INTRODUCTION

To generate a model of mammalian karyotype evolution and to extend our knowledge of the evolutionary relationships of mammals, it is helpful to deduce ancestral chromosomal arrangements. Of particular interest would be a global comparison among the 3 major extant groups of mammals, since this would reveal the most ancient arrangements of the mammalian genome and allow us to follow chromosomal rearrangements in each lineage.

Marsupials diverged from eutherian ("placental") mammals about 130 million yr ago (Figure 1), and monotremes about 170 million yr ago (Graves and Watson 1991). The genomic arrangement in a common ancestor could be deduced by comparing the ancestral genomes of the 3 groups deduced from interspecies and interordinal comparisons. However, it has been difficult to construct an ancestral mammalian chromosomal arrangement because of the wide variety of karyotypes observed in mammals. Neither comparative gene mapping nor chromosome banding studies are adequate for this task. Comparative chromosome banding has provided a powerful tool to establish interspecies chromosomal homologies within some orders (Yunis and Prakash 1982) and even between some orders (Nash and O'Brien 1982), but genomic shuffling within and between other eutherian orders is beyond the resolution of banding methods. Chromosome bands lack the detail necessary to establish homology except over large regions and are reliable only between closely related species.

Comparative mapping of many individual genes in several species provides a good test of genetic homology, which has revealed the presence of large chromosomal segments conserved in the vertebrate genome for up to 400 million yr (Wakefield and Graves 1996). However, it is time consuming, and whole chromosomal homology must be inferred from limited single-gene comparisons.

A new strategy for analyzing chromosomal evolution and for comparing genomes in mammals is comparative chromosome painting, performed by fluorescence in situ hybridization (FISH) using probes derived from flow-sorted or microdissected whole chromosomes or microdissected chromosome arms or segments. Chromosome painting provides information on chromosomal homology, chromosomal rearrangements, and syntenic groups of genes; this information greatly advances our understanding of genome evolution.

Comparative chromosome painting with human-derived DNA probes has been demonstrated in great apes (Stanyon and others 1992; Wienberg and others 1990), Old World monkeys (Koehler and others 1995; Wienberg and Stanyon 1998; Wienberg and others 1992) as well as in prosimian lemurs (Apiou and others 1996). It may also be used to demonstrate chromosomal homologies between more distantly related eutherians. Human paints have been hybridized to the chromosomes of nonprimate mammalian species such as horse (Raudsepp and others 1996), cattle (Solinas-Toldo and others 1995), pig (Frönicke and others 1996), muntjac (Yang and others 1997), cat (Rettenberger and others 1995) (Wienberg and Stanyon 1998), and even whale and mouse (Scherthan and others 1994) to elucidate chromosomal rearrange-
ments in relation to human. This has allowed us to establish chromosomal homologies across the Eutheria and should make it possible to establish an ancestral eutherian karyotype.

With our collaborators Professor M. A. Ferguson-Smith and coworkers, we ultimately hope to compare eutherian, marsupial, and monotreme karyotypes. At the time of this writing, it has not yet proved possible to cross-hybridize human chromosome paints directly onto chromosomes of marsupial or monotreme species. Until now, we have used cross-species chromosome painting to investigate chromosomal homologies between different marsupial species, as was done in the eutherian group. In this study, we aim to use chromosome paints from 1 or 2 model marsupial species for comparative painting across the entire marsupial group to establish the homologies between the karyotypes of all marsupials.

Marsupials, with their low diploid numbers and exceptionally large and well-differentiated chromosomes, have been exceptionally well studied by classic cytogenetic techniques. Marsupial karyotypes have shown a startling degree of homology even between distantly related groups. The presence of a Giemsa band-conserved 2n=14 karyotype in each of the marsupial superfamilies supports the concept of an ancestral 2n=14 marsupial karyotype, from which all others may be derived (Rofe and Hayman 1985). We now wish to embark on a thorough study of chromosomal homology within and between the major marsupial lineages. In our preliminary work, we have used paints from a model marsupial species that has been used extensively for comparative genetic studies. The tammar wallaby, *Macropus eugenii*, represents the family Macropodidae (kangaroos and wallabies).

**DEVELOPMENT OF CHROMOSOME PAINTS FROM TAMMAR WALLABY**

Professor M. A. Ferguson-Smith and coworkers generated chromosome paints by flow-sorting tammar wallaby (*M. eugenii*) chromosomes (karyotype 2n=16), singly sorting all the chromosomes except 4 and 5. The limited number of chromosomes in the tammar wallaby made it possible for single chromosomes and chromosomal regions to be microdissected (Toder and others 1997a,b), resulting in the separation of the tammar wallaby chromosomes 4 and 5 and Xp and Xq probes.

**COMPARATIVE CHROMOSOME PAINTING IN KANGAROOS**

The utility and efficacy of chromosome painting for comparative karyotypic analysis in marsupials has so far been demonstrated clearly in cross-species painting between macropodid species. The degree of homology among macropodid species is comparable with that observed by comparative painting of human chromosomes in great apes and Old World monkeys.

Within the chromosomally conservative marsupials, the kangaroos and wallabies (*Macropodidae*) are chromosomally diverse (2n=10, 11 to 24). A putative ancestral macropodid 2n=22 karyotype, common in macropodids and their relatives, can be derived from the 2n=14 marsupial ancestral karyotype (Rofe 1979). Among the macropodids, the rock wallaby group (genus *Petrogale*) is extremely variable, but some retain the putative ancestral macropodid 2n=22 karyotype as represented by species from the genus *Thylogale* (Eldridge and others 1992; Rofe 1979; Sharman and others 1990). It is particularly important to compare the chromosomes of species possessing the 2n=22 karyotype with our model macropodid, the tammar wallaby, to link the tammar chromosomes to a putative ancestral macropodid karyotype.

Comparative painting studies using tammar chromosome paints in 3 (2n=22) *Petrogale* species (*Petrogale lateralis*, *P. pearsonii*, and *P. xanthopus*) (O’Neill and others 1999) relate each of the *Petrogale* chromosomes directly to a tammar wallaby whole chromosome or chromosomal arm (Figure 2). This confirms the evolutionary scheme proposed by Rofe (1979), in which the tammar wallaby chromosome 1 is composed of a fusion of the ancestral chromosomes 1 and 10, chromosome 3 is composed of a fusion of the ancestral...
FIGURE 3 Fluorescence in situ hybridization of tammar wallaby chromosomes to metaphase chromosomes of the rock wallaby Petrogale inornata. Hybridizations of tammar wallaby chromosome 1 labeled with digoxigenin (Me 1 DIG—red), chromosome 2 labeled with biotin (Me 2 BIO—yellow), chromosome 3 labeled in DIG (Me 3 DIG—red), and chromosomes 4/5 labeled in BIO (Me 4/5 BIO—green).

chromosomes 5 and 8 (Figure 3), and chromosome 6 is composed of a fusion of the ancestral chromosomes 6 and 9.

At the other extreme of macropodid chromosome evolution lies the swamp wallaby (Wallabia bicolor). Compared with other macropods, this species has a highly derived karyotype since the chromosome number (2n=10 male; 11 female) is very low. It has only 4 autosome pairs and an XX/XY, Y2 system with a compound X chromosome. Comparative painting with tammar wallaby probes showed that there has been minimal genomic shuffling, and the unusually low diploid number in swamp wallaby is the result of tandem fusions (Toder and others 1997b). Some chromosomes did not change at all; for example, chromosome 3 in both species is equivalent, and tammar chromosome 5 is equivalent to swamp wallaby chromosome 4 (Toder and others 1997b). Other chromosomes represent tandem fusions. For instance, as shown in Figure 4 and by Toder and others (1997b), the swamp wallaby X is equivalent to the tammar wallaby X, 2 and 7; and the Y2 (an original autosome that was fused to the X) is equivalent to the tammar wallaby 2 and 7.

COMPARATIVE CHROMOSOME PAINTING IN MORE DISTANTLY RELATED MARSUPIALS

It will be particularly important to relate the chromosomes of marsupial species to those retaining the putative ancestral 2n=14 karyotype. For instance, Sminthopsis macroura (family Dasyuridae) shows very little deviation from the proposed ancestral marsupial 2n=14 karyotype. Dasyurids diverged from macropodids about 50 million yr ago (Figure 1), and therefore painting in this species with tammar wallaby chromosome paints would be expected to be more difficult. This attempt is comparable with the recent work published on comparative painting of human chromosomes in non-primate eutherians (Yang and others 1997). An improved
protocol was necessary to bridge this evolutionary distance between *M. eugenii* and *S. macroura*. Hybridization and washing conditions were modified from conditions used to hybridize human chromosome paints onto muntjak chromosomes. In our first attempt, tammar wallaby chromosome paints hybridized in large blocks onto the *S. macroura* chromosomes, so it seems that small rearrangements have occurred. For example, tammar chromosome 7 hybridizes to 2 regions on chromosome 2q and 6cen (Figure 5).

The successful hybridization of tammar paints onto the distantly related dasyurid chromosomes ensures that we can use the technique to contradict or confirm the hypothesis of the ancestral 2n=14 karyotype. We expect to extend the comparisons to more distantly related American marsupials and ultimately with the eutherian comparative mapping data. For this, we will use comparative gene mapping between human and tammar wallaby (still limited and therefore rough) to complement more refined FISH-painting techniques.

**REFERENCES**


