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Review

Kangaroo gene mapping and sequencing: insights into mammalian genome evolution

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Abstract. The deep divergence of marsupials and eutherian mammals 160 million years ago provides genetic variation to explore the evolution of DNA sequence, gene arrangement and regulation of gene expression in mammals. Following the pioneering work of Professor Desmond W. Cooper, emerging techniques in cytogenetics and molecular biology have been adapted to characterise the genomes of kangaroos and other marsupials. In particular, genetic and genomic work over four decades has shown that marsupial sex chromosomes differ significantly from the eutherian XY chromosome pair in their size, gene content and activity. These differences can be exploited to deduce how mammalian sex chromosomes, sex determination and epigenetic silencing evolved.

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Introduction

'Why would anyone want to map genes in a kangaroo?' I asked Des Cooper in exasperation. At Des' suggestion, I had recently arrived at La Trobe University, fresh from my Ph.D. at Berkeley and with new cell fusion technology ready to explore the control of DNA synthesis in mammals. I was quite dismissive of Australian scientists who worked on the 'local fauna', so I was nonplussed at Des' insistence that I use my somatic cell genetic techniques to map genes on the kangaroo X chromosome. But, like the outcome of many of my arguments with Des, he carried the day, and I have pursued his far-sighted vision ever since. With my colleague Rory Hope (another Adelaide contemporary), I spent the next 10 years developing rodent–marsupial cell hybrids and assigning genes to the X chromosome in several marsupial species. Mapping kangaroo genes took over my life and led to my participation in the Human Gene Mapping workshops that ultimately grew into mammalian (including marsupial) genome projects.

What Des propounded from our earliest interaction (as graduate students in Adelaide in the 1960s) was that marsupial and eutherian ('placental') mammals constitute independent experiments in mammal evolution. Marsupials last shared a common ancestor with placental mammals ~160 million years ago (Mya) (Luo *et al.* 2011). So there has been plenty of time – more than twice the time that humans and mice have been evolving separately – for the two groups of mammals to evolve different genome arrangements, novel genes and different ways of regulating them. Within marsupials there are also closely and distantly related species that can be analysed; for instance, kangaroo species radiated only ~18 Mya from an ancestral macropodid that diverged ~45 Mya from the dasyurid marsupials. Australian marsupials diverged ~70 Mya from American marsupials such as the opossum.

Of particular interest to Des, and to me, was the contributions marsupials could make to our understanding of sex chromosome function and evolution. The X chromosome of placental mammals is extremely conserved in gene content and gene order, and shares the same complex mechanism of epigenetic silencing that renders one X inactive in female somatic cells. The Y chromosome, on the other hand, contains few active genes and is quite variable in gene content, the result of loss of active genes from the Y as it degrades from its original identity with the X. Again, the very ancient divergence of marsupials might mean that the sex chromosomes have different gene content and different means of regulation and could provide clues about how the X and Y evolved in mammals and how they work to determine sex and to regulate activity. This approach proved more productive than either of us could possibly have imagined.

Marsupial chromosomes

With their low diploid number and large size, marsupial chromosomes have provided material for classic studies of chromosome structure, evolution and radiation biology. Even before the introduction of g-banding, careful observations of sizes and arm ratios strongly hinted that marsupial chromosomes are highly conserved across all 21 families, and the occurrence of modes at $2n = 14$ and $2n = 22$ (Sharman 1973; Hayman and Martin 1974) suggested that one or other might be ancestral. The observation that the $2n = 14$ mode was g-band identical (Rofe and Hayman 1985) meant that in Adelaide our money was on the

$2n = 14$ karyotype, but the presence of telomere sequences at junction points in South American marsupials offered an alternative view that the low number was secondarily derived from centric fusions in an Australian marsupial ancestor (Svartman and Vianna-Morgante 1999).

Marsupial chromosomes show some striking differences in behaviour, displaying low recombination in females rather than males (Bennett *et al.* 1986; van Oorschot *et al.* 1992), the opposite pattern from that observed in placental mammals.

Marsupial chromosomes also display some unusual structures. Marsupial centromeres are relatively short and simple, consisting of a few hundred kilobases, rather than the megabases of α repetitive DNA of the mouse centromere. Several types of repeat are interspersed (Carone and O'Neill 2010), and kangaroo centromeres have accumulated an endogenous retroviral sequence KERV, which is transcribed and has actively amplified in several macropodid lineages (Carone and O'Neill 2010; Ferreri *et al.* 2011). This element may facilitate the frequent Robertsonian fusions observed in macropodid marsupials (Bulazel *et al*. 2007).

The chromosome ends of one marsupial group are also unusual. Dasyurid telomeres have recently been found to exhibit a parent-specific control of length (Bender *et al.* 2012). This was first observed in Tasmanian devils as extremely heterogeneous binding of telomere-specific sequences to the two homologues of each chromosome: one haploid set had small telomeres, and the other set had enormous telomeres. Every animal examined showed the same effect, excluding hybridisation between a long- and a short-telomere subpopulation, and other dasyurids exhibit the same phenomenon. In male-derived cells the X had small, and the Y very large, telomeres, suggesting that telomeres are lengthened in the testis and shortened in the ovary. This parent-specific control of telomere length does not conform with either the well established telomerase mechanism (Blackburn *et al.* 2006), or the ALT method of telomere lengthening (Cesare and Reddel 2010). It is possible that a completely novel mechanism has evolved in this family to counteract stress in males that undergo frenetic mating and die after their first year.

Marsupials share with placental mammals an XX female : XY male system of chromosomal sex determination in which Y determines testis, although some other male phenotypes are independently determined by the X (O *et al.* 1988). The X chromosome is smaller than the 5% that is highly conserved in placental mammals (Hayman and Martin 1974), in accordance with 'Ohno's Law' (Ohno 1967). The Y is minute in some species. The X and Y do not undergo homologous pairing at male meiosis, but segregate from a proteinaceous plate (Fernandez-Donosa *et al.* 2010). Fusions between autosomes and sex chromosomes are rather common in marsupials, with several species displaying X_1X_2Y and XY_1Y_2 systems resulting from Y-autosome and X-autosome fusions.

In placental mammals, the dosage imbalance of X chromosomes in XX female and XY male is mitigated by an X-inactivation system in which one X chromosome becomes inactive in somatic cells of females (Lyon 1961). This process is random, complete, stable and somatically heritable in the mouse, and constitutes a splendid model system for studying epigenetic silencing. Des and I shared a fascination with marsupial X chromosome inactivation. My honours project was to determine whether in female marsupials as well as female humans and mice, one X showed delayed DNA replication, the cytological hallmark of inactivation. It did, and I set sail for Berkeley thinking that kangaroos are just like humans and mice in this respect (Graves 1967). But while I was away, Des, with colleagues from Macquarie University, showed that the kangaroo X chromosome differs fundamentally from that of placental mammals in that it is always the paternal X that is inactivated, the first demonstration of imprinting in a mammal (Richardson *et al*. 1971; Sharman 1971). Not only was the process in marsupials imprinted, but it was incomplete and tissue specific (Cooper *et al*. 1993; Deakin *et al*. 2009).

In order to use these differences to track how X inactivation evolved and how it works, it became important to know whether the marsupial and placental X chromosomes were monophyletic; hence Des' interest in kangaroo gene mapping.

Marsupial gene mapping

Des Cooper pioneered the mapping of genes in kangaroo species, discovering allozyme variation (revealed by his beloved starch gel electrophoresis) in several species. He was able to show that human sex-linked enzymes *PGK*, *G6PD* and *GLA* are sex linked also in kangaroos and dasyurid marsupials (Richardson *et al.* 1971; Cooper *et al*. 1971). Later the same markers were shown to be sex linked in the Virginia opossum and the grey short-tailed opossum (Samollow *et al.* 1987), suggesting that the X was completely conserved over all therian mammals, as predicted by Ohno (1967).

But the polymorphisms were scattered over several species and it was not possible to perform dihybrid crosses that would establish a linkage map of the kangaroo X. Des hoped that we might get further using somatic cell hybridisation, which depends on random loss of marsupial chromosomes (and the genes they bear) in hybrid clones. The many years I spent, in collaboration with Rory Hope, obtaining and analysing rodent–marsupial cell hybrids were frustrating in the extreme. Although we could fuse mouse and kangaroo cells and obtain heterokaryons, something awful seemed to happen to the marsupial chromosomes, which were ripped apart or thrown out very early in the hybrid's life.

We typed hundreds of struggling clones for allozyme markers, using Des' starch gel electrophoresis skills. We found that they retained the kangaroo form of the selected marker *HPRT*, but hardly any had other kangaroo markers. Out of some hundreds of hybrids that retained the *HPRT*, fewer than 10 retained any sign of marsupial chromosomes (Hope and Graves 1978). However, a crucial six hybrids retained a more-or-less intact marsupial X chromosome, and we could show by reversion analysis that three genes (*HPRT*, *PGK-A*, *G6PD*) that were located on the human and mouse X were all gained or lost with the marsupial X (Graves *et al.* 1979; Dawson and Graves 1986).

Since most hybrids retained only fragments of the X that contained the selected marker, we could use the frequency of retention of unselected markers to propose a gene order on the X; this was the forerunner of radiation hybrids, only our marsupial cells did not need to be irradiated (Dobrovic and Graves 1986).

Our attempts to map autosomal markers by the same strategy were even more frustrating, since most hybrids retained only fragments of the X chromosome, and rarely contained an

autosome (Dawson and Graves 1987). Gene mapping over the entire kangaroo genome therefore had to wait for *in situ* hybridisation. Radioactive *in situ* hybridisation, using heterologous probes (largely human cDNAs) provided the first detailed description of the marsupial X chromosome (Spencer *et al.* 1991*a*). The introduction of fluorescence *in situ* hybridisation was a dream come true. Now it was possible to screen a marsupial bacterial artificial chromosome (BAC) library for a large-insert clone bearing a particular gene. Aided by trace archive sequence of the tammar, we were able to design overgo probes to conserved genes and very efficiently clone positives (Deakin *et al.* 2012). From mapping three genes in three years, it became possible to map fifty in a week.

Chromosome painting also became available to us, thanks to the flow sorting of individual marsupial chromosomes by Malcolm Ferguson-Smith's laboratory in Cambridge, supplemented by microdissection in my laboratory. This made it possible to compare homologous regions in different species. In collaboration with Willem Rens and Malcolm Ferguson-Smith we compared chromosomes between closely related Australian marsupials, and then between Australian and American marsupials (Rens *et al*. 2001, 2003). Reciprocal painting between species from all the major marsupial families showed that all marsupial karyotypes comprise different arrangements of the same 19 conserved segments. This confirmed the classic observations of Hayman and Martin (1974) that the karyotypes of marsupials, unlike karyotypes of eutherian mammals, are highly conserved.

This high conservation of marsupial chromosomes was always held to be unusual, being very different from the great variety of eutherian chromosome numbers, sizes and morphology. However, in this conservation, marsupials are similar to birds and reptiles, whose karyotypes are strikingly homologous with each other – e,g. chicken, emu and even turtle have chromosomes that are homologous by painting (Graves and Shetty 2001); thus it is the highly variable karyotypes of placental mammals that are unusual, perhaps relating to the acquisition of mobile elements.

The Boden Conference and the Kangaroo Genome Centre

Marsupial (and monotreme) genetics was beginning to excite some international interest when, in 1988, with Des Cooper and Rory Hope, I made a bid for a small conference to gather the few experts in the world to share their knowledge of marsupial breeding, gene mapping and chromosomes. Out of this came a book 'Mammals from Pouches and Eggs' (Graves *et al*. 1990) edited by the three organisers (Fig. 1*a*, *b*). Marsupial gene mapping took centre stage when, with Marilyn Renfree and Des Cooper, I initiated a bid for a Centre of Excellence in Kangaroo Genomics in 2003. We were successful in securing limited support, with partner investigators bioinformaticist Terry Speed (Hall Institute) and genomicist Sue Forrest (Australian Genome Research Facility [AGRF]) (Fig. 1*c*).

We set about to complete a map of the entire tammar genome in preparation for sequencing the tammar genome. Since we already had information about X-borne genes, a start was made with the tammar X and Chromosome 5 (Alsop *et al.* 2005), but this was slow going.

A strategy was devised to efficiently map the entire tammar genome. First we identified blocks of genes shared between human and opossum, then we chose genes to mark each end of each group, pulled out BACs that contained them and mapped only these. A physical map of the tammar genome, containing 548 markers, was rapidly built up. Since we built the map from highly conserved genes with orthologues in all vertebrates, this map could be compared with maps of the opossum, human and even chicken. The comparative map of tammar relative to human shows that the genome has been rearranged at least 120 times; somewhat fewer than between mouse and human.

Combining comparative gene mapping with chromosome painting made it possible to establish detailed homologies between different marsupial species, and also between marsupials, eutherians and even birds. This work confirmed that all marsupials share 19 conserved segments that have undergone simple rearrangements. The arrangement of these segments was almost identical among more than 60 dasyurid species, as well as a family of South American opossums. Comparison with chicken enabled identification of ancestral gene arrangements in $2n = 14$ species and $2n = 22$ South American species. It was demonstrated that the $2n = 14$ karyotype common in Australian marsupials (but not the $2n = 22$ karyotype of South American marsupials) shared several gene arrangements with chicken, implying that these gene arrangements were present in the common ancestor of mammals and reptiles (Deakin *et al*. 2010). This is consistent with a $2n = 14$ ancestral marsupial karyotype. The more variable karyotypes of macropodids (kangaroos and wallabies) are easily derived by (largely Robertsonian) fusions between these segments, while the $2n = 22$ chromosomes of South American marsupials require several fissions and fusions.

At the same time, Des Cooper's group in Sydney (initially at Macquarie University, then at the University of New South Wales) had set up crosses and backcrosses between subspecies of tammar that differed in many fixed alleles, and produced the first tammar map based on microsatellites (McKenzie *et al*. 1997; Zenger *et al*. 2002). A microsatellite map of the opossum genome was later produced that could be anchored to opossum chromosomes (Samollow *et al*. 2007). The numbers of markers for the tammar map were greatly expanded by searching for microsatellites within conserved genes which could be mapped by FISH, and this strategy enabled the linkage maps to be anchored to physical chromosomes (Wang *et al*. 2011*a*). The physical and linkage maps could be combined into an integrated map of the tammar genome (Wang *et al*. 2011*b*).

Kangaroo sex chromosomes

Susumo Ohno predicted long ago that the gene content of the mammalian X chromosome would prove to be identical in all mammals, since rearrangement that disrupted the whole X-inactivation system would be selected against. Early demonstrations that *PGK* and *G6PD* were sex linked in several kangaroo species, dasyurids and opossum supported 'Ohno's Law'. Somatic cell hybridisation added *HPRT* to this list, and radioactive *in situ* hybridisation showed that several genes that lie on the long arm of the human X mapped onto the X in

Fig. 1. (*a*) Participants in the Boden Conference on Marsupial and Monotreme Genetics, Thredbo, Australia, 1988. Des Cooper is in the centre, in his trademark bow tie. (*b*) Editors of 'Mammals from Pouches and Eggs' – Rory Hope, Jenny Graves, Des Cooper. (*c*) Chief and Partner Investigators of the ARC Centre of Excellence for Kangaroo Genomics. From left: Sue Forrest, Marilyn Renfree (Deputy Director), Jenny Graves (Director), Terry Speed, Des Cooper.

kangaroos (Spencer *et al*. 1991*a*) and dasyurids. So far, Ohno's Law held.

However, Andrew Sinclair in my laboratory discovered that genes on the short arm of the human X chromosome were not, as we had expected, located on the marsupial X, but grouped on Chromosome 5 in tammar and Chromosome 3 in dasyrurids (Sinclair *et al*. 1987; Spencer *et al*. 1991*b*). This was confirmed by chromosome painting: the kangaroo X paint hybridised only to the long arm and pericentric region of the human X (Glas *et al*. 1999). This constituted the first breach of Ohno's Law. I happened to be visiting the City of Hope Medical Center in Los Angeles at the time, and took it upon myself to personally inform the great

man, whom I found in his office downstairs, composing music from DNA sequence. 'So?' he shrugged. A valuable lesson to me that laws are made to be broken.

Our finding could mean either that a piece of the X was lost from a large ancestral X, or that a piece of autosome was added to the X in placental mammals (Graves 1987). Comparison with orthologous genes on the chicken favoured the latter explanation, for the chicken orthologues of genes of the human X lie in two blocks, both autosomal. One (Chicken 4p) represents the region that is shared between the human and marsupial X, the other (Chicken 1) represents the region that is on the X in placentals but is autosomal in marsupials. It was proposed that the marsupial X represents the ancestral mammal X, to which an autosomal region was fused between 160 Mya, when marsupials and placentals diverged, and 105 Mya, when placentals radiated (Graves 1995). A recent study of the elephant X chromosome (Rodriguez Delgado *et al.* 2009) showed a correspondence between the order of genes on the human and elephant X, but placed the boundary of the conserved and added regions right at the centromere: this suggests that an original Robertsonian fusion occurred, and Afrotheria retain the original centromere; however, the ancestor of other mammals underwent a centric shift (Fig. 2).

We could go back further in time by mapping genes orthologous to those on the marsupial X (equivalent to the ancestral therian X) in birds and reptiles, frogs and fish. They formed autosomal blocks in all of these species (e.g. Nanda *et al.* 1999), implying that mammal sex chromosomes originated in an autosome some time between the divergence of mammals and reptiles, 310 Mya, and the divergence of marsupials and eutherians, 160 Mya. What was really surprising, however, was our finding that in the basal group of monotreme mammals, genes that are on the therian X are autosomal in platypus and echidna. The complex sex chromosomes of monotremes have homology, instead, to the bird ZW pair. This brings forward the time at which the therian sex chromosomes evolved to only 166–160 Mya (Veyrunes *et al.* 2008).

The marsupial Y chromosome, like the human Y, is expected to determine testis because XY and XXY embryos develop testes but XX and XO animals do not (even though some other secondary sexual characteristics are independently controlled – see O *et al.* 1988).

Thus marsupials furnished an important test of the identity of candidate human sex-determining genes: they should be shared by the marsupial Y. The autosomal location in marsupials of the first human candidate sex-determining gene was the first clue that *ZFY* was the wrong gene (Sinclair *et al.* 1988), and the trigger for finding the right gene *SRY* (Sinclair *et al.* 1990). The presence of *SRY* on the marsupial Y was an important piece of evidence that it was the right gene (Foster *et al.* 1992), and the discovery of *SOX3* on the X was the first indication that even Y-borne genes with a

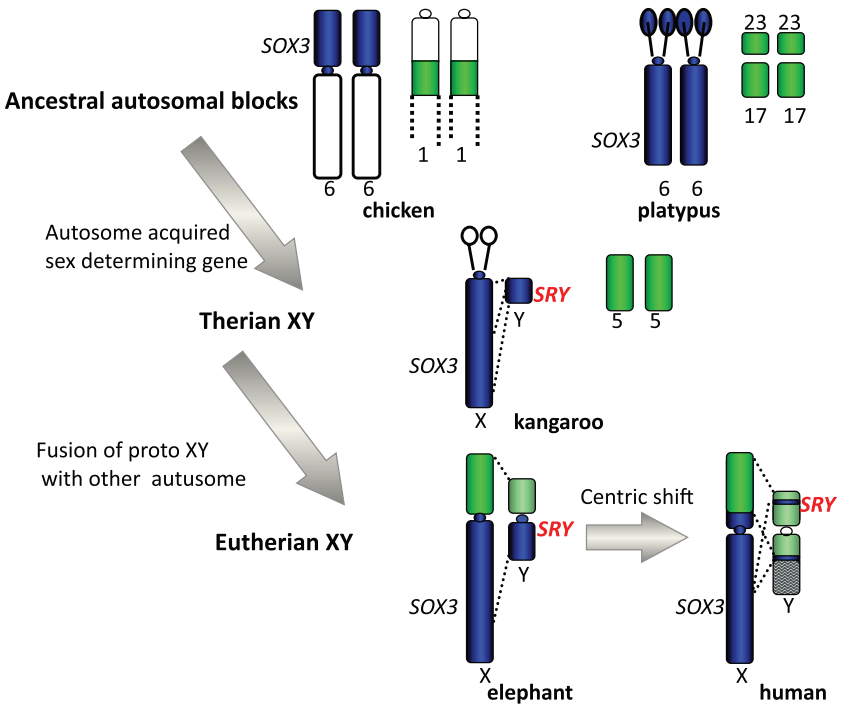

Fig. 2. Evolution of mammalian sex chromosomes from two conserved blocks (blue and green) that are autosomal in platypus as well as in birds and reptiles. In an ancestral therian 166–160 Mya, one autosome (blue) acquired a sex-determining gene *SRY*, which evolved from *SOX3*, and differentiated into an XY pair as the male-specific element degenerated. This ancient therian XY pair is retained by marsupials. In an ancestral placental mammal 160–105 Mya, there was a centric fusion between the shared region of the proto-XY and an autosome (green), a configuration retained by Afrotheria. A centric shift occurred in the ancestor of all other placental mammals. Most of the Y of placental mammals was derived from the added region (green).

function in males evolved from genes on the X (Foster and Graves 1994).

The tiny marsupial Y is completely isolated from the X and does not recombine with it; this 'minimal Y' is a good model system (Toder *et al.* 2000). It contains, as well as *SRY*, orthologues of three other human Y genes (*RBMY*, *UBE1Y*, *KDMCY*), all of which were shown to have X partners on the X from which they obviously diverged (Delbridge *et al.* 1999). However, all of the other 47 unique protein-coding genes on the human Y originated from the added region of the human X, so are autosomal in marsupials (Waters *et al.* 2001; Graves 2006).

Surprisingly, the little marsupial Y contains several other genes, all originating from the original therian X chromosome, but lost from the Y in placental mammals. *ATRY* was the first to be discovered (Pask *et al*. 2000). Others were found by screening a tammar BAC library with DNA from a microdissected Y (Sankovic *et al.* 2006). Sequencing Y-borne BACs yielded another seven tammar Y genes, six of which have paralogues on the tammar X chromosome, from which they clearly diverged (Murtagh *et al.* 2012), consistent with the hypothesis that the Y chromosome evolved by progressive degradation from an original proto–XY pair (Graves 2006).

These characteristics of marsupial sex chromosomes have led to major rethinks about the evolution of mammalian sex chromosome structure and function.

The kangaroo genome

When I was asked by the National Human Genome Research Institute (NHGRI) in 2002 to prepare a proposal ('White Paper') to sequence the genome of a model marsupial, I replied that of course it had to be a classic kangaroo. I enlisted Des Cooper and Marilyn Renfree and many other colleagues to prepare the case for sequencing (at the cost, still, of many millions of dollars) our model kangaroo, the tammar wallaby (Graves *et al.* 2002). However, the decision was to sequence the South American grey short-tailed opossum (because, in the absence of any financial support from Australia, 'it should be an American marsupial'). The opossum genome was published by a consortium that included 26 Australian marsupial researchers (Mikkelsen *et al*. 2007).

It was clear that a second marsupial genome was required to enable identification of marsupial-specific and eutherian-specific characteristics. Sue Forrest led a search for funding to commence sequencing the tammar genome. The Victorian State government, supplemented by Applied Biosystems and matched by NHGRI (thanks to Francis Collins) commenced Sanger sequencing in Brisbane (AGRF) and Houston (Baylor Genome Center, directed by Australian Richard Gibbs). The advances in sequencing technology (and the plummeting cost) meant that the 1.5X Sanger sequencing could be supplemented by SOLiD and Illumina sequencing. A hybrid assembly was published in 2011 (Renfree *et al*. 2011). Since that time, the genome of the Tasmanian devil has been reported (Murchison *et al*. 2012), and several other marsupials are being sequenced on the recommendation of the Genome 10K Community of Scientists (2009).

Even before the assembly, tammar sequence from trace archives proved important in providing handles to clone tammar genes of interest, for instance *HOX* genes (Yu *et al*. 2012), orthologues of imprinted genes (Suzuki *et al.* 2005; Rapkins *et al*. 2006) and the flanking markers to prove that *XIST* does not exist in marsupials (Hore *et al.* 2007). It greatly facilitated the identification of BACs that define conserved blocks for mapping.

The opossum and tammar sequence established that the size of marsupial genomes, and the size of the non-repetitive fraction, is within the range for eutherian genomes, but the composition of the repeats is very different. There are fewer segmental duplications, and more transposable elements, including more than 500 repeat families (many lineage-specific) especially enriched in LINE elements. The genome has a low %GC, perhaps related to the low recombination.

Like genomes of placental mammals, both marsupial genomes have at least 18 000 protein-coding genes, nearly all of which have orthologues in the human genome. Marsupial-specific genes are largely duplicates and pseudogenes, although a few prove to have homologues in chicken but not human, so represent ancient reptilian genes that were lost in the placental mammal lineage. Many marsupial-specific genes prove to be receptors or transcriptional regulators, and some have characteristics of milk proteins, as might be expected from the complex lactation in marsupials. Many gene families, such as olfactory receptor genes, cytokines, defensins, have been independently amplified in marsupials and eutherians, and may have evolved unique functions: for instance, in adaptation of smell to nocturnal marsupial life (Delbridge *et al*. 1999) and antimicrobial activity in the pouch to protect the altricial young (Wang *et al*. 2011*c*). The arrangement of genes within the MHC locus is different from that of placental mammals, and more similar to that of frog, and tammar is unique in distribution of Class I MHC genes around the genome rather than in a tandem array with other MHC genes (Siddle *et al*. 2009).

Comparison between orthologous regions of marsupial and eutherian genomes, such as the region containing *HOX* genes, efficiently identifies exons and conserved non-coding elements. Most of the sequences conserved across this 160-million-year interval proved to be non-coding sequences, many conserved also in birds, suggesting a vital function in all amniotes. This was confirmed by the overlap with functional elements and proximity to developmentally important genes. It is possible to date the origin of these elements by comparing their appearance in birds, marsupials, placentals, and to study both ancient amniote-specific and recently evolved placental- or marsupial-specific elements. The overlap of novel elements with transposable elements suggests that transposable elements furnished the raw material for the evolution of new elements (Mikkelsen *et al.* 2007; Renfree *et al*. 2011). The absence of accumulation of LINE1 elements on the X suggests that X chromosome inactivation in marsupials is not regulated in the same way as for the human and mouse X.

Conclusions

Des Cooper's foresight in exploring the marsupial genome has paid off in ways we could not have foreseen. When I started mapping kangaroo genes at his behest, other scientists told me I was wasting my time; some avowed that marsupials would be so different from human and mouse that comparison would be

meaningless, while others warned that the exercise was pointless as they would prove to be exactly the same as our familiar models.

They are neither just the same, nor too different to compare – in fact, they occupy an important middle ground that delivers informative genetic variation on conserved gene arrangements and regulation. The wide array of uses to which this knowledge has been put are summarised in the recent book 'Marsupial Genetics and Genomics' (Deakin *et al.* 2010). Sequence comparisons have proved to be efficient in spotting conserved genes and potential regulatory regions, and comparisons of gene arrangement have opened the way for a comprehensive look at mammalian chromosome evolution. This is particularly evident in the study of the organisation, function and evolution of sex chromosomes, where marsupials have provided a robust picture of the smaller ancestral X and Y, and documented steps in the building up of the complex epigenetic silencing of the X.

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