

MACROPODS

THE BIOLOGY OF KANGAROOS,
WALLABIES AND RAT-KANGAROOS



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Preface

This book arose from a symposium on the biology of kangaroos, wallabies and rat-kangaroos, collectively and colloquially known as macropods. The symposium was held on 6–7 July 2006 at the University of Melbourne, and was sponsored by the Australian Mammal Society. The symposium was attended by 117 macropod enthusiasts, and 55 papers were presented. This book contains 32 chapters derived from those papers, representing the work of 82 authors, refereed by at least two academic peers from Australia or overseas.

The Melbourne symposium was the second major scientific meeting on macropods. The first was held in 1988. It provided comprehensive reviews of our knowledge about this diverse group of Australasian marsupials at the time, and laid the foundations for work to follow (Grigg *et al.* 1989). The second was designed to build on the first, and bring together the many recent advances in the biology of macropods. However, the aim for the second symposium was not merely to update the earlier review papers, which remain valid and are still benchmarks in their areas. Instead, we aimed to highlight new developments in macropod biology and explore topics that have become prominent since the first symposium.

The subtitle for the Melbourne symposium was 'From genomics to GIS', and many authors took up the challenge. Deakin and Graves (Ch. 1) open the book with a summary of the release of the genome sequence of Australia's model marsupial, the tammar wallaby (*Macropus eugenii*); Chambers *et al.* (Ch. 25) describe the use of GIS technology to predict patterns of road-kill of the same species. Many other new techniques are covered: microsatellites (MacDonald *et al.*, Ch. 5), mitochondrial DNA (Hazlitt *et al.*, Ch. 8), stable isotopes (McMillan *et al.*, Ch. 14), assisted reproduction (Taggart *et al.*, Ch. 23), fertility control (Herbert *et al.*, Ch. 27) and population modelling (Pople *et al.*, Ch. 32).

Perhaps the most striking difference between the two macropod symposia is the balance of topics covered. Of the 61 chapters in Grigg *et al.* (1989), the majority were concerned with fundamental aspects

of the biology of macropods – evolution, ecology, energetics, reproduction, development, behaviour and population biology. Only five (8%) covered management issues. In this book, 34% of the chapters are directly concerned with the management of macropods and others have some management elements. This change in focus undoubtedly arises from real concerns about the future of macropods, ranging from threatened taxa to overabundant populations. In part, however, the switch also reflects the changing funding landscape, with its greater emphasis on applied research.

Part I, 'Genetics, reproduction and development', is primarily concerned with fundamentals of macropod biology. Deakin and Graves (Ch. 1) describe the phylogenetic significance of the tammar wallaby genome, and its value in tracing the evolutionary history of mammalian chromosome evolution. Siddle *et al.* (Ch. 2) show how the availability of these genomic resources facilitated the isolation and characterisation of macropodid immune genes, which, contrary to historical speculation, are comparable to those of eutherians. Hickford (Ch. 3) explains how the genomic resources have contributed to the *in vitro* culture of marsupial embryos and fetuses. Eldridge *et al.* (Ch. 4) review the applications of hypervariable genetic markers to the study of wild macropod populations, and MacDonald *et al.* (Ch. 5) outline the potential of such markers on the sex chromosomes to shed light on sex-specific population and evolutionary processes. Paplinska *et al.* (Ch. 6) apply a comparative morphological analysis of relative testis size to the study of macropodid mating systems. Miller *et al.* (Ch. 7) use microsatellites to assign paternity and demonstrate that male body size is an important determinant of dominance status and reproductive success in the tammar wallaby. Hazlitt *et al.* (Ch. 8) use mitochondrial DNA to show striking matrilineal clusters within a single colony of brush-tailed rock-wallabies (*Petrogale penicillata*).

Part II, 'Morphology and physiology', addresses more fundamental questions. Dawson and Webster (Ch. 9) describe macropods as among the most

athletic of mammals, with a combination of efficient locomotion and powerful skeletal muscles that enables them to sustain high speeds aerobically. Rose (Ch. 10) argues that skeletal muscle may allow the Tasmanian bettong (*Bettongia gaimardi*) to increase its metabolic rate by non-shivering thermogenesis. Munn and Dawson (Ch. 11) propose that juvenile red kangaroos (*M. rufus*) have high mortality rates during drought because they may be unable to meet their requirement for dietary fibre. Lentle and Hume (Ch. 12) argue that the occlusal forces involved in chewing fibre may not be as influential as nutritional factors in the progression of molars. Warburton and Prideaux (Ch. 13) analyse the pedal morphology of the extinct Pleistocene *Bohra*, confirming that tree-kangaroos occupied far broader geographic and climatic ranges than previously understood.

Vernes (Ch. 14) begins Part III, 'Ecology', with an analysis of the consumption of hypogeous ectomycorrhizal fungi in a community of four macropodid and two potoroid species; he concludes that the extent of mycophagy by macropods has been underestimated. McMillan *et al.* (Ch. 15) use stable isotope analysis of hair samples from a population of tammar wallabies to show their reliance on irrigated C4 grasses. Ritchie (Ch. 16) provides the only chapter on the ecology of a tropical macropod, the antilopine wallaroo (*M. antilopinus*), which has a broad but patchy distribution across northern Australia. Di Stefano *et al.* (Ch. 17) present a fine-scale study of space use by the swamp wallaby (*Wallabia bicolor*), showing that nocturnal use of harvested forest differs between males and females. At a large spatial scale, Pople *et al.* (Ch. 18) re-evaluate the population dynamics of red and grey (*M. fuliginosus* and *M. giganteus*) kangaroos from long-running aerial surveys, concluding that a number of intrinsic and extrinsic factors other than rainfall are influential. Chambers and Bencini (Ch. 19) highlight the influence of altered resources on tammar wallabies, which have higher population density, body weight and fecundity in modified habitats with abundant grass. Schmidt *et al.* (Ch. 20) demonstrate the role of fine-scale variation in habitat structure, rather than food resources, for coexistence of sympatric grey kangaroos and swamp wallabies. Finally, Beveridge *et al.* (Ch. 21) review the occurrence of helminth parasites (mostly nematodes) in macropods, showing that while helminth taxa are largely host-specific, some

are able to parasitise multiple species where their macropod hosts occur in sympatry.

The theme of Management, Part IV, splits into two poles of an abundance scale. Rare and threatened species of macropods have been the focus of much conservation effort. Finlayson *et al.* (Ch. 22) review the successes and failures of translocations of potoroids, and the management lessons that have been learned. Taggart *et al.* (Ch. 23) review the promising techniques of pouch young isolation and cross-fostering to provide stock for translocation of threatened macropods. Ramp (Ch. 24) summarises the impact of roads as a threatening process for many macropod species, Chambers *et al.* (Ch. 25) analyse traffic, roadside and seasonal effects on road-kills of tammar wallabies, and Lee *et al.* (Ch. 26) link the flight behaviour of red and grey kangaroos as well as euros (*M. robustus erubescens*) to their differing susceptibility to road-kill. The management of high-density macropod populations is discussed by Herbert *et al.* (Ch. 27), who review developments in fertility control techniques. Roberts *et al.* (Ch. 28) present a darting protocol for the capture of eastern grey kangaroos, and Higginbottom and Page (Ch. 29) describe the fate of eastern grey kangaroos that were captured and translocated to a more secure site. Morgan and Pegler (Ch. 30) describe and evaluate a culling program designed to simulate natural predation on western grey kangaroos in a national park, and Gowans *et al.* (Ch. 31) report a corresponding improvement in vegetation condition in the park. Pople *et al.* (Ch. 32) evaluate the potential for using harvesting statistics at a regional level to reduce the frequency of costly aerial surveys and provide more intensive coverage of kangaroo populations.

The breadth of chapters show how much progress has been made since the first macropod symposium. Nonetheless, many aspects of the biology of the ~73 macropod species are yet to be revealed. Indeed, as noted in the macropod species list contained in the Appendix, even the actual number of species is still unclear and evolutionary relationships among many taxa are yet to be fully resolved. There is an enormous wealth of new data from fossil macropods to be integrated into our understanding of macropod biology and evolution.

For living macropod species, there remain many imbalances in our understanding even of basic biology. Tropical macropods remain poorly known: our ignorance of the biology of the black wallaroo

(*M. bernardus*), most of the tropical rock-wallabies (*Petrogale* spp.) and all 17 endemic macropods (*Dendrolagus*, *Dorcopsis*, *Dorcopsulus* and *Thylogale* spp.) from New Guinea is almost complete; the reproductive biology of the antilopine wallaroo is unresolved; the ecology of a dominant herbivore, the agile wallaby (*M. agilis*), has received little attention; the still widespread spectacled hare-wallaby (*Lagorchestes conspicillatus*) and northern nailtail wallaby (*Onychogalea unguifera*) have been little studied for decades. Macropods of the arid zone have been equally neglected, aside from specific studies of threatened species, mostly in predator-free facilities. Temperate species, which overlap the distribution of most Australian universities and research institutes, have received uneven attention. Until recently, most research on the swamp wallaby had been dietary studies. The biology of the western brush wallaby (*M. irma*) is essentially unknown, despite its being relatively common in the south-west of Western Australia. Despite decades of ecological, physiological, reproductive and behavioural research, the mating system of the widespread and abundant large kangaroos remains unconfirmed. Many aspects of the biology of the model marsupial – the tammar wallaby – are well understood, but its ecology has been studied only on Garden Island, Western Australia. Similarly, while we may have the complete genomic sequence of a Kangaroo Island tammar wallaby, our knowledge of the distribution of

genetic diversity throughout the species' disjunct distribution, or even throughout Kangaroo Island, remains rudimentary. Indeed, the whole application of modern population genetics to macropods has been uneven, with most studies focusing on rare or threatened taxa. Very few widespread and common species have been examined, despite the enormous potential for this approach to reveal much about the species' ecology, reproduction, population biology and evolutionary history, especially when combined with detailed ecological, reproductive or behavioural data.

In light of these gaps and imbalances in our knowledge, the ongoing challenge for macropod biologists is to conduct high-quality research, in both pure and applied arenas, so our fundamental knowledge of this fascinating group of marsupials continues to advance, and macropods continue to prosper and intrigue us.

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1 Mapping genes on tammar wallaby target chromosomes

J.E. Deakin and J.A.M. Graves

SUMMARY

This is an exciting time for marsupial genomics, with the release of the sequences of two marsupial genomes. One of these is the tammar wallaby (*Macropus eugenii*), Australia's model kangaroo, which has been the model macropodoid species for marsupial genetic studies for over 30 years. The tammar, and other marsupials, occupy a phylogenetic 'sweet spot' between birds and eutherian mammals that is very valuable for genome comparisons. To anchor the tammar genome sequence, we are generating a physical map of genes on tammar chromosomes. Of particular interest to our laboratory are genes on chromosomes X and 5. Both of these chromosomes harbour genes that are found on the human X chromosome, many of which are essential to mammalian sex differentiation, reproduction and development. Mapping these genes has assisted in tracing the evolutionary history of the mammalian X chromosome, identifying ancient and recently added regions. In addition, mapping genes to the tammar X is permitting an intense study of marsupial X chromosome inactivation, a process that involves silencing one X chromosome in females to compensate for the difference in the number of X chromosomes between males and females. Marsupial X inactivation is different at the phenotypic and molecular level from inactivation in human and mouse, so investigations of the tammar X will help to deconstruct this complex process. Tammar chromosome 5 contains, in addition to genes found on the

human X, gene blocks from other human chromosomes; comparisons of gene organisation in this region between humans and other vertebrates have provided and will continue to provide insight into evolution of other regions of the mammalian genome.

VALUE OF MARSUPIALS IN COMPARATIVE GENOMICS

Comparative genomics is a powerful tool for identifying genes and regulatory sequences and for dissecting complex genetic pathways. The power of comparative genomic studies can be limited by the species included in the analysis. Genomes of closely related species are often too similar in sequence for meaningful comparisons, the high level of sequence similarity masking potentially important regulatory regions. Conversely, the comparison of very distantly related species is complicated by the difficulty in aligning their sequences, due to genome rearrangements, deletions or the level of sequence divergence. Marsupials fall at the phylogenetic 'sweet spot' in evolution, bridging the 310 million-year gap between birds and eutherians. Marsupial genomes are easily aligned to those of eutherians but have diverged enough for important conserved regions to be readily identified (Wakefield and Graves 2003).

Including marsupials in comparative genomic studies has made a major contribution to our understanding of the evolution of mammalian sex chromosomes (Graves 1995), X chromosome inactivation

(Davidow *et al.* 2007; Duret *et al.* 2006; Hore *et al.* 2007; Shevchenko *et al.* 2007), genomic imprinting (Edwards *et al.* 2007; Rapkins *et al.* 2006; Suzuki *et al.* 2005) and immune genes (Belov *et al.* 2006, 2007). Their genomes have also revealed previously unknown genes in human (Delbridge *et al.* 1999) and helped in the identification of regions that may be important for gene regulation (Deakin *et al.* 2006; Wakefield and Graves 2005).

One renowned example of the important role marsupials play in comparative genomics involves the discovery of the mammalian sex-determining gene. Many years of deletion mapping analysis on sex-reversed humans narrowed down the region responsible for testis determination to the short arm of the human Y chromosome. A gene cloned from this region, and thought to be the testis determining factor, was the zinc finger protein *ZFY*. The discovery that the marsupial orthologue of this gene mapped not to the Y chromosome but to a non-sex chromosome (autosome) provided the first evidence that *ZFY* was not the sex-determining gene (Sinclair *et al.* 1988). Subsequently, the *SRY* gene was discovered within the same region (Sinclair *et al.* 1990). Evidence for the role *SRY* plays in sex determination was demonstrated by the development of testes in XX mice transgenic for mouse *Sry* (Koopman *et al.* 1991). This gene was later mapped to the marsupial Y chromosome (Foster *et al.* 1992), confirming it as the mammalian sex-determining gene. Marsupials also provided information on the origin and evolution of *SRY* when a related gene (*SOX3*) was found on the X (Foster and Graves 1994).

Thus, the common ancestry of marsupials and eutherians provides a means to establish some of the ancestral mammalian genetic organisation and mechanisms. Similarly, comparisons between the two lineages can elucidate the evolution of unique marsupial features, such as the genes involved in their sophisticated lactation system and embryonic diapause.

MARSUPIAL GENOME PROJECTS

There are currently two marsupials whose genomes have been sequenced – the South American gray short-tailed opossum (*Monodelphis domestica*) and the tammar wallaby (*Macropus eugenii*). These two species diverged approximately 70 million years ago (Springer *et al.* 1994) so comparisons between the

opossum and tammar will be similar in evolutionary terms to the human/mouse comparison that has been critical in understanding eutherian genomes. It is anticipated that there will be many important and informative differences between the two marsupial genomes.

The opossum genome has been sequenced by the Broad Institute (US) on average six times over (i.e. to a depth of six-fold) and the sequence has been assigned to chromosomes (Mikkelsen *et al.* 2007). Comparative analysis of this sequence has revealed a number of important findings in regards to mammalian genome evolution. One major finding is that approximately one-fifth of eutherian conserved non-coding elements (conserved sequences not involved in encoding protein-coding genes) have emerged after marsupial/eutherian divergence, with a substantial proportion of these eutherian-specific conserved non-coding elements (CNEs) having arisen from sequence inserted by transposable elements (mobile pieces of DNA). CNEs are thought to be involved in gene regulation. Hence, transposable elements may have played a major role in the evolution of mammalian gene regulation (Mikkelsen *et al.* 2007).

Although the opossum genome analysis has been very informative, comparison between the opossum sequence and that of an Australian marsupial would not only allow the detection of marsupial-specific genes and non-coding elements, but would also permit the reconstruction of the genome of their common ancestor (Mikkelsen *et al.* 2007).

Australia's model kangaroo, the tammar wallaby, has been sequenced jointly by the Australian Genome Research Facility (AGRF) and Baylor College of Medicine Human Genome Sequencing Center (US) to provide an approximately 2× coverage of the genome (<http://www.genome.gov/12512299>). To anchor this genome sequence to tammar chromosomes, the ARC Centre of Excellence for Kangaroo Genomics (<http://kangaroo.genomics.org.au/>) is generating a physical map by localising genes to chromosomes.

MAMMALIAN SEX CHROMOSOMES

As a start on the physical map, we have chosen to focus on chromosomes X and 5. These chromosomes are of special interest because they harbour genes that are found on the eutherian X chromosome.

Therian (marsupial and eutherian) sex chromosomes, the X and the Y, are very different in size and gene content. The X is a large gene-rich chromosome; the Y is small, gene-poor and highly enriched in repetitive sequences. The X and Y arose from a pair of ordinary autosomes some time between the divergence of monotremes and therian mammals (Veyrunes *et al.* 2008). Differentiation between the X and Y occurred due to a suppression of recombination between the X and the Y, which has ultimately resulted in the progressive degradation of the Y chromosome. Comparisons between the sex chromosomes of eutherians, marsupials and monotremes have been important for tracing this process and for identifying ancient and recently added regions of the human X and Y.

Therian X and Y chromosomes have many special properties arising from their different representation in XX females and XY males. The gene content of both chromosomes is biased toward those that are essential to mammalian sex differentiation, reproduction and development (Graves *et al.* 2006). Mapping these genes in marsupials will assist in tracing the evolutionary history of the eutherian X chromosome. In addition, mapping genes to the tammar X will permit an intense study of marsupial X chromosome inactivation, a process that involves silencing one X chromosome in females to compensate for the 2:1 difference in the number of X chromosomes between males and females.

MAPPING GENES IN THE TAMMAR

The tammar genome is similar in size to the human genome (about 3.5 billion base pairs), but is packaged into seven pairs of autosomes and one pair of sex chromosomes, with XX females and XY males. Genes have been assigned to tammar chromosomes by two different approaches – linkage or physical mapping.

Linkage mapping uses the frequency of recombination between two loci to provide the relative positions of markers, either genes or anonymous DNA markers (e.g. microsatellites) on a chromosome, determined on the basis of how frequently these markers are inherited together. Linkage mapping requires these markers to be polymorphic between individuals. The generation of a tammar linkage map has been facilitated by using crosses of Kangaroo Island and Garden Island subspecies, with polymorphic markers found between the two subspecies

(Zenger *et al.* 2002). Linkage markers are currently being physically located onto chromosomes by FISH mapping to provide an integrated physical and linkage map.

The large and readily distinguishable chromosomes of the tammar are ideal for cytogenetic (physical) mapping. A skeleton physical map with 64 genes assigned to chromosomes was constructed with data collected over a 20-year period (Alsop *et al.* 2005). Many of the genes on this skeleton map were assigned to chromosomes by Radioactive *in situ* Hybridisation (RISH) using heterologous probes, typically human cDNA clones, or by Fluorescence *in situ* Hybridisation (FISH) using homologous probes.

Chromosome painting, a technique whereby individual chromosomes from one species can be hybridised to chromosomes from another species, has allowed comparison between distantly related marsupial genomes. For instance, chromosome-specific paints from the rufous rat-kangaroo (*Aepyprymnus rufescens*) (Rens *et al.* 2003) show that tammar chromosome 5 shares sequence with chromosomes 4 and 7 in the opossum (*M. domestica*). With the availability of the opossum genome sequence, it is possible to predict which genes will be on each tammar chromosome.

Although chromosome painting provides a global picture of the similarities between the tammar and opossum genomes, it is by physically mapping genes that a comparative map can be constructed between marsupials and other vertebrates. This will ultimately result in the determination of an in-depth evolutionary history of a region or chromosome. A new approach to mapping tammar genes is to use sequence from the tammar genome project to design small yet specific probes, called overgos, for screening a large insert genomic library (Bacterial Artificial Chromosome library). These large insert clones are perfect for FISH mapping. This new efficient approach has allowed dense gene maps of the tammar X chromosome and the 'neo-X' region on chromosome 5 to be generated (Deakin *et al.* 2008). Genes marking the ends of evolutionary blocks of genes conserved between opossum and human have been mapped to tammar chromosome 5 (Fig. 1.1). This has permitted a virtual map of the chromosome to be constructed, by extrapolating from the opossum genome to infer the location of genes between conserved end markers to be present on tammar chromosome 5. In total, 73 and 141 genes have been

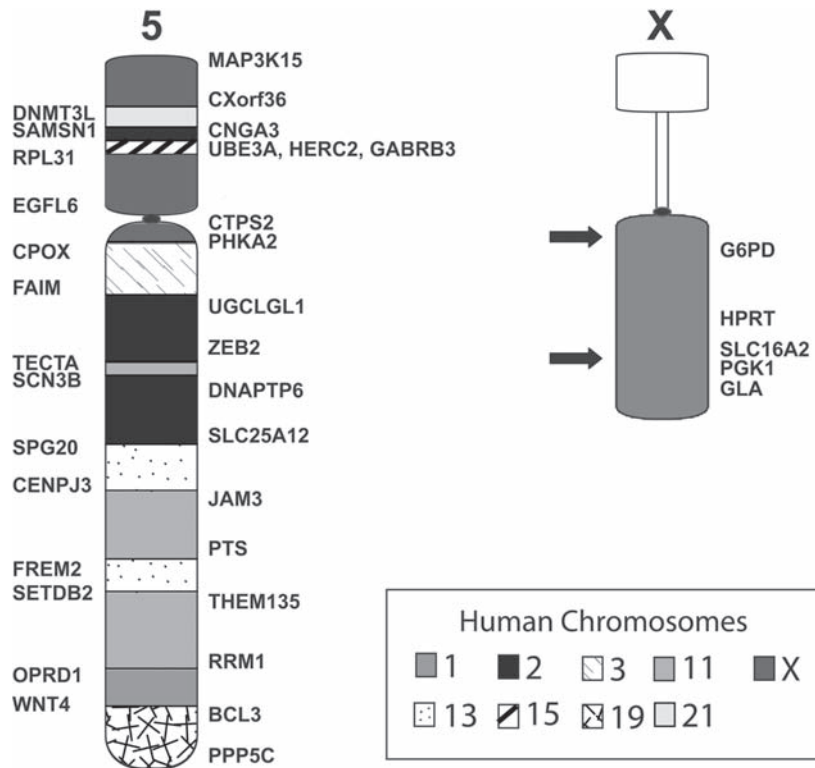


Figure 1.1: Tammar/human comparative map of tammar chromosomes 5 and X. Regions have been shaded based on the location of these genes in human. Genes located at the ends of evolutionary conserved blocks are indicated for chromosome 5. The five genes indicated on the X chromosome map are those for which XCI data is available in marsupials. Arrows indicate the position of genes flanking XIST in humans, demonstrating the disruption of this region in tammar.

assigned to the tammar chromosomes X and 5 respectively, with an additional 2320 genes virtually assigned to chromosome 5 (Deakin *et al.* 2008). This efficient approach is now being used to construct detailed physical and virtual maps of the remaining tammar autosomes.

By chromosome painting, tammar chromosome 5 was shown to consist of two segments conserved among marsupials, referred to as segments C11 and C12, with C11 spanning the entire short arm to just below the centromere and C12 covering the remainder of the chromosome (Rens *et al.* 2003). Conserved segment C11 corresponds to part of the short arm and the entire long arm of opossum chromosome 4. C12 spans the short arm and a small section of the long arm of opossum chromosome 7 (Fig. 1.2). Gene mapping on both tammar X and 5 has revealed internal rearrangements between tammar and opossum previously undetected by chromosome painting (Fig. 1.2) and provides a more accurate account of the extent of homology between tammar and opossum for these two chromosomes. Segment C12 does not actually span the entire short arm of tammar chromosome 5,

as indicated by chromosome painting, but is restricted to the region surrounding the centromere. The remainder of the short arm consists of genes from the short arm of opossum chromosome 4 (Deakin *et al.* 2008). Cross-species chromosome painting did not provide information on the homology of the short arm of opossum chromosome 4 with other marsupials, including tammar (Rens *et al.* 2003). In addition, two rearrangements were detected in segment C11 between tammar and opossum.

Gene mapping and chromosome painting between the three major mammalian lineages (eutherians, marsupials and monotremes) has revealed that the human X chromosome can be divided into an ancient region and a recently added region (Deakin *et al.* 2008; Glas *et al.* 1999; Graves 1995; Wilcox *et al.* 1996). Genes on the human X that also map to the tammar X chromosome are part of the ancient region, having been part of the mammalian X chromosome since marsupials and eutherians last shared a common ancestor. Chromosome painting indicates that the long arm of the tammar X shares homology with the opossum X (conserved segment

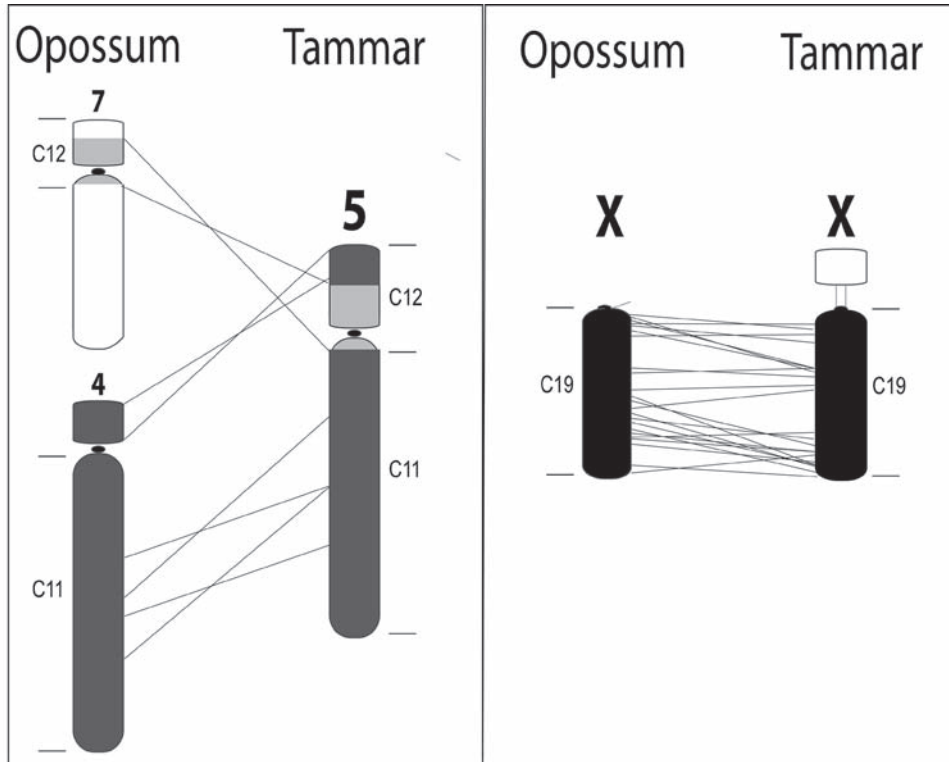


Figure 1.2: Rearrangements detected between tammar and opossum. Conserved segments C11, C12 and C19 as determined by chromosome painting are shown. Rearrangements are indicated by lines between chromosomes, revealing two large-scale rearrangements between these species within C11 and a highly rearranged gene order between the tammar and opossum X chromosomes.

C19) (Rens *et al.* 2003). Genes from this ancient region of the X are autosomal in birds and monotremes, being located on chromosome 4 in chicken and chromosome 6 in platypus (Veyrunes *et al.* 2008) (Fig. 1.3). Genes from the recently added region are located on chromosome 5 in tammar and were added to the eutherian X chromosome after the marsupial/eutherian divergence. Genes from this region are located on chromosome 1 in chicken and on chromosomes 15 and 18 in platypus (Edwards *et al.* 2007; Veyrunes *et al.* 2008). Gene mapping data have defined the limits of the ancient and recently added (neo-X) regions and narrowed down the fusion point of these regions to a 400kb region on the human X between genes *RP2* and *RBM10* (Deakin *et al.* 2008).

A comparison between the tammar and opossum gene order on the X chromosome has revealed many rearrangements between the opossum and tammar chromosomes (Fig. 1.2), rearrangements beyond the resolution of chromosome painting (Deakin *et al.* 2008). The highly rearranged order of genes on the X chromosome between the species was surprising,

given the very conserved gene order for the ancient region of the X among eutherians, a feature thought to result from the eutherian mode of X inactivation (Mikkelsen *et al.* 2007).

Marsupial X chromosome inactivation

Most genes within the ancient region of the human X are subject to X chromosome inactivation (XCI), which equalises the level of their expression between females with two X chromosomes and males with one (Graves and Gartler 1986). However, many genes within the recently added region of the human X escape inactivation (Carrel and Willard 2005; Johnston *et al.* 2008). The best explanation for this phenomenon is provided by the evolutionary history of the human X chromosome. Genes within the recently added region were previously autosomal, so did not require dosage compensation prior to marsupial/eutherian divergence. Following addition of the region to the X and Y, degradation of genes on the Y left genes on the X with dosage differences, and there was selection for recruitment into the XCI system (Graves *et al.* 1998; Graves and Schmidt 1992).

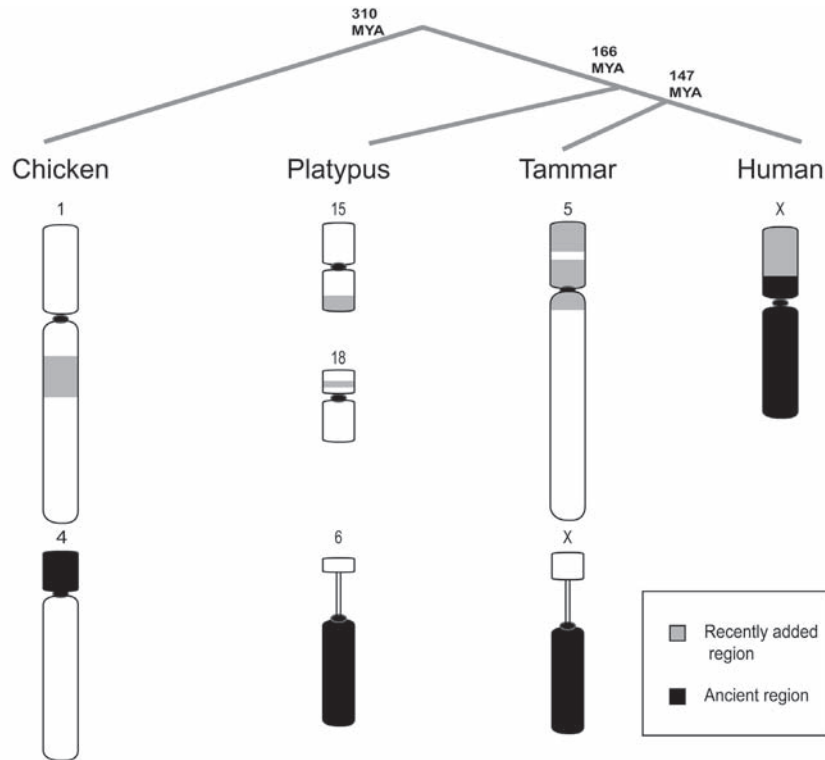


Figure 1.3: Evolution of the human X chromosome. The ancient region of the X (black) is located on the X chromosome in marsupials and eutherians but is autosomal in birds and monotremes. The recently added region (grey) is only found on the X chromosome in eutherians.

Relative to the human X, the mouse X has fewer genes that escape inactivation (Disteche *et al.* 2002), suggesting that recruitment to the inactivation system has been more rapid in the rodent lineage.

Eutherian XCI is a complex multi-stage process (Gartler *et al.* 1985), controlled by the *XIST* gene (X-Inactive Specific Transcript) which transcriptionally silences genes on one of the two X chromosomes in the somatic cells of females. Inactivation occurs in the early embryo and is random with regard to the parental origin of the X that is inactivated. It involves many variant histones and histone modifications such as deacetylation, and is stabilised by DNA methylation (Heard 2004). Marsupial XCI differs markedly from the random and fairly complete inactivation of eutherians. Marsupials have non-random X-inactivation, with the paternal X being preferentially silenced (Sharman 1971). It is tissue-specific, so that in some tissues both copies of an X-linked gene are active (Cooper *et al.* 1993). Variation in marsupial XCI exists between species; for example, the paternal *G6PD* allele is completely silenced in somatic tissues from two species of macropodids (*Macropus robustus* and *Macropus rufogriseus*) yet is partially

active in tissues from the North American Virginia opossum (*Didelphis virginiana*). Variation in marsupial XCI also exists between genes within a species. Methylation does not appear to play a role (Hornacker *et al.* 2007; Kaslow and Migeon 1987; Loebel and Johnston 1996) but some histone modifications do appear to be involved in the XCI process (Koina *et al.* 2009; Wakefield *et al.* 1997). However, the most striking difference between marsupial and eutherian XCI is the apparent lack of an *XIST* gene in marsupials, with *XIST* flanking genes mapping to completely different regions of the X chromosome in both the tammar (Deakin *et al.* 2008) (Fig. 1.1) and opossum (Davidow *et al.* 2007; Hore *et al.* 2007; Shevchenko *et al.* 2007).

Unfortunately, our understanding of marsupial XCI is largely based on results obtained for just five genes and there has been no consistency in the species used for these studies. Older studies determined the inactivation status of *G6PD*, *PGK*, *GLA* and *HPRT* in the common brushtail possum (*Trichosurus vulpecula*), *Didelphis virginiana*, *Antechinus* species and a number of different macropodid species (Cooper *et al.* 1993). Inactivation status of the house-