# The First Comprehensive Genetic Linkage Map of a Marsupial: The Tammar Wallaby (*Macropus eugenii*)

**Kyall R. Zenger,[1] Louise M. McKenzie[2] and Desmond W. Cooper**

*Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia*

Manuscript received April 24, 2002
Accepted for publication June 6, 2002

ABSTRACT

The production of a marsupial genetic linkage map is perhaps one of the most important objectives in marsupial research. This study used a total of 353 informative meioses and 64 genetic markers to construct a framework genetic linkage map for the tammar wallaby (*Macropus eugenii*). Nearly all markers (93.8%) formed a significant linkage (LOD > 3.0) with at least one other marker, indicating that the majority of the genome had been mapped. In fact, when compared with chiasmata data, >70% (828 cM) of the genome has been covered. Nine linkage groups were identified, with all but one (LG7; X-linked) allocated to the autosomes. These groups ranged in size from 15.7 to 176.5 cM and have an average distance of 16.2 cM between adjacent markers. Of the autosomal linkage groups (LGs), LG2 and LG3 were assigned to chromosome 1 and LG4 localized to chromosome 3 on the basis of physical localization of genes. Significant sex-specific distortions toward reduced female recombination rates were revealed in 22% of comparisons. When comparing the X chromosome data to closely related species it is apparent that they are conserved in both synteny and gene order.

MARSUPIAL and eutherian (placental) mammals diverged ~130 million years ago (Hope *et al.* 1990; Killian *et al.* 2001). The two groups are similar in most of their biology, but have distinctive attributes with regard to reproduction and cytogenetics. For example, marsupials are born at a very early stage of development equivalent to that of the end of embryogenesis in placentals, and marsupial development is completed usually in a pouch attached to a teat (Gemmell *et al.* 2002). Sex differentiation appears to be much more complex in marsupials than in placentals (Pask and Renfree 2001). While eutherians exhibit a wide range of chromosome numbers ($2n$ = 6–92; Wurster and Benirschke 1970; Schmid *et al.* 1988), marsupials are characterized by low numbers, ranging from $2n$ = 10–32 (Hayman 1990), and sex-specific recombination rates appear to be contrary to those observed in eutherians (Bennett *et al.* 1986; Samollow *et al.* 2000). Given these unique characteristics, marsupials have been regarded as ideal candidates for research encompassing reproductive biology, immunology, developmental studies, and comparative genomics (*e.g.*, Samollow and Graves 1998; Miller and Belov 2000; Pask and Graves 2001; Renfree and Shaw 2001).

The tammar wallaby (*Macropus eugenii*) is the Australian marsupial model for genetic mapping studies. Its value arises from a number of characteristics, including: (i) small numbers of chromosomes ($2n$ = 16) that are variable in both length and morphology and include a XX/XY female/male sex-chromosome dimorphism; (ii) a relatively small overall map length of 1172 cM (estimated from chiasmata data); (iii) delayed blastocysts, whereby removing the pouch young stimulates the reactivation of the blastocyst producing multiple offspring annually; (iv) small size (4.5–8.5 kg) relative to other macropodids and ease of breeding in captivity; and (v) subspecies that hybridize together, producing the perfect genetic pedigree system for genetic linkage mapping experiments (Sharp and Hayman 1988; Hinds *et al.* 1990; McKenzie and Cooper 1997; Renfree and Shaw 2000).

Genetic mapping progress in *M. eugenii* to date has proceeded primarily by physical approaches based on *in situ* hybridization methods (*e.g.*, Samollow and Graves 1998; Hawken *et al.* 1999; Waters *et al.* 2001), while linkage mapping in *M. eugenii* has produced four small linkage groups incorporating a total of nine genetic markers (van Oorschot and Cooper 1990; McKenzie *et al.* 1993, 1996). Traditionally, research has been directed at the in-depth comparisons of genome structure between metatherian and eutherian gene maps to better understand genome evolution. These differences can then be used as probes to explore the phylogenetic distinction of sex determination, gene expression, and other questions pertaining to the evolution of gene structure and function. However, gene mapping is of interest not only as a com-

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. AF503635 and AF503636.

[1]*Corresponding author:* Reprogen, Faculty of Veterinary Science, The University of Sydney, Camden, NSW 2570, Australia.
E-mail: kzenger@camden.usyd.edu.au

[2]*Present address:* The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609.

parative tool, but also for its intrinsic value for localizing genes and examining their function. Currently there is great interest in identifying trait loci connected with disease resistance and immune response within overabundant or endangered marsupials (*e.g.*, Buddle and Young 2000). In addition, the localization of unique marsupial trait loci, which could be of economic importance, has been the focus of recent attention (*e.g.*, Gellin *et al.* 2000). The production of a *M. eugenii* genetic linkage map would be the first necessary step to undertake these investigations.

In this article, we report the first comprehensive framework genetic linkage map of a marsupial (*M. eugenii*) consisting of coding (functional) and anonymous (microsatellites) genetic markers. The impetus for the development of this map has been primarily to develop a system for comparative and quantitative trait mapping. However, the application of this map should not be limited only to these objectives. In essence the map also provides a mechanism for investigating how different genetic control mechanisms evolved and how they function.

## MATERIALS AND METHODS

**Reference pedigrees:** The genetic data used to construct the *M. eugenii* genetic linkage map were obtained from a total of 353 informative meioses. The majority of these are derived from hybrid phase-known backcrosses from genetically distinct Kangaroo Island (KI) and Garden Island (GI) tammar wallabies bred at Macquarie University (see McKenzie *et al.* 1993). There are six different types of crosses (Figure 1), with the majority of informative data being derived from pedigrees 1, 2, and 3.

Separate male and female hybrid crosses were used so that independent information regarding male/female recombination rates and allelic transmission could be determined. Four informative hybrid males in pedigree 1 and pedigree 2 produced 105 progeny, while pedigrees 3–6 consisted of 21 informative females who generated 127 offspring. In addition, a number of KI or GI males in the female pedigrees were heterozygous, allowing for extra male informative crosses, which produced an additional 121 meiosis events. These males were originally phase unknown. However, their phase was inferred after careful consideration and examination with the original hybrid male results. Accordingly, all male hybrid-type crosses were combined to produce a total of 226 possible informative meiosis events. All DNA extractions were carried out according to the "salting-out" procedure described in Sunnucks and Hales (1996).

**Genetic markers and data integrity:** Sixty-four genetic markers were used for analysis in this study. Seventeen of these have previously been described and tested for genetic linkage (McKenzie *et al.* 1993, 1996; McKenzie 1994). These markers include 12 coding genes, 4 anonymous DNA markers, and 1 pseudogene (Table 1). Genotyping of these 17 markers was accomplished via allozyme electrophoresis (*MPI* and *NP*) according to Richardson *et al.* (1986), isoelectric focusing (*TF*) according to Righetti (1983), and Southern hybridization using $\alpha^{32}$-P-labeled probes (*AR*, *CASA*, *DBB*, *G6PD*, *HBB*, *LALBA*, *LLP*, *LPL*, *RNR*, *pB12*, *pB15*, *pB65*, *pB72*, and *PGK_9*) according to Sambrook *et al.* (1989). All probes were macropodid specific, except *HBB* and *RNR*, which were derived from *Dasyurus viverrinus* and *Xenopus laevis*. Although a small number of microsatellite loci have previously been tested (Taylor and Cooper 1998), they were tested again within this study due to limited numbers of meioses and inconsistencies within these data. The remaining 47 genetic markers are described in Table 1. All but 4 of these genetic markers were based upon anonymous macropodid-specific microsatellite genetic markers. These remaining four loci consisted of a gene-specific microsatellite marker located within interleukin 5 (*IL5*; Hawken *et al.* 1999), a hypoxanthine phosphoribosyltransferase (*HPRT*)-specific X-linked polymorphism (GenBank accession nos. AF503635 and AF503636) characterized by restriction fragment length polymorphism (RFLP) and two autosomal anonymous DNA polymorphisms identified as *pHPRT1* and *pHPRT2*, which were resolved by single-strand conformation polymorphism (SSCP).

All microsatellite loci and anonymous DNA markers were amplified via PCR in 10-μl reaction volumes containing 100–200 ng of genomic DNA, 2–3 mM $MgCl_2$, 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 0.1% Triton X-100, 0.1% Tween 20 and NP40, 200 μM each of dTTP, dCTP, and dGTP, 20 μM dATP, 0.05 μl of [$\alpha^{33}$-P]dATP at 1000 Ci/mmol, 1.0 μM of each primer, and 0.5 units of *Taq* polymerase (QIAGEN, Chatsworth, CA). PCR amplifications were carried out using an MJ Research (Watertown, MA) PTC100 thermocycler, with an initial 94° denaturation for 3 min, followed by "touchdown" cycles of 94° denaturation for 30 sec, annealing temperatures (60°, 58°, 56°, 54°, 52°, and 50°) for 45 sec, and an extension step of 72° for 1 min. The touchdown annealing temperatures decreased by 2° each cycle, whereby on completion of the last touchdown cycle another 30 cycles were performed at this annealing temperature with a final extension of 72° for 3 min. The amplified microsatellite PCR products were resolved on 6% denaturing polyacrylamide gels and visualized by autoradiography according to Taylor *et al.* (1994), while PCR-SSCP of *pHPRT1* and *pHPRT2* was carried out according to Sunnucks *et al.* (2000).

Preferential PCR amplification of introns 6–8 of the X-linked *HPRT* gene was accomplished using primers designed over exon/intron boundaries from closely related *M. robustus* sequences (Piper *et al.* 1993). PCR conditions are as above with the omission of [$\alpha^{33}$-P]dATP and the replacement of 20 μM dATP with 200 μM dATP. Characterization of the X-linked *HPRT* polymorphism was accomplished via RFLP using the restriction enzyme *Bsr*DI.

Even a minute proportion of genotypic errors in a data set can dramatically affect the precision of a linkage map. Several steps were carried out to ensure strict data integrity prior to map construction. This was accomplished by the identification and correction of errant typings by two different approaches. First, random samples of animals from previous typings were duplicated in subsequent analysis to ensure continuity and to allow for quality assessment of newly acquired data, and second, genotype data were inspected for typing inconsistencies via examination of Mendelian segregation patterns against known pedigrees. This second procedure was carried out manually twice at independent times to ensure correct genotyping of the markers. All discordant and non-Mendelian genotypes were either repeated or deleted from the primary data. A further step was carried out following the genetic linkage map construction. This entailed searching for potential errors by looking for dubious double crossovers using the "error detection" command of MAPMAKER version 3.0b with the default setting of 1% *a priori* probability of error (Lander *et al.* 1987; Lincoln and Lander 1992). This procedure was repeated at least three times for each linkage group. Once errors were identified and corrected, the linkage map was rederived.

Segregation distortions, which may be caused by gametic selection or postzygotic selection, have been observed frequently in divergent hybrid backcrosses (*e.g.*, Siracusa *et al.*

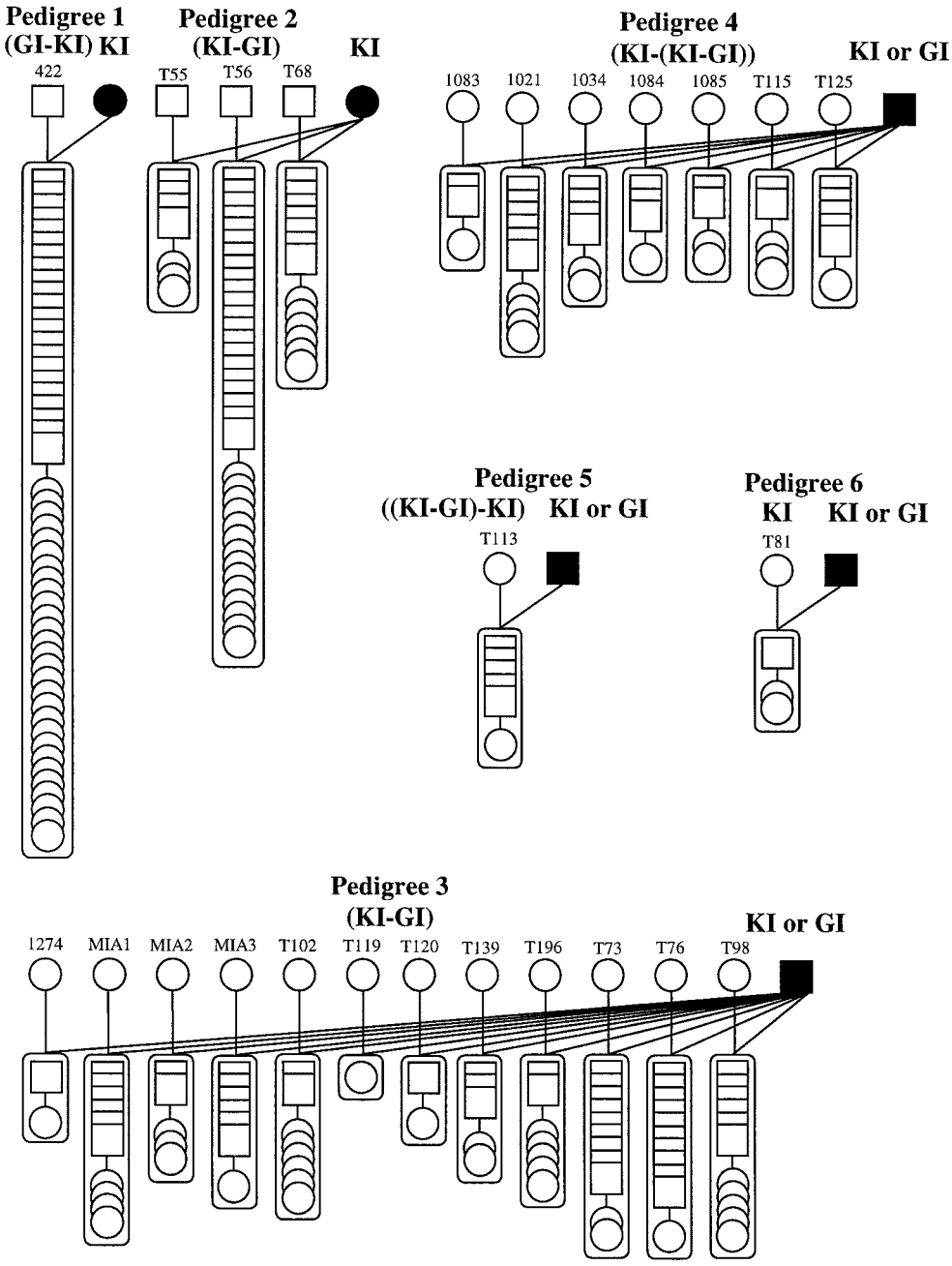


FIGURE 1.—Systematic representation of crosses derived from Kangaroo Island (KI) and Garden Island (GI) animals. Females are presented first in all parental genotypes, *e.g.*, ♀GI–♂KI and ♀KI–(♀KI–♂GI). Pedigree 4 and 5 mothers are the progeny of pedigrees 2 and 3, respectively. Solid symbols represent multiple individuals.

1991; XU *et al.* 1997; VOGL and XU 2000). To test if any of the loci within the hybrid (KI-GI) × KI crosses (excluding $F_1$ × GI individuals due to insufficient meioses) were expressing segregation distortions, chi-square tests with 100,000 randomizations were calculated (SOKAL and ROHLF 1995) using the "Monte Carlo" exact test function of SPSS version 10.0. This application calculated exact segregation heterogeneity for all 64 loci across pedigrees 1, 2, and 3. In addition, to examine if there were sex-specific differences in $F_2$ allelic inheritance, a Fisher's exact probability (SOKAL and ROHLF 1995) was calculated for all loci using SPSS version 10.0. After examination of the loci, the number of informative meioses per locus and the average number of informative meioses across the data set were calculated.

**Map construction:** Several strategies were carried out to ensure the most precise genetic linkage maps were obtained. This included the utilization of MAPMAKER version 3.0b (LANDER *et al.* 1987) and CARTHAGENE version 0.4 (SCHIEX and GASPIN 1997) linkage analysis programs, which employ different map-ordering algorithms, so that a confident consensus map could be derived. MAPMAKER uses EM algorithms to obtain multipoint maximum-likelihood estimations, while CARTHAGENE combines the multipoint maximum-likelihood criteria with local search techniques. In addition, before map construction commenced, $F_1$ hybrid individual heterogeneity was calculated for all locus pairs within and across all reference pedigrees of the same sex using two-point linkage analysis results. This was to ensure that all individuals were representative of the collective data within each of the sexes.

Map construction was performed by first grouping the markers together using a stringent minimum LOD score of 4.0 and a maximum recombination fraction of 0.4 as linkage thresh-

olds for both programs. Trial maps were obtained by inspection and by applying the two-point analysis method. Subsequent tests and validation of locus orders were performed using the more powerful maximum-likelihood methods incorporated in both MAPMAKER and CARTHAGENE. The Kosambi map function (Kosambi 1944) was used to convert recombination fractions to distances within both programs. Markers that did not maintain linkages with the original stringent LOD score threshold were placed within linkage groups only if their most likely position was supported by a LOD score of ≥3. A linkage map was considered fixed when loci within the linkage map could not be placed within 100:1 odds of their next maximum-likelihood positions. Loci within this range were placed in their maximum-likelihood positions for the final map. All tests were performed over male, female, and pooled reference pedigrees.

Once sex heterogeneity in recombination rates was taken into account, a consensus multipoint map was produced from the pooled data to ascertain a final sex-average genetic linkage map. Confidence intervals for the location of each marker were calculated using the $Z_{max} - 1$ criteria of Conneally *et al.* (1985) utilized in the LINKMAP program of the LINKAGE package (Lathrop and Lalouel 1988). To obtain confidence intervals, the location of each marker locus was varied (all other loci being fixed at their maximum-likelihood locations) to both the proximal and distal sides of its best location until the $\log_{10}$ likelihood value decreased by at least one LOD unit. This method was repeated for each marker to provide an ~90–95% confidence limit on each location in the multipoint map. The total multipoint map length and standard error of each linkage group and over the entire map were calculated according to Ott (1999).

**Heterogeneity:** Individual $F_1$ hybrid recombination heterogeneity within each reference pedigree was calculated using a likelihood ratio test, termed the M-test (Ott 1999), and follows a chi-square distribution with $(c - 1)$ degrees of freedom. Sex-specific differences of the intervals, linkage groups, and over the entire map were calculated on the basis of the difference in likelihood values of the combined male/female linkage data with the sex-pooled data using the same number of markers and map order in each. Tests of differences in specific linkage intervals were calculated according to Ott (1999). Differences in the linkage groups and across the entire map were tested by adding the chi-square values in each representative category, where the degree of freedom equals the number of intervals in each (Archibald *et al.* 1995). In addition, the ratios of female-to-male map distances ($R = X_f/X_m$) were calculated for each interval, linkage group, and over the entire map, where $X$ is the map distance.

## RESULTS

**Genotyping:** With the sexes combined 64 genetic markers were informative for analysis in this study; 59 of these were typed across male pedigrees, while 52 genetic markers were typed over the female pedigrees. The average number (±SE) of informative meioses across the 64 loci was 212 ± 13 for both sexes combined, while the average number for males and females was 139 ± 7 and 104 ± 4, respectively (Table 1).

The integrity of the newly acquired genotypic data was monitored repeatedly throughout this project. Among duplicate typings and with scrutiny of Mendelian inheritance there was a 98.4% concordance with the original data. Most of the discrepancies (95%) were due to genotyping errors, which were remedied once detected. The remaining errors resulted from inconsistencies in the Mendelian inheritance of the markers. Locus *T28-1* produced null alleles in 2 of the 21 female hybrids and their offspring, and six microsatellite loci exhibited a total of 11 stepwise mutations of one repeat unit. Following the detection of inconsistent inheritance of alleles, all discordant individuals were removed from the analysis with the exception of the mutations, which were reassigned inheritance where possible.

Analysis of segregation distortions within the sex-pooled data, separate male/female data, and sex-specific allelic inheritance data revealed no significant deviations following Bonferroni correction.

**Map construction:** The two-point linkage analysis of the sex-pooled data revealed a total of 60 markers (93.8%) that showed significant linkage with at least one other marker at a LOD score of 3 or greater (except *Me2* with LOD score of 2.58), while four markers (*G16-2*, *MPI*, *TF*, and *Y148*) did not show any linkage with any other markers.

Within the multipoint linkage analysis, both MAPMAKER and CARTHAGENE produced identical linkage groups and map-ordering results. On the basis of the consensus analysis of the sex-pooled data, nine groups were initially established with only locus *T19-1* not being placed into a group at the stringent LOD threshold of 4.0. However, the most likely position of this marker was supported with a LOD score of 3.33 when the threshold was reduced. Ultimately, nine linkage groups ranging from 15.7 ± 2.1 cM (linkage group 9) to 176.5 ± 9.8 cM (linkage group 1) were formed based on the two-point and multipoint analysis. These linkage groups almost certainly cover proportions of all seven autosomes and the X chromosome and have a total map length of 828.4 ± 23.2 cM with an average distance between adjacent markers of 16.2 ± 1.8 cM (Figure 2).

The sex-average multipoint linkage maps with approximate $Z_{max} - 1$ confidence intervals for each genetic marker are illustrated in Figure 2. Linkage groups 1and 4–9 exhibited comparatively discrete confidence limits attached to each marker's maximum-likelihood location. The minority of markers that exhibited confidence intervals that overlapped considerably with a neighboring marker were *Y105–T3-1T* and *DBB–G12-6* within linkage group 2 and *IL5–T28-1* and *LPL–Me28* within linkage group 3. In each respective pair both markers have confidence intervals that overlap with each other, probably resulting from their close proximity to each other and the relatively small numbers of informative meioses events observed between each pair. These departures were also observed during validation of the multipoint map gene orders. Consequently, linkage groups 2 and 3 were placed at their most-likelihood gene order position, while all others demonstrated gene orders greater than the 100:1 odds criteria.

**TABLE 1**

**Loci and number of informative meioses used within this study for linkage analysis**

| Locus | Reference | Informative meioses | | Locus | Reference | Informative meioses | |
|---|---|---|---|---|---|---|---|
| | | Male[i] | Female | | | Male[i] | Female |
| *AR*[a] | McKenzie (1994) | 0 | 71 | *Me1*[d] | Taylor and Cooper (1998) | 197 | 119 |
| *CASA*[a] | McKenzie (1994) | 61 | 0 | *Me2*[d] | Taylor and Cooper (1998) | 195 | 125 |
| *DBB*[a] | McKenzie (1994) | 88 | 0 | *Me14*[d] | Taylor and Cooper (1998) | 197 | 122 |
| *G6PD*[a] | McKenzie *et al.* (1996) | 0 | 71 | *Me15*[d] | Taylor and Cooper (1998) | 196 | 117 |
| *HBB*[a] | McKenzie (1994) | 70 | 0 | *Me16*[d] | Taylor and Cooper (1998) | 127 | 106 |
| *HPRT*[a] | This study[e] | 0 | 121 | *Me17*[d] | Taylor and Cooper (1998) | 171 | 114 |
| *LALBA*[a] | McKenzie *et al.* (1993) | 78 | 0 | *Me27*[d] | Taylor and Cooper (1998) | 60 | 52 |
| *LLP*[a] | McKenzie *et al.* (1993) | 100 | 47 | *Me28*[d] | Taylor and Cooper (1998) | 177 | 109 |
| *LPL*[a] | McKenzie *et al.* (1993) | 92 | 0 | *Pa55*[d] | Spencer (1996) | 99 | 101 |
| *MPI*[a] | McKenzie (1994) | 74 | 0 | *Pa297*[d] | Spencer *et al.* (1995) | 173 | 108 |
| *NP*[a] | McKenzie (1994) | 73 | 0 | *Pa593*[d] | Spencer *et al.* (1995) | 181 | 112 |
| *RNR*[a] | McKenzie *et al.* (1996) | 0 | 73 | *Pa595*[d] | Spencer *et al.* (1995) | 206 | 126 |
| *TF*[a] | McKenzie (1994) | 83 | 0 | *T3-1T*[d] | Zenger and Cooper (2001a) | 165 | 126 |
| *pB12*[b] | McKenzie (1994) | 79 | 0 | *T4-2*[d] | Zenger and Cooper (2001a) | 213 | 113 |
| *pB15*[b] | McKenzie (1994) | 91 | 0 | *T10-1*[d] | Zenger and Cooper (2001a) | 185 | 121 |
| *pB65*[b] | McKenzie *et al.* (1993) | 84 | 54 | *T15-1*[d] | Zenger and Cooper (2001a) | 190 | 122 |
| *pB72*[b] | McKenzie *et al.* (1993) | 45 | 0 | *T17-2*[d] | Zenger and Cooper (2001a) | 175 | 116 |
| *pHPRT1*[b] | This study[f] | 96 | 107 | *T19-1*[d] | Zenger and Cooper (2001a) | 195 | 116 |
| *pHPRT2*[b] | This study[f] | 97 | 110 | *T28-1*[d] | Zenger and Cooper (2001a) | 157 | 78 |
| *PGK_9*[c] | McKenzie (1994) | 85 | 0 | *T28-3*[d] | Zenger and Cooper (2001a) | 163 | 127 |
| *B87*[d] | Moritz (pers. comm.)[g] | 194 | 118 | *T30-1*[d] | Zenger and Cooper (2001a) | 214 | 123 |
| *B90*[d] | Pope *et al.* (2000) | 177 | 114 | *T31-1*[d] | Zenger and Cooper (2001a) | 196 | 120 |
| *B123*[d] | Moritz (pers. comm.)[h] | 175 | 118 | *T32-1*[d] | Zenger and Cooper (2001a) | 182 | 109 |
| *G12-6*[d] | Zenger and Cooper (2001b) | 20 | 58 | *T46-5*[d] | Zenger and Cooper (2001a) | 145 | 100 |
| *G15-4*[d] | Zenger and Cooper (2001b) | 141 | 91 | *T47-1*[d] | Zenger and Cooper (2001a) | 0 | 121 |
| *G16-1*[d] | Zenger and Cooper (2001b) | 215 | 118 | *T47-2*[d] | Zenger and Cooper (2001a) | 144 | 110 |
| *G16-2*[d] | Zenger and Cooper (2001b) | 71 | 87 | *Y105*[d] | Pope (pers. comm.)[j] | 37 | 32 |
| *G19-1*[d] | Zenger and Cooper (2001b) | 178 | 127 | *Y112*[d] | Pope (pers. comm.)[k] | 195 | 118 |
| *G20-2*[d] | Zenger and Cooper (2001b) | 141 | 119 | *Y148*[d] | Pope *et al.* (1996) | 100 | 123 |
| *G26-4*[d] | Zenger and Cooper (2001b) | 221 | 126 | *Y170*[d] | Pope *et al.* (1996) | 198 | 122 |
| *G31-1*[d] | Zenger and Cooper (2001b) | 182 | 119 | *Y175*[d] | Pope (pers. comm.)[l] | 163 | 106 |
| *G31-3*[d] | Zenger and Cooper (2001b) | 90 | 115 | *IL5*[a,d] | Hawken *et al.* (1999) | 77 | 34 |

Primer details for unpublished loci are indicated below with locus type and locus name. *AR*, androgen receptor; *CASA*, α-casein; *DBB*, MHC class II B-chain; *G6PD*, glucose-6-phosphate dehydrogenase; *HBB*, hemoglobin β-chain; *HPRT*, hypoxanthine phosphoribosyltransferase; *IL5*, interleukin 5; *LALBA*, α-lactalbumin; *LLP*, late lactation protein; *LPL*, lipoprotein lipase; *MPI*, mannose phosphate isomerase; *NP*, nucleoside phosphorylase; *RNR*, ribosomal RNA; *TF*, transferrin.

[a] Coding gene.
[b] Anonymous DNA marker.
[c] Pseudogene.
[d] Microsatellite marker.
[e] F-GGT AAA AGT AGC CAG GTA TGT CAT C and R-GTC TCA CTA ATG ACA CAG ACA TGC.
[f] F-AGA GGA TGA GGA GGA GAT AGA AGA G and R-TCT AGA GAT ATA TGC TCA ACT GG.
[g] F-ATG GTG GTC TTC GCA AGT TTG G and R-TCT GTG TAA GAG GGT GAA TGT CC.
[h] F-CCT TTG ATA GCA TGG GTT TAT T and R-TTA ACT TTG GAC TCT GCT ACC.
[i] Incorporates all male data.
[j] F-GGT AAT GAG TCA GTG TGA TGA GG and R-GGT AGG AGG AAA GGG AGA AAA G.
[k] F-CAT GTA CTG CTG AGA ATA GGC AC and R-CCT GGA GAA GTC TAT CTC CCA AC.
[l] F-TGG GAC ATT TCC TGA CCT AC and R-CCT CTT TAG GCT TCT TGA CCT AC.

**Recombination heterogeneity:** No significant deviations were observed between individuals for a particular recombination interval, providing evidence that the $F_1$ individuals are homogeneous within the sexes for recombination rates.

Sex-specific differences in recombination rates were evaluated for intervals, linkage groups, and over the entire map. Of the 37 intervals, 8 (22%) demonstrated significant sex-specific distortions ($P < 0.05$), all toward higher male recombination rates (Table 2). When investigating distortions over the linkage groups, five of the eight groups were significant ($P < 0.05$), with all significant groups incorporating at least 1 significant linkage interval (Table 2). Consequently, to determine

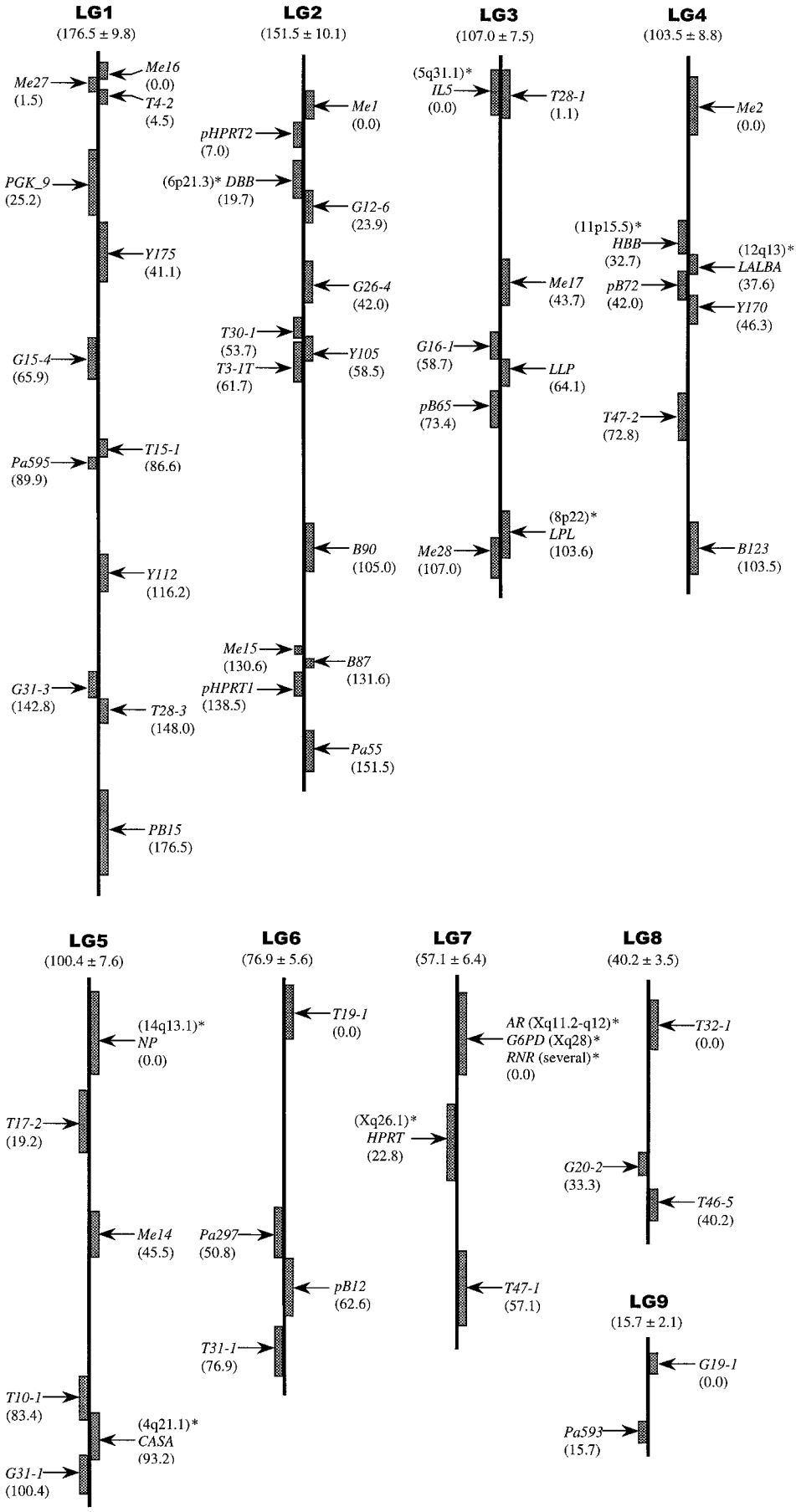


FIGURE 2.—Systematic representation of the sex-pooled multipoint linkage maps for LG1–9. Confidence limits ($Z_{max} - 1$) of maximum-likelihood locations for each locus are indicated in hatched bars. Distance (in centimorgans) of loci relative to first locus are indicated within parentheses as with total group map length (±SE). For comparison, human cytological positions of coding loci within linkage groups are indicated with an asterisk.

**TABLE 2**

**Significant likelihood-ratio test results for sex-recombination heterogeneity**

| Linkage group | Linkage intervals | Probability | *F/M* ratio |
|---|---|---|---|
| LG1 | *T4-2–Y175* | ** | 0.40 |
| | *T15-1–Pa595* | ** | 0.00 |
| | *G31-3–T28-3* | * | 0.22 |
| | Total | ** | 0.64 |
| | Total[a] | NS | 0.82 |
| LG2 | *T30-1–Y105* | * | 0.00 |
| | *T3-1T–B90* | * | 0.50 |
| | Total | * | 0.84 |
| | Total[a] | NS | 1.13 |
| LG3 | *T28-1–Me17* | * | 0.43 |
| | Total | * | 0.66 |
| | Total[a] | NS | 0.89 |
| LG6 | *T19-1–Pa297* | *** | 0.33 |
| | Total | *** | 0.49 |
| | Total[a] | NS | 1.02 |
| LG9 | *G19-1–Pa593* | * | 0.51 |
| Overall | Total | *** | 0.78 |
| | Total[a] | NS | 1.01 |

Calculated for linkage intervals, linkage groups, and overall.
[a] Calculated after removal of significant linkage intervals. NS, not significant. $0.01 < * < 0.05$; $0.001 < ** < 0.01$; $*** < 0.001$.

if these significant results were caused only by the significant intervals or from an overall distortion across the linkage groups, the significant linkage pairs were removed and the groups reanalyzed. No linkage groups were significant following the removal of the significant linkage intervals, indicating that the trend for higher male recombination occurs at specific intervals and not across the entire linkage group. This was also observed when the overall map length was investigated for sex-specific distortions (Table 2).

Although less informative due to sampling error, the *F/M* distance ratio was comparable to the likelihood ratio tests. Results indicated a *F/M* ratio distortion of 0.78 toward a larger male map compared to the female map when incorporating significant intervals. However, after removal of the eight significant sex-skewed intervals, the ratio was 1.01 with both sexes displaying equivalent overall map lengths (Table 2).

## DISCUSSION

**Genotyping:** This study describes the first comprehensive framework genetic linkage map for a marsupial, the tammar wallaby (*M. eugenii*). This map amends and extends previous tammar wallaby linkage data (McKenzie *et al.* 1993, 1996; Taylor and Cooper 1998) and contains 64 genetic markers, of which 43 are anonymous microsatellite markers, 14 are type-I coding genes, 6 are anonymous DNA markers, and 1 is a pseudogene.

The integrity of the genotypic data was high with an overall concordance of 98.4% when compared to duplicate typings and examination of Mendelian inheritance. Due to the relatively large amount of genetic divergence between the Kangaroo Island and Garden Island tammar wallaby populations (Oliver *et al.* 1979; Poole *et al.* 1991; McKenzie and Cooper 1997), it was a concern that null alleles may be present. However, examination of the pedigrees revealed only 2 female hybrid individuals and their offspring, which failed to amplify both alleles within locus *T28-1*. Considering that these null alleles were confined to only 2 of the 21 female hybrids, it is postulated that this is an individual characteristic rather than a hybrid effect.

**Segregation distortion:** Segregation distortions have been observed in numerous crosses between genetically divergent genomes (*e.g.*, Siracusa *et al.* 1991; Xu *et al.* 1997; Whitkus 1998; Chetelat *et al.* 2000). In this study, backcross progeny genotypic ratios were calculated to detect distortions in gametic proportions. Unfortunately, due to the nature of this backcross experimental design, it is not possible to distinguish between gametic selection and viability selection postfertilization. Consequently, in this study, segregation distortions were investigated to check for non-Mendelian inheritance of alleles and the accountability of these for errors in this mapping project. The segregation distortion analysis of the overall and sex-specific data revealed no skewed distortions from the expected Mendelian ratios. Consequently, the linkage map has not been compromised by segregation distortion arising from gametic selection or by postzygotic epistatic selection originating from the hybrid nature of the cross. This result also confirms the original work of McKenzie and Cooper (1997), who demonstrated hybridization between the two populations with no obvious loss of fitness.

**Map construction:** The number of markers within the sex-pooled data that showed significant linkages (LOD $> 3.0$) with at least one other marker was high (93.8%), suggesting that a large proportion of the genome had been mapped. The four markers (*G16-2*, *MPI*, *TF*, and *Y148*) that did not show any linkage with any other markers with the exception of *Y148* all had relatively low numbers of informative meioses and hence less power. The suggestion that a large proportion of the genome had been covered was strengthened when nine linkage groups of varying sizes were established (Figure 2). On the basis of the tammar wallaby karyotype ($n = 8$), these linkage groups almost certainly cover large proportions of all seven autosomes and the X chromosome. In fact, when comparing the estimated genome size of 1172 cM with 95% confidence intervals of 1145 and 1199 calculated from chiasmata data (Sharp 1984;

Sharp and Hayman 1988) to the total linkage map length of 828.4 ± 23.2 cM calculated in this study, ~71% of the total genome has been mapped. Interestingly, the estimated sex-average total map length of *M. eugenii* is considerably smaller when compared to sex-average human data (3600 cM; Ott 1999). Given that tammar wallabies have ~15% more DNA content than humans have (Hayman and Martin 1974), it is proposed that this difference reflects an overall lower *M. eugenii* recombination rate rather than a smaller physical genome size.

**Assignment of linkage groups:** All linkage groups excluding linkage group 7 can be placed on the autosomes on the basis of the Mendelian inheritance of markers. Using the inheritance pattern and physical assignment of genes, linkage group 7 is most certainly located on the X chromosome. In fact, all but locus *T47-1* has been physically assigned to this chromosome by *in situ* hybridization or somatic cell hybrids in *M. eugenii* or closely related species (Donald and Hope 1981; Robins *et al.* 1984; Dawson and Graves 1986; Spencer *et al.* 1991a,b).

Utilizing loci that have been physically assigned to *M. eugenii* autosomes, it can be proposed that linkage groups 2 and 3 reside on chromosome 1, while linkage group 4 is located on chromosome 3. The assignment of linkage groups 2 and 3 to chromosome 1 is derived from the *in situ* hybridization of the *DNA* gene, which is expected to closely flank the *DBB* gene (Slade *et al.* 1994), and from the location of the *IL5* gene anchored via fluorescence *in situ* hybridization (Hawken *et al.* 1999). Although another marker (*LLP*) was located within linkage group 3, which had been physically assigned by *in situ* hybridization using radiolabeled probes (Westerman *et al.* 1991), it was disregarded because of uncertain elements of its localization. The authors propose that, on the basis of grain density, *LLP* is located on chromosome 3, but they are unable to show clearly how they distinguished chromosome 3 from chromosome 4. However, they did attempt to distinguish them postlocalization by means of exclusion Southern blot mapping to hybrid cells, although this indicated only that *LLP* was not positioned on chromosome 4. Interestingly, when chromosome 3 was disregarded, the most likely position was on chromosome 1, as in this study. Evidence for the location of linkage group 4 on chromosome 3 is derived from the physical mapping of *HBB* performed by Sinclair and Graves (1991).

**X chromosome:** On the basis of Mendelian inheritance and *in situ* hybridization, five genetic markers (*AR*, *G6PD*, *RNR*, *HPRT*, and *T47-1*) have been assigned to the X chromosome (Figure 2). The gene order of these markers is p-{(*G6PD*, *RNR*, *AR*)–*HPRT*–*T47-1*}-q, with the most likely orientation being *G6PD*, *RNR*, and *AR* flanking the centromere, *HPRT* ~50% along the length of the long arm, and *T47-1* positioned distally on the long arm.

Data presented in this study indicate close linkage between *G6PD*, *RNR*, and *AR* on the basis of recombination rates. Previous work performed by McKenzie *et al.* (1996) suggests that this reduced or absent recombination may be a result of the genes being located closely together in heterochromatic regions flanking the centromere. Based on the *in situ* hybridization of *AR* and *RNR* in *M. eugenii*, this appears to be correct (Robins *et al.* 1984; Spencer *et al.* 1991b). Although *G6PD* has been tentatively positioned on the distal third of the long arm (Spencer *et al.* 1991a), *in situ* hybridization results on the closely related species *M. robustus* (Blair 1993) and somatic cell hybrid studies performed on *M. r. erubescens* and *M. giganteus* (Dawson and Graves 1986) contradict this placement and confirm the results in this study that *G6PD* is positioned near the centromere in close proximity to *AR* and *RNR*.

When incorporating all the data together, the linkage group presented for the X chromosome appears to be conserved in both synteny and gene order when compared to closely related species. The location of the *G6PD* and *HPRT* genes in *M. eugenii* within this linkage analysis is synonymous with *in situ* hybridization investigations in *M. robustus* (Blair 1993). In addition, somatic cell hybrid studies performed on *M. r. erubescens* and *M. giganteus* (Dawson and Graves 1986) indicate that *G6PD* forms a syntenic group with *HPRT* and *PGK1*, with both *G6PD* and *HPRT* located in analogous positions as indicated within this study. In fact, *PGK1* has also been localized to the same place in *M. eugenii* via *in situ* hybridization (Cooper *et al.* 1994).

**Recombination heterogeneity:** Sex-specific differences in recombination rates of *M. eugenii* were evaluated for intervals, linkage groups, and over the entire map. The data revealed significant distortions toward reduced female recombination rates in 8 of the 36 intervals (22%; Table 2). These were localized to specific intervals and were not a uniform characteristic across the linkage groups or across the entire map. In fact, when the 8 significant linkage intervals were removed from the analysis no significant distortions were discovered and the overall female/male map distance ratio was 1:1. This result is not unexpected and is consistent with other eutherian and marsupial linkage investigations (*e.g.*, Bennett *et al.* 1986; Ott 1999; Samollow *et al.* 2000), which also indicate interval sex-specific distortions.

The reduced rate of female recombination observed within this investigation is contrary to a previous *M. eugenii* linkage study that used small numbers of loci and individuals (McKenzie *et al.* 1995). Their results have since been amended within this investigation, with the disparity being attributable to the limited data in the earlier study. Consequently, there is now strong evidence for reduced female recombination when compared to males in *M. eugenii*. This sex-specific difference is also consistent in other marsupial linkage studies performed on *Sminthopsis crassicaudata* and *Monodelphis domestica* (Bennett *et al.* 1986; Samollow *et al.* 2000), but stands in contrast to eutherian studies where crossing over appears to be more frequent within the homogamatic sex

(XX). Accordingly, it can be postulated that reduced female recombination rates in marsupials may be of considerable antiquity, since the marsupial species investigated have been separated by as much as 60–80 million years (Springer *et al.* 1994; Kirsch *et al.* 1997). In addition, it can be suggested that other marsupial species may well show similar differences. Unfortunately, linkage data from more distantly related marsupial families and from distant mammalian groups (*i.e.*, monotremes) have yet to emerge.

Sex differences in recombination rates have been observed in numerous organisms (*e.g.*, mammals, insects, and birds). However, there has been no theoretical consensus regarding the mechanisms controlling these recombination differences. Early theories of Haldane (1922) and Huxley (1928) suggested that recombination tends to be reduced in the heterogametic sex. These views have been scrutinized on many occasions, both because there are some exceptions to the empirical generalizations and because the proposed explanations cannot account for the observed sex differences in many organisms (*e.g.*, Trivers 1988; Burt *et al.* 1991; Otto and Barton 1997; Barton and Charlesworth 1998; Lenormand and Otto 2000). However, these alternate hypotheses may attempt to provide improved explanations for the sex differences, but they all have failed to explain the differences seen within marsupials. In fact, most theorists ignore marsupial data altogether when developing models, either because previously there had been limited studies or because the data did not adhere to their hypotheses. Whichever of these is the case, marsupials must now be considered when evaluating theories regarding recombination differences between the sexes.

We are especially thankful to Lisa Pope and Craig Moritz for providing details of unpublished microsatellite loci. We are also grateful to Mark Eldridge and the two anonymous referees for reviewing this manuscript and providing much valued advice, Bill Poole for locating the original tammar wallaby hybrid pedigree data buried within archives for more than 10 years, Michael Baxter for compiling and debugging many of the programs used throughout this project, and Professor Trevor Tansley, Dean of Graduate Students, Macquarie University, and Professor D. Yerbury, Vice Chancellor, Macquarie University, for financial support.

## LITERATURE CITED

Archibald, A. L., C. S. Haley, J. F. Brown, S. Couperwhite, H. A. McQueen *et al.*, 1995 The PiGMaP consortium linkage map of the pig (*Sus scrofa*). Mamm. Genome **6:** 157–175.

Barton, N. H., and B. Charlesworth, 1998 Why sex and recombination? Science **281:** 1986–1990.

Bennett, J. H., D. L. Hayman and R. M. Hope, 1986 Novel sex differences in linkage values and meiotic chromosome behaviour in a marsupial. Nature **323:** 59–60.

Blair, I. P., 1993 X-linked genes in the kangaroo (*Macropus robustus*) by fluorescence *in situ* hybridisation. Honours Thesis, Macquarie University, Sydney, Australia.

Buddle, B. M., and L. J. Young, 2000 Immunobiology of mycobacterial infections in marsupials. Dev. Comp. Immunol. **24:** 517–529.

Burt, A., G. Bell and P. H. Harvey, 1991 Sex differences in recombination. J. Evol. Biol. **4:** 259–277.

Chetelat, R. T., V. Meglic and P. Cisneros, 2000 A genetic map of tomato based on BC(1) *Lycopersicon esculentum* × *Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. Genetics **154:** 857–867.

Conneally, P. M., J. H. Edwards, K. K. Kidd, J. M. Lalouel, N. E. Morton *et al.*, 1985 Report of the committee on methods of linkage analysis and reporting. Cytogenet. Cell Genet. **40:** 356–359.

Cooper, D. W., E. A. Holland, K. Rudman, J. A. Donald, R. Zehavi-Feferman *et al.*, 1994 Phosphoglycerate kinase pseudogenes in the tammar wallaby and other macropodid marsupials. Mamm. Genome **5:** 531–537.

Dawson, G. W., and J. A. M. Graves, 1986 Gene mapping in marsupials and monotremes. III. Assignment of four genes to the Z chromosome of the wallaroo and the euro (*Macropus robustus*). Cytogenet. Cell Genet. **42:** 80–84.

Donald, J. A., and R. M. Hope, 1981 Mapping a marsupial X chromosome using kangaroo-mouse somatic cell hybrids. Cytogenet. Cell Genet. **29:** 127–137.

Gellin, J., S. Brown, J. A. Marshall Graves, M. Rothschild, L. Schook *et al.*, 2000 Comparative gene mapping workshop: progress in agriculturally important animals. Mamm. Genome **11:** 140–144.

Gemmell, R. T., C. Veitch and J. Nelson, 2002 Birth in marsupials. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **131:** 621–630.

Haldane, J. B. S., 1922 Sex-ratio and unisexual sterility in hybrid animals. J. Genet. **12:** 101–109.

Hawken, R. J., P. Maccarone, R. Toder, J. A. Marshall Graves and J. F. Maddox, 1999 Isolation and characterization of marsupial IL5 genes. Immunogenetics **49:** 942–948.

Hayman, D. L., 1990 Marsupial cytogenetics. Aust. J. Zool. **37:** 331–349.

Hayman, D. L., and P. G. Martin, 1974 *Animal Cytogenetics, Vol. 4, Chordata 4.* Gebruder Bortraeger, Berlin.

Hinds, L. A., W. E. Poole, C. H. Tyndale-Biscoe, R. A. H. van Oorschot and D. W. Cooper, 1990 Reproductive biology and the potential for genetic studies in the tammar wallaby, *Macropus eugenii.* Aust. J. Zool. **37:** 223–234.

Hope, R. M., S. Cooper and B. Wainwright, 1990 Globin macromolecular sequences in marsupials and monotremes. Aust. J. Zool. **37:** 289–313.

Huxley, J. S., 1928 Sexual differences of linkage in *Gammarus chevreuxi.* J. Genet. **20:** 145–156.

Killian, J. K., T. R. Buckley, N. Stewart, B. L. Munday and R. L. Jirtle, 2001 Marsupials and eutherians reunited: genetic evidence for the Theria hypothesis of mammalian evolution. Mamm. Genome **12:** 513–517.

Kirsch, J. A. W., F. J. Lapointe and M. S. Springer, 1997 DNA-hybridisation studies of marsupials and their implications for metatherian classification. Aust. J. Zool. **45:** 211–280.

Kosambi, D. D., 1944 The estimation of map distances from recombination values. Ann. Eugen. **12:** 172–175.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

Lathrop, G. M., and J. M. Lalouel, 1988 Efficient computations in multilocus linkage analysis. Am. J. Hum. Genet. **42:** 498–505.

Lenormand, T., and S. P. Otto, 2000 The evolution of recombination in a heterogeneous environment. Genetics **156:** 423–438.

Lincoln, S. E., and E. S. Lander, 1992 Systematic detection of errors in genetic linkage data. Genomics **14:** 604–610.

McKenzie, L. M. 1994 Marsupial genetics studies in tammar, parma and black-striped wallabies: mapping, conservation and evolution. Ph.D. Thesis, Macquarie University, Sydney, Australia.

McKenzie, L. M., and D. W. Cooper, 1997 Hybridization between tammar wallaby (*Macropus eugenii*) populations from Western and South Australia. J. Hered. **88:** 398–400.

McKenzie, L. M., C. Collet and D. W. Cooper, 1993 Use of a subspecies cross for efficient development of a linkage map for a marsupial mammal, the tammar wallaby (*Macropus eugenii*). Cytogenet. Cell Genet. **64:** 264–267.

McKenzie, L. M., W. E. Poole, C. Collet and D. W. Cooper, 1995 Higher female than male recombination rates in a marsupial mammal, the tammar wallaby (*Macropus eugenii*). Cytogenet. Cell Genet. **68:** 64–66.

McKenzie, L. M., P. G. Johnston, M. D. Eldridge and D. W. Cooper, 1996 Close linkage between RNR and GPD genes on the tammar wallaby (*Macropus eugenii*) X chromosome. Cytogenet. Cell Genet. **72:** 197–199.

Miller, R. D., and K. Belov, 2000 Immunoglobulin genetics of marsupials. Dev. Comp. Immunol. **24:** 485–490.

Oliver, A. J., D. R. King and R. J. Mead, 1979 Fluoroacetate tolerance, a genetic marker in some Australian marsupials. Aust. J. Zool. **27:** 363–372.

Ott, J., 1999 *Analysis of Human Genetic Linkage*. The Johns Hopkins University Press, Baltimore.

Otto, S. P., and N. H. Barton, 1997 The evolution of recombination: removing the limits to natural selection. Genetics **147:** 879–906.

Pask, A., and J. A. Graves, 2001 Sex chromosomes and sex-determining genes: insights from marsupials and monotremes. EXS **91:** 71–95.

Pask, A., and M. B. Renfree, 2001 Sex determining genes and sexual differentiation in a marsupial. J. Exp. Zool. **290:** 586–596.

Piper, A. A., A. M. Bennett, L. Noyce, M. K. Swaton and D. W. Cooper, 1993 Isolation of a clone partially encoding hill kangaroo X-linked hypoxanthine phospho-ribosyltransferase: sex differences in methylation in the body of the gene. Somat. Cell Mol. Genet. **19:** 141–159.

Poole, W. E., J. T. Wood and N. G. Simms, 1991 Distribution of the tammar, *Macropus eugenii*, and the relationship of populations as determined by cranial morphometrics. Wildlife. Res. **18:** 625–639.

Pope, L. C., A. Sharp and C. Moritz, 1996 Population structure of the yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1854) inferred from mtDNA sequences and microsatellite loci. Mol. Ecol. **5:** 629–640.

Pope, L. C., A. Estoup and C. Moritz, 2000 Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. Mol. Ecol. **9:** 2041–2053.

Renfree, M. B., and G. Shaw, 2000 Diapause. Annu. Rev. Physiol. **62:** 353–375.

Renfree, M. B., and G. Shaw, 2001 Germ cells, gonads and sex reversal in marsupials. Int. J. Dev. Biol. **45:** 557–567.

Richardson, B. J., P. R. Baverstock and M. Adams, 1986 *Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies*. Academic Press, Sydney, Australia.

Righetti, P. G., 1983 *Isoelectric Focusing: Theory, Methodology and Applications*. Elsevier Biomedical Press, Amsterdam.

Robins, A. J., D. L. Hayman and J. R. E. Wells, 1984 Ribosomal gene reiteration in a marsupial species with an X-linked nucleolar organizer. Aust. J. Biol. Sci. **37:** 211–215.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Samollow, P. B., and J. A. M. Graves, 1998 Gene maps of marsupials. ILAR J. **39:** 203–224.

Samollow, P. B., C. M. Kammerer, J. L. VandeBerg and S. M. Mahaney, 2000 Linkage map development for the gray, short-tailed opossum, *Monodelphis domestica*. Gene Families Isozymes Bull. **33:** 45.

Schiex, T., and C. Gaspin, 1997 CARThAGENE: constructing and joining maximum likelihood genetic maps, pp. 258–267 in *Fifth International Conference on Intelligent Systems for Molecular Biology*. Porto Carras, Halkidiki, Greece.

Schmid, M., A. Fernández-Badillo, W. Feichtinger, C. Steinlein and J. I. Roman, 1988 On the highest chromosome number in mammals. Cytogenet. Cell Genet. **49:** 305–308.

Sharp, P. J., 1984 Cytological studies in Australian marsupials. Ph.D. Thesis, University of Adelaide, Adelaide, Australia.

Sharp, P. J., and D. L. Hayman, 1988 An examination of the role of chiasma frequency in the genetic system of marsupials. Heredity **60:** 77–85.

Sinclair, A. H., and J. A. Graves, 1991 Gene mapping in marsupials: detection of an ancient autosomal gene cluster. Genomics **9:** 581–586.

Siracusa, L. D., W. G. Alvord, W. A. Bickmore, N. A. Jenkins and N. G. Copeland, 1991 Interspecific backcross mice show sex-specific differences in allelic inheritance. Genetics **128:** 813–821.

Slade, R. W., P. T. Hale, D. I. Francis, J. A. Graves and R. A. Sturm, 1994 The marsupial MHC: the tammar wallaby, *Macropus eugenii*, contains an expressed DNA-like gene on chromosome 1. J. Mol. Evol. **38:** 496–505.

Sokal, R. R., and F. J. Rohlf, 1995 *Biometry: The Principles and Practice of Statistics in Biological Research*, Ed. 3. W. H. Freeman, New York.

Spencer, J. A., J. M. Watson and J. A. Graves, 1991a The X chromosome of marsupials shares a highly conserved region with eutherians. Genomics **9:** 598–604.

Spencer, J. A., J. M. Watson, D. B. Lubahn, D. R. Joseph, F. S. French *et al.*, 1991b The androgen receptor gene is located on a highly conserved region of the X chromosomes of marsupial and monotreme as well as eutherian mammals. J. Hered. **82:** 134–139.

Spencer, P. B., 1996 Coping with a naturally fragmented environment: a genetic and ecological study of the Allied rock-wallaby, *Petrogale assimilis*. Ph.D. Thesis, James Cook University, Townsville, Australia.

Spencer, P. B., D. M. Odorico, S. J. Jones, H. D. Marsh and D. J. Miller, 1995 Highly variable microsatellites in isolated colonies of the rock-wallaby (*Petrogale assimilis*). Mol. Ecol. **4:** 523–525.

Springer, M. S., M. Westerman and J. A. W. Kirsch, 1994 Relationship among orders and families of marsupials based on 12S ribosomal DNA sequences and the timing of the marsupial radiation. J. Mamm. Evol. **2:** 85–115.

Sunnucks, P., and D. F. Hales, 1996 Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus Sitobion (Hemiptera: Aphididae). Mol. Biol. Evol. **13:** 510–524.

Sunnucks, P., A. C. Wilson, L. B. Beheregaray, K. R. Zenger, J. French *et al.*, 2000 SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Mol. Ecol. **9:** 1699–1710.

Taylor, A. C., and D. W. Cooper, 1998 A set of tammar wallaby (*Macropus eugenii*) microsatellites tested for genetic linkage. Mol. Ecol. **7:** 925–926.

Taylor, A. C., W. B. Sherwin and R. K. Wayne, 1994 Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. Mol. Ecol. **3:** 277–290.

Trivers, R., 1988 Sex differences in rates of recombination and sexual selection, pp. 270–286 in *The Evolution of Sex: An Examination of Current Ideas*, edited by R. E. Michod and B. R. Levin. Sinauer Associates, Sunderland, MA.

van Oorschot, R. A., and D. W. Cooper, 1990 A conserved linkage between PI and GPI in a marsupial, the tammar wallaby (*Macropus eugenii*). Tenth international workshop on human gene mapping. Cytogenet. Cell Genet. **51:** 1097.

Vogl, C., and S. Xu, 2000 Multipoint mapping of viability and segregation distorting loci using molecular markers. Genetics **155:** 1439–1447.

Waters, P. D., P. J. Kirby and J. A. M. Graves, 2001 Assignment of the SMARCIF1 gene to tammar wallaby chromosome 5q by fluorescence in situ hybridisation. Cytogenet. Cell Genet. **93:** 315–316.

Westerman, M., J. Spencer and C. Collet, 1991 Chromosomal localization of the gene for late lactation protein (LLP) in the tammar wallaby (*Macropus eugenii*). Cytogenet. Cell Genet. **56:** 182–184.

Whitkus, R., 1998 Genetics of adaptive radiation in Hawaiian and Cook Islands species of Tetramolopium (Asteraceae). II. Genetic linkage map and its implications for interspecific breeding barriers. Genetics **150:** 1209–1216.

Wurster, D. H., and K. Benirschke 1970 Indian muntjac, *Muntiacus muntjak*: a deer with a low diploid chromosome number. Science **168:** 1364–1366.

Xu, Y., L. Zhu, J. Xiao, N. Huang and S. R. McCouch, 1997 Chromosomal regions associated with segregation distortion of molecular markers in $F_2$, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa L.*). Mol. Gen. Genet. **253:** 535–545.

Zenger, K. R., and D. W. Cooper, 2001a Characterization of 14 macropod microsatellite genetic markers. Anim. Genet. **32:** 166–167.

Zenger, K. R., and D. W. Cooper, 2001b A set of highly polymorphic microsatellite markers developed for the eastern grey kangaroo (*Macropus giganteus*). Mol. Ecol. Notes **1:** 98–100.

Communicating editor: N. Jenkins