all marsupial species of Figure 5.23 might be expected to show, as a heritage of their common ancestry, some similarity. These authors had also suggested that the major differences between the average superfamilial 2n=14 basic complements that they constructed on the basis of chromosome measurements, could be accounted for by pericentric inversions in chromosomes 2, 4 and 6 of Dasyuroidea and chromosome 1 of Phalangeroidea (the Phalangeroidea plus the Vombatoidea of this thesis).

The G-banded 2n=14 basic complements in Figure 5.23 are indeed extremely similar and do show deviations, which may be attributed to pericentric inversions, of chromosomes 2, 4 and 6 of the Dasyuroidea from the most common pattern. In addition chromosome 1 of the dasyurids deviates from the most common pattern, but most phalangeroid chromosomes (*Vu 1*, *Cc 1*, *Ap 1*) do not.

In defining the format of the 2n=14 basic complement Hayman and Martin also proposed that one of the two smallest pairs of autosomes "may have a satellited short arm". G-banding analysis has shown that pair 6 of *Perameles nasuta* has a satellited short arm, whereas for dasyurids this is clearly a feature of pair 5. Also, N-banding studies (Rofe, unpublished) of other species represented in Figure 5.23 indicate that the major N-banding sites, which are the visible secondary constrictions and sometimes other

sites, vary quite considerably in number and location between species. Sometimes they are restricted to the X chromosome or a single autosomal pair, and in other species they are present on a number of pairs in the complement. Thus, the location of visible secondary constrictions and of N-banding sites is *not* a constant feature of these otherwise very similar autosomal complements. This is not surprising as species of *Mus* with exactly similar G-banding patterns are reported to differ in the location of their N-bands (Hsu *et al.*, 1978).

However, in all important respects the G-banding data of Figure 5.23 are highly compatible with the 2n=14 basic complement proposal of Hayman and Martin (1974). Thus this concept may be redefined in terms of a "2n=14 basic G-banded autosomal complement" as ancestral for the four major superfamilies of Australian marsupials; namely Dasyuroidea, Perameloidea, Vombatoidea and Phalangeroidea. The G-banding patterns of the chromosomes of this complement are represented by the unmarked chromosomes (or "ancestral-types" referred to previously) in Figure 5.23. The G-banding patterns of the X and Y chromosomes of this "2n=14 basic G-banded complement" are undefined for the reasons given earlier.

2n=22 as the ancestral chromosome number

As already described, the data of Figure 5.23 are most simply interpreted in terms of a 2n=14 basic complement for the common ancestor of all four major superfamilies of Australian marsupials. However, as 2n=22 has also been favoured as the ancestral chromosome number for Australian marsupials, the simplest interpretation of the G-banding patterns of Figure 5.23 in terms of a 2n=22 ancestral complement is also presented.

Apart from whole chromosomes, the largest, most common and most diversely represented G-banded units evident from Figure 5.23, are the homologously banded chromosome arms possessed by the unmarked chromosomes.

Therefore the most straightforward inference of a 2n=22 ancestral type is one with an autosomal complement with two submetacentric or metacentric chromosome pairs corresponding in G-banding pattern to two pairs (unmarked chromosomes) of Figure 5.23 and eight acrocentric chromosomes essentially corresponding in G-banding pattern to the G-banded arms of four pairs (unmarked chromosomes) in Figure 5.23. As no particular format has been proposed for the putative 2n=22 ancestral complement, the acrocentrics of this proposed G-banded complement may correspond to the long and short arms of any four of the six autosomal pairs of Figure 5.23. To take a particular example, it will be assumed that the eight acrocentric pairs of the proposed 2n=22 G-banded ancestral complement correspond in G-banding pattern to essentially pairs 1, 2, 3 and 4 (unmarked chromosomes) of Figure 5.23. This putative G-banded 2n=22 complement is shown in Figure 5.26. The G-banded 2n=14 complements of present day animals are also represented schematically in this figure and it is now necessary to account for these by rearrangements of the proposed 2n=22 G-banded ancestral complement.

The Dasyuridae are considered first: Pair 5 is of ancestral type and pair 6 is derived by pericentric inversion. Pair 3 shows banding pattern homology with pairs 3q and 3p of the 2n=22 complement such that it may be considered as derived by Robertsonian fusion. The other three chromosomes, pairs 1, 2 and 4, are homologous with 1q, 2q and 4q respectively, for only a portion of their long arms, and their short arms are homologous with only a portion of 1p, 2p and 4p respectively. These dasyurid metacentric chromosomes can each be related most simply to the respective acrocentrics of the proposed ancestral type by Robertsonian fusion, 1p with 1q, 2p with 2q, 3p with 3q, 4p with 4q, followed by pericentric inversion of each chromosome. These proposals are consistent with the differential G-banding patterns of the centromeric segments of the acrocentrics of the 2n=22 putative ancestral complement and the

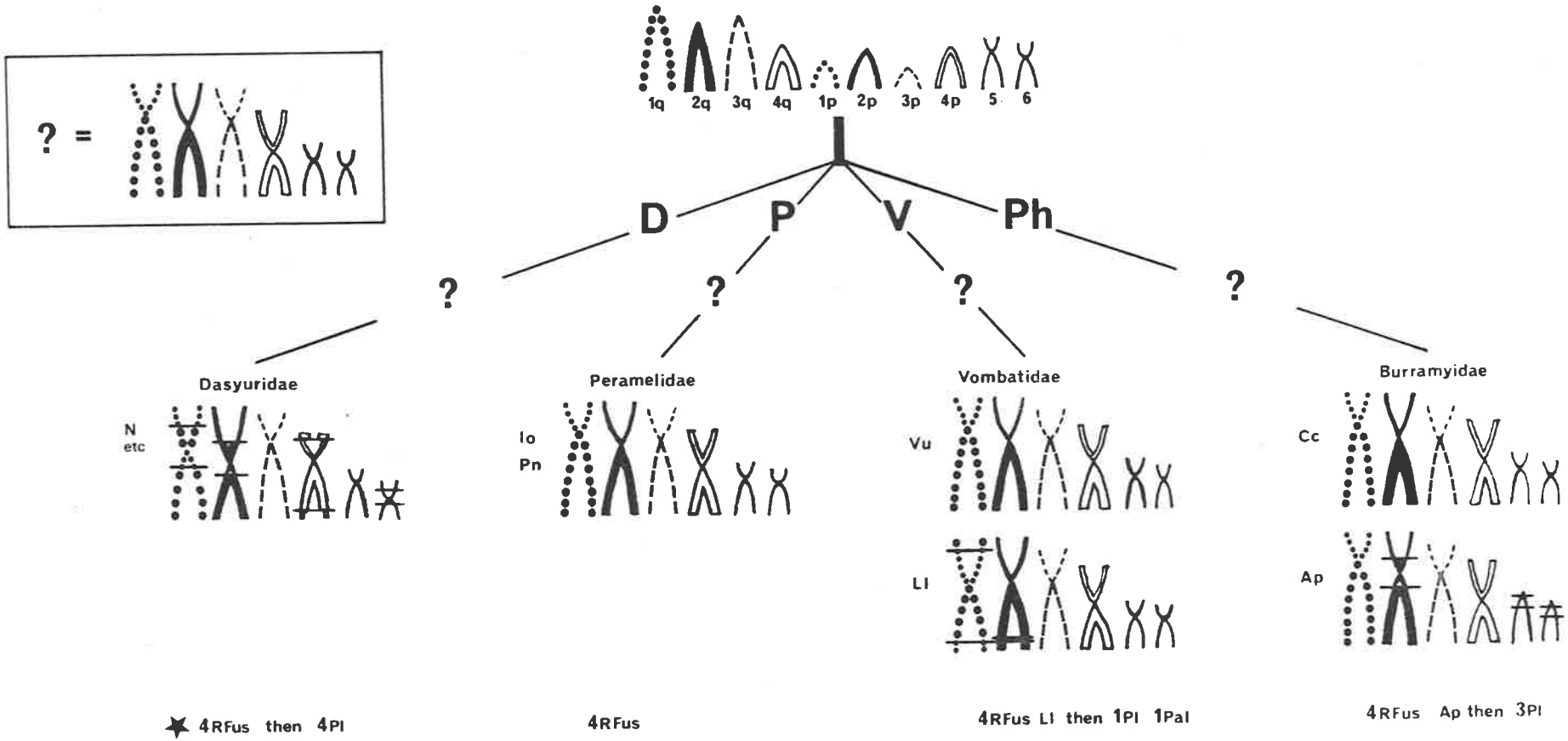
Figure 5.26

A hypothetical G-banded 2n=22 complement as ancestral for the marsupials with G-banded 2n=14 basic complements.

D	Dasyuroidea	*N* etc.	the *Ningaui* species and the other 7 species of dasyurids studied
P	Perameloidea	*Io*	*Isoodon obesulus*
		Pn	*Perameles nasuta*
V	Vombatoidea	*Vu*	*Vombatus ursinus*
		Ll	*Lasiorhinus latifrons*
Ph	Phalangeroidea	*Cc*	*Cercartetus concinnus*
		Ap	*Acrobates pygmaeus*

* rearrangements of the hypothetical G-banded 2n=22 complement required to produce the G-banded 2n=14 complements of present day marsupials

RFus Robertsonian fusion
P pericentric inversion) the inverted segments are
Pa paracentric inversion) indicated by lines


? =
1q 2q 3q 4q 1p 2p 3p 4p 5 6
D
P
V
Ph
?
?
?
?
Dasyuridae
N
etc
Peramelidae
Io
Pn
Vombatidae
Vu
LI
Burramyidae
Cc
Ap
4RFus then 4PI
4RFus
4RFus LI then 1PI 1PaI
4RFus Ap then 3PI

corresponding metacentrics of the dasyurid complements. These differential segments are shown (between the lines) on the dasyurid chromosomes in Figure 5.26.

In the Peramelidae, the G-banded complements of both present day species can be simply related to the proposed 2n=22 ancestral form by four Robertsonian fusions, 1p with 1q, 2p with 2q, 3p with 3q and 4p with 4q.

For Vombatidae, the complement of *Vombatus ursinus*, *Vu*, can be derived from the 2n=22 ancestral type by Robertsonian fusions 1p with 1q, 2p with 2q, 3p with 3q and 4p with 4q. The G-banding pattern of the complement of *Lasiorhinus latifrons*, *Ll*, can be accounted for pericentric inversion in a *Vu*-like complement subsequent to the Robertsonian fusions.

In the Burramyidae the G-banded pairs *Ap 1*, *Cc 1*; *Cc 2*; *Ap 3*, *Cc 3*; *Ap 4*, *Cc 4* can be derived from the proposed ancestral types by Robertsonian fusions 1p with 1q; 2p with 2q; 3p with 3q; 4p with 4q respectively and subsequently *Ap 2*, *Ap 5* and *Ap 6* are formed by pericentric inversions.

Thus with the G-banded 2n=22 complement shown in Figure 5.26 as ancestral, the changes proposed in each superfamily (see * in Figure 5.26) include the formation of an intermediate 2n=14 complement (?s and the rectangle in Figure 5.26). These intermediates are all (except that of the Dasyuridae which may have a metacentric pair 6) of the "2n=14 basic G-banded complement" form (in terms of the proposed 2n=22 G-banded ancestral complement of Figure 5.26, 1p with 1q, 2p with 2q, 3p with 3q, 4p with 4q and 5 and 6 of ancestral type).

These considerations have assumed that the four largest pairs (unmarked chromosomes) shown in Figure 5.23 were the eight acrocentric components of the proposed ancestral 2n=22 complement. However, if any four pairs (unmarked chromosomes) of this figure are chosen to represent the eight acrocentric chromosomes, the same intermediate 2n=14 complement will most simply relate the G-banded complements of Figure 5.23 to the

proposed 2n=22 ancestral type; since four of the six pairs of G-banded dasyurid autosomes and some of each of pairs 1, 2, 5 and 6 from other families represented require pericentric inversion subsequent to Robertsonian fusion to most simply account for the G-banding data in terms of such a 2n=22 ancestral complement.

If any 2n=22 complement with a format different to those already considered is proposed, many more and much more complex rearrangements are necessary to interpret the present data.

Also, any proposed ancestral complement with a diploid chromosome number greater than 2n=14 will most simply account for the present data only when an intermediate karyotypic form the same as in Figure 5.26, the "2n=14 basic G-banded complement", is used.

Thus unless there is independent evidence for 2n=22 as ancestral, it is illogical on the basis of the data of Figure 5.23 to argue for any complement other than the "2n=14 basic G-banded complement" as commonly ancestral for all these marsupials and thus as a common ancestor of Dasyuroidea, Perameloidea, Vombatoidea and Phalangeroidea.

Nevertheless if an ancestral chromosome number of 2n=22 is argued on some independent basis, it is then necessary to explain the extremely similar G-banding patterns of the chromosome complements of these diverse marsupials. These would be most easily explained if a 2n=22 ancestral karyotype of the form shown in Figure 5.26 was proposed. However, as the "2n=14 basic G-banded complement" is a requisite intermediate, this proposal would necessitate explanation of the production, independently in each superfamily, of this karyotypic form. That is, the formation of the same four fusion chromosomes with particular G-banding patterns, from 2n=22 G-banded complements that did not differ significantly from the putative ancestral form, needs to be accounted for. Such a situation cannot be ascribed to chance and thus requires selection which is extremely

specific for karyotypic format and which is capable of independently producing the same end result in the separate lineages of very different Australian marsupials.

The G-banded 2n=14 complements - the expectations and the results

It was expected (Sharman, 1973; Hayman and Martin, 1974) that periodic-banding analysis of the 2n=14 basic complements might provide better definition of the similarity or otherwise of these karyotypic forms, and that if these G-banded complements were either very similar or very different that the results might provice evidence for either 2n=14 or 2n=22 as the more likely ancestral number for Australian marsupials.

In retrospect it is easy to see that only if the G-banded 2n=14 complements had differed in specific ways would the results have been destructive of the 2n=14 hypothesis (for example, if the G-banded 2n=14 basic complements differed only in different combinations of the same arm components reciprocal translocation of chromosome arms may have explained the differences). It is also interesting to notice that it is not so much the extreme similarity of the G-banding patterns of these complements, but *this together with their small specific differences* that have provided the most positive evidence for 2n=14, and against 2n-22 as the ancestral chromosome number for most living Australian marsupials.

CHAPTER 6

CHROMOSOME EVOLUTION IN MACROPODIDAE (KANGAROOS AND WALLABIES)

6.1 Introduction

There are approximately fifty six species (from seventeen genera) of living kangaroos and wallabies and the family Macropodidae displays more karyotypic diversity than any other family of Australian marsupials (Figure 3.1). Thirty nine species (from fourteen genera) have been studied cytologically to some extent and chromosome lengths measurements (most of them corrected for nuclear DNA content) are available for thirty two species of thirteen genera.

The chromosome complements and their proposed relationships

The general stained chromosomes of kangaroos and wallabies have been described by Hayman and Martin (1974) in six groups,

> "within which we perceive relationships and between which there are no clear-cut similarities".

Group 1 consists of species (from eight genera) with morphologically similar 2n=22 and related complements. It includes *Petrogale* and *Thylogale* species.

Group 2 contains *Macropus* species and *Wallabia bicolor*, all with less than 2n=22 chromosomes.

Group 3 comprises *Dendrolagus* species with 2n=12 and 2n=14 chromosomes; Group 4, two *Bettongia* species with 2n=22 mostly metacentric chromosomes; Group 5, *Aepyprymnus rufescens* with 2n=32 chromosomes; and Group 6, *Potorous tridactylus* with 2n=12♀, 13♂ chromosomes.

On the basis of the common possession of 2n=22 chromosomes by species of several genera, namely *Lagorchestes*, *Thylogale*, *Petrogale*, *Setonix*,

Bettongia and *Hypsiprymnodon* a complement with this number of chromosomes was proposed as ancestral for Macropodidae (Martin and Hayman, 1966). The New Guinea macropod, *Dorcopsis veterum*, also has 2n=22 chromosomes (Hayman, personal communication).

This 2n=22 complement was proposed to have been produced by chromosome fission from a 2n=14 basic burramyid-like complement and pericentric inversions of acrocentric chromosomes were invoked to account for the different 2n=22 complements possessed by the present day macropods of Groups 1 and 4.

The particular 2n=22 karyotypic form (like that of *Thylogale billardierii*) commonly possessed by many Group 1 species, was proposed as ancestral for Group 2 species as well as those of Group 1. A pericentric inversion of one chromosome of this 2n=22 complement was postulated to have produced a complement like that of *Setonix brachyurus* and from this, by one tandem fusion and two centric fusions, a 2n=16 complement was formed that was ancestral for all *Macropus* species and *Wallabia bicolor*. The 2n=20 complement of *M. rufus* was proposed as derived from this 2n=16 complement by two Robertsonian fissions, and the 2n=10♀, 11♂ complement of *W. bicolor* by several translocations. Further relationships were suggested for species of Group 2 on the basis of known species hybridization and small differences in the morphologically similar 2n=16 complements of many *Macropus* species.

Nuclear DNA content and C-banding

The nuclear DNA values of macropodid marsupials vary quite substantially from that of *Thylogale billardierii* male, 84.5 arbitrary units, to that of *Macropus parryi* male with 124.6 units. Even within the genus *Macropus* there is considerable variation, with the values ranging from 97 units for *M. agilis* female to 126 for *M. parryi* female (Hayman and Martin, 1974).

These differences in DNA content were suggested to be at least

partly attributable to differences in the amount of C-banding material (Hayman and Martin, 1974), as the complement of *M. rufogriseus* (DNA = 116 units) showed more C-banding on all chromosomes than that of *M. fuliginosus* (DNA = 100 units). Consistent with this proposal, the results of *in situ* hybridization studies (Dunsmuir, 1976; Venolia, 1977) have shown that macropodid satellite DNAs are located at the C-banding regions of macropodid chromosomes.

Secondary constrictions, nucleolar-organizer activity and N-bands

In macropod marsupials the X chromosome (and usually only the X chromosome) is marked by a characteristic secondary constriction and this region has been shown to be the major site of ribosomal DNA cistrons in *Potorous tridactylus* (Hsu *et al.*, 1975).

Although in female macropods the paternal X chromosome is largely late replicating and genetically inactive (Sharman, 1971; Richardson *et al.*, 1971; Cooper *et al.*, 1971), and the appearance of the secondary constriction is different in the two X chromosomes (opinions differ as to whether it is the early-or late-replicating chromosome that shows the larger constriction, Hayman and Martin, 1965b; Graves, 1967; Sharman and Johnston, 1977), the secondary constriction is not included in the differentially replicating segment of the X chromosomes (Sharman, 1971; Hayman and Rofe, 1977). Furthermore, as most macropodid males do not show a secondary constriction on the Y chromosome, the observation of an achromatic region on the Y chromosome of *Macropus parryi* presented

> "interesting possibilities for the study of regulation in this region compared to the situation in other macropods."
>
> Hayman and Martin (1974)

In an examination of the possibility of differences in the number of active nucleolar organizers in macropods, Hayman and Rofe (1977) have reported that the maximum number of N-bands per metaphase (where each visible secondary constriction corresponds to a single N band) is correlated with the maximum number of nucleoli observed per interphase cell. Therefore macropodid females would appear to possess twice as many active nucleolar organizer regions (*nors*) as most males. However, the significance of these findings for dosage regulation of *nor* activity between the sexes in macropods is not clear since by the conditions of the *in situ* hybridization study in *Potorous tridactylus*, there would appear to be a number of copies of ribosomal DNA genes; and therefore if dosage regulation does occur the form it takes may be quite different to that of single copy genes.

X and Y chromosomes

Kangaroos and wallabies show great variation in the size (corrected for nuclear DNA value) and morphology of their X chromosomes, and species with large X chromosomes tend also to have large Ys (Hayman and Martin, 1969, 1974).

It has been suggested (Hayman and Martin, 1974) that the larger X and Y chromosomes may have increased amounts of repetitive DNA, with such regions staining darkly after C-banding, or that they may possess translocated autosomal material.

Hayman and Rofe (1977), after G-, C-, N-banding and late DNA replication studies of the chromosomes of a number of macropodid species, concluded that their observations, together with others (Graves, 1967; Sharman, 1971; Hayman and Martin, 1974) were consistent with the concept of a basic unit of the X-chromosome in Macropodidae. The C-bands replicated synchronously in both X-chromosomes of female macropods and were not a part of this basic region. Changes in the amount of C-banding were largely responsible for the considerable variation in the size of the

X chromosomes. In the four macropodid species (*Macropus fuliginosus*, *M. parryi*, *M. rufus* and *Wallabia bicolor*) studied with G-banding, the basic X-chromosomal region appeared to show a similar G-banding pattern.

The present study

In this chapter C-, N- and G-banding studies of the chromosomes of ten species of kangaroos and wallabies are presented and discussed. These macropods are from groups 1 and 2 of Hayman and Martin (1974). The species, animals and methods used, together with the source of the chromosome preparations, are shown in Table 6.1.

6.2 The C-banded chromosomes

C-banding analysis of these macropodid complements was considered necessary before their G-banding patterns were compared as the nuclear DNA values for these species are so variable.

The two C-banding methods (see, Table 6.1) gave generally similar C-banding patterns. However, the non-centromeric C-bands of X and Y chromosomes and the bands flanking the secondary constrictions were often more pronounced after treatment with barium hydroxide than with sodium hydroxide. Prolonged exposure to alkali resulted in smaller C-bands. Unless otherwise indicated, the following descriptions and comparisons of C-banding patterns refer only to the spectrum of bands shown after a standard alkali treatment.

The C-bands of these macropodid complements are usually distributed symmetrically about the centromere. However, in species with large C-bands these regions are often visibly composite (Figure 6.1 a, b, c) and it is sometimes hard to pinpoint the centromere. The apparent size of the C-band also varies from cell to cell. Thus with such background variation it is difficult to ascertain true chromosome pair heteromorphism in C-band symmetry and size. Also, where two or three chromosome pairs are

NSW	New South Wales.
SA	South Australia.
Q	Queensland.
Z	samples obtained from animals at the Adelaide Zoological Gardens, localities unknown.
Z(SA)	as for Z, but known to be from South Australia.
Z(WA)	" " " " " " " " Western Australia.
CAH	animal obtained from the Central Animal House, University of Adelaide; known only to be from South Australia.
MG	cell culture obtained from Dr. J.A. Marshall Graves.
C	" " " " Professor D.W. Cooper.
CSL	" " " " Commonwealth Serum Laboratories.

L	short-term lymphocyte culture.
F	culture of fibroblast-like cells.

C-	C-banding.
B	" " with barium hydroxide.
N	" " with sodium hydroxide.

N-	N-banding.
NHP	" " with NaH_2PO_4.
T	" " with TCA.
Ag	" " with $AgNO_3$.

G-	G-banding.
G	animals represented in G-banding karyotypes or charts.

(x)	x is the number, when less than 15, of cells recorded.

*	when there is more than one entry per species per C- or N-banding technique, * marks the method used to band chromosome complements shown in Figure 6.2.

Table 6.1

The macropods studied and the chromosome banding methods used.

2n	Species	Sex	Source or locality	Culture type	Chromosome banding method C-	N-	G-
22	*Petrogale penicillata* the brush-tailed rock wallaby	♂	Jenolan Caves, NSW	L	B		G
22	*Thylogale billardierii* the red-bellied pademelon	♀	Z	L	B		
		♂	Z	L	B*	NHP	G
20	*Macropus rufus* the red kangaroo	♀	CAH	L	N	T	G
		♂1	Z	L	N,B	T*	G
		♂2	Z	L	N,B*	T(6)	
16	*Macropus fuliginosus* the western grey kangaroo	♂1	Z	L	N(9),B	T	
		♂2	Z	L	B*	NHP*	G
16	*Macropus giganteus* the eastern grey kangaroo	♀	MG	F	B	T	G
16	*Macropus eugenii* the Kangaroo Island wallaby	♀1	Kangaroo	L		T(12)*	
		♀2	Island,	L	B*	T	G
		♂	SA	L	B		
16	*Macropus parryi* the whip-tailed wallaby	♀	CSL	F	N	T	G
		♂	C	F	B*	T,Ag(14)*	G
16	*Macropus rufogriseus* the red-necked wallaby	♀	Z	L	N,B*	T*,NHP	G
16	*Macropus robustus* the euro	♀	Z(SA)	L	B*	NHP	G
		♂	Z(WA)	L	B(8)		
10♀,11♂	*Wallabia bicolor* the swamp wallaby	♂1	Pechey, Q	L	N	T*	
		♂2	Z	L	B*	T	G

Figure 6.1

Banded macropodid chromosomes.

a-e C-banding
f,g N-banding
h G-banding

a the complex C-band of *Petrogale penicillata* chromosome 2 - barium hydroxide.

b complex C-bands in the autosomes of the Western Australian *Macropus robustus* - barium hydroxide.

c the complex C-band of an autosome of *Macropus rufogriseus* - sodium hydroxide.

d the heteromorphic C-banded X-chromosomes of the female *Thylogale billardierii*, showing the multi-banded appearance of the secondary constriction region - barium hydroxide.

the dots indicate centromere positions.

e the C-banded heteromorphic pair 2 of the female *Macropus rufus* - sodium hydroxide.

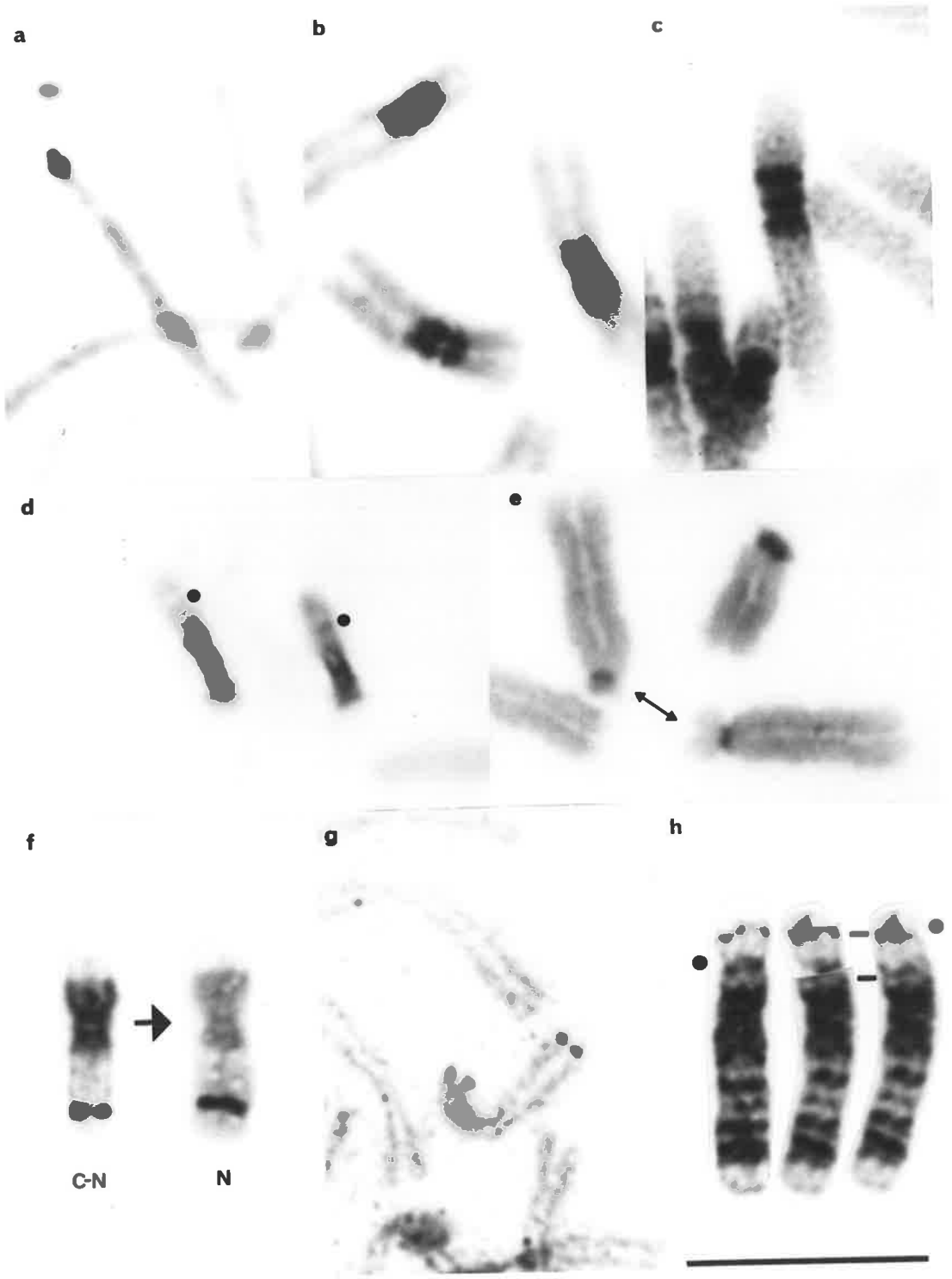
f a progression of C- and N-band to predominantly N-band display with increasing exposure of the *Macropus rufogriseus* X chromosome to NaH_2PO_4.

g a metacentric Y chromosome, with silver N-bands near the end of each arm, of the *Macropus parryi* cell line.

h the outermost chromosomes are the G-banded heteromorphic pair 2 of the female *Macropus rufus*.

the dashes indicate the breakpoints of the postulated pericentric inversion which produced the more metacentric chromosome (centre and left).

the dots indicate centromere positions.


a
b
c
d
e
f
g
h
C-N
N

morphologically very similar (for example, the general stained pairs 3 and 4 of most *Macropus* species with 2n=16 chromosomes) only relatively large C-banding differences between the homologues of a pair can be detected. For these reasons the chromosome pair heteromorphism recorded in the following pages is undoubtedly an underestimate of the variation that may be present.

With the possible exception of some *Petrogale penicillata* chromosomes, all acrocentric chromosomes of the macropods studied were clearly biarmed after C-banding and the short arms were at least terminally capped with non C-banding material. That is, no completely C-banding short arms were observed for macropodid chromosomes, although some of these short arms are of the size (between 0.1 and 0.6 of the length of the chromosome relative to the total X-containing haploid genome length) that Imai (1975) has argued are entirely C-banded in most mammals. Thus the C-banding patterns of these macropodid chromosomes suggest that the growth or deletion of completely C-banding short arms (Duffey, 1972; Pathak *et al.*, 1973b) has not featured in the evolution of these complements.

All Y chromosomes appeared to be biarmed, but because of their small size their exact morphology (for example, submetacentric or acrocentric) could not be defined. The response of Y chromosomes to C-banding was very variable. Often they appeared homogeneously grey with a stain intensity intermediate to that of the centromeric bands and the unbanded chromosome arm. The centromere of the Y chromosome was frequently marked by a small dark band. In other metaphases Y chromosomes were completely darkly stained.

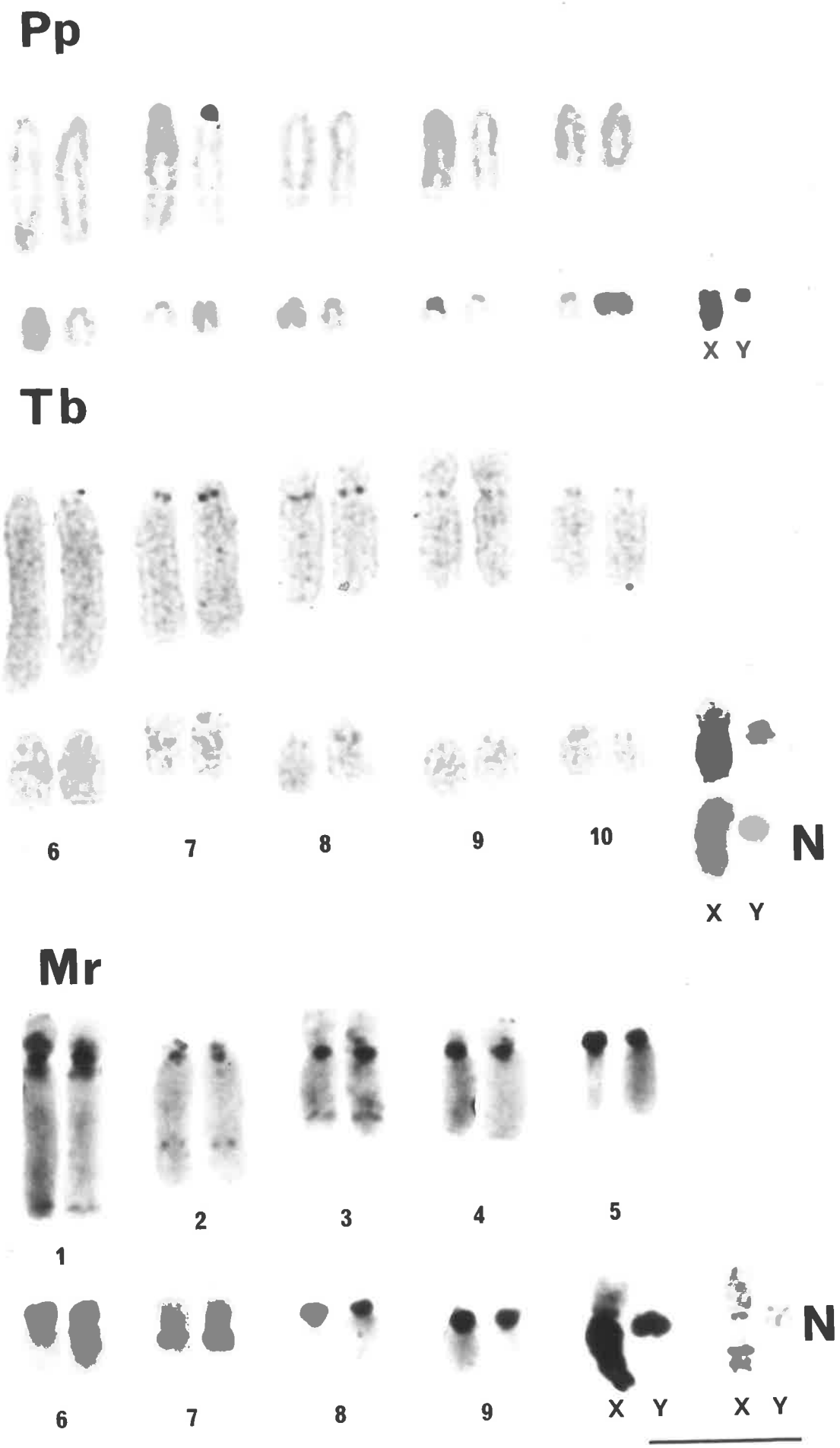
Figures 6.2 *Pp* to *Wb* are the C-banding karyotypes for each species studied. The method used to produce the C-bands of each complement in Figure 6.2 is shown in Table 6.1. For all species detailed patterns of X chromosome banding are shown in Figures 6.12 and 6.13. The following descriptions of the C-banding patterns of the chromosomes of each

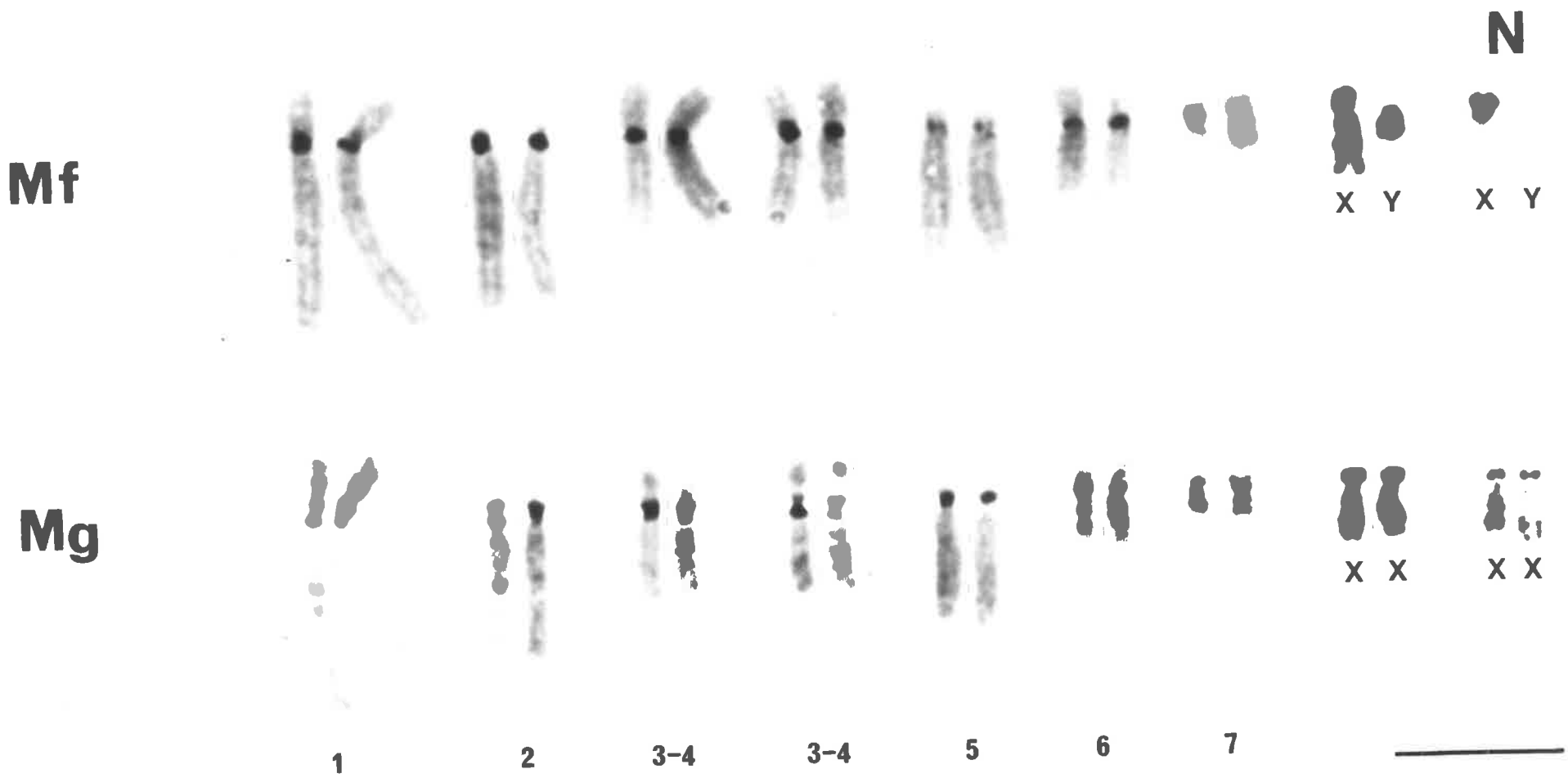
Figure 6.2

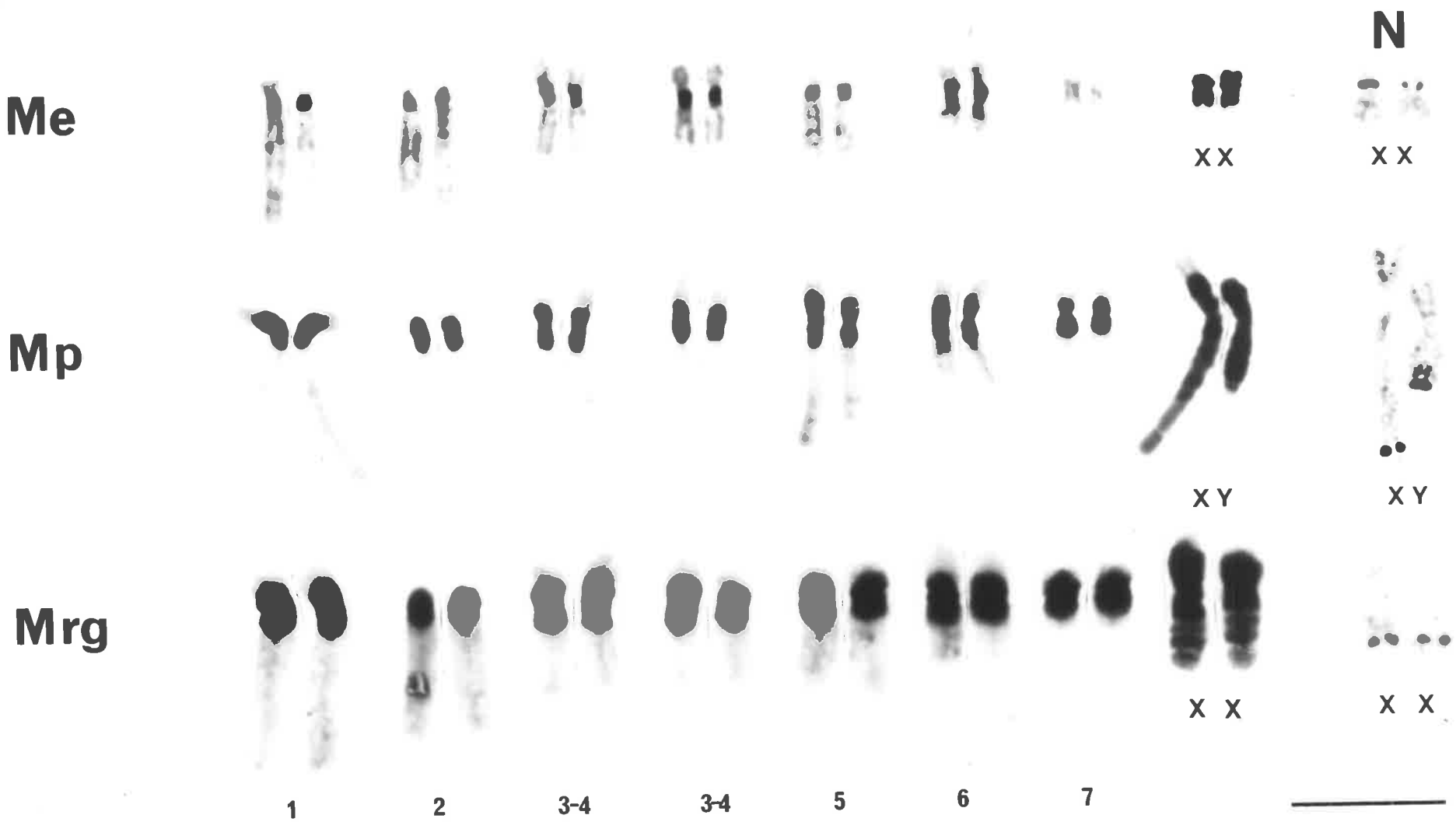
The C-banding karyotypes and N-banded chromosomes of macropods.

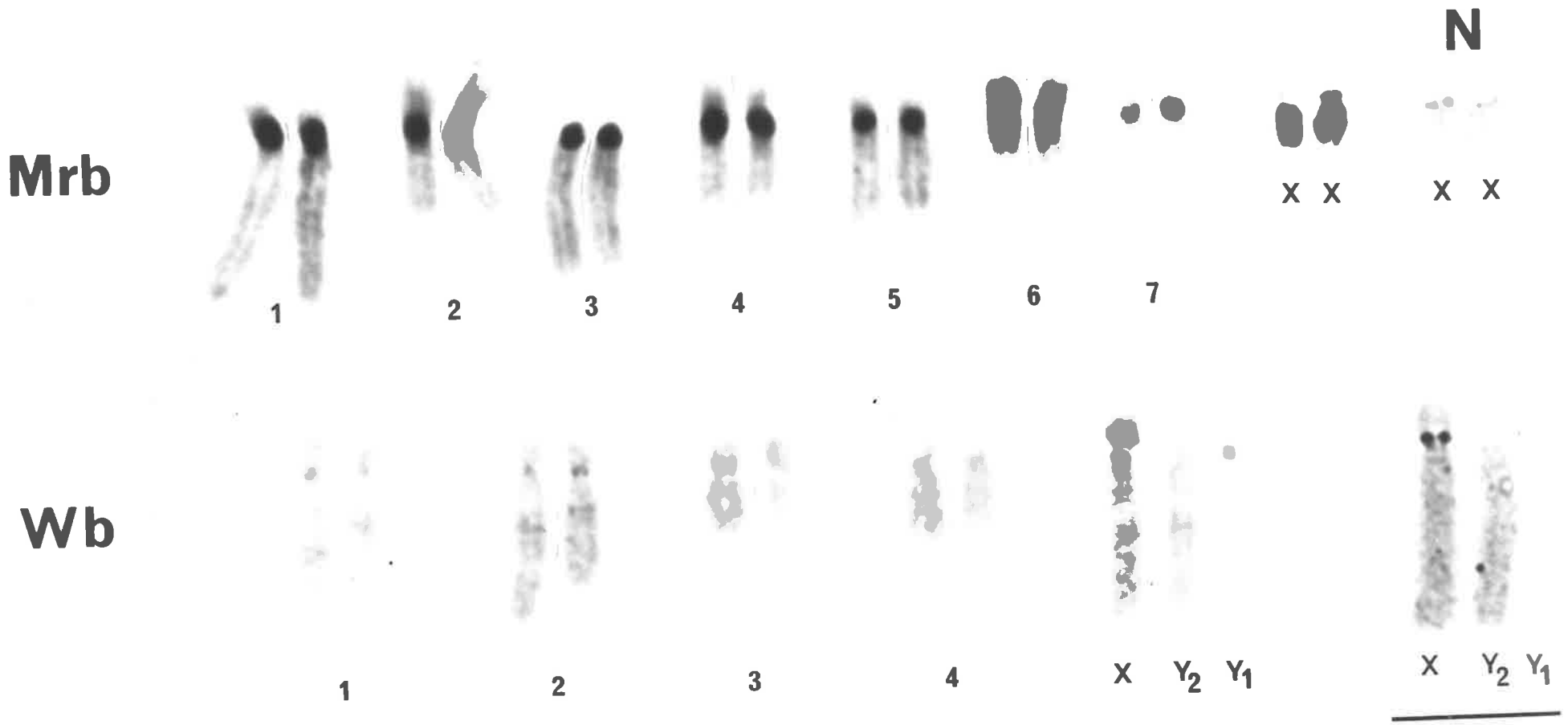
Pp	*Petrogale penicillata*
Tb	*Thylogale billardierii*
Mr	*Macropus rufus*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Mrb	*Macropus robustus*
Wb	*Wallabia bicolor*
N	N-banded chromosomes

The methods used to C- and N-band these chromosomes are shown with *s in Table 6.1.


Pp
X Y
Tb
6
7
8
9
10
N
X Y
Mr
1
2
3
4
5
6
7
8
9
X Y
X Y
N


N
Mf
X Y X Y
Mg
X X X X
1
2
3-4
3-4
5
6
7


N
Me
Mp
Mrg
XX
XX
XY
XY
X X
X X
1
2
3-4
3-4
5
6
7


N
Mrb
X X
X X
1
2
3
4
5
6
7
Wb
1
2
3
4
X
Y2
Y1
X
Y2
Y1

macropodid species document only intra-specific differences and particular points of interest.

Petrogale penicillata - Figure 6.2 *Pp*

Centromeric C-bands are regularly seen on chromosome pairs 2, 5 to 10 inclusive and the X chromosome, with very small C-bands infrequently visible on the other autosomes. Most chromosomes (including pair 2) can be seen in some cells to possess very small, non C-banding short arms. The large C-bands of chromosome 2 (see Figure 6.1 a) and the X are complex structures, with the X-band appearing more than double in extended chromosomes.

Thylogale billardierii - Figure 6.2 *Tb*

All chromosomes of both animals have very small, often indistinct C dots at the centromeres, and are visibly biarmed. The X chromosome has a very large C-band corresponding to the secondary constriction region. In the female animal studied this C-band is twice as large in one X chromosome as it is in the other, and in extended chromosomes a number of smaller bands can be seen within this larger C-band (Figure 6.1 d). The trabant of the X chromosome may appear grey.

Macropus rufus - Figure 6.2 *Mr*

This is the only species where interstitial C-bands were frequently seen. They appear in four autosomal pairs (1, 2, 3 and 7) and also in the short arm of the X-chromosome after C-banding with barium hydroxide or short sodium hydroxide treatment. The short arms of chromosome pairs 5, 6, 8 and 9 appear almost completely dark, although distally somewhat greyer than at the centromere. Figure 6.2 *Mr* shows a karyotype of male-2 after such mild alkali C-banding. After stronger treatment the pattern is different with no interstitial bands and the C-bands being restricted

to the immediate centromeric region of all autosomes; pairs 1, 3 and 4 having the most C-banding and 2 and 7 the least.

The X chromosome usually displays a small centromeric C-band with the C-banding pattern of the long arm being quite complex in extended chromosomes. Near the centromere two dark bands may be visible followed by two paler bands more distally. The interbands are greyer than the short arm. These four bands in the long arm can sometimes be further resolved into minor bands. In contracted X chromosomes the proximal half of the long arm is darkest. The short arm of the X chromosome shows a faint median band after mild alkali C-banding.

The female *M. rufus* studied has a heteromorphic pair 2, the two homologues having different arm ratios (Figure 6.1 e, h). Both male *M. rufus* are homozygous for the chromosome with the more terminal centromere.

Macropus fuliginosus - Figure 6.2 *Mf*

In both animals studied, pair 5 shows noticeably less C-banding than all other chromosomes. The centromeric C-band of the X chromosome of male-2 is the largest in the complement, and is slightly larger than that of male-1. The X chromosomes also show a pronounced C-band distal to the secondary constriction in the short arm and two non-centromeric bands in the long arm; one median, the other at the distal end.

These patterns are generally similar to that of a C-banded metaphase preparation from *M. fuliginosus* published by Hayman and Martin, 1974.

Macropus giganteus - Figure 6.2 *Mg*

Chromosome pair 5 has the smallest centromeric C-bands and pairs 1 and 6 are heteromorphic for C-banding pattern. For pair 1, a similar amount of C-banding material is differently distributed in the two homologues and the chromosome has almost all the C-band on the short arm,

while the other chromosome is more symmetrically banded. For pair 6, one chromosome has C-banding only in the short arm, while the other has more C-banding material, and it is present on both arms. The X chromosome has a centromeric band of average size and a C-band distal to the secondary constriction in the short arm. Median interstitial and terminal C-bands are visible on the long arm.

Macropus eugenii - Figure 6.2 *Me*

In the male wallaby, the C-band of pair 1 is mainly in the long arm, whereas these bands are more symmetrically distributed in the corresponding chromosomes of the female studied with C-banding. This female animal also has very small C-bands on pair 7, smaller than those of the male. In both animals the X chromosome has a centromeric C-band of average size, which extends into the long arm. There is also a C-band distal to the secondary constriction in the short arm of the X chromosome.

Macropus parryi - Figure 6.2 *Mp*

In both animals the second chromosome has approximately the least C-banding material. The X chromosome of *M. parryi* is the largest X in marsupials that is not a component of a multiple sex chromosome system, and more than three-quarters of its length is C-banded. There is a block of particularly dark C-bands around the centromere and also a dark band in the middle of the short arm. The C-bands of the remainder of the X chromosome are lighter. The Y chromosome appears completely darkly stained, except for a secondary constriction near the end of the long arm. The smallest autosomal pair in the male may have a heteromorphic distribution of C-bands.

Macropus rufogriseus - Figure 6.2 *Mrg*

The C-banding regions of the chromosomes of this species are massive (Hayman and Martin, 1974; Dunsmuir, 1976) and appear to correspond with the regions of heterochromatin described by Fredga (1964).

Pair 2 consistently shows the least C-banding material and appears heteromorphic in most metaphases, with one homologue having a smaller C-band or staining less darkly than the other.

In agreement with Hayman and Martin, the long arm of the X chromosome is not completely banded. Distal to the large centric C-band in this arm there are three separate bands, one each side of the secondary constriction and another between the centromeric band and the secondary constriction. The distal one-quarter of the short arm is unbanded. The X chromosome has the largest centromeric C-band of the complement.

Macropus robustus - Figure 6.2 *Mrb*

Pair 7 has the smallest centromeric C-bands of the complement in both the South and Western Australian euros. These two animals differ in the size distribution of their C-bands. In complements of the Western Australian euro, pairs 2 and 4 have more C-band material than pair 1, whereas for the South Australian animal the reverse is true. Also, the C-band distal to the secondary constriction in the short arm of the X chromosome of the Western Australian animal is darker than that in the South Australian euro. In the X chromosomes of both animals the centromeric C-band is completely in the long arm and grades into a greyish C-band extending midway down the arm.

Wallabia bicolor - Figure 6.2 *Wb*

The autosomes show very little centromeric C-banding. The X chromosome of both animals is C-banded at the centromere and also distal to the secondary constriction in the short arm. Male-1 has a centromeric

C-band on the Y_2 chromosome comparable in size to that of the X, but the Y_2 chromosome of male-2 (Figure 6.2 *Wb*) has less C-banding, even less than the autosomes.

The most striking feature of the C-banded macropodid complements is their pattern variability. The amount and distribution of C-banding material shows considerable variation between species and although only a maximum of three animals of each species were studied, there are some apparent intra-specific differences.

C-bands and nuclear DNA content

The amount of C-banding material (as estimated visually) appears to be positively correlated with nuclear DNA content where values are available for these species. If the species are grouped in order of increasing amounts of C-banding, which is considered approximately the same within the brackets, (*Thylogale billardierii*, *Wallabia bicolor*), (*Macropus eugenii*, *M. fuliginosus*, *M. rufus*, *M. robustus*), (*M. parryi*, *M. rufogriseus*), the corresponding nuclear DNA values taken from Hayman and Martin (1974) are [98(♂), 84(♂)], [98(♂), 100(♀), 104(♂)], [125(♂), 116(♂)].

6.3 The N-banded chromosomes

In all complements N-bands prominently marked only the visible secondary constrictions of macropodid chromosomes. Thus all X chromosomes, but the Y chromosome of only *Macropus parryi*, were N-banded. Representative N-banded X chromosomes and the *M. parryi* Y chromosome are shown in Figure 6.2.

Some cells of the *M. parryi* male possessed Y chromosomes that N-banded at each end (Figure 6.1 g). As these chromosome preparations were made

from cultures of fibroblast-like cells, it is possible that they are isochromosomes or some other culture-related aberration.

Two of the N-banding techniques, *NHP* and *Ag* in Table 6.1, also stained the centromeric region of all chromosomes to some extent; but only faintly when the secondary constriction N-bands were most prominent (Figure 6.1 f). The three different N-banding techniques (see Table 6.1) gave consistent results, whether applied to chromosomes of the same preparation, individual or species.

In many metaphases of chromosome preparations from female macropods, one of the two X chromosomes showed a much larger N-band than the other. In a smaller proportion of cells only one X was banded. The X with the larger N-band appears to correspond to the chromosome with the more pronounced secondary constriction. Since both X chromosomes of the female seem able to organize a nucleolus (Hayman and Rofe, 1977), the band difference may be explained simply by a difference in contraction or in the activity of the nucleolar organizer region or even in the number of ribosomal DNA copies at that site.

In most macropods studied (except *M. parryi*, *M. rufus* and *Thylogale billardierii*) the N-banding site appears to be flanked by a single C-band. For *M. parryi* the secondary constrictions of both the X and Y chromosomes are flanked on both sides by a more extended C-banding region. In *M. rufus* the N-bands correspond to two C-interband areas (Hayman and Rofe, 1977). The N-banding pattern of the *T. billardierii* X chromosome is characteristically granular and covers the entire secondary constriction (Figure 6.2 *Tb*). This same area shows a number of C-bands and interbands in extended chromosomes (Figure 6.1 d). Thus it is possible that in all these macropodid species the N-banding regions are immediately flanked by C-bands. As C-bands correlate with sites of constitutive heterochromatin, such observations probably do little more than reflect the long known association of the nucleolar organizing region of chromosomes with heterochromatin.

6.4 The G-banded chromosomes

For each species (except *Macropus rufogriseus* and *M. robustus*) a G-banding karyotype (Figures 6.3 *Pp* to *Wb*) and chart (Figures 6.4 to 6.11) is presented. For *M. rufogriseus* and *M. robustus* only G-banding karyotypes are shown (Figures 6.3 *Mrg* and *Mrb*) as only six and nine (respectively) well G-banded metaphases were available for analysis. More detailed G-banding patterns of X chromosomes are shown in Figure 6.12 and 6.13 and these are discussed, together with their C-banding in section 6.6. Where G-banded Y chromosomes are missing from karyotypes and charts representatives are also shown in Figures 6.12 and 6.13.

As usual, the chromosomes in karyotypes and charts are numbered according to the measurements of Hayman and Martin (1974). However, although some autosomal pairs could not be distinguished by arm ratio and length measurements of general stained chromosomes, they could be after G-banding. The rationale adopted for the numbering of such G-banded chromosomes is given below.

For *Thylogale billardierii* pairs 8 and 9 were assigned G-banding patterns as in Figures 6.3 *Tb* and 6.5.

For *Petrogale penicillata* and *Macropus rufus* where autosome pairs could not be clearly assigned numbers by size and morphology they were given the same number as the *T. billardierii* chromosome with the corresponding G-banding pattern. The pattern of pair 7 of *P. penicillata* was not at all clear after G-banding analysis (Figure 6.4) and therefore this pair was numbered by exclusion.

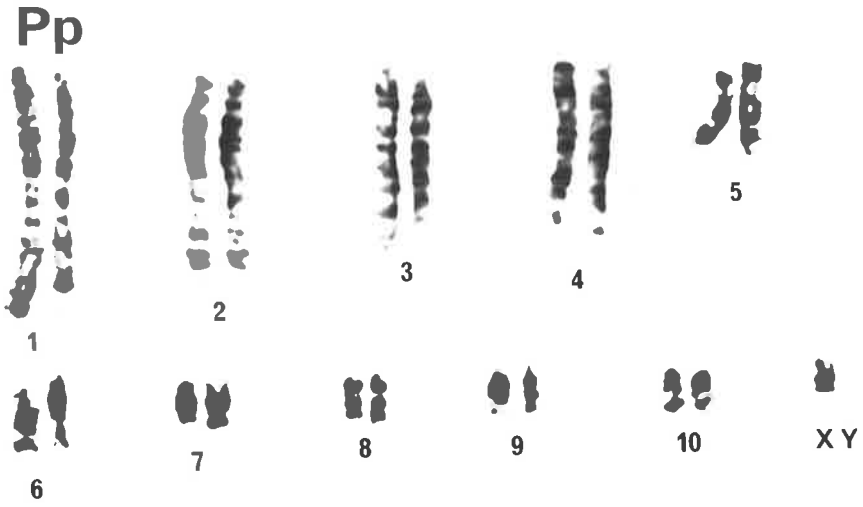
For *Macropus fuliginosus*, *M. giganteus*, *M. eugenii*, *M. parryi* and *M. rufogriseus* the general stained chromosome pairs 3 and 4 are of equal arm ratio and total length. The G-banded chromosomes with a pattern corresponding to that of *T. billardierii* pair 4 was chosen as pair 4 for all these macropods.

Figure 6.3

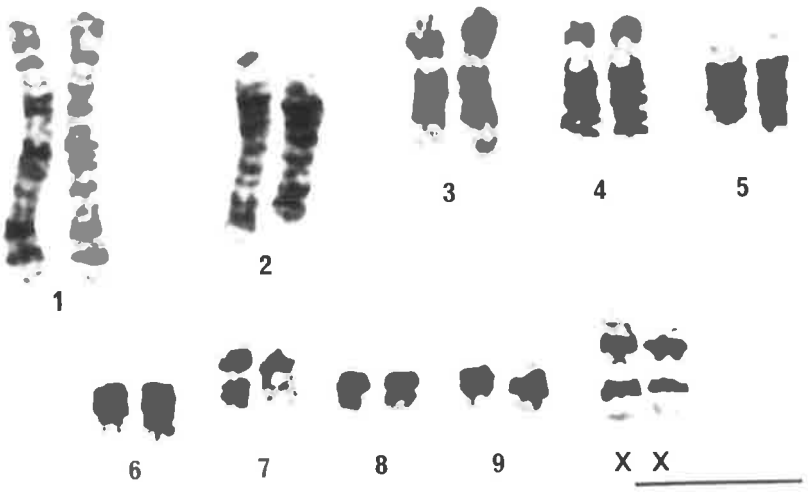
The G-banding karyotypes of macropods.

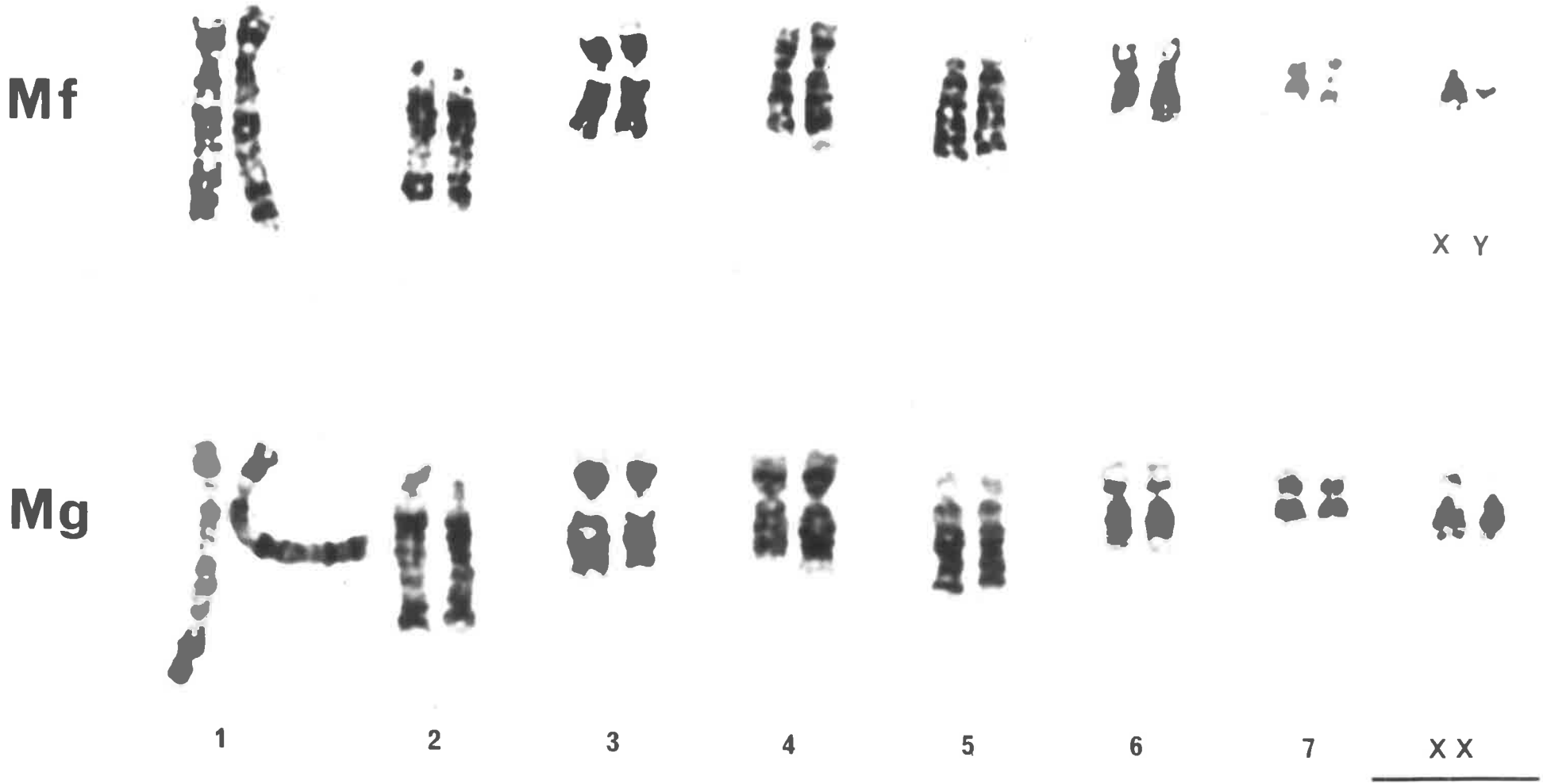
Pp	*Petrogale penicillata*
Tb	*Thylogale billardierii*
Mr	*Macropus rufus*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Mrb	*Macropus robustus*
Wb	*Wallabia bicolor*

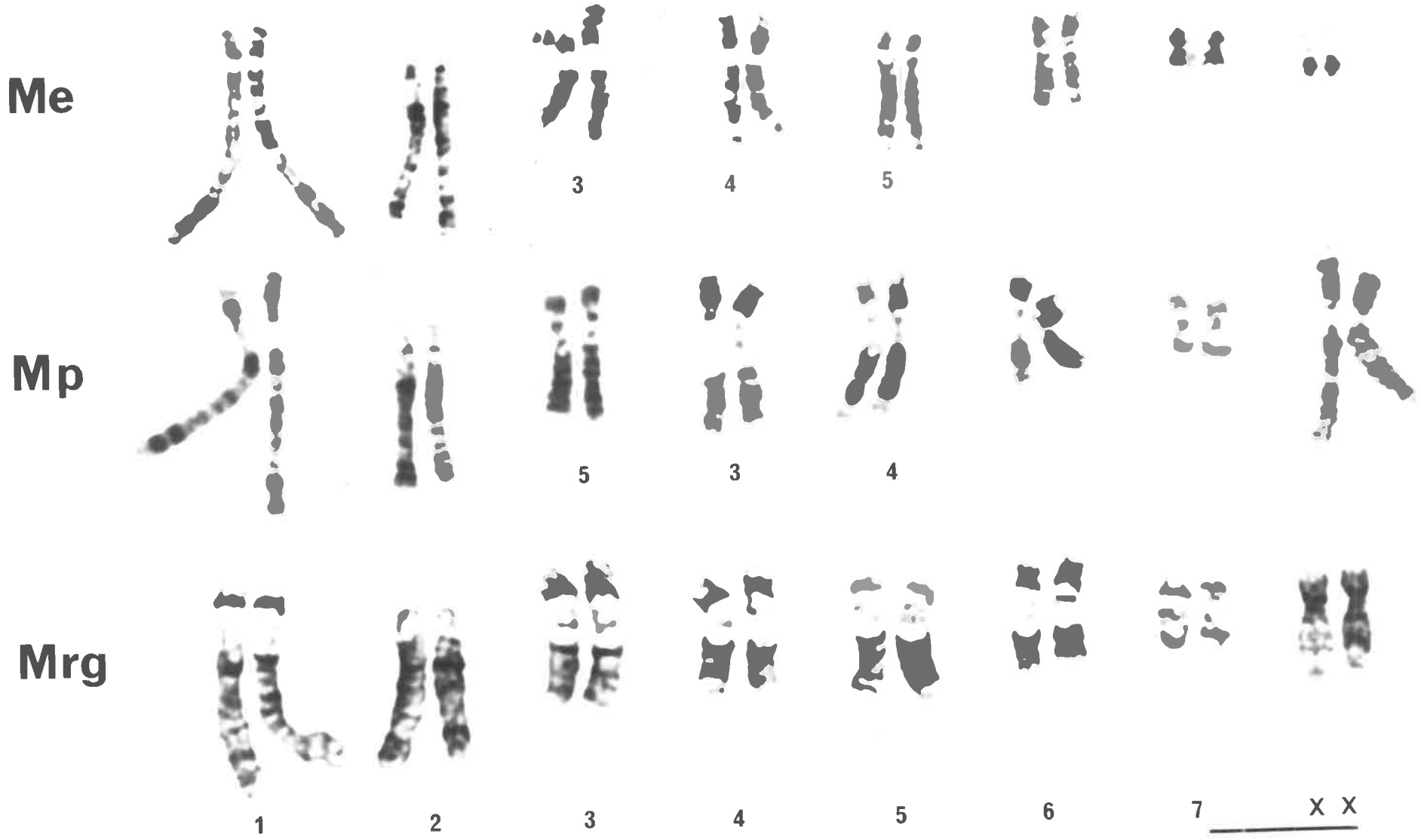
Where Y chromosomes are missing from the karyotypes of male macropods, G-banded representatives are shown in Figures 6.12 and 6.13.

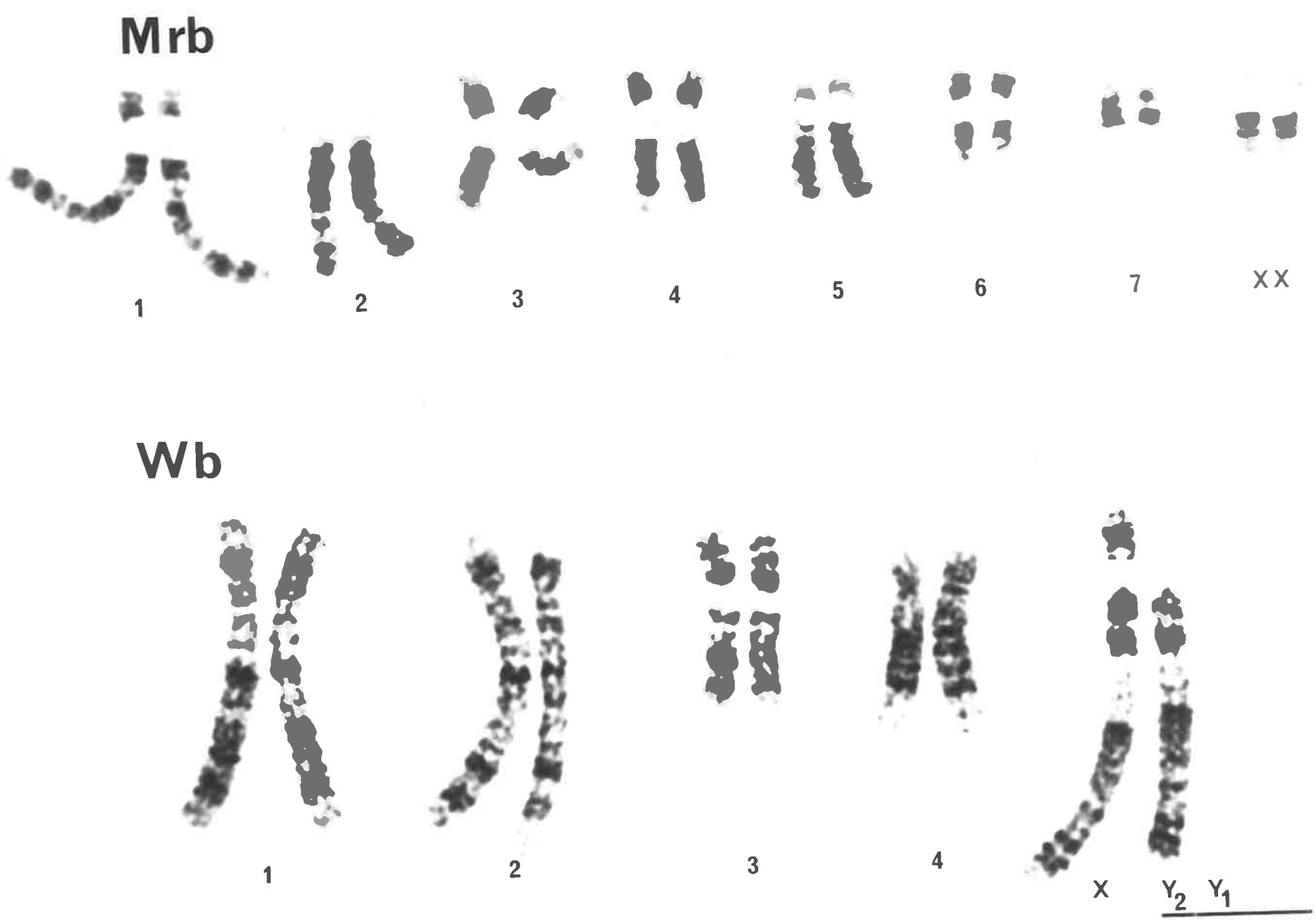

Pp
1
2
3
4
5
6
7
8
9
10
X Y


Tb
1
2
3
4
5
6
7
8
9
10
X


Mr
1
2
3
4
5
6
7
8
9
X X


Mf
X Y
Mg
1
2
3
4
5
6
7
X X


Me
Mp
Mrg
3
4
5
5
3
4
1
2
3
4
5
6
7
X X


Mrb
1
2
3
4
5
6
7
XX
Wb
1
2
3
4
X
Y2
Y1

Figures 6.4 to 6.11

The G-banding charts for macropods.

6.4	*Pp*	*Petrogale penicillata*
6.5	*Tb*	*Thylogale billardierii*
6.6	*Mr*	*Macropus rufus*
6.7	*Mf*	*Macropus fuliginosus*
6.8	*Mg*	*Macropus giganteus*
6.9	*Me*	*Macropus eugenii*
6.10	*Mp*	*Macropus parryi*
6.11	*Wb*	*Wallabia bicolor*

Where Y chromosomes are missing from the charts of male macropods, G-banded representatives are shown in Figures 6.12 and 6.13.

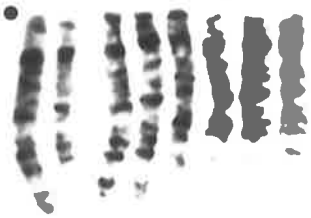
Pp

1

2

3

4



5 

6 

7 

8 

9 

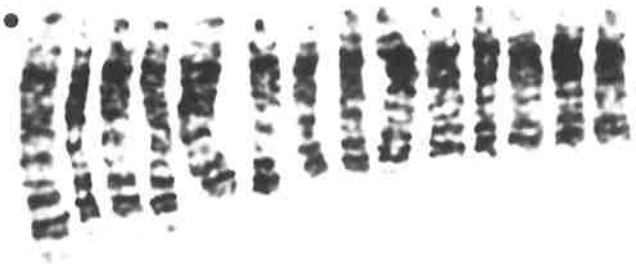
10 

X 

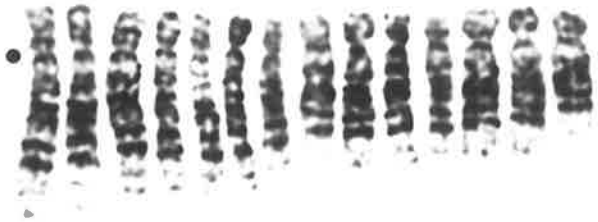
Tb

1

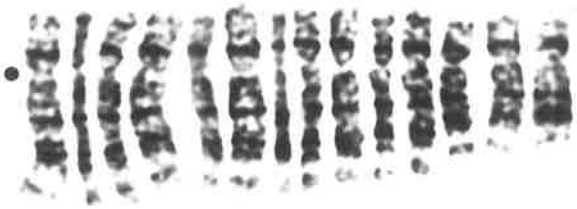
2



3



4



5

6

7

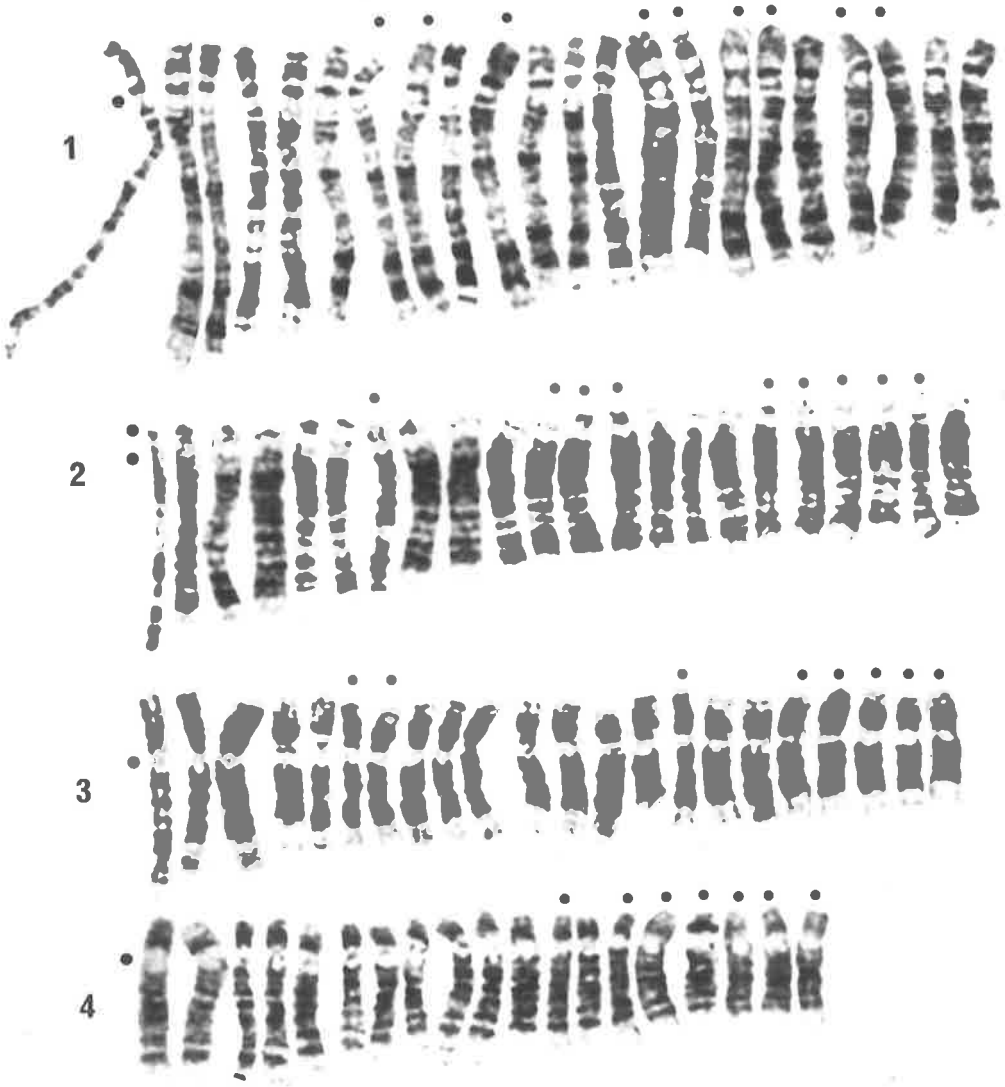
8

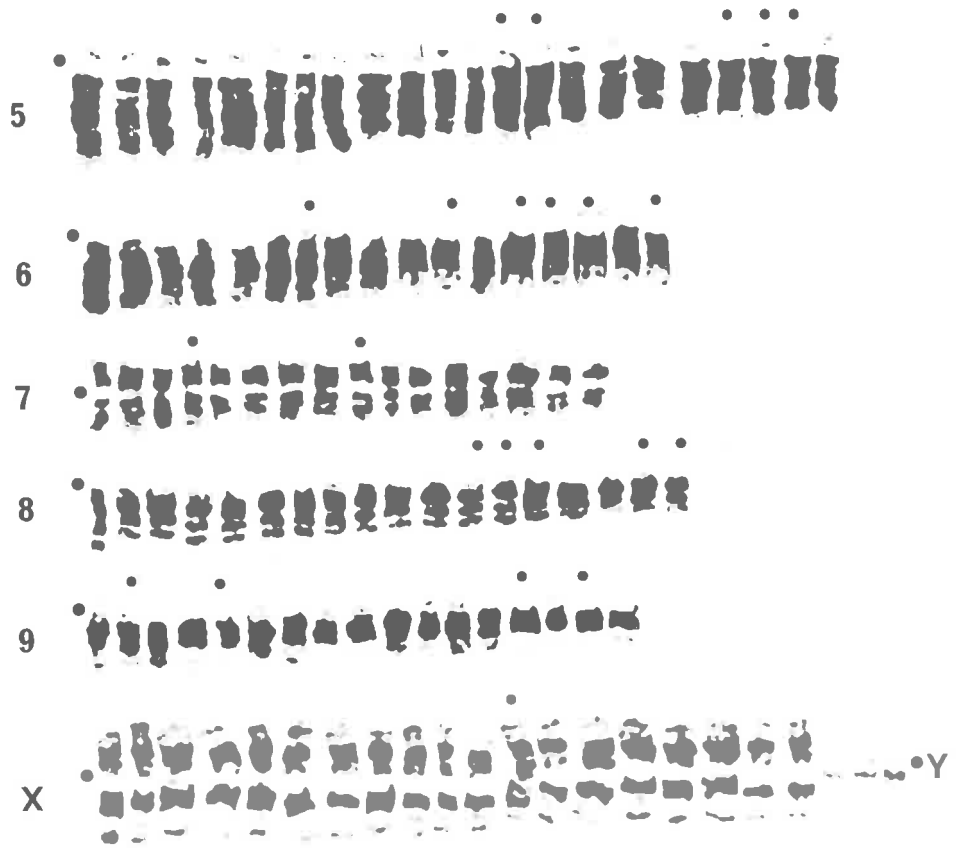
9

10

X

Mr

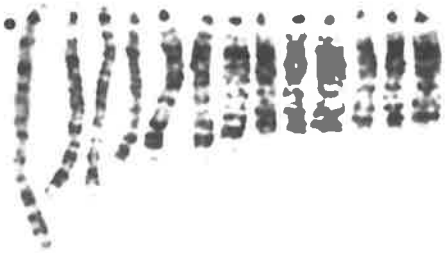



5
6
7
8
9
X
Y

Mf

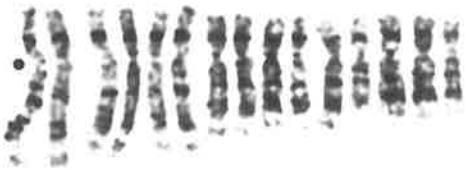
1

2



3

4



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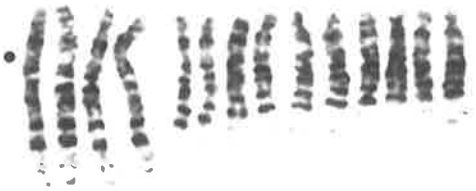


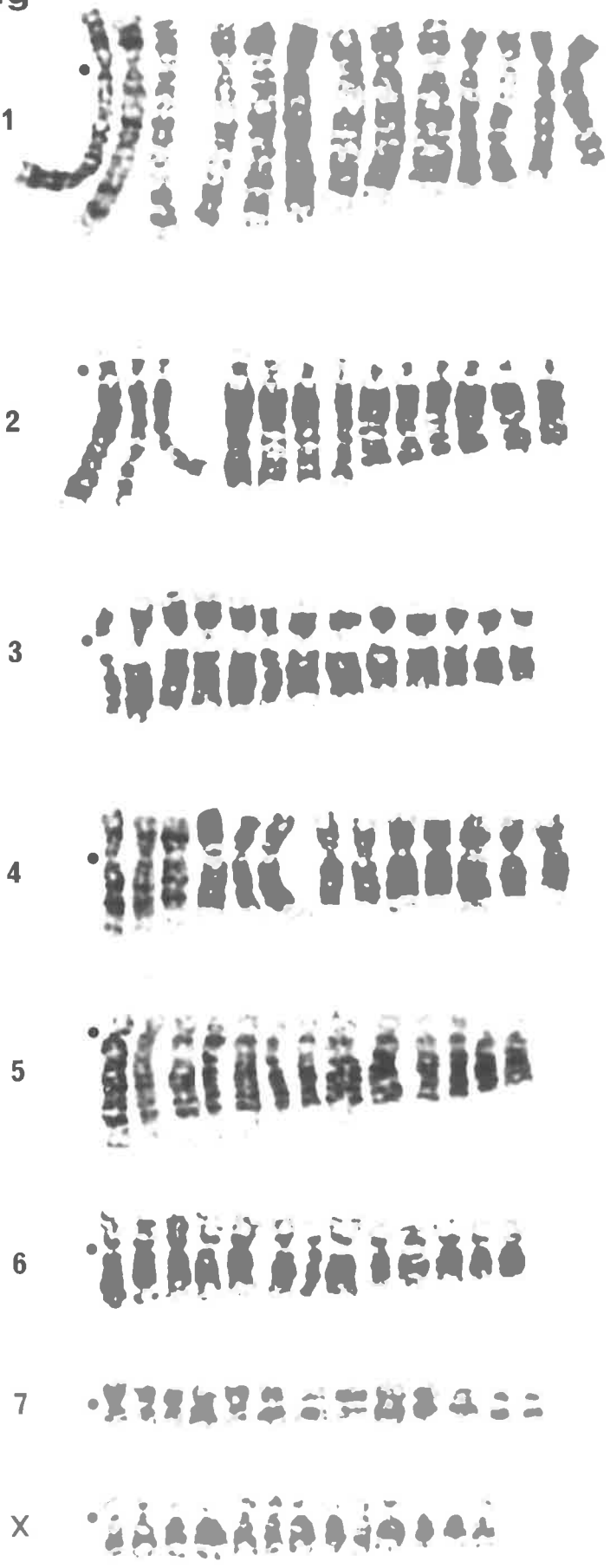
6

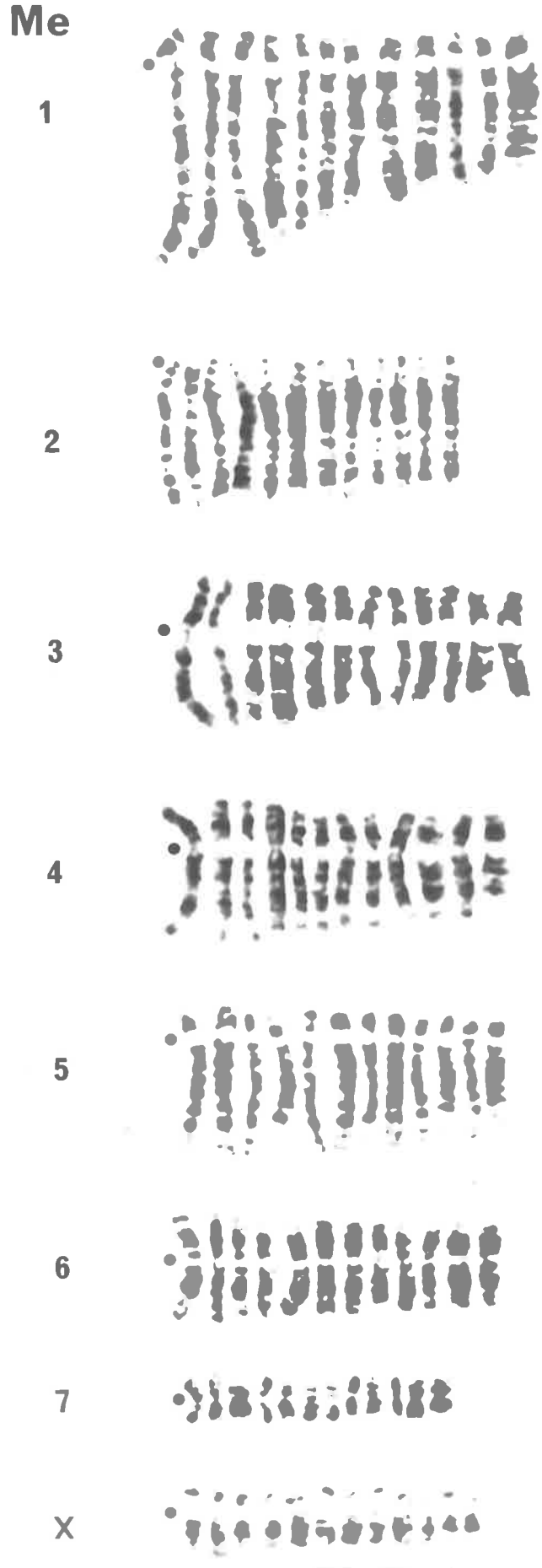
7

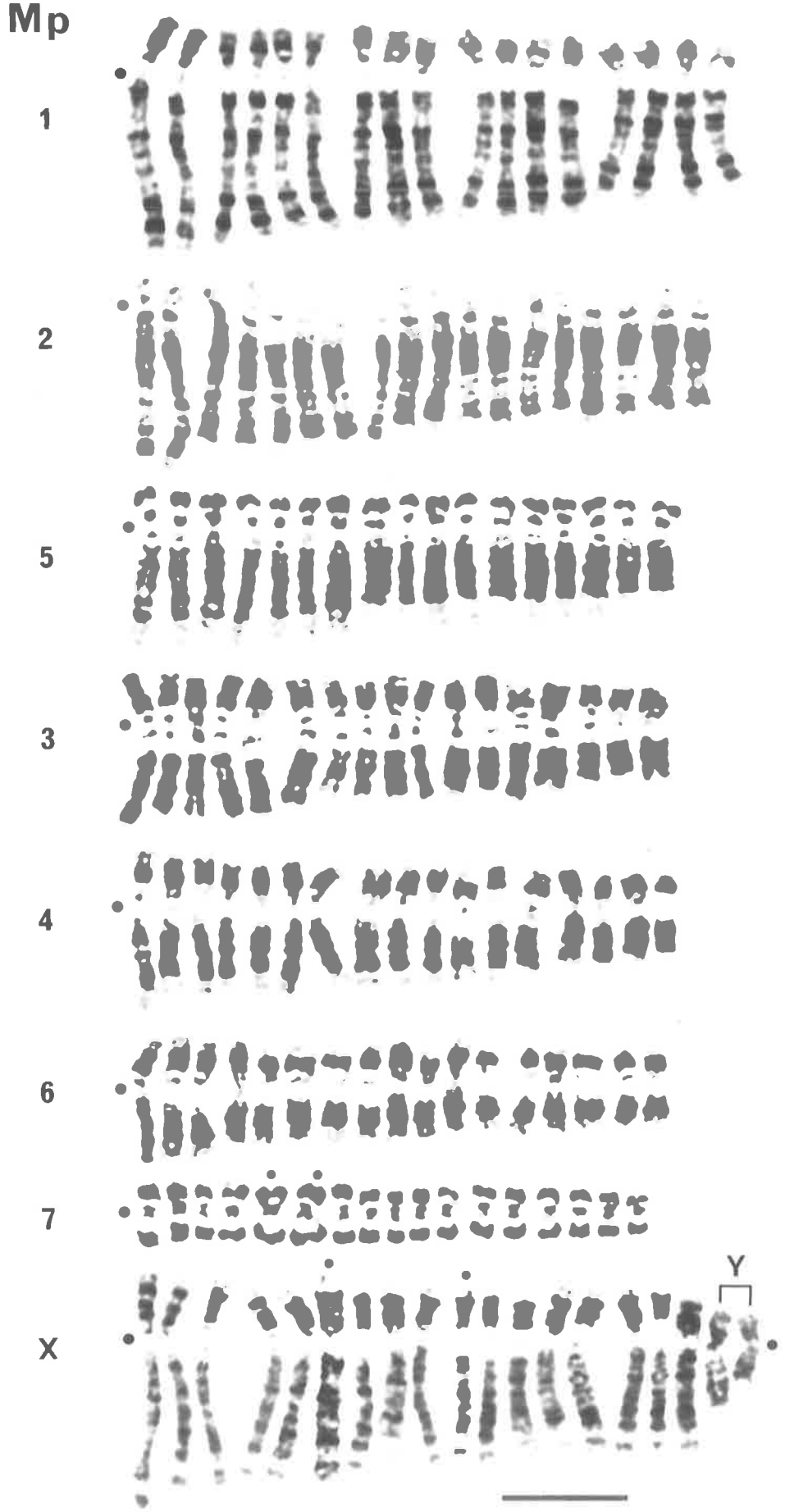
X

Y

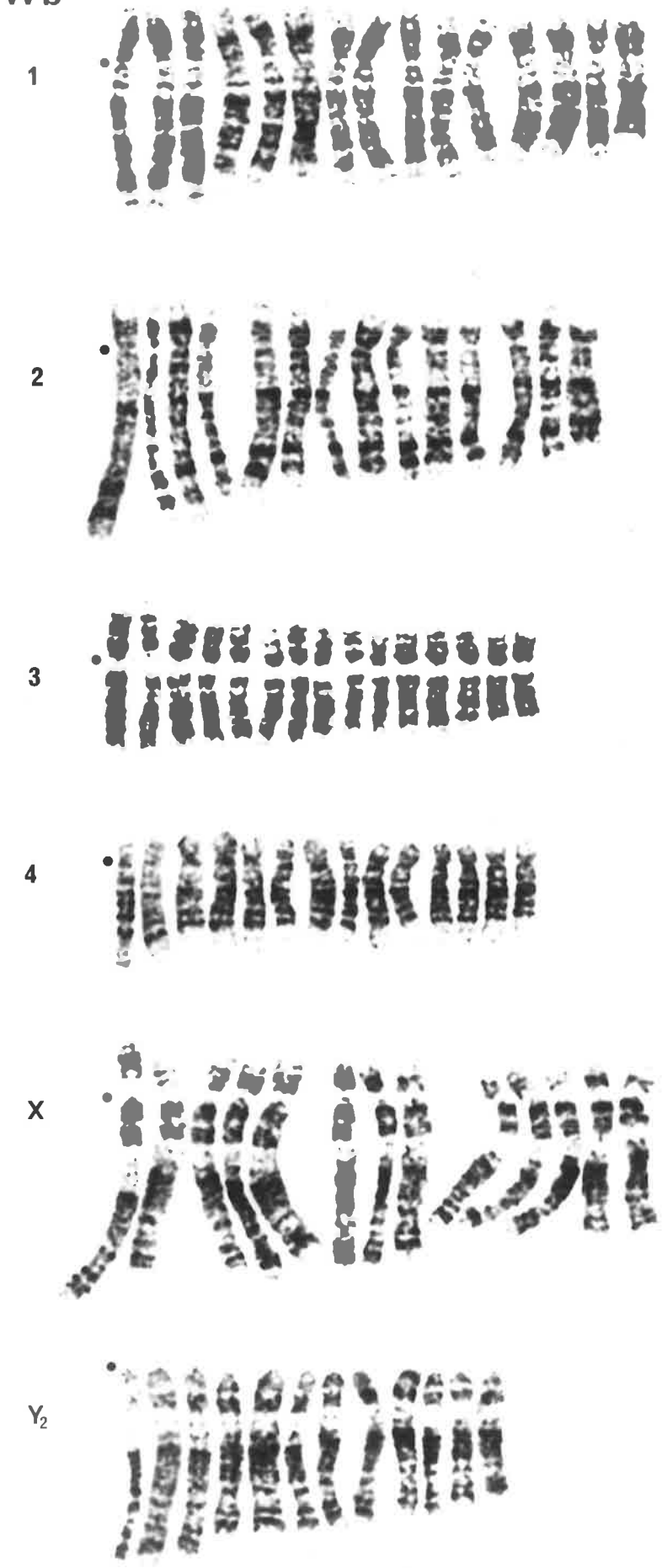
Mg




Me
1
2
3
4
5
6
7
X


Mp
1
2
5
3
4
6
7
X
Y

Wb



6.5 The G- and C-banded chromosomes

No chromosome preparations were sequentially G- and then C-banded. The following observations were made by comparing the G- and C-banding patterns of different cells of the same animal or species. (See Figures 6.2, 6.3 and 6.4 to 6.11)

Most C-bands correspond to very lightly G-banded areas. The large C-banding regions of the chromosomes of *Macropus rufogriseus* and *M. robustus* are particularly devoid of stain after G-banding. Some C-banding areas, although pale, do show discrete G-bands (for example those of *fuliginosus* and *M. giganteus*), while others, particularly the long arm of the X chromosome of *M. parryi*, do not show any clear G-banding pattern. The centromeric C-banding region of the *M. rufogriseus* X chromosome stains very darkly after G-banding.

Not only is there variation in the response of C-band areas to G-banding between the chromosome complements of different species, but also between chromosomes within a complement. This is well illustrated by the differential G-banding of the centromeric C-banding regions of *M. rufogriseus* autosomes and the X chromosome. The centromeric C-band of the X chromosome also responds diffcrently to those of the autosomes when stained with quinacrine (Pearson *et al.*, 1971) and following *in situ* hybridization of *M. rufogriseus* satellite DNAs (Dunsmuir, 1976).

C-banding regions within a particular chromosome may also G-band differently. For example, the C-banded long arm of the *M. rufus* X chromosome shows, after G-banding, a white region near the centromere with grey bands more distally.

The C-banding patterns of autosomes with homologous G-banding patterns in the complements of different species (section 6.7) have also been compared. Only specific points of interest are documented.

For individual chromosomes with G-banding pattern homology in

different *Macropus* species, chromosomes corresponding to *Thylogale billardierii* pairs 2, 3 and 7 often appear to have smaller C-bands than all other autosomes. These chromosomes are three of the four pairs that have single homologously G-banded chromosome counterparts in all *Macropus* species.

There are two groups of species with complements that show G-banding pattern homology for all autosomes. *M. fuliginosus* and *M. giganteus* comprise one group, and the C-banding regions of the autosomes of these species respond similarly to G-banding (grey with grey bands), but differently to those of all other species. Chromosome pair 5 shows the least C-banding in the complements of both species. *M. eugenii*, *M. parryi* and *M. rufogriseus* also group after G-banding. Two of these species, *M. parryi* and *M. rufogriseus*, have less C-banding on pair 2 than all other autosomes, but this is not so for *M. eugenii*. This latter species shows a much reduced C-band (at least in one of the two animals studied) in pair 7. *M. parryi* and *M. rufogriseus* both have very large areas of C-banding material.

In view of the large differences in the amount and distribution of C-bands between species grouped by G-banding pattern similarity, and also of the extent of intra-specific C-banding variation, which is likely to approach inter-specific differences for particular chromosomes, no phylogenetic relationships are proposed on the basis of C-banding patterns.

6.6 The X and Y chromosomes

A G-banded X chromosome from each macropodid species and some G-banded Y chromosomes are shown in Figures 6.12 and 6.13. The Y chromosomes are included to illustrate that even small Y chromosomes show G-banding differentiation, and also to provide examples of Y chromosome G-banding for species where these chromosomes are not shown in G-banding karyotypes

Figure 6.12

The G-banded X chromosomes of macropods.

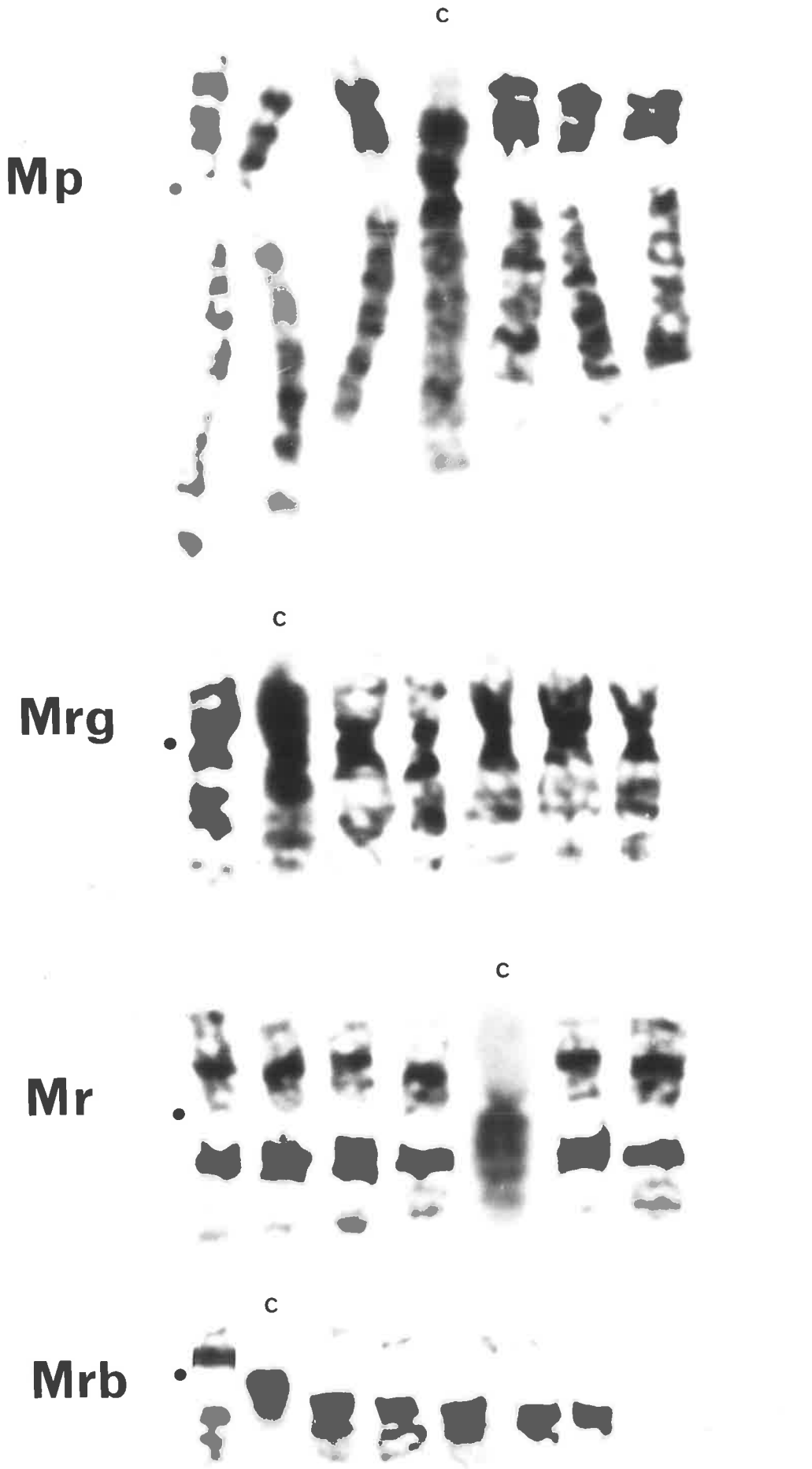
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Mr	*Macropus rufus*
Mrb	*Macropus robustus*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Tb	*Thylogale billardierii*
Pp	*Petrogale penicillata*

A G-banded X chromosome of *Wallabia bicolor* is shown in Figure 6.13 together with a G-banded Y and a C-banded X chromosome of this species.

C C-banded X-chromosomes, which are included to facilitate comparisons of the G-banding patterns of these chromosomes.

ys G-banded Y chromosomes that are not represented in karyotypes or charts.

The dots indicate centromere position.


c
Mp
c
Mrg
c
Mr
c
Mrb

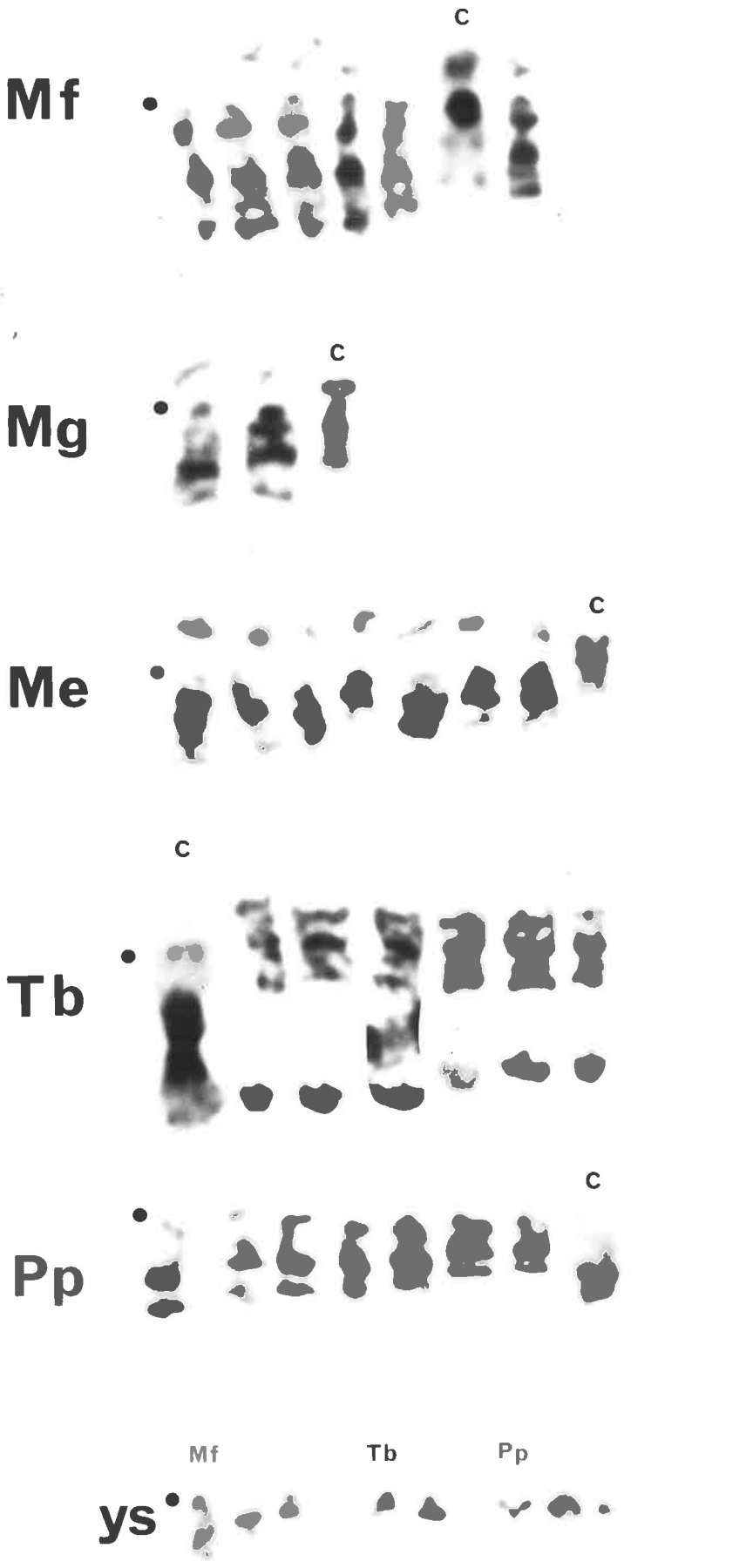

Mf
C
Mg
C
Me
C
Tb
C
Pp
C
Mf
Tb
Pp
ys

Figure 6.13

A comparison of the G-banded X chromosomes

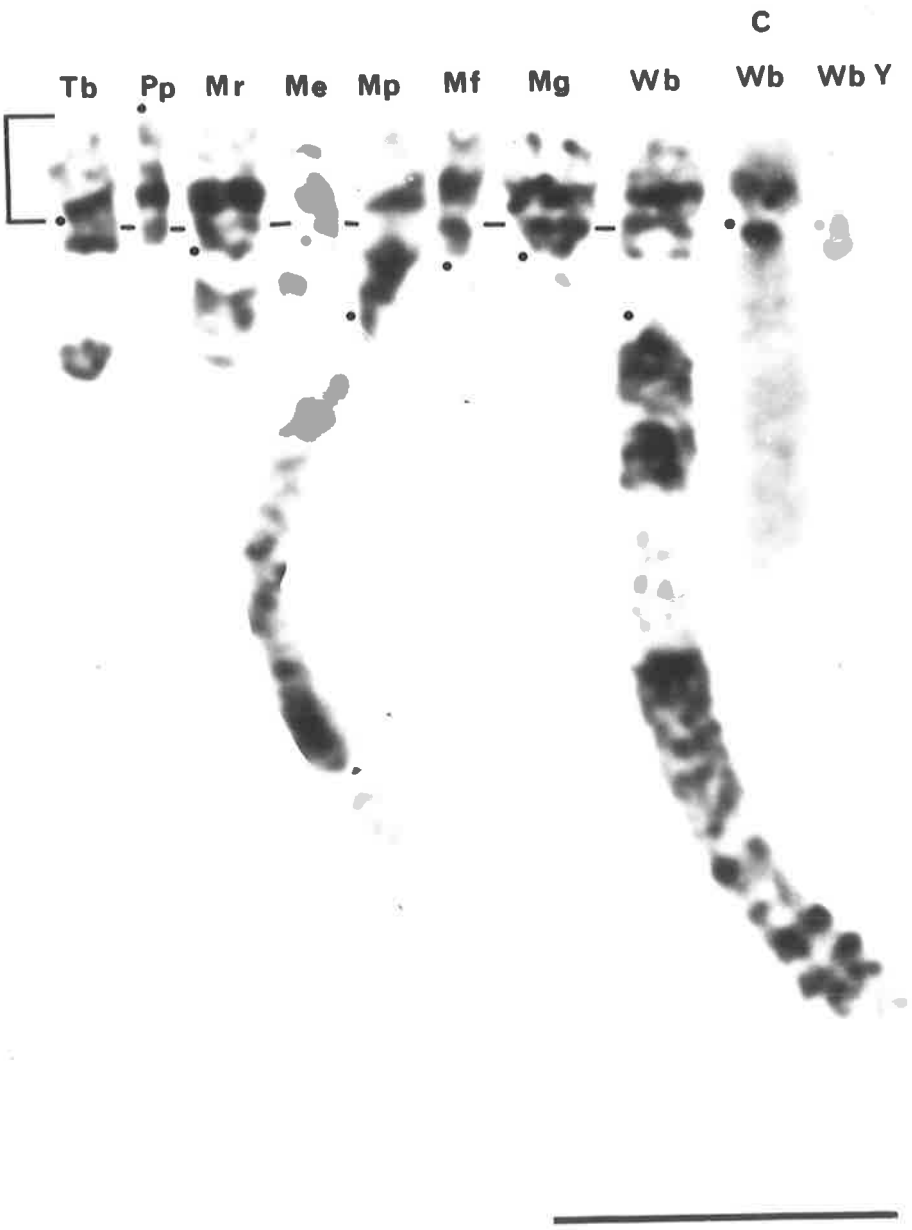
of macropodid marsupials.

The bracket delineates the regions which show a similar sequence of G-bands in different X chromosomes.

The X chromosomes of *Macropus robustus* and *Macropus rufogriseus* are not included as these chromosomes do not display this G-banding sequence (see Figure 6.12).

A G-banded Y and a C-banded X chromosome of *Wallabia bicolor* are included, as this species is not represented in Figure 6.12.

Centromere positions are marked by small dots.


C
Tb
Pp
Mr
Me
Mp
Mf
Mg
Wb
Wb
Wb Y

and charts. A single C-banded X chromosome from each species is included in Figures 6.12 and 6.13 for easy reference.

Inspection of these figures shows that, as suggested in section 6.1, differences in the amount of C-banding material can largely account for the substantial size differences between the X chromosomes of these species. (The X chromosome of *Wallabia bicolor* is part of an XY_1Y_2 sex chromosome system and the original X corresponds to the short arm of this chromosome.)

Therefore, apart from the characteristic secondary constriction and its trabant (which are variably located), the basic X-region may be defined by the non C-banding areas. For convenience "basic X-region" in the following text excludes the secondary constriction and trabant.

In X chromosomes where the non C-banding, or the basic X-region, is in a single uninterrupted block, its G-banding pattern appears to consist of one dark and approximately three grey bands. However, where the basic X-region is immediately adjacent to the secondary constriction, there appears to be an extra dark G-band at the junction (see the X chromosomes of *Thylogale billardierii*, *Petrogale penicillata* and *W. bicolor*). Also, interstitial C-bands interrupt the continuity of the basic region in the X chromosome of *Macropus parryi* and there is often a faint interstitial C-band visible at the site of the largest G band of the basic X-region of *M. rufus*, *M. fuliginosus* and *M. giganteus*. The G-banding pattern of the basic X-region cannot be ascertained for *Macropus rufogriseus*, Figure 6.12 *Mrg*, where it appears to be in at least two small sections, the distal half of the short arm and the middle of the long arm.

Although given the above variation, and the small size of the basic X-region, it is possible that G-banding pattern homologies of these regions may be largely superficial, there appears to be a similar sequence of G-bands in the basic X-region of many of the macropods studied; see Figure 6.13. This region, which is shown as bracketed in the figure,

is slightly larger than that previously proposed as basic (Hayman and Rofe, 1977). It includes one additional grey band.

The basic X-regions of *P. penicillata* and *T. billardierii* have similar G-banding patterns to those of most other macropods, differing from the common pattern only in centromere position. The basic X-region of *M. robustus* shows a different G-band sequence to that of all other macropods. All three differences can be accounted for by pericentric inversions.

The Y chromosomes of these macropodid species vary in size and C-banding pattern (section 6.2). No comparison of their G-banding patterns is attempted.

6.7 The relationships of the G-banded autosomes

The autosomal G-banding patterns of all macropods studied are most simply described in terms of the G-banding karyotype of *Thylogale billardierii*, the red-bellied pademelon. Therefore a brief description of the G-banding patterns of the autosomes of this species is now given.

The G-banded autosomes of *Thylogale billardierii* - Figures 6.3 *Tb* and 6.5

All but two chromosomes have unique and distinctive G-banding patterns, and pairs 6 and 9, with similar patterns, can be readily distinguished by their difference in size. Only the 'land-mark' features of each autosome pair are described and for acrocentric chromosomes 1, 2, 5, 8, 9 and 10, the descriptions refer to the long arms only.

Pair 1

The two dark bands in the distal third and the dark band about one-third of the arm's length from the centromere, are the 'land-mark' features of this chromosome arm. Between the centromere and the most proximal dark

'land-mark' band there is a pale region bisected by a grey band, otherwise the arm appears rather evenly banded.

Pair 2

The proximal half of the arm is dark. The distal half is striped; four or five bands alternating with white interbands.

Pair 3

The short arm is grey with several bands, one flanking the centromere. In the long arm there is a pale proximal region followed by a number of evenly spaced bands. Four of these are dark and occur in doublets in which each two bands are separated by white interbands. There is a large white distal region with two faint bands.

Pair 4

The short arm is grey with a dark band in the proximal half. In the long arm there is a dark band and a broad dark region, at a third and near two-thirds of the arm's length from the centromere respectively. This chromosome also has a large pale distal end to the long arm, but with a definite median grey band.

Pair 5

There is a white region near the centromere and then three large dark bands equally spaced along the arm. Between the most proximal and the median large bands there is often a discrete band.

Pair 6

This is a grey arm, with a darker proximal half.

Pair 7

This chromosome is grey, with two or three small bands on each arm.

Pair 8

This G-banded chromosome arm has a very striped appearance. There are three equally spaced dark bands in the long arm, the most proximal interband is grey, the other white. The terminal region is white.

Pair 9

This pair has a similar pattern to pair 6; however, the contrast between the two halves of the long arm for pair 9 is greater and unlike pair 6 there is a grey band near the distal end.

Pair 10

The arm is pale, with a band near the centromere and two others; one median, the other near terminal.

Accounting for C-banding

The variation in C-banding pattern of the complements of these kangaroos and wallabies is taken into account when their G-banding patterns are compared. The way this is done is illustrated in Figure 6.14 for a comparison of the G-banded autosomes of *Macropus parryi*, the whip-tailed wallaby (2n=16), with those of *Thylogale billardierii* (2n=22).

If the C-banding regions are deleted from the G-banded autosomes of the whip-tailed wallaby, these chromosomes can be compared with the G-banded autosomes of *T. billardierii* which has virtually no centromeric C-bands. Thus the G-banding patterns of chromosomes 2, 4, 5 and 7 of *M. parryi* correspond to those of chromosomes 2, 3, 4 and 7, respectively, of *T. billardierii*; and chromosomes 1, 3 and 6 of *M. parryi* correspond to Robertsonian or centric fusions, 1 with 10 (1/10), 5 with 8 (5/8), and 6 with 9 (6/9) of *T. billardierii* acrocentric chromosomes.

The G-banding regions near the very large C-bands of *M. parryi* chromosomes are often distorted (see Figure 6.10). Such distortion is also quite marked for G-banded chromosomes of *M. rufogriseus* (Figure 6.3

Figure 6.14

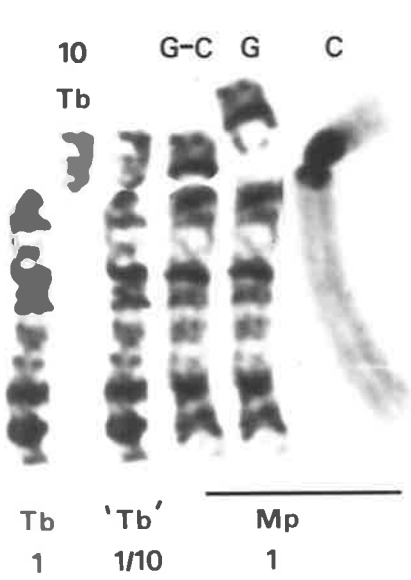
Accounting for C-banding in a comparison of the G-banded autosomes of *Thylogale billardierii*, 2n=22 (with very little centromeric C-banding) with those of *Macropus parryi*, 2n=16 (with large areas of C-banding).

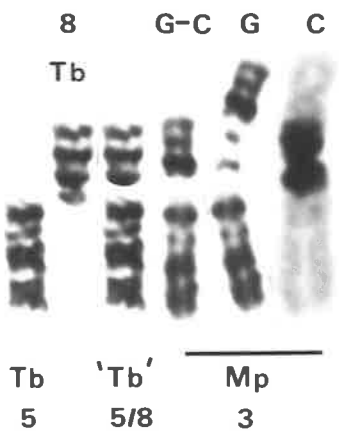
Tb *Thylogale billardierii* chromosomes

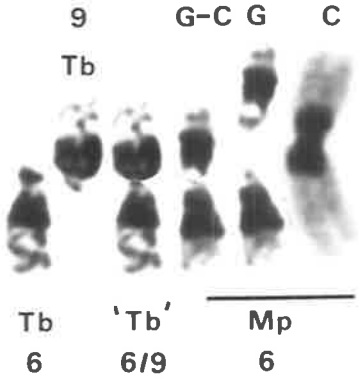
'*Tb*' artificial chromosomes constructed from the long arms of *Thylogale billardierii* acrocentric autosomes

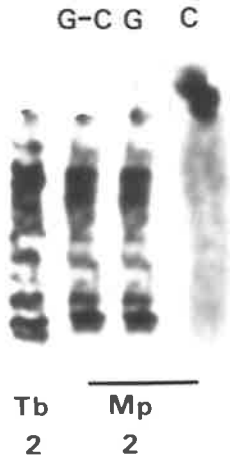
Mp *Macropus parryi* chromosomes

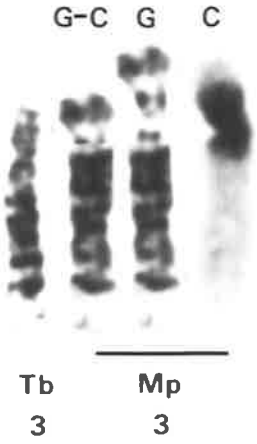
C C-banded chromosome
G G-banded chromosome
G-C G-banded chromosome with the C-banding region deleted

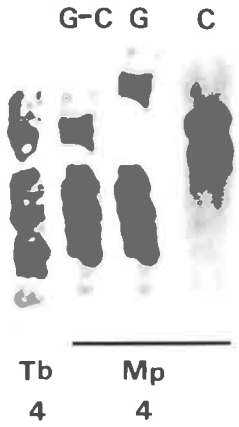

10
Tb
G-C
G
C
Tb
1
'Tb'
1/10
Mp
1

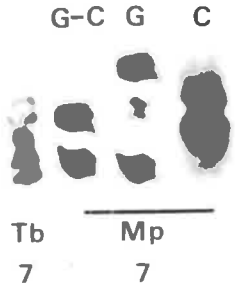

8
Tb
G-C
G
C
Tb
5
'Tb'
5/8
Mp
3


9
Tb
G-C
G
C
Tb
6
'Tb'
6/9
Mp
6


G-C
G
C
Tb
2
Mp
2


G-C
G
C
Tb
3
Mp
3


G-C
G
C
Tb
4
Mp
4


G-C
G
C
Tb
7
Mp
7

Mrg) which also has large C-bands. For both these species most of the G-banding detail of the smallest chromosome is lost.

The autosomal G-banding patterns of all other macropodid species studied also show complete correspondence to the *T. billardierii* patterns if C-bands are deleted. The following descriptions of autosomal homologies refer to these conserved G-banding regions only.

The G-banded macropodid complements in terms of *T. billardierii* equivalents

Figures 6.16, 6.17
6.18, 6.19 and 6.20

Each G-banded autosome (with the exception of pair 7) of *Petrogale penicillata* (2n=22 chromosomes) is homologously banded to a single autosome of *Thylogale billardierii*. Pairs 1, 2, 5, 6, 8, 9 and 10 of both species have very similar morphology and G-banding patterns. Pairs 3 and 4 have similar G-banding patterns but differ in centromere position. Centromere shift by pericentric inversion is compatible with the G-banding sequence of pairs 3 of these species, but not with that of pair 4 (Figure 6.15).≠ This difference requires that a segment be broken out of the chromosome and re-inserted at the third break-point. The translocated segment may or may not have included the centromere. As the G-banding pattern of pair 7 of *P. penicillata* is not clearly defined (see Figure 6.4) no comparison can be made with that of *T. billardierii* pair 7.

In all *Macropus* species studied, there are four autosomes with very similar G-banding patterns to those of pairs 2, 3, 4 and 7 of *T. billardierii*. There is only one chromosome of *Wallabia bicolor* that shows G-banding pattern homology with a single chromosome of *T. billardierii*, namely *T. billardierii* pair 3.

There are some small differences between these autosomes with corresponding G-banding patterns; for example, between the size of the

≠ Figure 6.15 accompanies Figure 6.18.

short arm of pair 2 in *M. giganteus* (see Figure 6.8) and that of many other species (see Figures 6.4 to 6.11). However, these are very minor differences in G-banding pattern which can be accounted for by small pericentric inversions. Chromosome pair 2 of the female *M. rufus* studied is heterozygous for such a difference, as shown in Figure 6.1 h. No small differences in the size of short arms are entirely attributable to C-banding.

All other *Macropus* metacentric autosomes and pairs 2 and 3 of *W. bicolor* can be described as metacentric chromosomes produced by the combination of the long arms of two *T. billardierii* acrocentric autosomes. These chromosomes will be called 'fusion' chromosomes to distinguish them from the other submetacentric or metacentric chromosomes of these complements.

The remaining *W. bicolor* chromosomes each correspond to other combinations of *T. billardierii* chromosomes; pair 1 to three and the Y_2 to two *T. billardierii* autosomes. As expected, the G-banding pattern of the long arm of the *W. bicolor* X chromosome corresponds to that of the Y_2 chromosome. The junction points of *T. billardierii* autosome equivalents in the long arm of the *W. bicolor* X chromosome do not appear to correspond to the late DNA-replicating regions (Hayman and Martin, 1965b; Hayman, 1977) of this arm.

If the autosomal complement of *T. billardierii* is represented as in Figure 6.16, those of *Macropus* species and *W. bicolor* can be represented in terms of *T. billardierii* equivalents, in Figure 6.17.

There are three different G-banded autosomal karyotypes for *Macropus* species with 2n=16 chromosomes. Each of these karyotypes has two or three different centric 'fusion' chromosomes. G-banding thus classifies these 2n=16 *Macropus* species with morphologically similar chromosome complements, into three groups: *M. robustus*, the euro, with 'fusion'

Figures 6.16 and 6.17

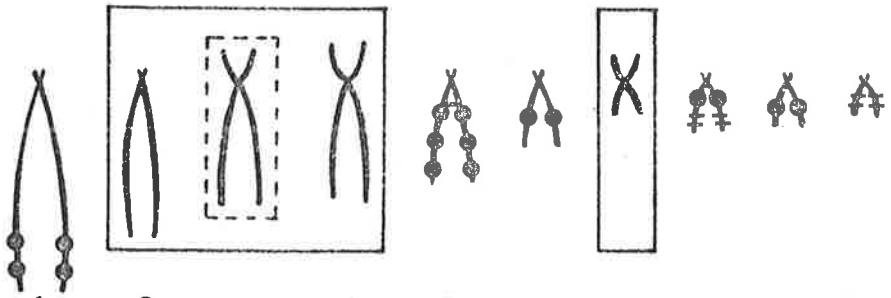
Ideograms of -

6.16 *Thylogale billardierii* G-banded autosomes

6.17 *Macropus* species and *Wallabia bicolor* autosomes in terms of *Thylogale billardierii* G-banded arm equivalents.

the squares indicate common chromosomes

M.r	*Macropus rufus*
M.rb	*Macropus robustus*
M.e	*Macropus eugenii*
M.p	*Macropus parryi*
M.rg	*Macropus rufogriseus*
M.f	*Macropus fuliginosus*
M.g	*Macropus giganteus*
W.b	*Wallabia bicolor*



	2n		
(a)	20	M.r	1/10, 5, 6, 8, 9, 4 prs
(b)	16	M.rb	1/10, 5/6, 8/9, 4 prs
(c)	16	M.e M.p M.rg	1/10, 5/8, 6/9, 4 prs
(d)	16	M.f M.g	1/8, 5/9, 6/10, 4 prs
(e)	10♀ 11♂	W.b	4/6/10, 1/9, 5/8, 2/7/X neo X, 2/7 neo Y, 1 pr

chromosomes (in terms of *T. billardierii* long arm equivalents) 1/10, 5/6 and 8/9; *M. fuliginosus* and *M. giganteus*, the grey kangaroos, both with 1/8, 5/9 and 6/10 chromosomes and *M. eugenii*, *M. parryi*, *M. rufogriseus*, the brush wallabies, with 1/10, 5/8 and 6/9 chromosomes.

Figures 6.18, 6.19 and 6.20 show the G-banding pattern homologies between the complements of all macropods studied. They include a representative of each G-banded autosome for each species. Where the C-banding regions of these chromosomes are large they have been deleted from the chromosomes used in these figures, as was done for the comparison of the G-banded autosomes of *M. parryi* and those of *T. billardierii* (Figure 6.14). In Figures 6.19 and 6.20, whenever the G-banding pattern of a metacentric chromosome corresponds to those of the long arms of two *T. billardierii* acrocentric autosomes, the particular G-banded autosomes of *T. billardierii* are included singly, for example, in Figure 6.19, *Tb 1* and *Tb 10*, and artificially fused '*Tb*'*1/10*. This has been done to more clearly show the relationships.

Figure 6.18 shows the chromosomes that have single G-banded chromosome equivalents in all macropods studied (with the exception of *W. bicolor*). These chromosomes have G-banding patterns corresponding to those of pairs 2, 3, 4 and 7 of the *T. billardierii* karyotype. *Pp 7* has been omitted from the figure, as the G-banding pattern of this chromosome cannot be discerned, and *Mp 7* and *Mrg 7* which are included for completeness, show that most G-banding pattern detail is lost in small chromosomes with very large centromeric C-bands.

Figure 6.19 shows all centric 'fusion' chromosomes of the *Macropus* species, and also, below the lines, chromosomes of *P. penicillata* and *M. rufus* that show G-banding pattern homology with single chromosomes of *T. billardierii*.

The G-banding pattern homologies of the chromosomes of *W. bicolor* and *T. billardierii* are illustrated in Figure 6.20.

Figure 6.18

The pattern homologies of the macropodid chromosomes that have single G-banded equivalents in *Petrogale penicillata*, *Thylogale billardierii* and the *Macropus* species studied.

Where the C-banding regions of these chromosomes are extensive they have been deleted from the G-banded chromosomes.

As the G-banding pattern of *Petrogale penicillata* pair 7 is undefined, this chromosome has been omitted from the figure.

Pp	*Petrogale penicillata*
Tb	*Thylogale billardierii*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Mr	*Macropus rufus*
Mrb	*Macropus robustus*

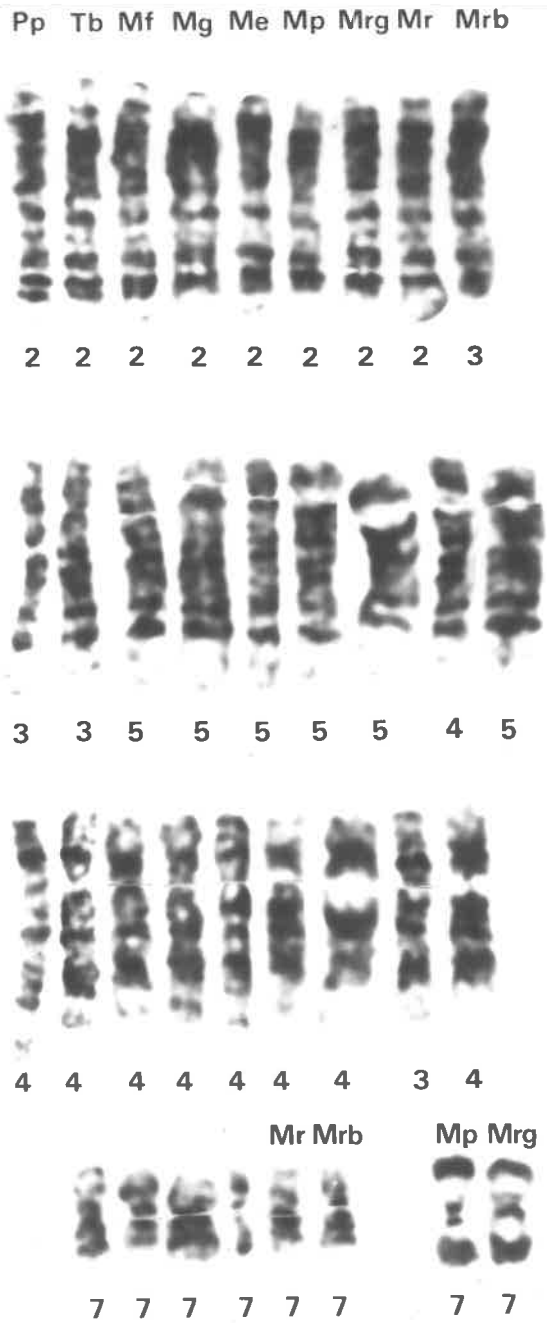
Figure 6.15

The relationships of the G-banded pairs 3 and 4 chromosomes of *Petrogale penicillata* and *Thylogale billardierii*.

Pp 3 and *Tb 3* can be related by a pericentric inversion, the breakpoints of which are indicated on *Tb 3*.

Pp 4 and *Tb 4* differ by a centromere shift which cannot be accounted for by a single pericentric inversion.

Centromere positions are indicated by dots.


Pp Tb Mf Mg Me Mp Mrg Mr Mrb
2 2 2 2 2 2 2 2 3
3 3 5 5 5 5 5 4 5
4 4 4 4 4 4 4 3 4
Mr Mrb
Mp Mrg
7 7 7 7 7 7 7 7

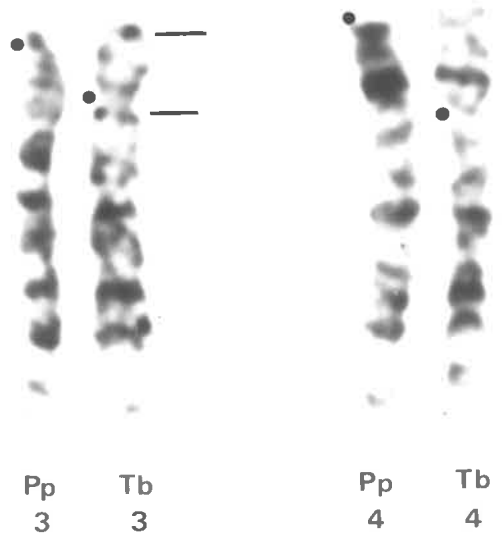

Pp Tb
3 3
Pp Tb
4 4

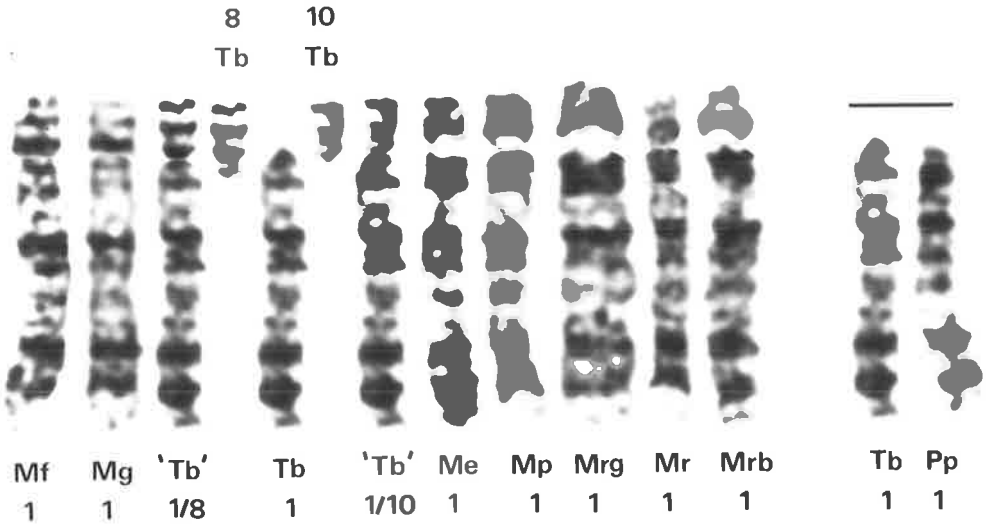
Figure 6.19

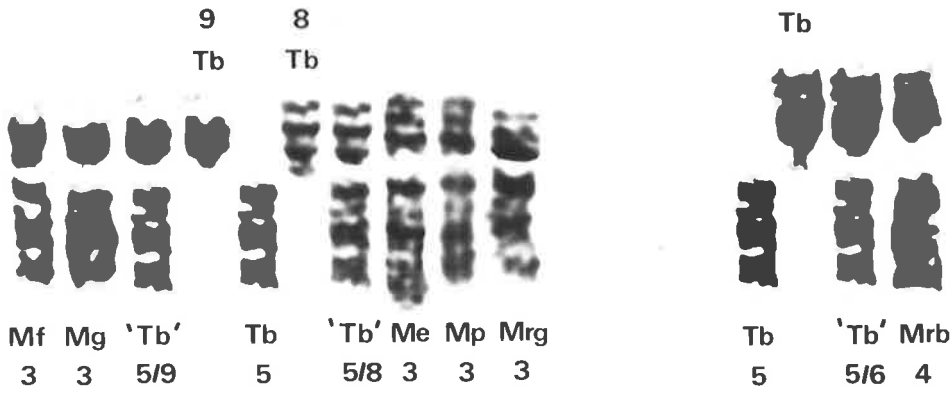
The G-banded centric 'fusion' chromosomes of *Macropus* species and, below the lines, chromosomes which have single G-banded equivalents in *Thylogale billardierii*, *Petrogale penicillata* and *Macropus rufus*.

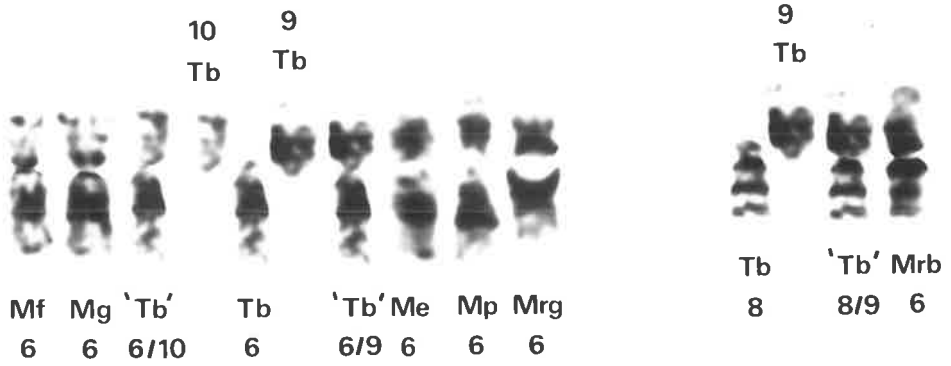
Where the C-banding regions of these chromosomes are extensive they have been deleted from the G-banded chromosomes.

'Tb'	artificial chromosomes constructed from the long arms of *Thylogale billardierii* acrocentric autosomes

Tb	*Thylogale billardierii*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Mr	*Macropus rufus*
Mrb	*Macropus robustus*


8 Tb
10 Tb
Mf 1
Mg 1
'Tb' 1/8
Tb 1
'Tb' 1/10
Me 1
Mp 1
Mrg 1
Mr 1
Mrb 1
Tb 1
Pp 1


9 Tb
8 Tb
6 Tb
Mf 3
Mg 3
'Tb' 5/9
Tb 5
'Tb' 5/8
Me 3
Mp 3
Mrg 3
Tb 5
'Tb' 5/6
Mrb 4


10 Tb
9 Tb
9 Tb
Mf 6
Mg 6
'Tb' 6/10
Tb 6
'Tb' 6/9
Me 6
Mp 6
Mrg 6
Tb 8
'Tb' 8/9
Mrb 6

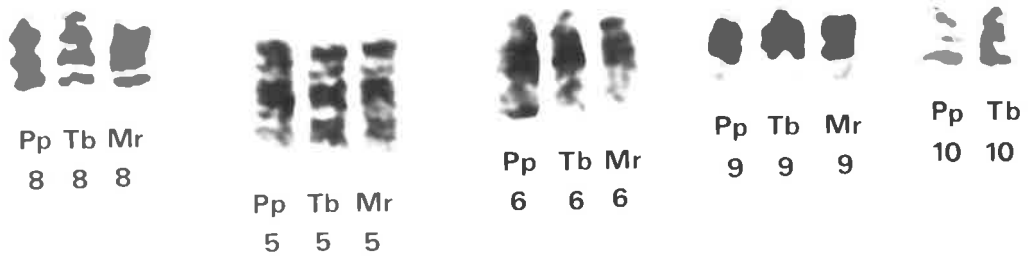

Pp 8
Tb 8
Mr 8
Pp 5
Tb 5
Mr 5
Pp 6
Tb 6
Mr 6
Pp 9
Tb 9
Mr 9
Pp 10
Tb 10

Figure 6.20

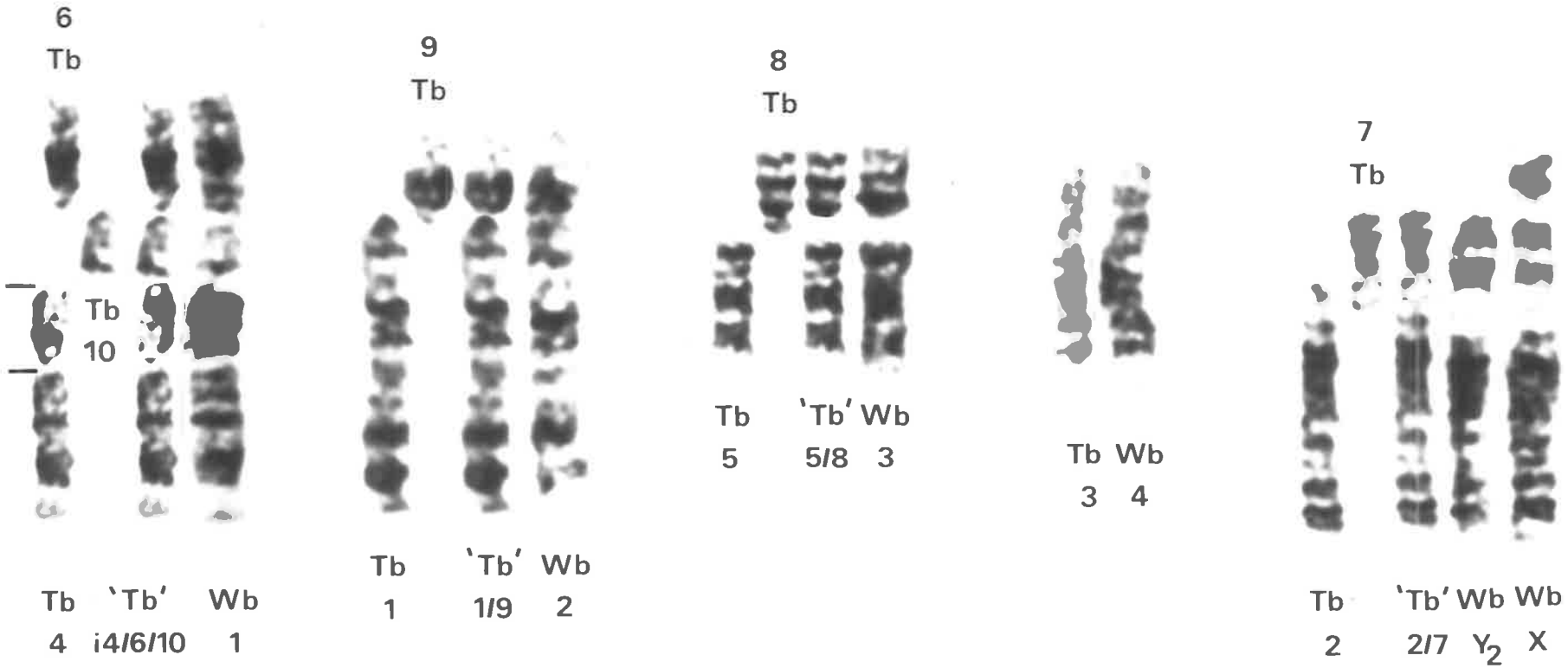
A comparison of the G-banded autosomes of *Thylogale billardierii* (2n=22) and *Wallabia bicolor* (2n=10♀, 11♂).

'*Tb*' artificial chromosomes constructed using *Tb* chromosomes.

The lines indicate the breakpoints of a proposed pericentric inversion of *Tb 4*.

Tb *Thylogale billardierii*

Wb *Wallabia bicolor*


6
Tb
Tb
10
Tb 'Tb' Wb
4 i4/6/10 1
9
Tb
Tb 'Tb' Wb
1 1/9 2
8
Tb
Tb 'Tb' Wb
5 5/8 3
Tb Wb
3 4
7
Tb
Tb 'Tb' Wb Wb
2 2/7 Y2 X

6.8 Mechanisms of chromosomal evolution

A 2n=22 complement of the same general format as that of *Thylogale billardierii* has been proposed as ancestral for *Thylogale*, *Petrogale* and *Macropus* species and *Wallabia bicolor* (section 6.1). Consistent with this hypothesis, of the G-banded macropodid complements of this thesis, those of *T. billardierii* and *P. penicillata* are most like the G-banded complements suggested as ancestral for phalangeroid marsupials in Chapter 8, and are also most similar to "the 2n=14 basic G-banded complement" of Chapter 5. Also, the G-banded macropodid complements of this chapter can be related most simply if a complement of G-banded *T. billardierii* form is assumed as commonly ancestral for all these species.

Given this G-banded complement as ancestral for the species studied, G-banding shows that the units of karyotypic evolution for these species have been relatively large segments, most frequently whole chromosome arms. Therefore chromosome rearrangements that have been fixed have often involved near centric events. However, the G-banding data do not indicate the detailed mechanisms of such rearrangements. Thus the biarmed chromosomes that have been earlier referred to as "centric 'fusion'" chromosomes, may have been produced in a number of ways.

Production of 'fusion' chromosomes

They may all have been produced by Robertsonian or centric fusion, for which there are a number of possible mechanisms as discussed in Chapter 1 (Figure 1.1). Two of these ways, (a) a Mullerian, and (b) a non-Mullerian method of producing a metacentric from two acrocentric chromosomes, are shown again in Figure 6.21 (i).

However, once one biarmed fusion chromosome has been produced, others may be generated by reciprocal arm exchange between the fusion chromosome and an acrocentric chromosome, Figure 6.21 (ii), and when two centric 'fusion' chromosomes, each with different arm components are present, a

Figure 6.21

Ways of producing new biarmed chromosomes.

(a) Mullerian
(b) non-Mullerian

near centric chromosome breakage is assumed for (a), and near centric or centric breakage for (b).

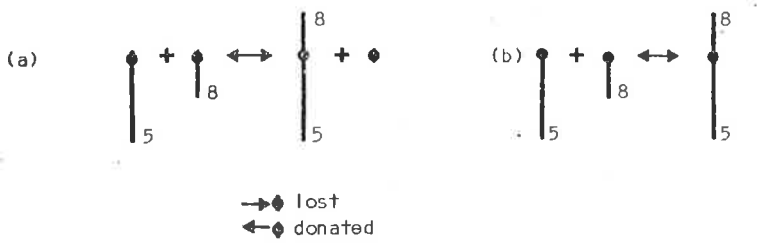
Figure 6.22

Ways of producing *Wallabia bicolor* compound chromosomes from *Thylogale billardierii*-like autosomes.

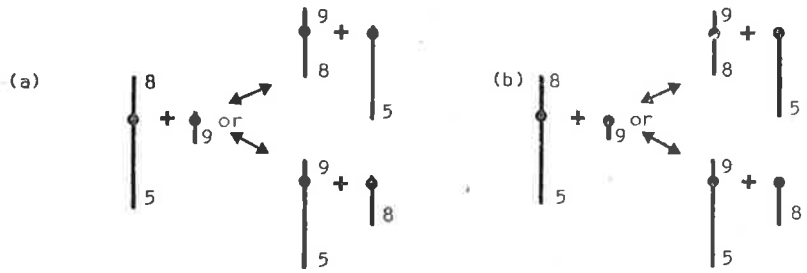
(a) for chromosomes X and Y_2 of *W. bicolor*
(b) (i) alternative ways of producing chromosome 1
(ii) of *W. bicolor*

rearrangements involve only pericentric inversion, i, and Robertsonian fusion.

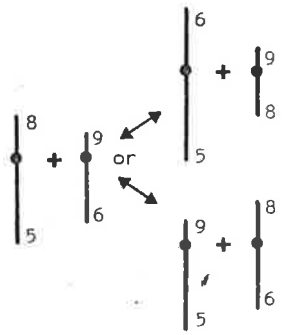
(I) Type 1

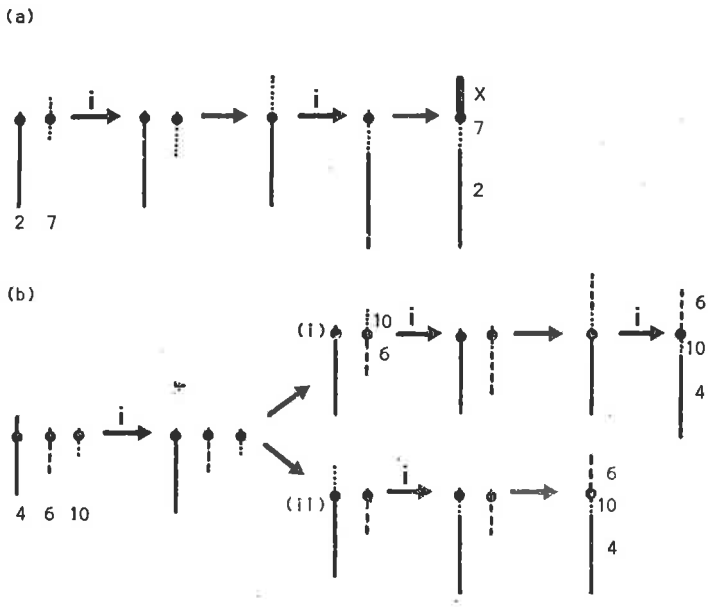


(II) Type 2



(III) Type 3





further two with novel components can be formed in a similar manner, Figure 6.21 (iii). These ways of producing 'fusion' chromosomes will be called Type 1, Type 2 and Type 3 mechanisms respectively.

Although the end-products of these three different mechanisms of producing new biarmed fusion chromosomes may be indistinguishable, new 'fusion' chromosomes produced by these mechanisms may fare quite differently in the initial heterozygous condition. The meiotic configurations of chromosomes in heterozygotes for a Type 1, Type 2 or Type 3 rearrangement are different. If chisamata occur in each chromosome arm, meiotic cells heterozygous for a Type 1 fusion will show a chain of three chromosomes at metaphase I, heterozygotes for products of Type 2 and 3 rearrangements different associations of four chromosomes. In each case the chromosomes must disjoin in particular ways to produce balanced gametes and viable offspring. It is quite likely that heterozygotes for Types 1, 2 and 3 biarmed 'fusion' chromosomes will produce gametes with balanced sets of chromosomes with different frequencies. Therefore even given similar selective values, the probability of spread and subsequent fixation in the population of biarmed 'fusion' chromosomes produced by these mechanisms may well be different.

As will be shown in section 6.10, the G-banded chromosome complements of the macropods studied can be simply related not only by assuming exclusive Type 1 (Robertsonian fusion) production of biarmed 'fusion' chromosomes, but also by assuming a combination of Type 1, Type 2 and Type 3 events.

There is at present very little definitive knowledge of the relative contribution of Type 1, 2 and 3 mechanisms to the general formation of new metacentric chromosomes. Biarmed chromosomes proposed to have been produced by Type 1 mechanisms (and in particular Robertsonian fusion) have been commonly documented as intrapopulation polymorphisms and interspecific differences (for examples, see White, 1973a), and in many cases Type 1

production of these chromosomes is clearly the simplest interpretation of the data.

For example, in marsupials the Type 1 production of a fusion chromosome readily accounts for the observation of one male spectacled hare-wallaby *Lagorchestes conspicillatus* with 2n=14 chromosomes among seven animals (Martin and Hayman, 1966). The other animals possessed all acrocentric autosomes, the two females having 2n=16 and the four males 2n=15 ($X_1X_1X_2X_2$ ♀; X_1X_2Y ♂ sex chromosome system). The autosomal trivalent in the meiosis of the heterozygous male appeared to show only 70% regular disjunction, but all secondary spermatocytes examined at metaphase II had balanced chromosome complements. The acrocentric chromosomes corresponding to those involved in the fusion do not have obvious short arms, therefore if a Type 1 (a) event has occurred in the production of this metacentric chromosome, very little autosomal material has been lost.

Type 1 events can also easily explain many inter-specific karyotypic differences in marsupials, for example, Robertsonian fusion differences between *Petrogale* species (Hayman and Martin, 1969).

Differences in the amount and distribution of C-banding material have also been reported between metacentrics and acrocentrics that are related by Type 1 events (Bruere *et al.*, 1974; Buckland and Evans, 1978b). Bruere *et al.* have also suggested that recently produced Robertsonian metacentrics may differ in centromeric C-banding properties from the other metacentrics of the complement and Niebuhr (1972) using fluorochrome- and C-banding, has indicated the dicentric nature of some Robertsonian fusion chromosomes. The C-banded macropodid complements of this chapter do not suggest any such correlation of Type 1 formation of 'fusion' chromosomes with C-banding properties, as no consistent differences between the C-banding patterns of acrocentric chromosomes and metacentric chromosomes with corresponding G-banding pattern, or between 'fusion' and other metacentric chromosomes

were observed. Although *Macropus* chromosomes with G-banding patterns homologous to those of *Thylogale billardierii* pairs 2, 3 and 7 (that is three of the four chromosomes not involved in the 'fusions' of these species) often show smaller C-bands than most other autosomes (6.5), this is not true for chromosomes with G-banding patterns homologous to that of *T. billardierii* pair 4 (the fourth 'constant' chromosome).

While G-banding has not indicated either Type 1 or a combination of Type 1, Type 2 and Type 3 mechanisms as a much simpler interpretation of the production of 'fusion' chromosomes in the macropods of this chapter, it has favoured Type 1 mechanisms in some other mammalian groups. For example, the karyotypic differences between G-banded sheep and goat complements (Nadler *et al.*, 1974) and between the G-banded complements of black rats (Yosida and Sagai, 1972) may be most simply interpreted as the progressive fixation of chromosomes produced by Type 1 events in the phylogeny of each of these groups of animals. In addition Type 1 mechanisms of formation of new metacentrics can account for the differences between the G-banded complements of Apennine *Mus* (Capanna *et al.*, 1976) much more simply than can Type 2 or Type 3 mechanisms.

There are as yet few reports of metacentric chromosomes produced by Type 2 and Type 3 mechanisms. However this may be due in part to the difficulty of ascertaining the components of general stained 'fusion' chromosomes and to the dominance of the concept of Robertsonian fusion as a perpetrator of chromosomal change. Type 2 and Type 3 mechanisms have been proposed for the production of metacentrics in Diptera, in chironomid midges (see White, 1973a) and some species of black flies (Rothfels and Freeman, 1966), where the karyotypic differences were defined using polytene chromosome banding patterns.

Production of the compound chromosomes of *Wallabia bicolor*

As has been discussed, there are several ways the centric 'fusion' chromosomes of *Macropus* species and *Wallabia bicolor*, the swamp wallaby, may have been produced from a *Thylogale billardierii*-like complement. There are also many ways the compound chromosomes, 1, X and Y_2 of the swamp wallaby may have been formed from the chromosomes of such a complement. Figure 6.22 shows some examples where only Robertsonian fusion of acrocentrics and pericentric inversion, i, are invoked. All rearrangements shown are consistent with the G-banding pattern differences between the chromosomes of *T. billardierii* and the swamp wallaby (see Figure 6.20). Apart from the schemes shown in Figure 6.22, there are numerous other ways these G-banded chromosomes may have been formed, for example, tandem translocation and centromere inactivation or three breakpoint rearrangements.

6.9 The 2n=16 *Macropus* karyotype and karyotypic orthoselection

The 2n=16 karyotypes of *Macropus* species are morphologically very similar after general chromosome staining. However, G-banding has shown that three of the seven autosomal pairs of these complements may consist of completely different 'fusion' chromosomes (see Figure 6.17).

Although small differences between the general stained chromosomes of the similar karyotypes of *Macropus* species had been recognized (Sharman, 1973; and Hayman and Martin, 1974, who used these small differences together with known species hybridization to subdivide 2n=16 *Macropus* species), these differences were not, and could not have been used to formulate the same groupings as are obvious after G-banding. This is because the variation in C-banding, which is not correlated with these groupings (section 6.5), contributes much more substantially to the variation in size and morphology of the general stained chromosomes than does the differential arm composition of the three 'fusion' chromosomes.

The similar general format of these differently G-banded 2n=16 complements appears largely due to four pairs of chromosomes that are common to all *Macropus* species and which show very little morphological variation. These are the chromosomes with G-banding patterns corresponding to pairs 2, 3, 4 and 7 of *Thylogale billardierii*. In contrast it appears that the G-banded chromosome arms that show patterns corresponding to those of the long arms of *T. billardierii* acrocentric pairs 1, 5, 6, 8, 9 and 10 may be represented in random combinations in the 'fusion' chromosomes of *Macropus* species. Given a *T. billardierii*-like complement as ancestral for these species, the possible combinations of these arms in 'fusion' type chromosomes are 1/5, 1/6, 1/8, 1/9, 1/10, 5/6, 5/8, 5/9, 5/10, 6/8, 6/9, 6/10, 8/9, 8/10, 9/10. *Macropus* species display 1/8, 1/10, 5/6, 5/8, 5/9, 6/9, 6/10, 8/9 and *Wallabia bicolor* has a 1/9 chromosome.

This possibility of the random combination of *T. billardierii*-like chromosome arms in 'fusion' chromosomes may be better evaluated when more G-banding data become available, as there are a number of genera that possess general stained chromosome complements that have been proposed (as have those of *Macropus* species and *Wallabia bicolor*) as derived from a complement of the same general format as that of *T. billardierii* (see section 6.1).

However, if these combinations have been random and if the 2n=16 complement of the general format possessed by most *Macropus* species has been selectively favoured, this selection has involved conservation of four pairs of G-banded autosomes while otherwise favouring the fixation of rearrangements giving rise to three pairs of 'fusion' autosomes with random combinations of arm components.

White (1973a) has suggested that similar general stained karyotypes may be produced by a process of "karyotypic orthoselection", which he defines as:

> "the tendency for the same type of rearrangement to occur over and over again in different chromosomes of the same species".
>
> White, 1978

He does not believe that this is the result of particular types of rearrangements occurring more frequently than others, but suggests that explanations may involve similar rearrangements having similar effects on the phenotype; the need for the sizes, shapes and numbers of the chromosomes to be dimensionally appropriate to cells and "possible regularities in the architecture of the interphase nucleus".

However, *if* the common 2n=16 general karyotypic format for *Macropus* species *has* been favoured, and *if* the combination of arms in the 'fusion' chromosomes of these complements *is* random, then clearly the sizes and shapes of the individual 'fusion' chromosomes themselves are not important, although the formation of any metacentric chromosome from these particular components may be.

Such random combinations of arms of specific G-banding pattern and size has also been suggested for the Robertsonian metacentrics of *Mus musculus* populations in the Italian Alps and the Apennines (Capanna *et al.*, 1976), and when more information is available on the composition of complements reputedly produced by "karyotypic orthoselection", the possible ways selection has acted to fashion these complements will be better defined.

6.10 Pathways of karyotypic evolution and the phylogeny and taxonomy of macropods

The G-banded complements

The present G-banding data are consistent with the proposal that a *Thylogale billardierii*-like G-banded complement was ancestral for the species studied. However, as there is no inherent sequence in the

rearrangements proposed from the chromosomal differences in G-banding pattern (in the way that there is for the overlapping inversions of *Drosophila* species), pathways of karyotypic evolution can only be deduced if it is assumed that each rearrangement occurred and was fixed before the next. This may be an unwarranted assumption; for example, *Macropus* species and *Wallabia bicolor* are closely related species (many produce viable hybrids) and it is possible that they have been derived from an ancestral population that was polymorphic for most of the 'fusion' chromosomes.

Nevertheless, if this assumption is made, and also that chromosomal rearrangements and their fixation are rare events, a number of relationships can be proposed for the macropodid species studied on the basis of their G-banded chromosomes. (As the C-banding patterns of these species are so variable they are not considered when determining possible relationships - see section 6.5.) The resulting pathways of chromosomal evolution also depend on the assumed mechanisms of the rearrangements.

Table 6.2 lists the rearrangements proposed for these macropods in terms of *T. billardierii* G-banded equivalents. As the G-banding pattern of *Petrogale penicillata* pair 7 was not clear, the difference in centromere position between this chromosome and that of the *T. billardierii* pair 7 is referred to as r7. In recognition of the possibility that an intermediate in the formation of the X and Y_2 ([i(r7/2)]/X and i(r7/2) in Table 6.2 respectively) of *W. bicolor* may have been of *P. penicillata* pair 7 form, r7 is also included in the list of rearrangements differentiating the chromosomes of *W. bicolor* from those of the ancestral *T. billardierii*-like complement. Rearrangements proposed for chromosomes 1, X and Y_2 of *W. bicolor* are those of the schemes in Figure 6.22 where *1 corresponds to a pathway including (b) (i) and *2, (b) (ii).

Table 6.2

Chromosome rearrangements of the nine macropods relative to the *Thylogale billardierii* karyotype.

2n	Species	
22	*Thylogale billardierii*	-
22	*Petrogale penicillata*	i3, CS4, r7
20	*Macropus rufus*	1/10
16	*M. robustus*	1/10, 5/6, 8/9
16	*M. eugenii*	1/10, 5/8, 6/9
16	*M. parryi*	" " "
16	*M. rufogriseus*	" " "
16	*M. fuliginosus*	1/8, 5/9, 6/10
16	*M. giganteus*	" " "
10♀,11♂	*Wallabia bicolor*	1/9, i4, 5/8, r7, r7/2, i(r7/2), [i(r7/2)]/X, 6/10, i(6/10), i4/[i(6/10)], i[i4/[i(6/10)]]
		or 1/9, i4, 5/8, r7, r7/2, i(r7/2), [i(r7/2)]/X, i4/10, i(i4/10), 6/[i(i4/10)]

r7 rearranged chromosome 7

r7 in *P. penicillata* may not be the same as r7 in *W. bicolor*

i pericentric inversion

CS centric shift

The proposed relationships for *Macropus* species and *W. bicolor* are also largely dependent on the assumed mechanisms of formation of the 'fusion' chromosomes of these species.

Figure 6.23 shows the simplest relationships that may be proposed for all species if 'fusion' chromosomes are formed exclusively by Robertsonian fusion or the combination of two acrocentric chromosomes (a Type 1 mechanism of Figure 6.21). Depending whether the r7s of the *W. bicolor* and *P. penicillata* lineages are assumed the same (as in (a) but not in (b)), and whether 6/10 does ((i), *1) or does not ((ii), *2) occur in the *W. bicolor* lineage, different relationships may be proposed. Where more than two lines intersect in the figure this indicates that no particular order of divergence is proposed for these lineages.

In a similar way Figure 6.24 shows the simplest relationships that may be proposed if the 'fusion' chromosomes are assumed products of combinations of the Type 1 or Type 2 or Type 3 rearrangements of Figure 6.21.

Figures 6.23 and 6.24 show that there are many ways these species, especially the 2n=16 *Macropus* species, may be karyotypically related. Accordingly G-banding has not substantially contributed to knowledge of the phylogenetic relationships of these species.

However, G-banding has classified the 2n=16 *Macropus* species into three groups on the basis of the components of their 'fusion' chromosomes. This classification assumes that the common possession of similarly G-banded 'fusion' chromosomes is indicative of recent common ancestry. The groups are (as shown in Figure 6.23 and 6.24) *M. robustus* (with 'fusion' chromosomes 1/10, 5/6 and 8/9), *M. fuliginosus* and *M. giganteus* (1/8, 5/9 and 6/10), and *M. eugenii*, *M. parryi* and *M. rufogriseus* (1/10, 5/8 and 6/9).

Figure 6.23

Most parsimonious karyotypic relationships for the macropods studied, assuming Type 1 formation of 'fusion' chromosomes.

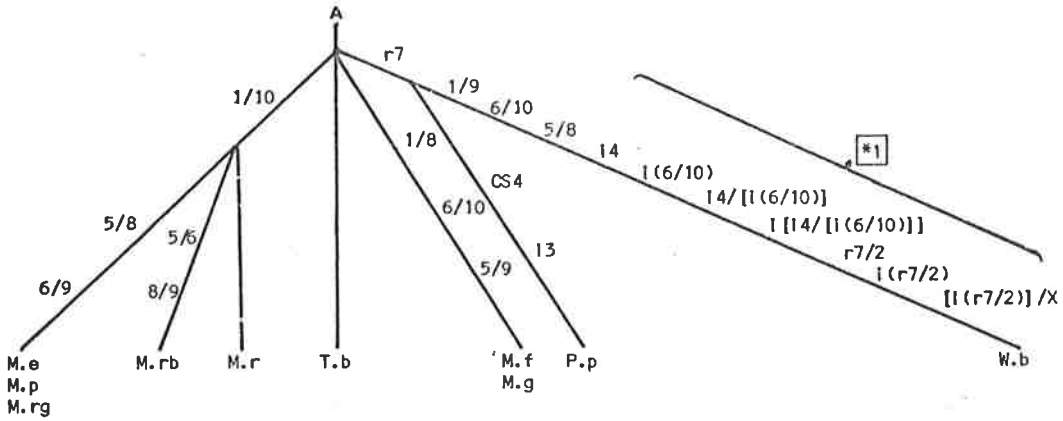
(a) rearrangement of chromosome 7 (r7) occurs once
(b) r7 occurs twice, each by a different mechanism
(i) 6/10 occurs in W.b lineage ((b)(i) of Figure 6.22)
(ii) 6/10 does not occur in W.b lineage ((b)(ii) of Figure 6.22)

A ancestral macropod with T.b like karyotype
i pericentric inversion
CS centric shift

T.b *Thylogale billardierii*
P.p *Petrogale penicillata*
M.r *Macropus rufus*
M.rb *Macropus robustus*
M.e *Macropus eugenii*
M.p *Macropus parryi*
M.rg *Macropus rufogriseus*
M.f *Macropus fuliginosus*
M.g *Macropus giganteus*
W.b *Wallabia bicolor*

(a)

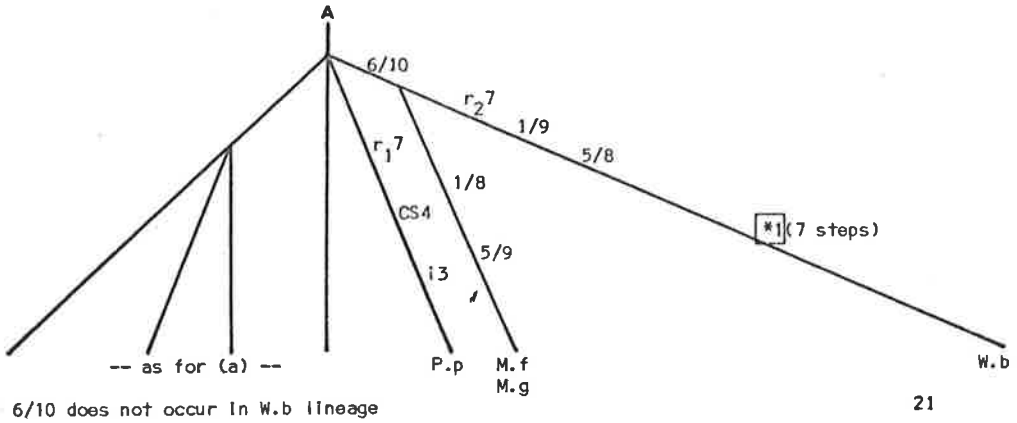
(I) 6/10 occurs in W.b lineage — Number of steps: 21



(II) 6/10 does not occur in W.b lineage — 20

(b)

(I) 6/10 occurs in W.b lineage — 21



(II) 6/10 does not occur in W.b lineage — 21

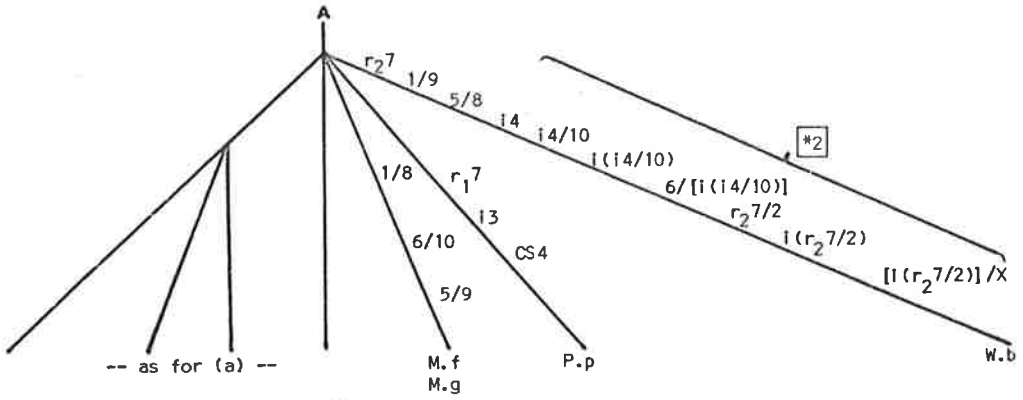


Figure 6.24

Most parsimonious karyotypic relationships for the macropods studied, assuming Type 1, Type 2 and Type 3 formation of 'fusion' chromosomes.

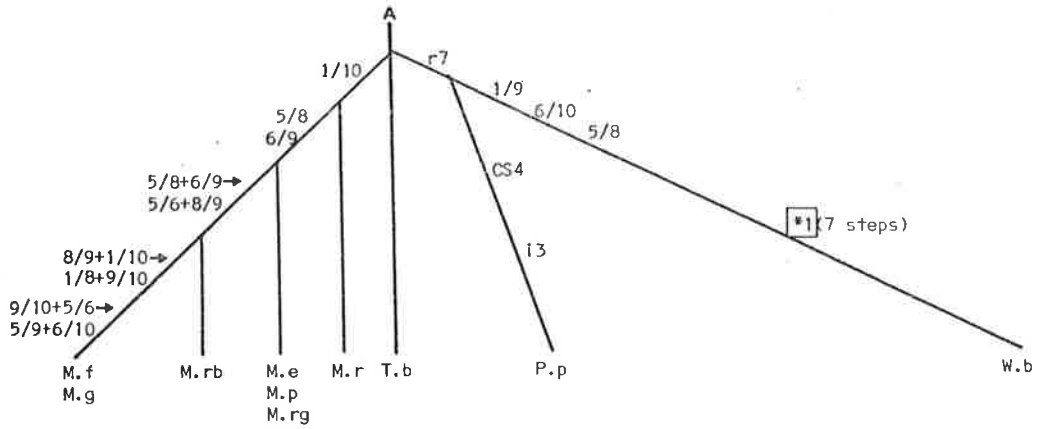
(a) rearrangement of chromosome 7 (r7) occurs once
(b) r7 occurs twice, each by a different mechanism
(i) 6/10 occurs in W.b lineage ((b)(i) of Figure 6.22)
(ii) 6/10 does not occur in W.b lineage ((b)(ii) of Figure 6.22)

*1 and *2 and other abbreviations as for Figure 6.23

(a)

Number of steps

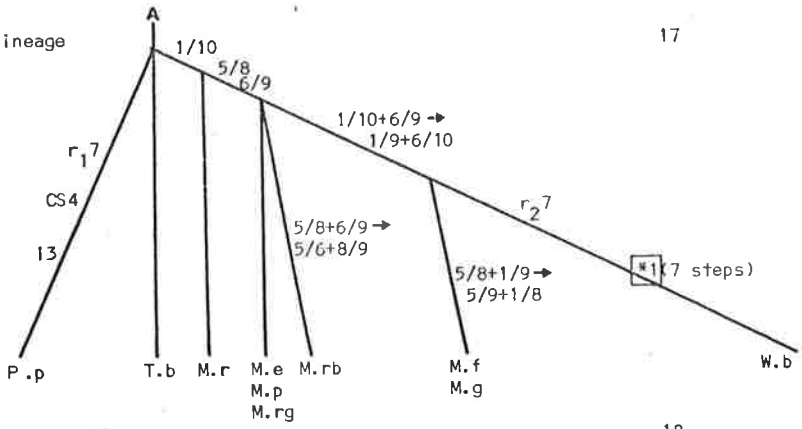
(i) 6/10 occurs in W.b lineage — 19



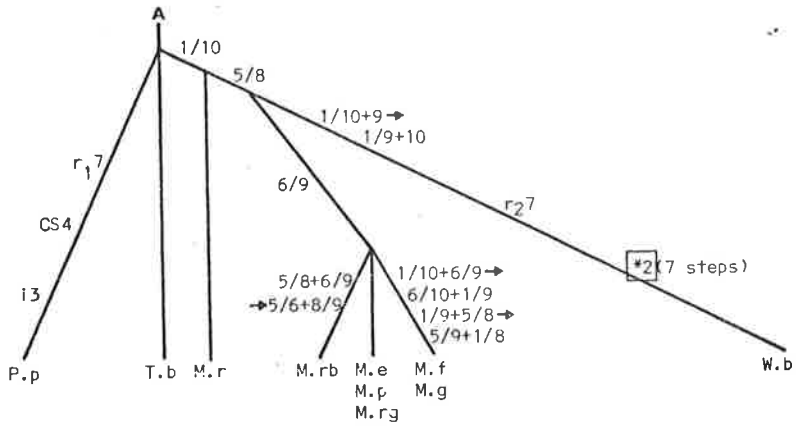
(ii) 6/10 does not occur in W.b lineage — 18

(b)

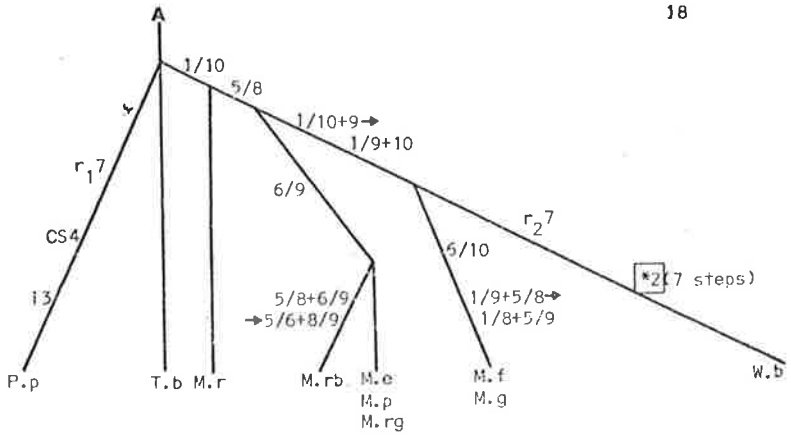
(i) 6/10 occurs in W.b lineage — 17



(ii) 6/10 does not occur in W.b lineage — 18



18



It is interesting that the same complements that have been suggested (Sharman, 1961; Calaby, 1966) as a means of defining *Macropus* species (by their *similarity* after general chromosome staining) can now be used (on the basis of their *differences* in G-banding patterns) to subdivide *Macropus*.

The three groups defined for *Macropus* species and *Wallabia bicolor* on the basis of general chromosome staining (excluding *M. bernardus*, Kirsch and Calaby, 1977; for which a chromosome number or a karyotype has not been published) are shown in Figure 6.25, together with groupings for the same species after G-banding analysis.

The *Macropus* species have also been variously grouped by other methods. Bartholomai (1975), on the basis of morphological and fossil evidence, has grouped 2n=16 *Macropus* species into a number of subgenera; the wallaroos *Macropus* (*Osphranter*), the brush wallabies *Macropus (Prionotemnus)* and *Macropus* (*Macropus*) of which the only living species appear to be the grey kangaroos. The red kangaroo, *M. rufus*, has been classified in another subgenus, *Macropus* (*Megaleia*) (Tate, 1948). *Wallabia bicolor* is generically distinct. These groupings and also those of Richardson and McDermid (1978) on the basis of electrophoretic protein differences are in agreement with those proposed for *Macropus* species and *W. bicolor* on the basis of the present G-banding data, see Figure 6.25. They differ from the groupings determined by Kirsch (1977a), who used serological affinity to cluster species (see also Figure 6.25).

Apart from *M. greyi* which is probably extinct, there are six *Macropus* species that were not studied with G-banding in this thesis. Five of these have 2n=16 chromosomes. If these species are shown to possess G-banded chromosomes like those of the *M. eugenii*, *M. parryi*, *M. rufogriseus* group, this would constitute evidence for the validity of the taxa proposed by Bartholomai.

Figure 6.25

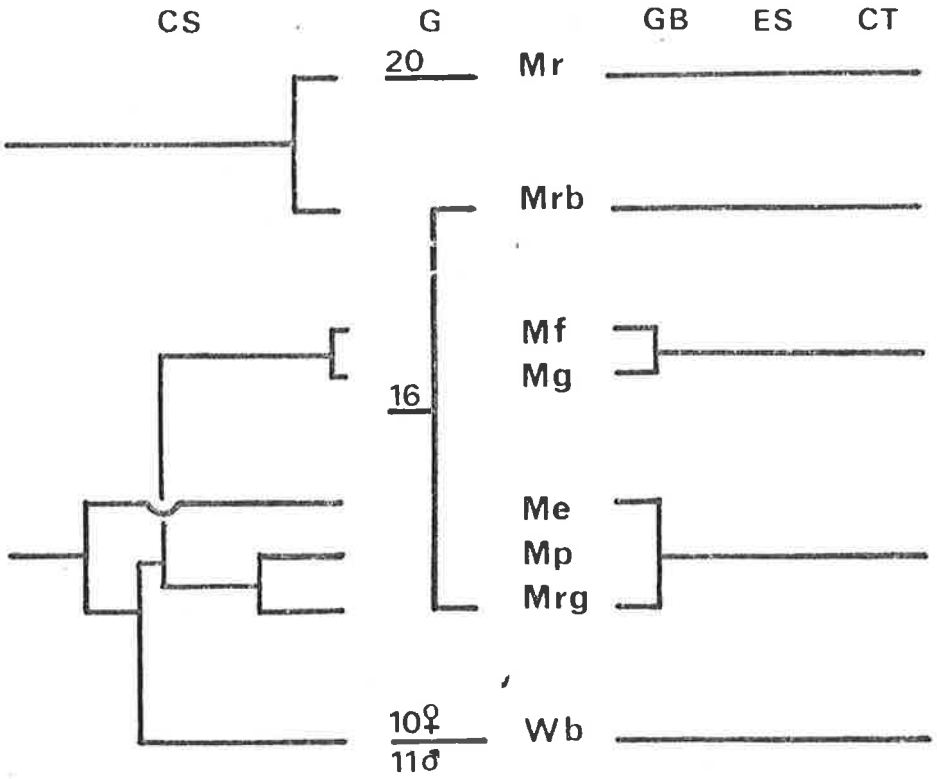
Classifications for the *Macropus* species studied

and *Wallabia bicolor*.

on the basis of	comparative serology	CS
	general stained chromosomes	G
	G-banded chromosomes	GB
	electrophoretic studies	ES
	conventional taxonomy	CT

numbers are diploid chromosome numbers

Mr	*Macropus rufus*
Mrb	*Macropus robustus*
Mf	*Macropus fuliginosus*
Mg	*Macropus giganteus*
Me	*Macropus eugenii*
Mp	*Macropus parryi*
Mrg	*Macropus rufogriseus*
Wb	*Wallabia bicolor*


CS
G
GB
ES
CT
20
Mr
Mrb
Mf
Mg
16
Me
Mp
Mrg
10♀
11♂
Wb

Before G-banding analysis most *Macropus* species with 2n=16 chromosomes were considered more similar karyotypically to each other than to the red kangaroo, with 2n=20 chromosomes. Therefore Hayman and Martin (1974) considered this 2n=20 complement and the 2n=10♀, 11♂ complement of *Wallabia bicolor* to have been derived from 2n=16 complements.

The relationships of the G-banded complements of these species (Figures 6.23 and 6.24) suggest that the 2n=20 complement of the red kangaroo is most simply directly derived from that of an ancestral *T. billardierii*-like complement by the centric fusion of one pair of acrocentric autosomes, and that it represents an intermediate in the formation of some 2n=16 *Macropus* complements. The differences between some of these 2n=16 complements are also such that the complement of the red kangaroo is no more different to those of 2n=16 *Macropus* species than they are to each other; as the 1/10 'fusion' chromosome of the red kangaroo is shared with some other *Macropus* species, *M. robustus*, *M. eugenii*, *M. parryi* and *M. rufogriseus*, which do not share any fusion chromosomes with others, namely *M. fuliginosus* and *M. giganteus*. Thus karyotypically the red kangaroo is just another *Macropus* species. Also, on the basis of the present G-banding data, the complement of *Wallabia bicolor* is more readily derived from a 2n=18 or 2n=20 complement shared with *Macropus* species or a 2n=22 *T. billardierii*-like complement than it is from the G-banded complement of any *Macropus* species studied.

Other macropodid complements

Although a *T. billardierii*-like complement appears to have been ancestral for *Macropus* species, *Wallabia bicolor* and, by inference from the general stained complements in other genera, for many macropods, only further G-banding studies may determine whether such a complement has been ancestral for all present-day macropods. However, a number of hypotheses

for pathways of karyotypic evolution within Macropodidae may be advanced on the basis of the present data.

The 2n=22 chromosome complement of *Setonix brachyurus* does not appear to represent an intermediate between the *T. billardierii* and 2n=16 *Macropus* like complements as Hayman and Martin 1974) have suggested. This complement is most simply related to the *T. billardierii* type by a change in the centromere position of pair 1. The *S. brachyurus* pair 1 would appear to be derived rather than ancestral, not only because karyotypes of the general *T. billardierii* format are present in other macropodid genera, but also as the published G-banding of the chromosomes of a *Potorous tridactylus* cell line (Brown and Cohen, 1973) suggests that the long arm of one of the largest biarmed chromosome in cells of this culture corresponds to the G-banded pair 1 of the *T. billardierii* complement. As *P. tridactylus* is classified in a different subfamily of the Macropodidae to all other kangaroos and wallabies studied in this thesis, providing this pattern homology is real and not an artifact of the continuous culture of potoroo cells, it is likely that this G-banded chromosome arm has been a unit of the chromosome complement of a macropod ancestral to both subfamilies of kangaroos and wallabies.

On the basis of chromosome size the multiple sex chromosome system of *Lagorchestes conspicillatus* (2n=16♀, $X_1X_1X_2X_2$; 2n=15♂, X_1X_2Y) has been derived from the *T. billardierii*-like ancestral complement independently to that of *Wallabia bicolor* (2n=10♀, XX; 2n=11♂, XY_1Y_2). The G-banded autosomal components of the multiple sex chromosome system of *W. bicolor* correspond to pairs 2 and 7 of the G-banded *T. billardierii* complement. However, the general stained complement of *L. conspicillatus* is most simply related to that of *L. hirsutus* (Sharman, 1961) and to that of *T. billardierii* by assuming that the smallest autosomal pairs of a *T. billardierii*-like complement (at least four of pairs 6, 7, 8, 9 and 10)

have been involved in the formation of these multiple sex chromosomes. It is therefore not possible that *L. conspicillatus* and *W. bicolor* had a common ancestor with an XX/XY_1Y_2 sex chromosome system like that of the G-banded *W. bicolor* complement.

Other than the possibility that the long arm of pair 1 of the *T. billardierii*-like G-banded complement may be a unit of karyotypic evolution for all macropods, no other useful predictions can be made concerning the components of karyotypes in groups 3, 4, 5 and 6 (see section 6.1) of Hayman and Martin (1974).

CHAPTER 7

THE G-BANDED CHROMOSOMES OF THE BRUSH-TAILED POSSUM *TRICHOSURUS VULPECULA*, PHALANGERIDAE AND THE SQUIRREL GLIDER *PETAURUS NORFOLCENSIS*, PETAURIDAE.

With the presentation of the G-banding patterns of the chromosomes of these two species all major families of Australian marsupials have been sampled in the G-banding study of this thesis (see Tables 2.1 and 3.1). In addition to the Macropodidae, the Petauridae is the only Australian taxonomic family of marsupials in which 2n=22 (as possessed by *P. norfolcensis*) is known to occur. The highest chromosome number known for Phalangeridae is 2n=20 (as possessed by *T. vulpecula*). The chromosome complements of the two species studied in this chapter are also of particular interest as Hayman and Martin (1974) have suggested that both may be simply related to a hypothetical 2n=22 complement which was produced by fission of four chromosomes of a 2n=14 basic burramyid-like complement in a common phalangeroid ancestor.

7.1 *Trichosurus vulpecula*, Phalangeridae

There are eleven species (three genera) of living phalangerid marsupials. The monotypic *Wyulda* has not been examined cytologically. The *Trichosurus* possums all have 2n=20 chromosomes, while the *Phalanger* species (cuscuses) that have been karyotyped have 2n=14 chromosomes. Hayman and Martin (1974) have proposed that these complements were both produced by fusion from a 2n=22 hypothetical fission-product complement; with a fusion of two fission chromosomes to produce the 2n=20 mostly acrocentric complement of *Trichosurus vulpecula* and four separate fusions to produce the 2n=14 non-basic complement (four large metacentric and three acrocentric chromosomes) of the two *Phalanger* species.

The chromosomes of one male *T. vulpecula*, brush-tailed possum, from suburban Adelaide were G-banded. Chromosome preparations were made from fibroblast cultures.

Very few chromosome pairs can be unequivocally identified after general staining of brush-tailed possum chromosomes. In several cells there was a suggestion of a secondary constriction in the short arm of the X-chromosome. This was clearly seen in a partially G-banded cell with very extended chromosomes, Figure 7.1.

The G-banding karyotype and chart for the brush-tailed possum are shown in Figures 7.2 and 7.3.

In G-banded metaphases each autosome pair and the X and Y chromosomes have characteristic G-banding patterns. Pairs 2, 3 and 4 and pairs 6, 7 and 8 are of very similar size in G-banded complements. Numbers as in Figures 7.2 and 7.3 have been arbitarily assigned to these chromosomes. (This numbering departs from that of Hayman and Martin (1974), as the autosome with the longest short arm, here designated pair 4, corresponds to their third longest chromosome.)

The centromeric regions of all autosomes are unstained after G-banding. These areas are C-banding positive in this animal (Rofe, unpublished). G-banding accentuates the differences in short arm length between these chromosomes and autosome pairs listed in order of decreasing G-banded short arm length are 4, 7, (5, 6), (3, 9), 8, 2 and 1.

The G-banding patterns of brush-tailed possum pairs 1, 2, 4, 5, 6, 7, 8 and 9 may be described as the same as the patterns of whole chromosome arms of the *Acrobates pygmaeus* G-banding karyotype. Pair 3 of the brush-tailed possum corresponds to a combination of the arms of two autosome pairs of the *A. pygmaeus* karyotype. The G-banding patterns of the X and Y chromosomes of the possum are briefly described.

Figure 7.1

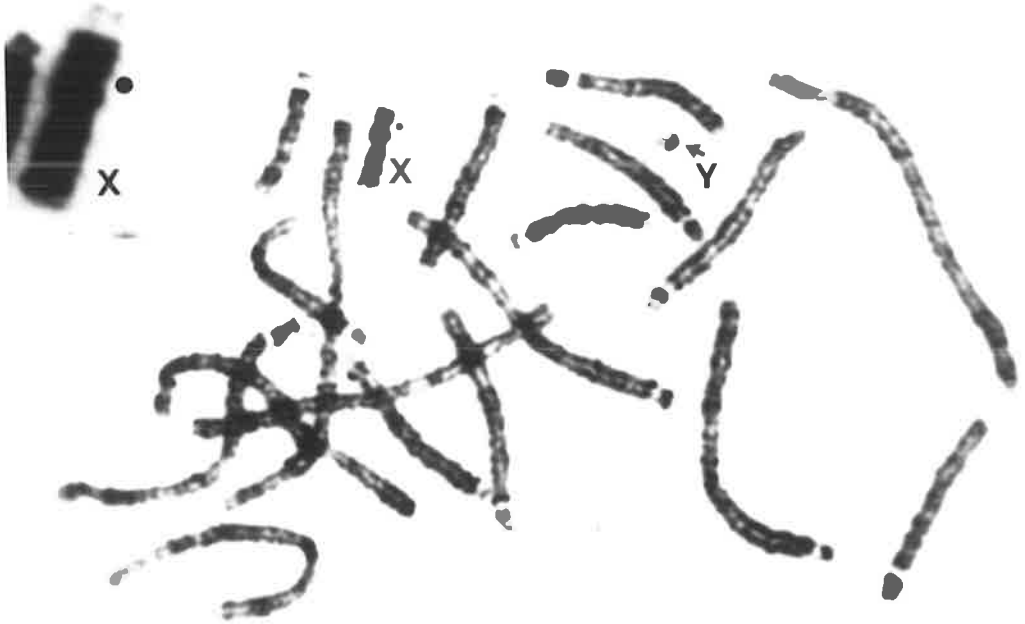
Partially G-banded chromosomes of *Trichosurus vulpecula*, with an enlargement of the X chromosome showing the secondary constriction and small satellite of the short arm.

The centromeres of the X chromosome are indicated by dots.

Figure 7.2

The G-banding karyotype of *Trichosurus vulpecula*.

The Y chromosome was missing from this cell. A representative G-banded Y can be seen in Figure 7.1 above.


X
X
Y

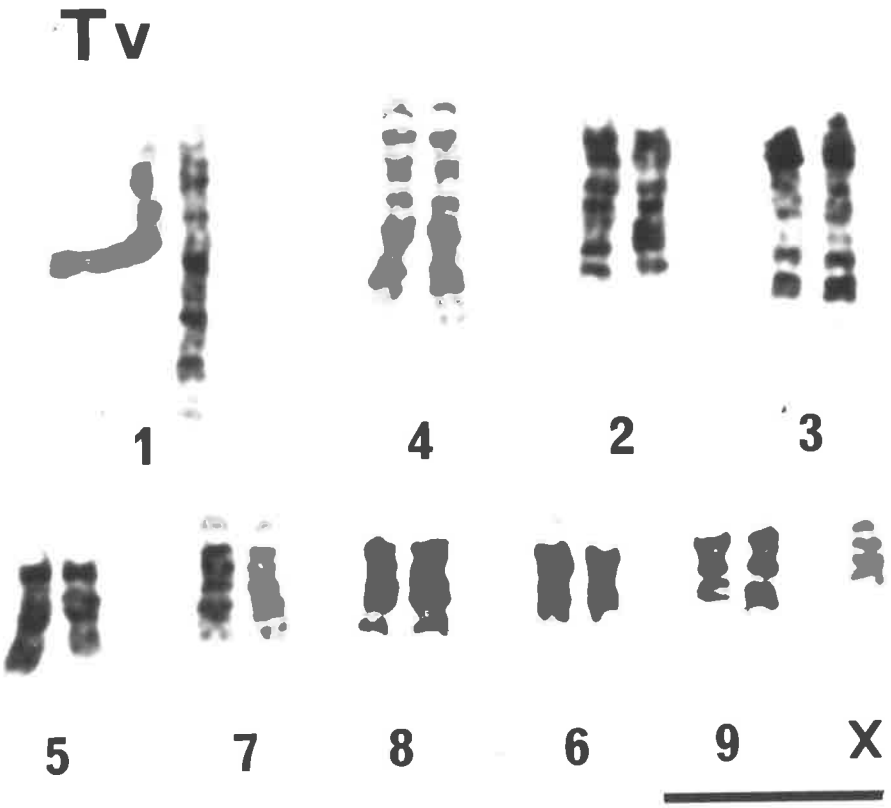

Tv
1
4
2
3
5
7
8
6
9
X

Figure 7.3

The G-banding chart for *Trichosurus vulpecula*.

Tv

The X chromosome

This chromosome is characteristically striped. There is one G-band in the short arm. In the long arm a dark band flanks the centromere and another marks the middle of the arm. These bands are separated by a white interband. The remainder of the long arm is grey with a near terminal band and an unstained distal end.

The Y chromosome

A faint G-band marks the centromere of this very small acrocentric chromosome. There is a dark G-band near the end of the long arm.

7.2 *Petaurus norfolcensis*, Petauridae

There are twenty species (five genera) of living petaurids. Chromosome numbers have been recorded for ten species (five genera) and range from 2n=10 to 2n=22. Hayman and Martin (1974) have proposed that 2n=22 petaurid karyotypes are related to the hypothetical 2n=22 fission product ancestral phalangeroid complement by "centromere shifts" which converted the acrocentrics to the metacentric chromosomes that predominate in petaurid complements.

The following research was carried out in conjunction with Dr. P.R. Baverstock and Ms. M. Gelder.

A single male squirrel glider, *Petaurus norfolcensis*, was obtained from the Adelaide Zoological Gardens, and no other source information is available. The chromosome preparation was from the short-term culture of the lymphocytes of a sample of whole blood obtained by cardiac puncture.

The karyotype of this species has not been previously reported - it is similar to that of the sugar glider (*P. breviceps*). The chromosome number is 2n=22 and all chromosomes are submetacentric or metacentric. There is no obvious secondary constriction on any chromosome. The largest fourteen chromosomes could be paired quite readily in general stained

karyotypes. However, it was often difficult to pair several of the smaller chromosomes. The X and Y of *P. norfolcensis* appear to be the two smallest chromosomes, as these correspond in general size and C-banding pattern to the chromosomes known to be the X and Y in *P. breviceps* (Rofe, Baverstock and Gelder, unpublished) and squirrel and sugar gliders are known to produce fertile hybrids (Fleay, 1947). This designation of the X chromosome for *P. breviceps* differs from that of Hayman and Martin (1974) who show the X chromosome as the fourth largest chromosome.

The C-banding karyotype of the squirrel glider is shown in Figure 7.5. Apart from the X and Y chromosomes there are at least four small chromosomes that cannot be paired on the basis of size and C-banding pattern; these correspond to those labelled 8 and a and b. Of these, chromosomes a and b show both centromeric and interstitial C-bands.

The G-banding karyotype and chart for the squirrel glider are shown in Figures 7.4 and 7.7. The G-banded chromosomes of the squirrel glider complement formed eight pairs, each with a characteristic G-banding pattern. Apart from the X and Y, four chromosomes were unpaired; a, b and those of 8. The G-banding patterns of the six smallest autosomes and the X chromosome are shown in greater detail in Figure 7.6.

The four small autosomes with large C bands, chromosomes 7, one of 8 and a, appear to correspond to autosomes with large non G-banding centromeric regions. The G- and C-banding patterns of chromosomes 8 indicate these to be members of a heteromorphic C-banding pair. Of the remaining three small autosomes, chromosomes 9 show homologous G-banding patterns and therefore two small autosomes with homologous C-banding patterns are also designated as pair 9.

[It is recognized that the relationships between these C- and G-banded chromosomes cannot be regarded as absolute in the absence of sequential G- and C-banding. This was attempted, but was unsuccessful.]

Figure 7.4

The G-banding karyotype of *Petaurus norfolcensis*.

There are four chromosomes that remain unpaired after G-banding and the Y chromosome, which is minute (see Figure 7.5) was missing from this cell.

Figure 7.5

The C-banding karyotype of *Petaurus norfolcensis*.

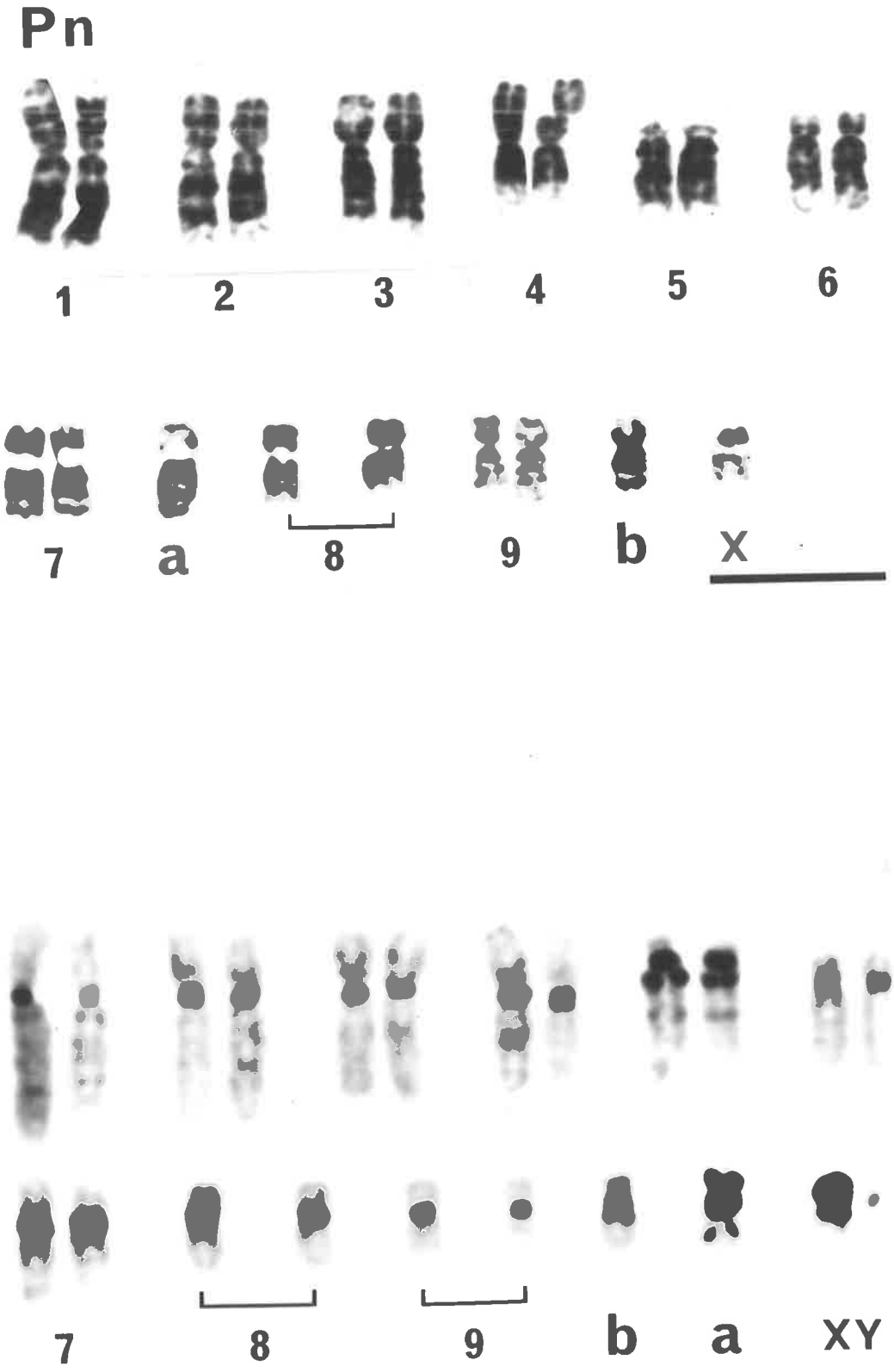

Pn
1
2
3
4
5
6
7
a
8
9
b
X
7
8
9
b
a
XY

Figure 7.6

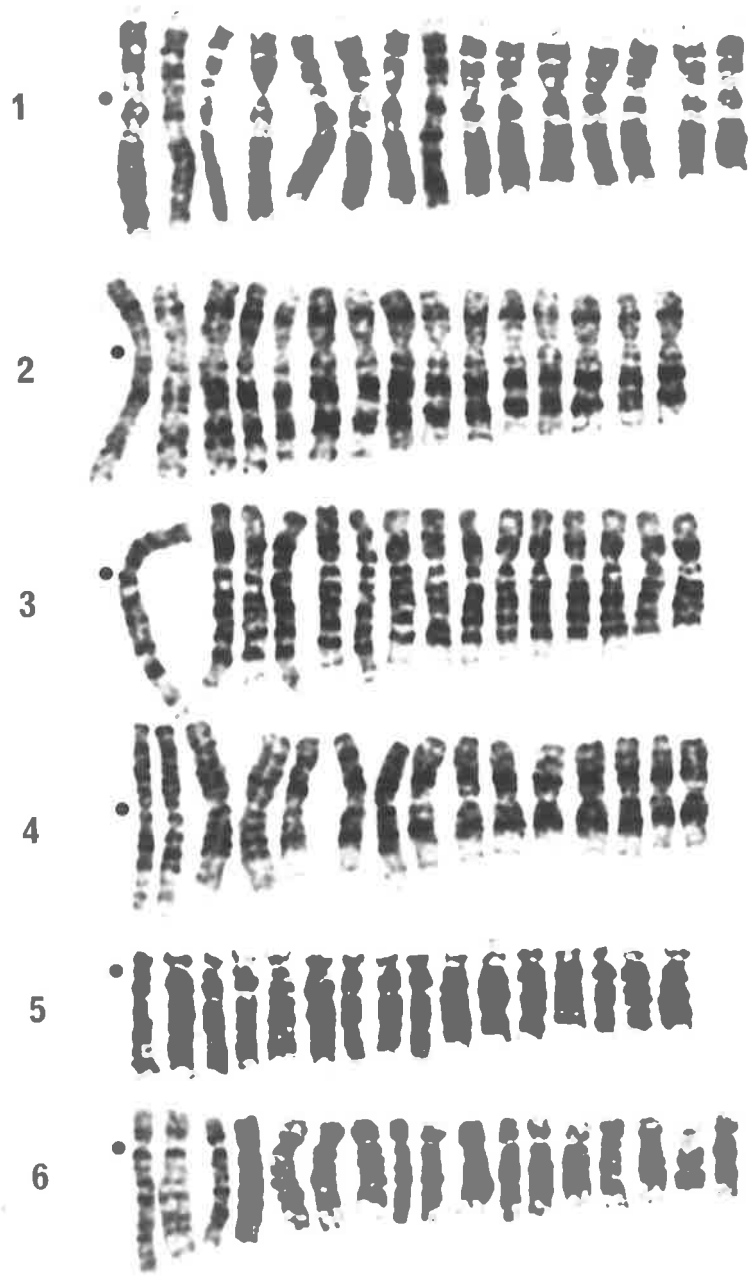
The detailed G-banding patterns of chromosomes 8, 9, a, b and X of *Petaurus norfolcensis*.

7
a
8
9
b
X

Figure 7.7

The G-banding chart for *Petaurus norfolcensis*.

Pn



Thus after C- and G-banding analysis two chromosomes, a and b, remain unpaired.

It is possible that the size discrepancy between a and b may be accounted for by the much greater area of C-banding material shown by chromosome a, and that if all C-banded regions were deleted from the G-banded chromosomes that the residual G-banding patterns of a and b would match. However, a and b are relatively small chromosomes and the large number of differences (including interstitial C-bands) they possess makes any demonstration of residual G-banding homologies quite equivocal. The meiosis of the one male squirrel glider was not studied.

A relevant observation may be that of a C-banding heteromorphic pair similar to that of the C-banded a/b in a number of sugar gliders, *P. breviceps*. In this species eleven bivalents were regularly observed in the meiosis of heteromorphic animals (Hayman and Sharp, personal communication). Unfortunately no *P. breviceps* have as yet been successfully G-banded, and therefore it is not known whether this species also shows heteromorphic chromosomes with G-banding patterns corresponding to those of chromosomes a and b of the squirrel glider, *P. norfolcensis*.

The G-banding patterns of several squirrel glider chromosomes can be described in terms of those of some *Trichosurus vulpecula* autosomes; these are *P. norfolcensis* chromosome pairs 1, 2, 3, 5, 6 and 9. The G-banding patterns of pairs 4, 7, 8 and of chromosomes a, b, X and Y of the squirrel glider are briefly described.

Pair 4

The most metacentric of the larger chromosomes, this pair is mostly dark after G banding, with a large pale distal area marking the long arm.

The distal half of the short arm is grey with two grey bands, a wide dark band follows, then a grey region near the centromere. In the

long arm there is a discrete band, a grey area, then a broad dark band followed by the pale distal region in which one or two faint bands are often visible.

Pair 7

The short arm is dark, but lighter more distally, where a discrete band may be visible. There is a large white centromeric region which is flanked in the long arm by a band and a dark region containing a wide doublet band. This is followed by a pale interband then a grey doublet band. The ends of both arms are white.

Pair 8

The only apparent difference between the G-banding patterns of the two homologues of this pair is that a larger centromeric white area is possessed by one chromosome. Otherwise two G-bands mark the short arm, the more proximal often being visibly double. There are four bands in the long arm, a wide dark band most proximally, then two grey bands, with a third grey band sometimes visible distal to the wide dark band. The distal ends of the arms are unstained

Pair 9

These chromosomes display a distinctive striped G-banding pattern. Five bands are regularly observed, two grey bands in the short arm (one of these near terminal) and three dark bands in the long arm. The latter three are regularly spaced with pale interbands, the near-centromeric band is broadest and blackest. The end of the long arm is unstained.

Chromosome a

This chromosome possesses a very characteristic G-banding pattern. There is a large white centromeric region flanked by discrete dark bands

in both arms and a third dark band in the long arm, two thirds of the arm's length from the centromere.

The short arm shows a near-terminal band. In the long arm two bands are regularly seen between the two dark bands. Just proximal to the more distal dark band there is a pale interband. A larger pale area flanks this dark band distally, followed by a band near the end of the long arm. The end of the arm is marked by a small band.

Chromosome b

There are three bands in the short arm, the most distal being near terminal and grey, the next darker and median. A thin dark band flanks the small unstained centromeric strip. In the long arm there is a dark band adjacent to the centromere, a pale interband, a broad dark near-median band, a pale interband and a grey band. The distal end is marked by a small band.

The X chromosome

A large dark band covers most of the short arm. The centromere is marked by a small faint band. In the long arm there is a clear region most proximally, followed by a dark band and two grey bands.

The Y chromosome

This chromosome which is not present in G-banding karyotypes and charts, is minute (see Figure 7.5) and no G-banding pattern detail can be seen. It stains palely.

CHAPTER 8

G-BANDED CHROMOSOMES AND CHROMOSOMAL EVOLUTION IN PHALANGEROIDEA

The chromosome complements of phalangeroid marsupials represent most of the karyotypic diversity of Australian marsupials (see Figure 3.1). The superfamily Phalangeroidea is composed of four taxonomic families of living marsupials, Burramyidae, Macropodidae, Phalangeridae and Petauridae. The G-banded chromosome complements of two burramyids (Chapter 5.4), ten macropods (Chapter 6), one phalangerid and one petaurid (Chapter 7) have been presented in earlier chapters and these are now compared.

8.1 G-banding pattern homologies in Phalangeroidea

The autosomes

The G-banded autosomes of the 2n=14 basic complement of *Acrobates pygmaeus* (Burramyidae) are used as a convenient point of reference.

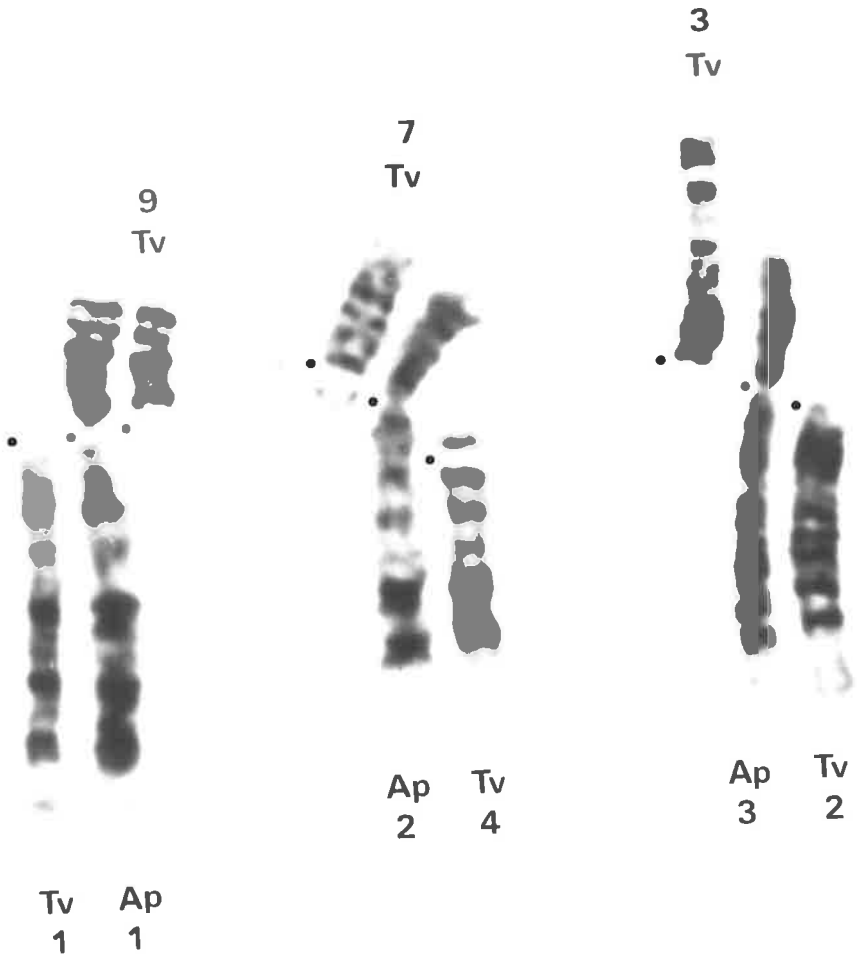
As shown in Chapter 5.4, the G-banded autosomal complements of the two burramyids studied (*A. pygmaeus* and *Cercartetus concinnus*) are very similar and are considered to differ by three pericentric inversions. Of all other G-banded phalangeroid complements, with 2n ranging from 10♀, 11♂ for *Wallabia bicolor*, to 2n=22 for *Thylogale billardierii*, *Petrogale penicillata* and *Petaurus norfolcensis*; that of *Trichosurus vulpecula* (Phalangeridae) with 2n=20 chromosomes, is most like that of *A. pygmaeus*.

The G-banding pattern homologies of the chromosomes of these two species are shown in Figure 8.1, where each G-banded autosomal pair of both species is represented by a chromosome (see also Figures 5.20 and 7.3). Of the nine G-banded autosomes of *T. vulpecula* (*Tv* in Figure 8.1), *Tv 5* and *Tv 8* have homologous patterns to two chromosomes of *A. pygmaeus* (*Ap*), *Ap 5* and *Ap 6* respectively and *Tv 1*, *Tv 9*, *Tv 7*, *Tv 4*, *Tv 2* and *Tv 6*

Figure 8.1

The G-banding pattern homologies of the autosomes of *Acrobates pygmaeus*, *Ap*, and *Trichosurus vulpecula*, *Tv*.

Centromeres are indicated by dots.


3
Tv
7
Tv
9
Tv
Ap
2
Tv
4
Ap
3
Tv
2
Tv
1
Ap
1
Tv
6
Ap
4
Tv
3
Ap
5
Tv
5
Ap
6
Tv
8

each correspond to an arm of three *A. pygmaeus* autosomes *Ap 1q*, *Ap 1p*, *Ap 2p*, *Ap 2q*, *Ap 3q* and *Ap 4q* respectively. The remaining *T. vulpecula* autosome, *Tv 3*, corresponds to *Ap 3p* and *Ap 4p* tandemly linked. Thus the G-banded autosomes of these two species show complete G-banding pattern homology. The G-banding patterns of most chromosomes and chromosome arms are distinctive and therefore the pattern homologies are very clear. The least convincing is the correspondence of *Tv 7* with *Ap 2p*, as the pattern of these segments is more even. Nevertheless, inspection of Figures 5.20 and 7.3 shows that these regions share a number of G-banding features.

The G-banding pattern homologies of the autosomes of these and of other phalangeroid marsupials are shown in Figure 8.2. For convenience *Ap 2*, *Ap 3* and the phalangeroid chromosomes with patterns corresponding to those of the short arms of these chromosomes are shown (third row) below the main figure. Phalangeroid chromosomes that have not been matched with any others in the figure are enclosed by the rectangle.

Many G-banded autosomes of *Petaurus norfolcensis*, *Pn*, (Petauridae), with 2n=22 chromosomes, each show homologous G-banding patterns to single chromosomes of *T. vulpecula* (see also Figures 7.7 and 7.3). Pericentric inversions can account for the pattern differences between *Pn 9* and *Tv 9*, *Pn 2* and *Tv 4*, *Pn 3* and *Tv 2*, and *Pn 5* and *Tv 5*. The G-banding pattern of *Pn 1* corresponds to that of an *A. pygmaeus* autosome, *Ap 4*, with the differences being simply interpreted as the result of pericentric inversion. *Pn 6* corresponds to *Tv 7* and parts of *Pn 4* and *Pn 7* have some G-banding homology with *Tv 1* and *Tv 8* respectively. All *P. norfolcensis* autosomes that cannot be fully matched to other G-banded autosomes in Figure 8.2 are shown in the rectangle; these are *Pn 4*, *Pn 7*, *Pn 8*, *Pn a* and *Pn b*.

Of the kangaroos and wallabies (Macropodidae) studied, the G-banded autosomes of *Thylogale billardierii*, *Tb*, and of *Petrogale penicillata*, *Pp*,

Figure 8.2

The pattern homologies of the G-banded automomes

of phalangeroid marsupials.

For convenience the autosomes with G-banding patterns homologous to those of *Ap 2p* and *Ap 3p* are shown below the main figure.

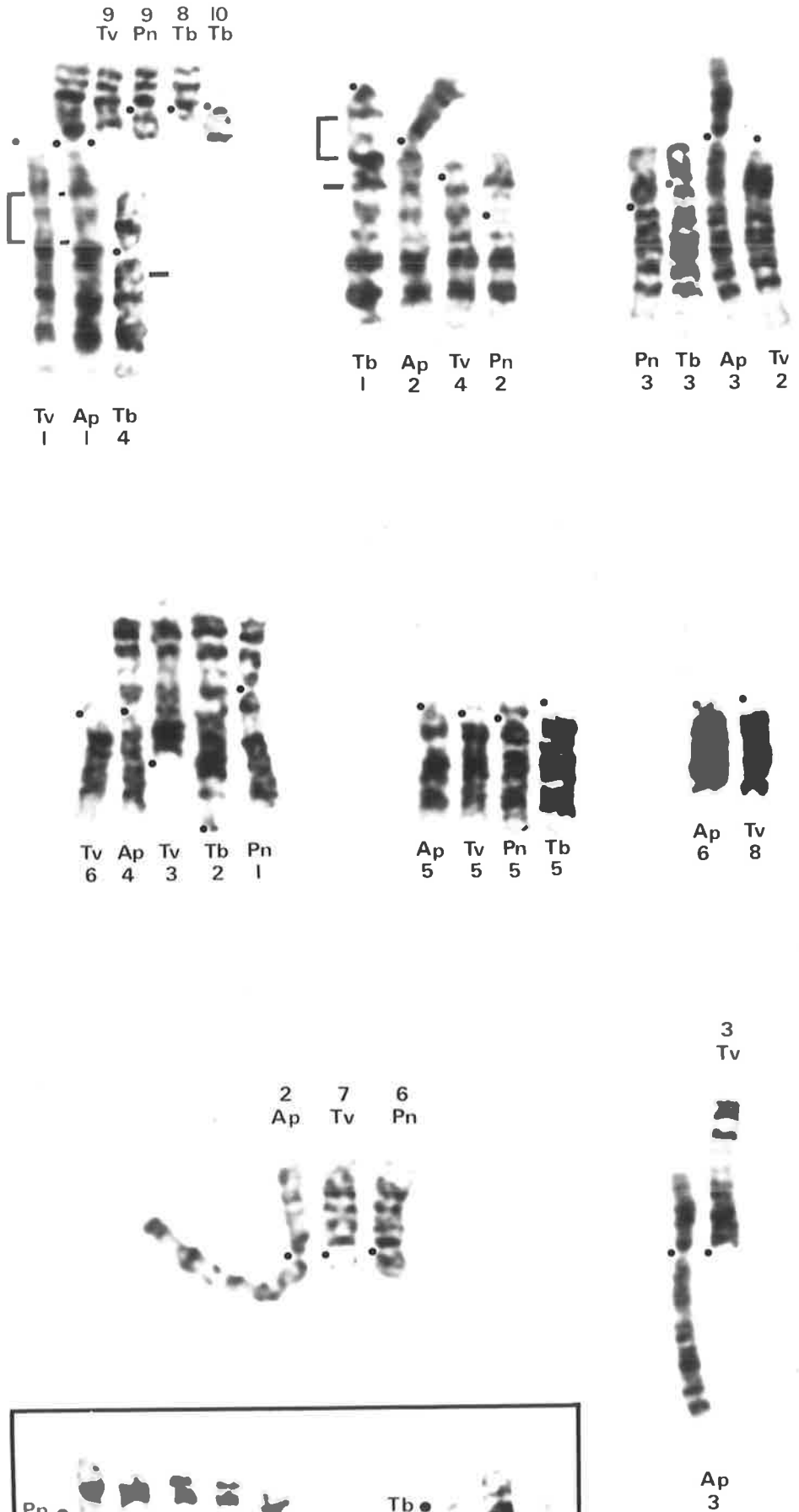
Chromosomes in the rectangle show no clear pattern homology with any autosome of the other species.

The bracketed regions are homologously banded areas of *Tv 1*, *Ap 1* and *Tb 1* chromosomes.

Below the dashes, *Tb 4* and *Tb 1* chromosomes show pattern homology with the chromosomes with which they are aligned.

Centromeres are marked with 'dots.

Ap	*Acrobates pygmaeus*	Burramyidae
Tv	*Trichosurus vulpecula*	Phalangeridae
Pn	*Petaurus norfolcensis*	Petauridae
Tb	*Thylogale billardierii*	Macropodidae


9 Tv
9 Pn
8 Tb
10 Tb
Tv 1
Ap 1
Tb 4
Tb 1
Ap 2
Tv 4
Pn 2
Pn 3
Tb 3
Ap 3
Tv 2
Tv 6
Ap 4
Tv 3
Tb 2
Pn 1
Ap 5
Tv 5
Pn 5
Tb 5
Ap 6
Tv 8
2 Ap
7 Tv
6 Pn
3 Tv
Ap 3
Pn
4
7
a
8
b
Tb
6
7
9

are most like the G-banded autosomes of *A. pygmaeus* (and *T. vulpecula* and *P. norfolcensis*). This is not shown in Figure 8.2, and can be seen most easily for all macropods by inspection of Figures 6.18, 6.19 and 6.20 together with Figure 8.2. The G-banded autosomal complement of *P. penicillata* would appear to match more closely to those of *A. pygmaeus* or *T. vulpecula* than that of the very similar *T. billardierii* complement since *Pp 3* has a near terminal centromere, whereas *Tb 3* does not (Figure 6.15). The other differences between the complements of *P. penicillata* and *T. billardierii*, a centric shift in pair 4 and a rearrangement involving the centromere in pair 7, cannot be used to indicate which of these complements is more similar to those of the other phalangeroids shown in Figure 8.2, as the differential part of chromosome 4 (which is above the line drawn for *Tb 4* in Figure 8.2) and all of chromosome 7 of these two species cannot be matched to the other phalangeroid chromosomes. Given that a karyotype of *Tb* form has been proposed as ancestral for a number of macropodid genera (Martin and Hayman, 1966), including *Thylogale* and *Petrogale*, and given also that there are many differences in G-banding pattern between either *Pp* and *Tv* or *Ap* chromosomes, or *Tb* and *Tv* or *Ap* chromosomes, the difference in centromere position between *Tb 3* and *Pp 3* is not considered indicative of a closer relationship of the *Pp* karyotypic form to *Ap* or *Tv* than that of the *Tb* form to *Ap* or *Tv*. Only the G-banded chromosomes of *T. billardierii* (see also Figure 6.5) are compared with those of *Ap*, *Tv* and *Pn* in Figure 8.2.

Tb 5 has a G-banding pattern corresponding to that of *Ap 5* and *Tv 5*. *Tb 3* shows pattern homology with *Tv 2* and *Pn 3*, and *Tb 2* with *Ap 4* and *Pn 1*. *Tb 3* is more simply related to *Tv 2* than to *Pn 3*, likewise *Tb 2* to *Ap 4* rather than *Pn 1*. The particular small differences between these chromosomes with otherwise homologous G-banding patterns indicate that *Tb 3*- and *Pn 3*-like ('*Pn 3*') chromosomes were formed from '*Tv 2*' by different

and independent pericentric inversions, likewise '*Tb 2*' and '*Pn 1*' from '*Ap 4*'. G-banding also suggests that *Tb 8* and *Tb 10* correspond in pattern to the long and short arms of *Pn 9* and also to *Ap 1p* and *Tv 9*. These pattern homologies are indicative of the following rearrangement sequence:

$$\text{'}Ap\ 1\text{'} \underset{fu}{\overset{fi}{\rightleftharpoons}} \text{'}Ap\ 1p\text{'}+\text{'}Ap\ 1q\text{'},\ \text{'}Ap\ 1p\text{'} \overset{p}{\longleftrightarrow} \text{'}Tv\ 9\text{'} \overset{p}{\longleftrightarrow} \text{'}Pn\ 9\text{'} \underset{fu}{\overset{fi}{\rightleftharpoons}} \text{'}Tb\ 8\text{'}+\text{'}Tb\ 10\text{'};$$

where *p* is pericentric inversion, *fi* Robertsonian fission, and *fu* Robertsonian fusion. The distal three-fifths of the long arm of *Tb 1* (below the line in Figure 8.2) shows pattern homology with all but the most proximal part of the long arms of *Ap 2*, *Tv 4* and *Pn 2*. The long arm of *Tb 4* (below the line) shows homology with *Tv 1* and *Ap 1q*. *Tb 6* may have pattern homology with *Ap 6* and *Tv 8*, however the band sequence is slightly different and therefore *Tb 6* is shown in the rectangle. There is also apparent homology between a segment of *Tb 1* and of *Tv 1* and *Ap 1q* (shown bracketed). This homology may extend to the centromere of each of these arms, and a little further distally than the bracketed regions. *Tb* chromosomes (*Tb 6*, *Tb 7*, *Tb 9*) that are unmatched are shown in the rectangle. The segments of the G-banded *Tb* complement that do not show any obvious homology with *Ap*, *Tv* or *Pn* chromosomes are, part of *Tb 1*, the short arm and most proximal region of the long arm of *Tb 4*, and the chromosomes within the rectangle. For both *Tb* and *Tv* the non-matching segments, according to the measurements of the general stained complements of these species (Hayman and Martin, 1974), constitute approximately 10% of the total haploid chromosome length of female animals.

The presently available G-banding data for phalangeroid marsupials represented in Figure 8.2 suggests that some G-banded autosomal segments have changed very little relative to others. This is particularly true

of chromosomes with G-banding patterns homologous to *Ap 5* and to segments homologous to *Ap 3q*, and to a lesser extent of regions with patterns corresponding to much of *Ap 2q*.

The X and Y chromosomes

The G-banded X chromosomes of a number of phalangeroid marsupials representing all taxonomic families are shown in Figure 8.3 (only two X chromosomes are shown for Macropodidae - see also Figure 6.13). The C-banding patterns of all these chromosomes are also represented. As discussed in sections 5.4 and 6.6, there are possible G-banding pattern homologies between the non C-banding regions of X chromosomes within the families Burramyidae and Macropodidae. It is also conceivable that there is some G-banding homology for the non C-banding portions of the lower arms of the X chromosomes of all these phalangeroid marsupials (below the dotted lines in Figure 8.3). A slightly larger region including the next most proximal dark band may be common to the macropodid, phalangerid and petaurid chromosomes. However, given the variation in general morphology, and C-banding patterns of the X chromosomes of these taxonomically distinct marsupials, these putative homologies in G-banding patterns are regarded as equivocal.

In all phalangeroid families there are some species possessing secondary constrictions (which N-band) on the X chromosome (there are also sites on other chromosomes). The possible phylogenetic significance of these observations (Rofe, unpublished) are unknown.

The Y chromosomes of the phalangeroid species studied also show substantial variation in size, shape, C- and G-banding pattern, with many being minute. Thus no G-banding comparisons were attempted.

Figure 8.3

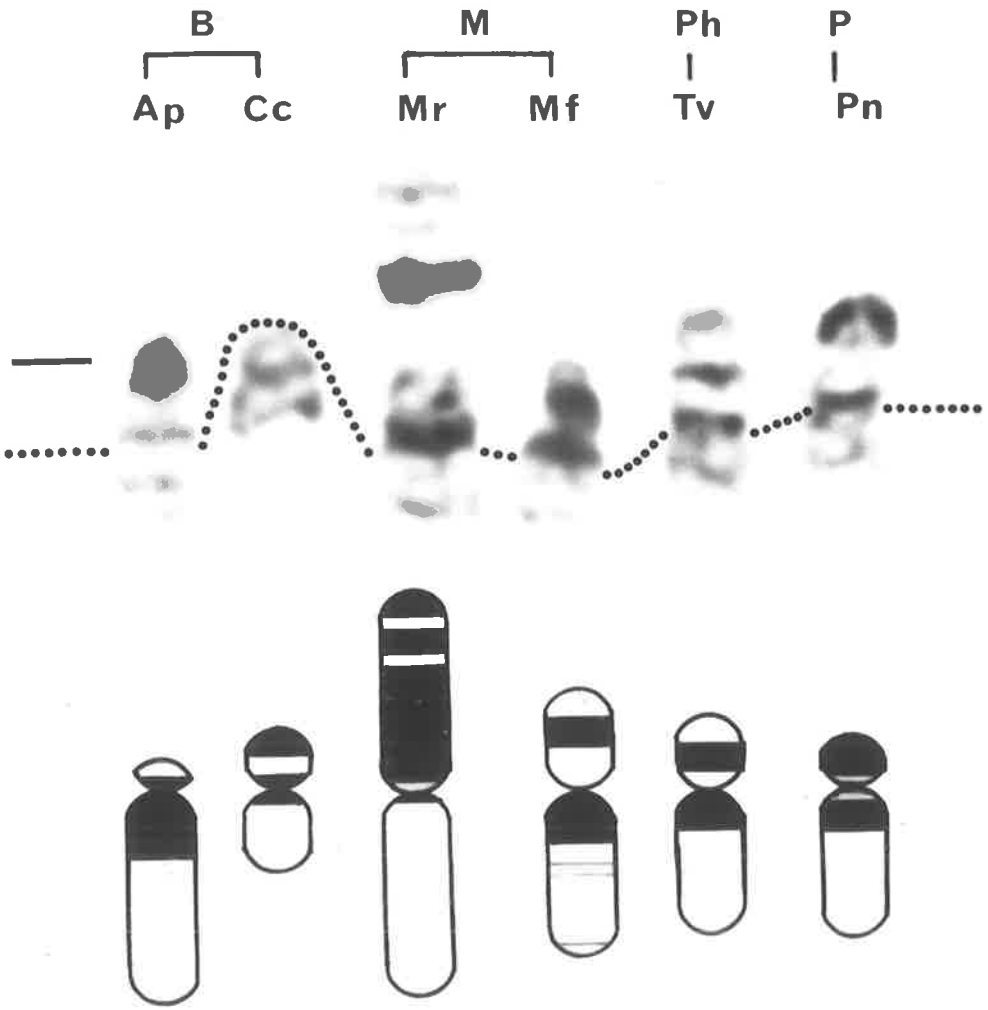
The G-banded X chromosomes of phalangeroid marsupials.

There are possible G-banding pattern homologies for the chromosome regions below the dotted line.

The C-banding ($Ba(OH)_2$) patterns are shown below the G-banded chromosomes.

The dash indicates centromere position.

B	Burramyidae
Ap	*Acrobates pygmaeus*
Cc	*Cercartetus concinnus*
M	Macropodidae
Mr	*Macropus rufus*
Mf	*Macropus fuliginosus*
Ph	Phalangeridae
Tv	*Trichosurus vulpecula*
P	Petauridae
Pn	*Petaurus norfolcensis*


B
M
Ph
P
Ap
Cc
Mr
Mf
Tv
Pn

8.2 Pathways of chromosomal evolution

Pathways of chromosomal evolution for the phalangeroid marsupials studied may be constructed using their similarities and differences in autosomal G-banding pattern. These pathways are regarded as tentative as they are based on interpretations of G-banding pattern homologies, are limited by the extent of the present data, and are also deduced on the following conditions.

Only differences that involve large chromosomal segments (and so may be readily defined) and only changes that may be interpreted as single Robertsonian changes or pericentric inversions have been used. Thus such variation as is present between chromosomes with G-banding patterns corresponding to those of *Ap 5* and *Ap 6* (Figures 5.19 and 8.2) and can be attributed to intrachromosomal rearrangements, has been ignored. Also large complex differences, such as those between most *Pn* chromosomes (those in the rectangle of Figure 8.2) and those of other phalangeroids are not used in determining relationships and are referred to only as "many other changes". Only particular G-banded complements that can be related most simply to the G-banded complements in other superfamilies of Australian marsupials are proposed as ancestral for phalangeroid marsupials. There are three of these and each possible complement is considered in turn.

A 2n=14 complement as ancestral

If an ancestral complement of essentially the form of the G-banded complement of *Acrobates pygmaeus* (Burramyidae) is proposed as ancestral for the phalangeroid marsupials studied, then the present G-banding data can be simply interpreted as indicating the pathways of chromosomal evolution shown in Figure 8.4 (a). [For the reasons given above the exact format of chromosomes corresponding in pattern to those of *Ap 5* and *Ap 6* are undefined.]

Figure 8.4

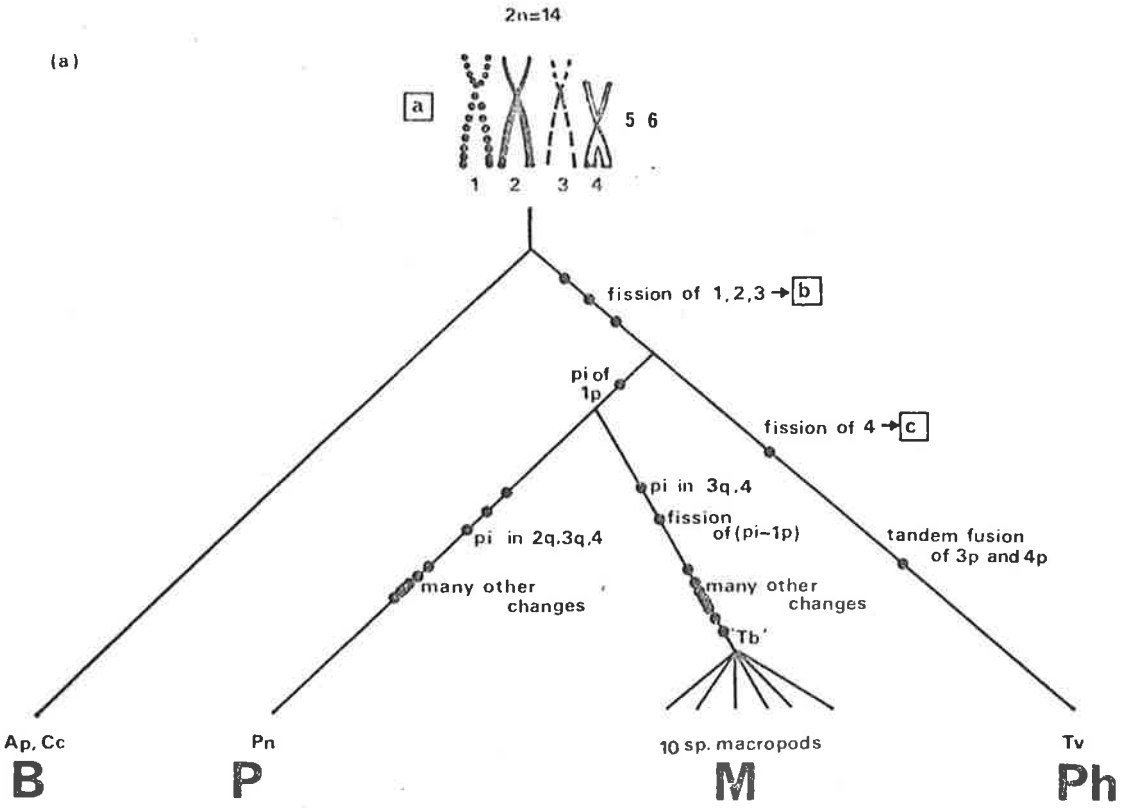
Hypothetical G-banded ancestral complements and pathways of chromosomal evolution for phalangeroid marsupials.

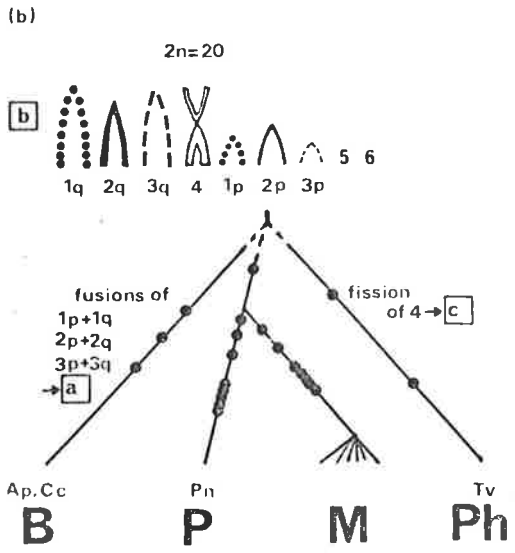
(a) a 2n=14 ancestral complement, a
(b) a 2n=20 " " b
(c) a 2n=22 " " c

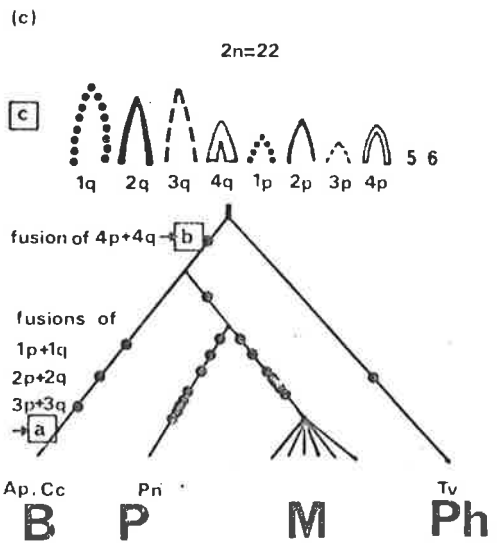
Only the autosomes of the ancestral complements are shown.

Chromosomal rearrangements are indicated by dots and where these are not specified in (b) and (c) they are the same as in (a).

pi	pericentric inversion
'*Tb*'	a *Thylogale billardierii*-like complement
B	Burramyidae ,
Ap	*Acrobates pygmaeus*
Cc	*Cercartetus concinnus*
P	Petauridae
Pn	*Petaurus norfolcensis*
M	Macropodidae
Ph	Phalangeridae
Tv	*Trichosurus vulpecula*


(a)
2n=14
a
5 6
1 2 3 4
fission of 1,2,3 → b
pi of 1p
fission of 4 → c
pi in 3q,4
fission of (pi-1p)
pi in 2q,3q,4
tandem fusion of 3p and 4p
many other changes
many other changes
'Tb'
Ap, Cc
Pn
10 sp. macropods
Tv
B
P
M
Ph


(b)
2n=20
b
5 6
1q 2q 3q 4 1p 2p 3p
fusions of
1p+1q
2p+2q
3p+3q
→ a
fission of 4 → c
Ap, Cc
Pn
Tv
B
P
M
Ph


(c)
2n=22
c
5 6
1q 2q 3q 4q 1p 2p 3p 4p
fusion of 4p+4q → b
fusions of
1p+1q
2p+2q
3p+3q
→ a
Ap, Cc
Pn
Tv
B
P
M
Ph

The lineage of the burramyids is proposed to have diverged first from the phalangeroid stock. Secondly, after Robertsonian fission of the three largest chromosomes (producing acrocentrics like *Tv 1*, *Tv 4*, *Tv 2*, *Tv 9*, *Tv 7* and *Ap 3p* - see Figure 8.1), the lineages of the present day *Trichosurus vulpecula* (Phalangeridae) diverged from that of *Petaurus norfolcensis* (Petauridae) and the macropods (Macropodidae). Fission of the fourth autosomal pair of the *Ap*-like complement then occurred in the lineage of *T. vulpecula* to produce chromosomes like *Tv 6* and *Ap 4q*. Following this fission, a tandem fusion of '*Ap 4q*' and '*Ap 3p*' occurred to produce *Tv 3* and the 2n=20 complement of the present day *T. vulpecula*.

The *P. norfolcensis* and *Thylogale billardierii* lineages diverged from each other after the pericentric inversion of '*Tv 9*' to produce '*Pn 9*'. A large number of chromosomal rearrangements then occurred in the separate *P. norfolcensis* and *T. billardierii* lineages. These included, (see Figure 8.2) for *P. norfolcensis*, pericentric inversions of '*Tv 4*', '*Tv 2*', '*Ap 4*' to produce *Pn 2*, *Pn 3*, *Pn 1*, and numerous other changes, and for *T. billardierii*, pericentric inversions of '*Tv 2*', '*Ap 4*', to produce *Tb 3* and *Tb 2*, fission of '*Pn 9*' to produce *Tb 8* and *Tb 10*, and many other rearrangements. The chromosome complements of the other macropods studied were produced from a '*Tb*' complement as described in Chapter 6.

A 2n=20 complement as ancestral

The G-banded complements of these phalangeroid marsupials may also be simply related if a 2n=20 complement equivalent to that proposed as commonly ancestral for *P. norfolcensis* and the macropods studied (and thus logically for Petauridae and Macropodidae), *b* in Figure 8.4 (a), is assumed as ancestral for all phalangeroid marsupials. This is shown in Figure 8.4 (b). The plan of this figure is essentially that of Figure 8.4 (a) with the point of phalangeroid ancestry changed; the sequence of divergence of the burramyid, phalangerid and petaurid-

macropodid lineages is undefined, but the petaurids and macropods again diverge from each other only after the divergence of all other lineages. Otherwise (b) differs from (a) of Figure 8.4 in having three less fissions (which were present in a common lineage of Phalangeridae, Petauridae and Macropodidae in (a)) and three more fusions (in the burramyid lineage).

A 2n=22 complement as ancestral

A 2n=22 complement of the form of *c* in Figure 8.4 (a) and (b) is shown as ancestral for phalangeroid marsupials in Figure 8.4 (c). The lineage of Phalangeridae diverges first from the common phalangeroid stock. However, as for (a) and (b), the lineages of Petauridae and Phalangeridae diverge from each other after the divergence of all other familial lineages. In (c) there are four less fissions (nevertheless one fission is still proposed in the '*Tb*' lineage - see (a)) and four more fusions than in (a).

As has been noted, a common feature of all three schemes of Figure 8.4 is the more recent common ancestry of the familial lineages of Petauridae and Macropodidae relative to the other familial lineages of Phalangeroidea. This relationship is based only on the proposed G-banding pattern homologies between *Pn 9* and *Tb 8* and *Tb 10* which, given the many other differences between the G-banded complements of *P. norfolcensis* and *T. billardierii*, must be regarded as highly tentative. Further G-banding studies in these two phalangeroid families, particularly the Petauridae, may clarify the situation. (For example, the demonstration of a '*Pn 9*' chromosome in several petaurid genera would substantiate the proposal of this relationship.)

The pathways of chromosomal evolution that have been proposed by Hayman and Martin (1974) for Phalangeroidea on the basis of general stained

chromosomes are shown in Figure 8.5.

The scheme of Figure 8.4 (a) resembles that of Hayman and Martin, with the G-banding data being consistent with their proposals of the occurrence of fission of four pairs of the ancestral 2n=14 complement, and of a tandem fusion following these fissions in the lineage of *T. vulpecula*. The G-banding data are also in accordance with their suggestions that pericentric inversions have occurred in the separate petaurid and macropodid lineages.

However, the scheme of Figure 8.4 (a) departs from that of Figure 8.5 in showing fission of only three pairs of the 2n=14 complement before the divergence of the lineages of Petauridae, Macropodidae and Phalangeridae, and in indicating the petaurid and macropodid lineages to have more recent ancestry with each other than either does with Phalangeridae. Also, although G-banding indicates, as Hayman and Martin had proposed, a number of pericentric inversions in the lineages of present day petaurids and macropodids with 2n=22 chromosomes, it has further shown that there have been many other chromosomal changes (including at least one increase in chromosome number) in each of these lineages. Thus, in contrast to Figure 8.5, the 2n=22 petaurid and macropodid complements cannot be simply related to each other or to the proposed 2n=14 ancestral complement.

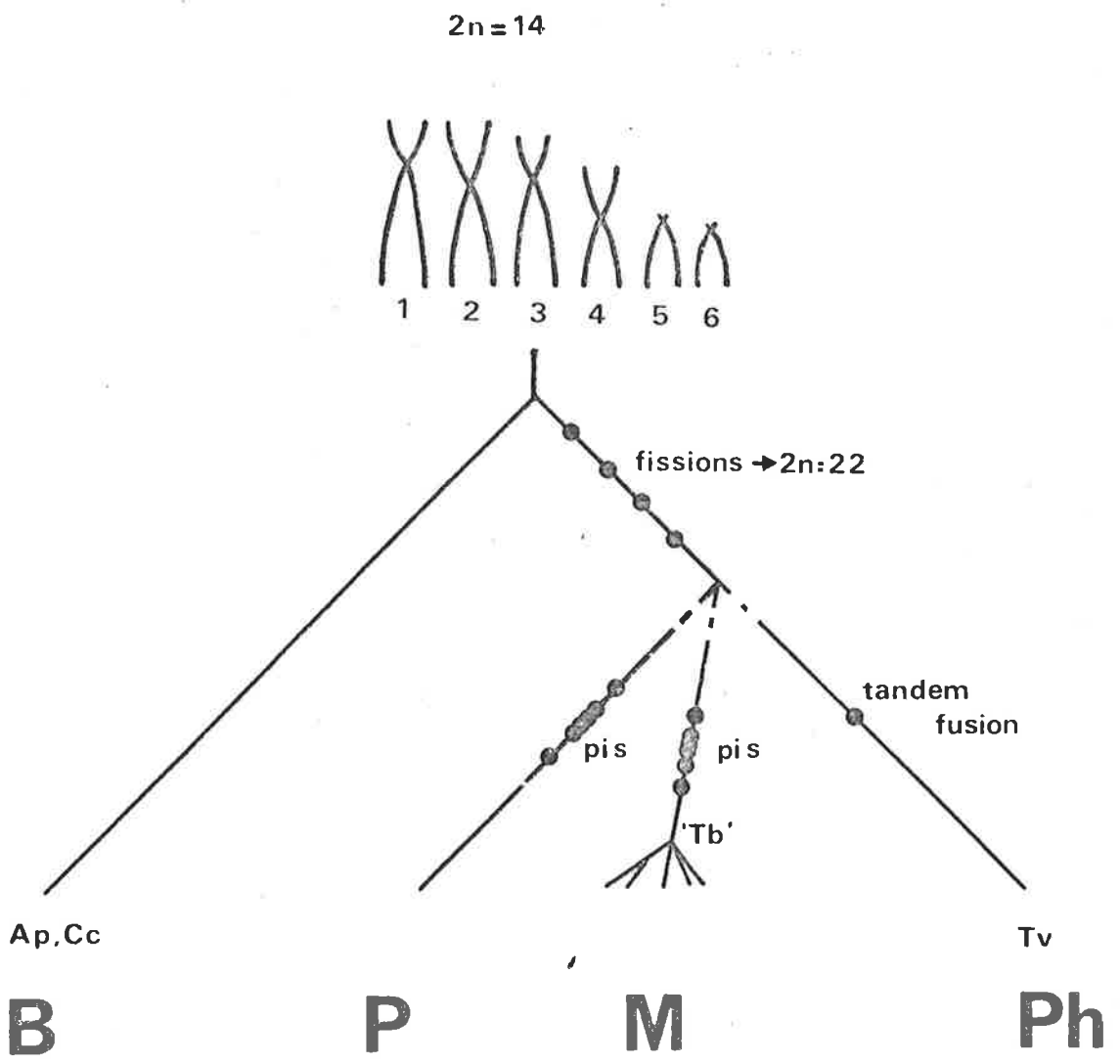
There is at present no evidence for the sequence of divergence of phalangeroid familial lineages available from the fossil record or any other source. On the basis of *the cytogenetic data of this Chapter alone* the choice between these three schemes appears to be a choice of more or less fission or fusion, with 2n=14 as ancestral, Figure 8.4 (a), requiring the greatest number of fission events and 2n=22, Figure 8.4 (c), the least.

Figure 8.5

The general stained ancestral complement and pathways of chromosomal evolution proposed for phalangeroid marsupials by Hayman and Martin (1974).

Only the autosomes are shown.

Abbreviations as for Figure 8.4.


2n = 14
1 2 3 4 5 6
fissions → 2n: 22
pis
pis
'Tb'
tandem
fusion
Ap, Cc
Tv
B
P
M
Ph

CHAPTER 9

G-BANDED CHROMOSOMES AND CHROMOSOMAL EVOLUTION IN AUSTRALIAN MARSUPIALS

G-banding has demonstrated the presence of large homologously banded segments in marsupial karyotypes of very different general format and thus provided much greater resolution of the modes and pathways of chromosomal evolution in these Australian animals than was previously possible.

9.1 Conservation of G-banding pattern

The conservation of G-banding pattern in marsupial complements is particularly obvious for autosomal segments, with more pattern variation often demonstrable between the X chromosomes of different marsupial species (for example, the X chromosomes of 2n=14 basic complements, Figure 5.24, and of the similar 2n=16 complements of *Macropus eugenii*, *M. parryi* and *M. rufogriseus*, Figure 6.12) than between their autosomes (Figures 5.23; and 6.3 *Me*, *Mp* and *Mrg* respectively). These differences between X chromosomes may be partly or wholly due to differences in the amounts of C-banding or other non-basic X-chromosomal material. Nevertheless, such observations indicate that the G-banding patterns of X-chromosomes should be compared very carefully. Also, as periodic-banding pattern homology and conservation of structural gene loci have been correlated in Primates (for example, Finaz *et al.*, 1977) it is possible that widespread and stringent conservation of genetic loci in particular chromosomal segments which has previously only been proposed for X chromosomes (and then as a consequence of the special gene dosage properties envisaged for X-linked genes) is likely for autosomal regions. Thus Ohno's hypothesis (1967) of genic and basic size conservation for

X chromosomes should not be used to justify inter-ordinal comparisons of the G-banding patterns of X chromosomes, as has been done by Pathak and Stock (1974).

These authors compared the G-banding patterns of the X chromosomes of sixty species from many mammalian orders, without specific reference to possible translocated autosomal material or to the heterochromatic regions of these particular chromosomes. They present a diagram of the G-banded X chromosome of the macropodid marsupial, *Potorous tridactylus*, without any recognition of the fact that this chromosome is a component of an $XX♀$, $XY_1Y_2♂$ sex chromosome system (Sharman *et al.*, 1950) and thus largely composed of translocated autosomal material.

As the G-banding patterns of the X (and Y) chromosomes of the marsupials studied in this thesis were so variable, and the differences difficult to define, the following discussion of chromosomal evolution will be confined (as in previous chapters) to the autosomes.

9.2 The nature of the karyotypic differences

Apart from the differences in the amount and distribution of C-banding material in the macropods, almost all other differences between the G-banded complements of the marsupials studied can be accounted for by Robertsonian changes and intrachromosomal rearrangements that may be simply interpreted as pericentric inversions. The other changes include two paracentric inversions (see Figures 5.11 and 5.15 (b)) and one centric shift (Figure 6.15). The more complex differences that cannot be explained by single events of the above types may be products of several such re-arrangements.

As in many other chromosome banding studies in animals (for examples see Chapter 1), the relationships between the G-banded marsupial complements are not always as proposed from studies of general stained chromosomes:

for example, some of the morphologically similar 2n=16 complements of *Macropus* species possess three different pairs of autosomes, and the G-banded complements of *Thylogale billardierii* (2n=22) and *Acrobates pygmaeus* (2n=14) cannot be related as simply as proposed by Hayman and Martin (1974). Most importantly, although the types of karyotypic differences found between the G-banded marsupial complements are the same as those usually observed in eukaryotes, namely differences consistent with proposals of Robertsonian changes and pericentric inversions, there is a single, significant, difference. Whereas Robertsonian differences have often been interpreted, by no inherent requirement of the cytological data, as centric fusion differences, the relationships of the G-banded chromosome complements of the marsupials studied in Chapter 5 show that Robertsonian *fission* merits serious consideration as a major mechanism of karyotypic evolution in Australian marsupials.

9.3 An ancestral complement for Dasyuroidea, Perameloidea, Vombatoidea and Phalangeroidea

As discussed in Chapter 2, the best choice of a putative ancestral complement for any group of organisms is the karyotypic form that is present in the greatest number and variety of taxa. However, accurate deduction of this ancestral type is only possible if a number of taxonomically diverse living marsupials have chromosome complements which are substantially unchanged from this ancestral conformation. Using this reasoning alone, the general stained 2n=14 basic complement appeared to be the best candidate for the ancestral complement of all marsupials (Hayman and Martin, 1974, and also Sharman, 1973).

G-banding has shown that the 2n=14 basic complements of marsupials from four taxonomic superfamilies of Australian marsupials are very similar (section 5.5) and thus substantiated the proposal (Martin and

Hayman, 1967) of "2n=14 basic" as ancestral for these particular marsupials. In contrast the present G-banding study has also shown that the 2n=22 complements of species from the only two families of Australian marsupials in which this number is presently known to occur, the Macropodidae and Petauridae, are very different and can be most simply related by a common 2n=20 complement (see Figure 8.4).

Nevertheless, comparison of the G-banded complements of phalangeroid marsupials has indicated that there are three putative ancestral complements for Phalangeroidea that may more simply relate the presently G-banded phalangeroid complements and these to the G-banded 2n=14 complements of other superfamilies studied, than any other complement. These were shown in Figure 8.4 and will now be considered as possible ancestral complements for the four major superfamilies of Australian marsupials sampled in this thesis, namely Dasyuroidea, Perameloidea, Vombatoidea and Phalangeroidea. The format of the autosomes corresponding in G-banding pattern to the two smallest pairs of the 2n=14 basic G-banded complement was previously undefined for ancestral complements of Phalangeroidea but will now be considered to be of the same morphology as pairs 5 and 6 of the 2n=14 basic G-banded complement of Figure 5.23, as this is consistent with the simplest interpretation of the total G-banding data.

Not only is the choice between the 2n=14, 2n=20 and 2n=22 complements as ancestral for these four superfamilies accompanied by the proposition of more or less fission or fusion, or, more or less increases or decreases in chromosome number (as in Chapter 8 and see also Table 2.1 for other superfamilies), but the close similarity and particular small differences between the G-banded 2n=14 basic complements possessed by marsupials in different superfamilies requires explanation.

If Robertsonian fission (and increases in chromosome number) and

Robertsonian fusion (and decreases in chromosome number) are considered equally feasible chromosomal changes then the proposal of any complement other than the 2n=14 basic G-banded complement as ancestral for all major superfamilies of Australian marsupials is untenable (as argued in section 5.5).

However, if Robertsonian fusion, which has been used in the past to favour 2n=22 as ancestral, is used to argue for either of the hypothetical 2n=20 or 2n=22 G-banded complements (given the G-banding data 2n=20 would be the more parsimonious choice) as ancestral for all marsupials, it is necessary to invoke some type of selection for karyotypic format to account for the similarly G-banded 2n=14 complements.

The principle of "karyotypic orthoselection" (see section 6.9) has been proposed to explain the formation of general stained complements of similar format in other animal groups (White, 1973a). However, it is difficult to envisage how such selection could explain the formation of the identically G-banded 2n=14 "intermediate" complements of Figure 5.26, especially as G-banding studies of morphologically similar complements from other animal groups (for example, *Mus*, Capanna *et al.*, 1976, and *Macropus* species with 2n=16 chromosomes, this thesis) have indicated that similarly sized metacentrics may be formed by random combinations of arm components. Given that selection for general karyotypic format in these groups of *closely related* species has involved such *non-specific* formation of metacentrics, it is unlikely that karyotypic orthoselection can explain the *specific* similarity of the complements of such *diverse* marsupials as those with similarly G-banded 2n=14 complements.

Thus the proposal of a G-banded complement of either the 2n=20 or 2n=22 form of Figure 8.4 as ancestral for Australian marsupials requires extraordinary parallel evolution. In addition, if any other 2n=20 or 2n=22 complement is proposed as ancestral the G-banding data of this thesis cannot be simply interpreted.

One of the major criticisms (Sharman, 1973) of 2n=14 as ancestral for marsupials has been the lack of many demonstrable cases of increases in chromosome number in marsupials. The present G-banding study of phalangeroid marsupials indicates that Robertsonian fission and increases in chromosome number have been more prevalent than could be judged on the basis of the general stained chromosomes. For example, with the 2n=22 complement of Figure 8.4 (c) as ancestral, increases in chromosome number are nevertheless necessary in each of the lineages of *Petaurus norfolcensis* (Petauridae) and *Thylogale billardierii* (Macropodidae) to simply account for the G-banded 2n=22 complements of these species. Robertsonian fission is implicated in the *T. billardierii* lineage.

In summary, it is clear that the 2n=14 basic G-banded complement of Figure 5.23 is by far the best candidate for the putative chromosome complement of a common ancestor of the four major superfamilies of Australian marsupials, the Dasyuroidea, Perameloidea, Vombatoidea and Phalangeroidea. It is thus also apparent that Robertsonian fission and increases in chromosome number have been significant means of karyotypic evolution in Australian marsupials.

G-banding analysis may also be useful for evaluating the possible directionality of Robertsonian change in other animal groups. For example, Matthey (1973) considers the similar general stained lizard karyotypes with 12 metacentrics and 24 microchromosomes to be derived by Robertsonian fusion in different lineages, but Gorman (1973) regards this taxonomically widely distributed karyotypic form as "a primitive condition with evolution away from 12V+24m".

It is also possible that G-banding studies of the complements of groups such as these lizards may eventually unequivocally establish a general bi-directionality of Robertsonian change.

9.4 Conservation of "the 2n=14 G-banded basic complement" and long-term conservation of karyotypic form in animals

With the 2n=14 G-banded basic complement as ancestral for the four major superfamilies of Australian marsupials it follows that there has been long term conservation of this karyotypic form. The fossil evidence indicates that the lineages of the four families of marsupials with 2n=14 basic G-banded complements have been separately evolving for at least twelve million years (Archer and Bartholomai, 1978) and it is believed that they have been independent for fifty to sixty million years (Stonehouse, 1977).

The feasibility of the long term conservation of this karyotypic form is well illustrated by the conservation of the 2n=14 dasyurid basic G-banded complement (section 5.1). Unquestionably this karyotypic form has been commonly ancestral for all dasyurids whose chromosomes have been studied with G-banding in this thesis. Thus this particular "2n=14 basic" complement has remained virtually unchanged since the divergence of most modern dasyurid genera, approximately ten million years B.P. (Archer and Kirsch, 1977). This G-banded complement is probably even more conservative than indicated by the present study, as the numbat, which has been classified in a separate dasyuroid family, Myrmecobiidae (see Table 2.1) possess a 2n=14 complement with general stained autosomes of very similar morphology to those of dasyurids (Sharman, 1961).

There have been a number of reports indicating long-term conservation of the G-banding patterns of particular chromosomes and autosomal components in other animals: in birds, Takagi and Sasaki, 1974; Stock *et al.*, 1974; Stock and Mengden, 1975: in carnivores and seals, Wurster-Hill and Gray, 1975; Arnason, 1977: in Artiodactyls, Buckland and Evans, 1978a: and in Primates, Dutrillaux *et al.*, 1978. The report (Takagi and Sasaki) of G-banding pattern homologies

between birds and reptiles can be regarded as demonstrably erroneous (Stock and Mengden).

The inter-ordinal G-banding pattern homologies in birds involve only a small number of chromosomal components, and the reports of homologies between the mammalian orders Carnivora and Pinnepedia (Wurster-Hill and Gray; Arnason) and between families of seals, Pinnepedia (Arnason) must be regarded as preliminary, as no direct comparisons of the detailed G-banding patterns of these chromosomes have been published.

However, Wurster-Hill and Gray have shown G-banding pattern homology for a number of chromosomes between species from two different superfamilies of carnivores. Superfamilial conservation of G-banding pattern has also been demonstrated for some chromosomal components of bovoid species with those of one species each of Giraffoidea and Cervoidea, and between many of the chromosomes of the baboon and Man.

Nevertheless conservation of the general morphology of each G-banded autosome of an entire complement has only been suggested for the 2n=36 complement of seals and sea-lions (Pinnepedia, family Otariidae). If this report is substantiated, then this particular G-banded complement has remained superficially unchanged for at least fourteen million years (Arnason, 1977).

Thus, as shown by the G-banded complements of Dasyuridae and indicated by studies in other animals, conservation of the temporal order required to account for the similarity of the G-banded 2n=14 complements of diverse Australian marsupials is a feasible proposition.

9.5 Rationale for chromosome changes in Australian marsupials

Given 2n=14 as ancestral for the four major superfamilies of Australian marsupials there appear to have been no decreases in chromosome number without intervening increases in chromosome number. The five species of marsupials known to possess chromosome number less than 2n=14 all occur in Petauridae and Macropodidae (see Table 2.1 or Figure 3.1) and thus can be accounted for by number reduction from a commonly ancestral 2n=20 complement (see Figure 8.4). Therefore not only do Australian marsupials differ from many other animal groups which have a normal distribution of chromosome number, but the proposed ancestral number is near minimal, and not of the mean or of higher number (Matthey, 1973).

It is conceivable that the demonstrable conservatism of the 2n=14 basic complement provides the explanation, not only for the relative abundance of complements with 2n=14 chromosomes, but also for the deficiency of those with chromosome numbers less than 2n=14. There are very few differences between the G-banded 2n=14 basic complements of animals from different superfamilies of Australian marsupials, given the longevity of their separation. Thus it is possible that the only substantial release from the rigidity of this selectively favoured karyotypic form was via Robertsonian fission, and the widespread occurrence of increases in chromosome number in the evolution of the chromosome complements of Australian marsupials may not simply be a reflection of the spontaneous frequency of such events, but their selective fixation.

This difference between the relative occurrence and fixation of particular chromosomal rearrangements is well illustrated in Man, as although non-Robertsonian reciprocal translocations are the most commonly occurring chromosome rearrangements that are compatible with life, there

are extremely few such differences between the karyotypes of Man and the hominoid apes (Miller, 1977). Thus Mullerian fission need not be an impediment to increasing chromosome number.

Any increase in chromosome number also offers an increase in the reassortment of genes and thus is a potential avenue for the extra release of genetic variability (Darlington, 1939). In a situation of adaptive radiation, as when marsupials entered Australia) such an increase in genetic variability (and therefore fission as an immediate agent of such release) may have been advantageous. Todd (1970) has in fact proposed that mammalian evolution is largely dependent on extensive chromosome fissioning via misdivision of a number of centromeres in a single germ cell. He has presented theoretical cases (1970, 1975) for karyotypic fissioning in canid and artiodactyl phylogeny and has related presumed episodes of such fissioning to known periods of explosive speciation and adaptive radiation. However, it does not seem necessary to assume synchronous fissioning events in marsupials (for Mullerian fission this certainly would be impossible), as the high frequency of 2n=22 in Australian marsupials may largely be explained by the retention of an ancestral *Thylogale billardierii* karyotypic form in Macropodidae (ten species of macropods are known to possess general stained 2n=22 complements closely related or like that of *T. billardierii*, see also Figure 3.1).

However, it may be significant that the Macropodidae, which of the families of Australian marsupials, shows the most karyotypic diversity (Figure 3.1), also shows the greatest taxonomic diversity (see Table 2.1). Chromosomal rearrangements have been proposed as agents of incipient speciation (White, 1973a), and Bush *et al.* (1977) have suggested that the rate of speciation in a given vertebrate group is positively correlated with the rate of chromosomal evolution. However, the proposals of Bush

et al. are based on rates of karyotypic evolution estimated from changes in the number and gross morphology of the general stained chromosomes of whole orders of vertebrates, and it is very doubtful that such an average rate has any real or useful meaning. For example, it is quite clear in Australian marsupials that the rates of karyotypic evolution (as ascertained from G-banded chromosomes) may vary quite markedly within a superfamilial group (Phalangeroidea).

Bush *et al.* have also suggested that both the rate of speciation and of chromosomal evolution may be expedited by small effective population size, which is highest when populations are divided into small demes. Consequently they proposed that karyotypically diverse genera may be those with the most marked subdivision of the population into small demes, and that this subdivision may be greatly influenced by the social structure of the population.

It would thus seem potentially useful to examine the deme structure of the natural populations of a number of different marsupial species with 2n=14-basic G-banded complements or compare the social structure of populations of marsupial species of the same superfamily possessing 2n=14-basic and complements of higher number. However, despite Kaufmann's (1974) assertion that

> "The Macropodidae are the most social marsupials, and the whiptail wallaby is the most social macropod"

there have been so few detailed studies of the social structures of natural populations of marsupials that no attempt at correlating the rate of karyotypic evolution with deme size for these animals can presently be made.

9.6 An ancestral chromosome number for all marsupials

The G-banding studies of this thesis have suggested that the 2n=14 basic G-banded complement was ancestral for four superfamilies of Australian marsupials. General stained complements of this format are possessed by marsupials in both American superfamilies and as discussed in Chapter 2, Hayman and Martin (1974) have proposed that such a complement was ancestral for all living marsupials.

The chromosomes of some American marsupials have been studied with periodic-banding techniques and the Q-banding karyotype of a didelphid marsupial, *Marmosa mitis*, with a 2n=14 basic chromosome complement has been published (Curcuru-Giordano *et al.*, 1974). However, the linear differentiation of these chromosomes with the fluorochrome was poor and no comparisons with the G-banded complements of this thesis are possible. G-banding karyotypes of the American opossum *Didelphis virginiana*, which has 2n=22 chromosomes have also been published (Sinha and Kakati, 1976). Although these chromosomes are very contracted and the resolution of the banding does not approach that of this thesis, there are some similarities in the trypsin G-banding patterns of these chromosomes and those of "the 2n=14 basic G-banded complement" defined in this thesis for Australian marsupials, which are unmistakable to eyes familiar with the G-banded chromosomes of Australian marsupials.

In Figure 3 of the paper of Sinha and Kakati, chromosomes 1, 2, 3, 4, 5 and 6 display patterns which are clearly similar to those of the following segments of the 2n=14 basic G-banded complement (Figure 5.23) of this thesis; 1q, 2q, 3q, 5, 4q and 4p respectively. The patterns of chromosomes 7, 8, 9 and 10 of the published figure are not discordant with those of the remaining autosomal arms of the 2n=14 basic G-banded complement, namely 1p, 6, 2p and 3p respectively.

These observations are consistent with the proposal of "the 2n=14 basic G-banded complement" as ancestral for all marsupials. However,

they are also consistent with a 2n=22 ancestral complement of the form in Figures 5.26 and 8.4. High resolution G-banding analysis, particularly of the 2n=14 and 2n=22 complements of American marsupials is necessary, not only to substantiate these intercontinental similarities in G-banding pattern, but also to directly compare the 2n=14 complements of Australian and American marsupials.

Given the possibility that the G-banded 2n=14 and 2n=22 complements of American marsupials may be essentially the same as the 2n=14 basic G-banded complement and the hypothetical G-banded 2n=22 complement of Figure 8.4, a proposal of 2n=14 as ancestral for all marsupials would require independent production of the same 2n=22 karyotypic form in American and Australian marsupials.

There is very little karyotypic diversity in American marsupials (see Figure 2.1) and it is reasonable to assume that the three different karyotypic forms with 2n=14, 2n=18 and 2n=22 chromosomes include two non-independent derivations of an ancestral (either 2n=14 or 2n=22) complement. Thus with 2n=14 as ancestral it follows that only four Robertsonian fissions have been fixed during the evolution of the karyotypically known American marsupials. Given, as was suggested for Australian marsupials, that fission is the only significant rearrangement of the 2n=14 complement that has been selectively favoured, the possibly similar format of the G-banded 2n=22 complements of both Australian and American marsupials may otherwise be ascribed to chance. There are only a limited number of complements that may be produced by fission of the autosomes of a 2n=14 complement (in contrast to the number that may be produced by fusion of the acrocentric autosomes of a 2n=22 complement).

9.7 Suggestions for further G-banding studies

No doubt the most immediately interesting cytogenetic question remaining unanswered in marsupials is the possible relationship of the G-banded 2n=14 and 2n=22 complements of American and Australian marsupials.

Nevertheless, there are many Australian marsupials with chromosome complements that could profitably be studied with G-banding, especially since the present study was designed to evaluate the 2n=14 basic complement proposal and therefore did not sample much of the karyotypic diversity.

These species (see Table 9.1) include *Notoryctes typhlops*, the marsupial mole, and *Tarsipes spencerae*, the honey possum, which constitute the two superfamilies of Australian marsupials (Notoryctoidea and Tarsipedoidea respectively) not studied in this thesis. These animals possess 2n=20 and 2n=24 chromosomes respectively and the relationship of these to the 2n=14 basic G-banded complement would be of particular interest.

There are also four other families of living Australian marsupials that were not studied here, Myrmecobiidae, Thylacinidae, Thylacomyidae and Phascolarctidae. *Thylacinus cynocephalus* is probably extinct and is karyotypically unknown. *Myrmecobius fasciatus*, the numbat, is a dasyuroid marsupial with a general stained 2n=14 basic complement very like that of the dasyurids; G-banding would be useful in evaluating this apparent similarity. The Thylacomyidae and Phascolarctidae each consist respectively of two and one living species, with one species of each known to possess more than 2n=14 chromosomes. G-banding of these complements would better define their relationships to the 2n=14 chromosomes possessed by all other karyotypically known marsupials in their respective superfamilies (Perameloidea and Vombatoidea) and provide information on the possibility of the convergent occurrence of the same fissions in

Table 9.1

Species of Australian marsupials

suggested for further G-banding studies

SUPERFAMILY	FAMILY	SPECIES	2n
Dasyuroidea	Myrmecobiidae	*Myrmecobius fasciatus*	14
Perameloidea	Thylacomyidae	*Macrotis lagotis*	18♀,19♂
Phalangeroidea	Phalangeridae	*Phalanger* species	14
	Petauridae	*Pseudocheirus* species)	10
		)	16
		Dactylopsila species	18
	Macropodidae	*Bettongia* species	22
		Aepyprymnus rufescens	32
		Potorous tridactylus	12♀,13♂
		Dendrolagus species	14
Vombatoidea	Phascolarctidae	*Phascolarctos cinereus*	16
Tarsipedoidea	Tarsipedidae	*Tarsipes spencerae*	24
Notoryctoidea	Notoryctidae	*Notoryctes typhlops*	20

these and the lineages of other marsupial families (see Table 2.1).

Within the Australian families there are also many species with karyotypes of quite different general format to those of the marsupials presently studied. (These include the species with 2n=14 non-basic complements.) Knowledge of the G-banding patterns of the chromosomes of these complements (particularly those of Petauridae and Macropodidae, families which show several distinctive karyotypic forms) will have considerable implications for pathways of chromosomal evolution and thus for the phylogeny of Australian marsupials.

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