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Intraspecific variation, sex-biased dispersal and phylogeography of the eastern grey kangaroo (*Macropus giganteus*)

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Genetic information has played an important role in the development of management units by focusing attention on the evolutionary properties and genetics of populations. Wildlife authorities cannot hope to manage species effectively without knowledge of geographical boundaries and demic structure. The present investigation provides an analysis of mitochondiral DNA and microsatellite data, which is used to infer both historical and contemporary patterns of population structuring and dispersal in the eastern grey kangaroo (*Macropus giganteus*) in Australia. The average level of genetic variation across sample locations was one of the highest observed for marsupials ($h = 0.95$, $H_E = 0.82$). Contrary to ecological studies, both genic and genotypic analyses reveal weak genetic structure of populations, where high levels of dispersal may be inferred up to 230 km. The movement of individuals was predominantly male-biased (average $N_em = 22.61$, average $N_fm = 2.73$). However, neither sex showed significant isolation by distance. On a continental scale, there was strong genetic differentiation and phylogeographic distinction between southern (TAS, VIC and NSW) and northern (QLD) populations, indicating a current and/or historical restriction of gene flow. In addition, it is evident that northern populations are historically more recent, and were derived from a small number of southern founders. Phylogenetic comparisons between *M. g. giganteus* and *M. g. tasmaniensis* indicated that the current taxonomic status of these subspecies should be revised as there was a lack of genetic differentiation between the populations sampled.
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Keywords: kangaroos; genetic diversity; dispersal; mtDNA; microsatellites

Introduction

Many native species pose a difficult challenge for wildlife managers, and lead to a dichotomy in population management strategies. Many Australian marsupial species have declined because of habitat loss and fragmentation, competition from introduced herbivores and predation by introduced carnivores, while many others have become locally overabundant. Regardless of whether a species is considered as a natural resource to be conserved or a pest to be controlled, a species cannot be managed effectively without knowledge of its biological boundaries and demic structure. Traditional ecological approaches for establishing population management strategies use intensive fieldwork and monitoring of marked individuals to provide detailed information on current demography, rate of recruitment and movement. However, there are significant limitations associated with these approaches (Moritz and Lavery, 1996): (1) ecological studies are usually a snapshot in time, and usually do not encompass stochastic environmental effects that have significant long-lasting effects on community organization; (2) ecological studies are often very limited in space, because they are focused on a small part of the range of the species or community and (3) ecological studies based on marked individuals may be restricted to specific age, sex or size classes to which accessibility is the easiest.

The recent application of high-resolution genetic markers has proven to be a highly effective tool in the investigation of evolutionary processes and population dynamics within species (Sunnucks, 2000). While traditional ecological studies have yielded invaluable insights into behavioral patterns in animal populations, molecular markers have advanced our ability to identify new information about the biology of the species including; genetic structure, dispersal, social structure and identification of individuals within unique populations.

The eastern grey kangaroo (*Macropus giganteus*) was one of the first kangaroo species to be described by members of Captain James Cook's expedition in 1770 (Frith and Calaby, 1969). *M. giganteus* is also one of the largest and most commonly observed macropod species, having a wide and almost continuous distribution down the east coast of Australia, where annual rainfall is more than 250 mm (Figure 1; Poole, 1982). Two subspecies are recognized, based on differences in skull morphology and pelage; *M. g. giganteus* from mainland Australia (QLD, NSW, SA and VIC), and *M. g. tasmaniensis* from northeast Tasmania (TAS) (Figure 1; Kirsch and Poole, 1972). Although these subspecies have been distinguished by morphological differences, no genetic data exist to test these classifications.

Group home ranges have been identified from a handful of ecological studies for *M. giganteus* (Kaufmann, 1975; Jarman and Taylor, 1983; Johnson, 1989; Jaremovic and Croft, 1991), and comprehensive informa-

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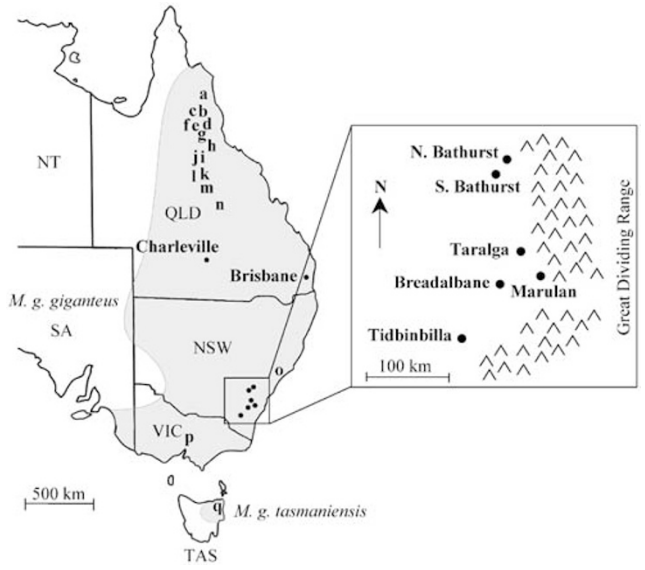

Figure 1 Map of Australia showing geographical distribution (from Poole, 1995) and sampling locations of *M. giganteus*. Populations within 'box' signify samples used to determine fine-scale population structure and dispersal. Sample locations identified by a 'filled circle' constitute populations used to determine broad-scale population genetic differentiation (refer to Table 1 for sample sizes). Sample localities represented by letters constitute additional individuals used in mtDNA variability and phylogeography analysis. Sample sizes for these are; a = 1, b = 1, c = 2, d = 2, e = 1, f = 1, g = 2, h = 1, i = 1, j = 1, k = 2, l = 1, m = 2, n = 1, o = 6, p = 3 and q = 10.

tion of dispersal is sketchy and unbalanced. However, from these limited studies, females have demonstrated strong site fidelity where they tend to live out their lives close to female relatives, while males range widely with observed range length increasing with the size of the animal, probably reflecting their feeding needs or their greater reproductive activity. In general, these data suggest that both sexes are relatively sedentary, with only a few individuals dispersing distances up to 17 km.

M. giganteus groups have been shown to vary in size and sex/age composition, throughout different parts of Australia (Jarman and Taylor, 1983; Johnson, 1989; Jaremovic and Croft, 1991), which is partly related to habitat structure and resource availability. Populations have now become overabundant in many parts of Australia including rural areas and nature reserves (Hill *et al*, 1988; Coulson, 1998; Coulson *et al*, 1999).

Management of wildlife populations can be divided into three areas: pest control, conservation and sustained yield harvesting (Caughley and Sinclair, 1994). Whichever is the case, the management of *M. giganteus* requires population monitoring and a comprehensive understanding of the biology of the animal. This project aims to provide analysis of genetic data, which will be used to infer both historical and contemporary patterns of population structuring and dispersal.

Methods

Sample collection and DNA extraction

Detailed information on contemporary population structure and dispersal patterns was obtained using six southern New South Wales (NSW) populations sampled at increasing distances. In addition, individuals from two localities within southern Queensland (QLD) were also sampled to evaluate broad-scale population genetic differentiation (Figure 1). At each location, animals were sampled within a discrete area of approximately 3 km^2, which typically contained several social groups. To investigate species diversity and phylogeography, additional *M. giganteus* ear tissue samples were opportunistically obtained from 24 localities throughout the range of *M. g. giganteus* and from one population of *M. g. tasmaniensis* (Figure 1). DNA extractions were carried out according to the 'salting-out' procedure described in Sunnucks and Hales (1996).

Microsatellite amplification and screening

Microsatellite genetic diversity was assessed using 10 heterologous macropodid loci derived from the tammar wallaby (*M. eugenii*), Me14, Me17, Me28, T19-1 and T46-5 (Taylor and Cooper, 1998; Zenger and Cooper, 2001a); allied rock-wallaby (*Petrogale assimilis*), Pa55, Pa297 and Pa597 (Spencer *et al*, 1995; Spencer, 1996) and yellow-footed rock-wallaby (*P. xanthopus*), Y148 and Y151 (Pope *et al*, 1996). Microsatellite loci were amplified via PCR in 10 µl reaction volumes containing; 100–200 ng of genomic DNA, 2.5 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 dCTP, dGTP and dTTP, 20 µM dATP, 0.05 µl of [α^{33}-P]dATP at 1000 Ci/mmol, 1.0 µM of each primer and 0.5 U *Taq* polymerase (Qiagen). PCR amplifications were carried out using an MJ Research PTC100 thermocycler, with an initial 94°C denaturation for 3 min, followed by 'touchdown' cycles of 94°C denaturation for 30 s, annealing temperatures (60, 58, 56, 54, 52 and 50°C) for 45 s and an extension step of 72°C for 1 min. On completion of the last touchdown cycle another 30 cycles were performed at 50°C annealing temperature with a final extension of 72°C for 5 min. The amplified PCR products were resolved on 6% denaturing polyacrylamide gels with a standardized DNA size reference marker and visualized by autoradiography according to Taylor *et al* (1994).

Mitochondrial DNA amplification and screening

Mitochondrial DNA (mtDNA) control region variation was revealed using the single-stranded conformation polymorphism (SSCP) technique (Sunnucks *et al*, 2000). The mtDNA control region was amplified by PCR using marsupial-specific primers (mt15999L and mt16498R; Fumagalli *et al*, 1997). PCR reactions were carried out as described above, except that a single annealing temperature of 60°C was used. The amplified PCR products were resolved on a nondenaturing polyacrylamide gel according to Sunnucks *et al* (2000). To investigate sequence differences among the unique mtDNA haplotypes, new PCR products (devoid of [α^{33}-P]dATP) from three independent individuals for each identified haplotype were purified, sequenced using BigDye termination (Perkin-Elmer Applied Biosystems) and resolved on an ABI 377 sequencer. In addition, three western grey kangaroo (*M. fuliginosus*) individuals were sequenced to provide a comparative outgroup in the phylogenetic analysis. All resultant sequences have been

deposited in GenBank under accession numbers AF443122 to AF443175.

Diversity indices

Allelic diversity (A) and heterozygosity (observed H_O and Hardy–Weinberg expected H_E) of the microsatellite loci were estimated using the BIOSYS program (Swofford and Selander, 1981). Exact tests for Hardy–Weinberg equilibrium and genotypic linkage disequilibrium were calculated across all microsatellite loci using the Markov chain method with 1000 iterations calculated in GENEPOP version 3.1 (Raymond and Rousset, 1995). The level of inbreeding and/or the presence of null alleles were assessed by measuring F_{IS} and its significance (10 000 permutations) for all loci within all populations using FSTAT version 2.9.1 (Goudet, 2000). When performing multiple simultaneous comparisons, the sequential Bonferroni procedure (Rice, 1989) with $\alpha = 0.05$ was used to adjust the statistical significance level.

Estimates of mtDNA haplotypic diversity (h) and nucleotide diversity (π) within populations and nucleotide divergence (d_A) among populations were calculated using the software package REAP (McElroy *et al*, 1991). Divergence at the sequence level was calculated using the Kimura 2-parameter (K2P) genetic distance (Kimura, 1980) with a gamma distribution value of $\alpha = 0.5$ (Wakeley, 1993) within MEGA version 2.1 (Kumar *et al*, 2001). Evidence for population expansion was evaluated using the mismatch distribution analysis (Schneider and Excoffier, 1999) calculated in ARLEQUIN version 2.0 (Schneider *et al*, 1997).

Population structure

Levels of genetic structure derived from microsatellite data were evaluated using both genic and genotypic methods. Firstly, pairwise values of θ_{ST} (an unbiased estimator of F_{ST}) and their significance were calculated using FSTAT version 2.9.1 (Goudet, 2000), incorporating 10 000 permutations. Secondly, mutilocus genotype data were used to infer population structure via a model-based Bayesian clustering method implemented within the STRUCTURE software package of Pritchard *et al* (2000). The results presented are based on a modeling approach to infer the number of populations (K), deduced by posterior probability using 10^6 iterations following a burn-in period of 30 000 simulations. Finally, overall and sex-specific pairwise relatedness values were compared within and among populations to investigate contemporary fine-scale structuring and sex-biased dispersal. All possible pairwise relatedness values were calculated using the relatedness coefficient (R) within the program KINSHIP version 1.2 (Goodnight *et al*, 1998). The significance of within- versus among population relatedness and male versus female relatedness was calculated using a resampling statistic (10 000 permutations), performed within the program RESAMPLING STATS version 5.0.2 (Simon, 1999).

Patterns of mtDNA geographical structuring were examined using two approaches. Firstly, pairwise Φ_{ST} values (K2P with gamma at 0.5) were calculated with associated significant levels (10 000 permutations) across all populations using the ARLEQUIN version 2.0 package (Schneider *et al*, 1997). Secondly, a nested hierarchical analysis of genetic differentiation was calculated using the AMOVA application (Excoffier *et al*, 1992) performed within the ARLEQUIN version 2.0 package (Schneider *et al*, 1997). This program estimates the proportion of total genetic variation within and among the different hierarchical levels based on distribution of haplotypes and pairwise distances. This analysis was calculated using three models: (i) NSW populations as one group; (ii) QLD populations as one group and (iii) NSW and QLD populations combined as two groups.

Gene flow and geographic distance

Pairwise distance values were used to estimate the number of individuals that migrate between each pair of sampling locations per generation. The relations $N_e m = [(1/\theta_{ST}) - 1]/4$ was used to estimate the net migration rates using microsatellite nuclear markers (Wright, 1951), while $N_f m = [(1/\Phi_{ST}) - 1]/2$ was used to investigate the relative amount of female migration using mtDNA data (Slatkin, 1993; Baker *et al*, 1994).

Analyses of correlation between gene flow and geographic distances were carried out using the Mantel (1967) permutation test executed by the ISOLDE routine (10 000 permutations) in GENEPOP 3.1 (Raymond and Rousset, 1995). All pairwise genetic distance indices including microsatellite θ_{ST}, mtDNA Φ_{ST} and mean relatedness values (R) (male and female) were compared with geographical distance (km) between localities.

Phylogenetic analysis

Evolutionary relationships among the mtDNA haplotypes were inferred using both Neighbor-Joining (NJ) distance and Maximum-Parsimony (MP) methods for phylogenetic reconstruction calculated in PAUP 4.0b8 (Swofford, 2000). The NJ distance method used the genetic distance as previously described (see Diversity indices), while a heuristic search with gaps identified as a fifth state was utilized in the MP method. Statistical support for the branching topology was estimated by bootstrap replications of 1000 for both methods. Both methods used *M. fuliginosus* sister taxa sequences as the outgroup.

Results

Microsatellite genetic variability

A total of 171 individuals (76 males and 95 females) were genotyped from eight localities (Table 1) using the 10 microsatellite loci. All of the loci were highly polymorphic and exhibited eight to 23 alleles. Two of the loci (Me14 and Y151) indicated the presence of null alleles during genotyping (ie, faint and missing bands). This was verified by large positive F_{IS} values (0.129 and 0.130 respectively) that deviated significantly ($P < 0.001$) from Hardy–Weinberg equilibrium. Both of these loci were subsequently removed from the remainder of the analysis. There was no evidence of linkage disequilibrium between any of the loci.

Allelic diversity (A) and heterozygosity (H_O and H_E) across the eight remaining microsatellite loci averaged 5.6–10.4 alleles per locus, with observed heterozygosity ranging from 0.766 to 0.852 (Table 1). Under Hardy–Weinberg equilibrium only Marulan demonstrated a significant departure ($P < 0.05$) with a positive F_{IS} value (0.078), indicating a mild Wahlund effect (Table 1).

Table 1 Microsatellite and mtDNA diversity indices for each population.

Sample location (n)	*Microsatellite diversity*				*mtDNA diversity*		
	A (±*se*)	H_O (±*se*)	H_E (±*se*)	F_{is}	*No. haplotypes*	% *h* (±*sd*)	% π
TID (48)	10.4 (±1.4)	0.773 (±0.024)	0.810 (±0.027)	0.046	7	70.68 (±4.55)	2.50
BRE (29)	10.1 (±1.3)	0.789 (±0.044)	0.799 (±0.046)	0.013	5	59.61 (±9.50)	0.70
MAR (15)	8.9 (±1.1)	0.781 (±0.053)	0.845 (±0.034)	0.078*	8	91.21 (±4.86)	2.68
TAR (11)	7.4 (±0.9)	0.807 (±0.032)	0.837 (±0.029)	0.037	5	76.36 (±10.66)	2.03
S. BA (35)	9.6 (±1.3)	0.814 (±0.050)	0.819 (±0.034)	0.005	7	76.11 (±4.34)	2.57
N. BA (11)	8.1 (±1.2)	0.852 (±0.064)	0.827 (±0.055)	−0.032	4	69.09 (±12.76)	1.80
BRI (10)	5.6 (±0.7)	0.766 (±0.064)	0.781 (±0.041)	0.021	5	85.71 (±10.82)	1.01
CHA (12)	8.9 (±1.0)	0.813 (±0.078)	0.822 (±0.048)	0.012	6	85.45 (±8.52)	0.81

Sample size is shown in parentheses next to sample location. Average number of alleles (*A*), average observed and expected heterozygosity (H_O and H_E) and F_{IS} shown for microsatellite data after omitting loci with null alleles. Number of unique haplotypes, haplotypic diversity (*h*) and nucleotide diversity (π) shown for mtDNA data. TID=Tidbinbilla, BRE=Breadalbane, MAR=Marulan, TAR=Taralga, S. BA=South Bathurst, N. BA=North Bathurst, BRI=Brisbane and CHA=Charleville. *$P<0.05$ for Hardy–Weinberg deviation.

mt DNA variability

Estimates of mtDNA diversity within and between populations were derived from eight localities incorporating between 10 and 48 individuals (Table 1). Haplotypic diversity (*h*) and nucleotide diversity (π) within populations averaged 76.78 and 1.76%, respectively (Table 1). Nucleotide divergence (d_A) among populations ranged between 0.06 and 3.85%. Hierarchical comparisons revealed that the greatest nucleotide divergence occurs between geographical regions (NSW and QLD; mean = 2.90%, SD = 0.53%), while comparisons within regions demonstrated the least divergence (mean = 0.46%, SD = 0.35%). On average, there was five times the amount of nucleotide diversity within populations as among populations in the same geographic region.

Divergence at the sequence level was derived from 51 haplotypes obtained from 202 individuals sampled across the range of *M. giganteus* (Figure 1). Of 648 bp of sequence, 81 sites (12.5%) were variable, 68 of which were parsimony informative (Figure 2). Haplotypic diversity (*h* ± SD) across the species range was extremely high (94.83 ± 0.66%). Sequence divergence between all haplotypes varied from 0.16% to 6.88% with a mean (±SE) of 3.33 ± 0.50%. When comparing subspecies, there was more sequence divergence within *M. g. giganteus* (mean 3.36 ± 0.50%), than between the taxa (mean 2.99 ± 0.53%). *M. g. giganteus* haplotypes within southern Australia (NSW and VIC) demonstrated the greatest mean sequence divergence (3.23 ± 0.48%), while haplotypes within northern Australia (QLD) shared the least (0.86 ± 0.17%). The mean sequence divergence between the two regions was high (4.49 ± 0.74%). Mean sequence divergence between *M. giganteus* and *M. fuliginosus* was 14.96 ± 1.97%.

Population structure

Both pairwise θ_{ST} and Φ_{ST} values revealed significant differences ($P<0.05$) between populations both before and after Bonferroni correction, with θ_{ST} results varying between –0.0037 and 0.0967 and Φ_{ST} values ranging from 0.0395 to 0.8511 (Table 2). Not surprisingly, comparisons between QLD and NSW populations produced the greatest differences, while pairwise comparisons involving Marulan and N. Bathurst on average demonstrated the least significant differences (Table 2).

The model-based clustering method, for inferring the number of populations (*K*) based on posterior probability (Pritchard *et al*, 2000), indicated that samples from NSW and QLD could only be assigned to two separate clusters ($P = 0.999$). When analyzing each of these groups separately, both produced clustering patterns consistent with a single population ($P = 0.999$). For both analyses, all other values of *K* were deemed highly improbable ($P<0.0001$). Consequently, no structure could be inferred among the sampled populations within NSW or QLD.

Analysis of mtDNA differentiation using the nested analysis of variance approach (Excoffier *et al*, 1992) revealed that 49% of total molecular variance ($\Phi_{CT} = 0.486$; $P<0.05$) was partitioned between NSW and QLD when analyzed together as separate groups. In addition, the analysis distributes 40% of the variation ($\Phi_{ST} = 0.594$; $P<0.01$) within populations, while the remaining 11% was among populations within groups ($\Phi_{SC} = 0.211$; $P<0.01$). When analyzing NSW and QLD groups separately, the majority (>79%) of the variation was distributed within populations ($\Phi_{ST} = 0.214$ and 0.148, respectively; $P<0.01$).

Based on the differentiation between NSW and QLD populations and the small number of sites sampled in QLD, the remaining genotypic analyses were confined to the six NSW populations. Female pairwise relatedness analysis demonstrated a marginal but significant ($P<0.001$) greater mean within-population relatedness when compared to mean among-population relatedness, while males demonstrated none ($P = 0.212$; Figure 3). Although it is evident that females have a higher within-population relatedness values than males (0.042 and 0.026 respectively), there was no significant difference between them ($P = 0.402$). In general, mild structuring was only evident for females, with all mean relatedness values only slightly deviating from zero (Figure 3). These results are suggestive of females having higher site fidelity.

Gene flow and geographical distance

The average number of migrants (N_em) among NSW populations was high at 22.61 individuals per generation, with a range of 8.17–59.30 (excluding infinity; Table 2). When examining the average number of

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            Variable sequence position
                 1111111122222222222333333333333333333333333333333333344444444444445555555556666
            2559945566789000567778880001112333333344555566677788888990000111236770111112223612
Haplotype   6271825658099027760680343794597045678937235757828924689152348236345589234602814 62
N1          TTTTTACCCTTTCCTATGATACCTATTTCTAAGAACTTCAC-ACTCCACCTACGTTCCGCATGACTCTCTTCTTTATCTAC
N2          .....................................T-.........................T.............
N3          ......................................-.............................C.......
N4          .....G..............................C...-.........................T.............
N5          ....................................G.A....T.TT......T...........T.............
N6          ..........C.T.........................-.....................................
N7          ................A...........C...........-.T....T..............T................
N8          ........................C.............-....T.....TA..........................
N9          ..................C.....................-...........A..T....C..................
N10         ..................CG...................T-...........A..T....C..................
N11         ................A.....................-....T.................C...............
N12         ..............C.......................-...................A.T.............C..
N13         ..........C.............................-.......T.............................
N14         ..........C......A......................-.....................................
N15         ..............C.......................-.....................................C.......
N16         ......................................-.............T.......T.....................
N17         ......................................-.....................................T.............
N18         ............................G.....C....-......T.......T.................C.......
N19         ............................G.....C....-......T.......T.....................
N20         ......................................-.....................................T...
N21         ..............................G.......-.............T.......T.....................
N22         ......................................-.....................................CG......
N23         ......................................-........C...C...........T......C.......
S1          ..............GCAG...T....C.............--.C.T..T..GA..T.......T.......C.......T
S2          .........C......CA....TC..C.TCG.A..TC.T.TT-.CT..TTC.G...TTAT....T.............T...
S3          .........C......CA....TC..C.TCG.A..TC...TT-.CT..TTC.GA..TTAT....T.............T.G.
S4          .......TTC..T...CA....TC..C.TCG.A...C.T.TT-.C...TTC.G...TTAT....TC..T....C.G.T...
S5          ...C..T..C..T...CA....TC......G.A...C.T..C-.C.T..TC.G.CCTTAT....T...T..T....CT...
S6          ...C..T..C..T...CA...TTC......G.A...C.T.TC-.C.T..TC.G.C.TTAT....T...T.CT.....T...
S7          ..............GCA....T....C......G..C...--.C.T..T..GA..T.......T.......C.......T
S8          .........C......CA....TC..C.TCG.A..TC...TT-.C...TTC.GA..TTAT....T.............T.G.
S9          ...CC.T..C..T...CA....TC......G.A...C.T..C-.C.T..TC.G.CCTTAT....T...T..T....CT...
S10         .......T.C..T...CA..G.TC..C.TC..A...C.T.TT-.C...T...G...TTAT....TC.......C...T...
S11         .........C......CA....TC..C.TCG.A..TC.TGTT-.CT..TTC.G...TTAT....T.............T...
S12         .......T.C..T...CA....TC..C.TCG.A...C.T.TT-TC...TTC.G...TTAT....TC..T....C...T...
S13         .CCC..T..CC.T...CA...TT.G.....G.....C.T.TC-.C.T..TC.AA..TTAT....T...T.CT.....T...
S14         ...C..T..C..T.C.CA...TTC......G.A...C.T.TC-.C.TG.TC.G...TTATG..GT..CT.CT.....T...
S15         .....G...C......CA....TC..C.TCG.A..TC...TT-.CT..TTC.GA..TTAT....T.............T.G.
S16         ...C..T..C..T.C.CA....TC......GGA.G.C.T.TC-.C.TG.TCGG...TTATG..GTC..T.CT.....T...
S17         ...C..T..C..T.C.CA...TTC......G.A...C.T.TC-.C.TG.TC.G...TTATG..GT...T.CT.....T...
S18         .................CA....T..C....G.....C....--.C.T..T...A..T.....A.TC...C..C.....C..
S19         .......TTC..T...CA....TC..C.TCG.A...C.T.TT-.C...TTC.G..CTTAT....TC..T....C...T...
S20         ..............GCA....T....C.............--.C.T.TT..GA..T.......T.......C.......T
S21         C.............GCAG...T....C.............--.C.T..T..GA..T.......T.......C.......T
S22         .........C......CA....T...C.TCG.A..TC.T.TT-.CT..TTC.G...TTAT....T.............T...
S23         ...C..T..C..T...CA...TTC......G.A...C.T.TC-.C.T..TCGAA..TTAT..A.T...T..T.....T...
S24         .......T.C..T...CA..G.TC..C.TCG.A...C.T.TC-.C...T...G...TTAT....TC.......C...T...
S25         ..............GCA....T....C..G.......T..--.C.T..T..GA..T.......T.......C.......T
S26         .................CA....T..C..........C....--.C.T..T...A..T.......T....C..C.....C..
T1          ..............GCA....T....C..G..........--...T.TT..G...T.......T.......C.......T
T2          ..............GCA....T....C..G..........--...T..T..G...T.......T.......C.......T
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Figure 2 Variable sites of a 648-bp segment of mtDNA control region from 202 eastern grey kangaroos. Variable nucleotide positions are relative to the beginning of the sequence. Dots represent identical bases to the first haplotype. Haplotypes are labeled relative to their geographical region, that is, 'northern' (eg, N1), 'southern' (eg, S1) or 'Tasmanian' (eg, T1).

female immigrants per generation within NSW, $N_f m$ was 2.73 with a range of 0.60–12.16 (Table 2). Although females demonstrate smaller migration rates compared to the sexes combined, the values are still comparatively high. These results are consistent with previous data, which suggest little genetic differentiation and sex-biased dispersal among *M. giganteus* populations. As expected, comparisons between NSW and QLD populations produced the smallest values (Table 2).

Analysis of isolation by distance across NSW using the Mantel test, revealed no significant relation between any of the three pairwise genetic distance indices (θ_{ST}, Φ_{ST} and R) and geographical distance ($P = 0.25$, 0.88 and 0.74, respectively). When comparing pairwise relatedness

Table 2 Differentiation between populations

	TID	*BRE*	*MAR*	*TAR*	*S. BA*	*N. BA*	*BRI*	*CHA*
(a) *Pairwise Φ_{ST} values for mtDNA data below diagonal and pairwise θ_{ST} for microsatellite data above diagonal*								
TID	—	0.0206*	0.0155*	0.0184*	0.0154*	0.0135*	0.0915*	0.0485*
BRE	0.2440*	—	0.0130*	0.0212*	0.0289*	0.0102	0.0967*	0.0530*
MAR	0.1340*	0.2846*	—	0.0065*	0.0047*	−0.0037	0.0690*	0.0370*
TAR	0.0395	0.2552*	0.1533*	—	0.0297*	0.0129	0.0752*	0.0487*
S. BA	0.1685*	0.4531*	0.1299*	0.2883*	—	0.0042	0.0700*	0.0342*
N. BA	0.0729	0.0883	0.0875	0.0970	0.2368*	—	0.0809*	0.0413*
BRI	0.4977*	0.8406*	0.5815*	0.6601*	0.4762*	0.6640*	—	0.0406*
CHA	0.5271*	0.8511*	0.6215*	0.6962*	0.5119*	0.7013*	0.1386*	—
(b) *Pairwise number of migrants per generation between populations for mtDNA data ($N_f m$) below diagonal and for microsatellite data ($N_e m$) above diagonal*								
TID	—	11.88	15.88	13.34	15.94	18.26	2.48	4.90
BRE	1.55	—	18.98	11.54	8.40	24.26	2.33	4.47
MAR	3.23	1.25	—	38.21	52.95	Inf.	3.37	6.50
TAR	12.16	1.46	2.76	—	8.17	19.44	3.07	4.88
S. BA	2.47	0.60	3.35	1.23	—	59.30	3.32	7.06
N. BA	6.36	5.16	5.21	4.65	1.61	—	2.84	5.80
BRI	0.50	0.09	0.36	0.26	0.55	0.25	—	5.91
CHA	0.45	0.09	0.30	0.22	0.48	0.21	3.11	—

*Significance prior to Bonferroni correction at $P<0.05$. Underlined values are significant after Bonferroni correction. Inf.=infinite.

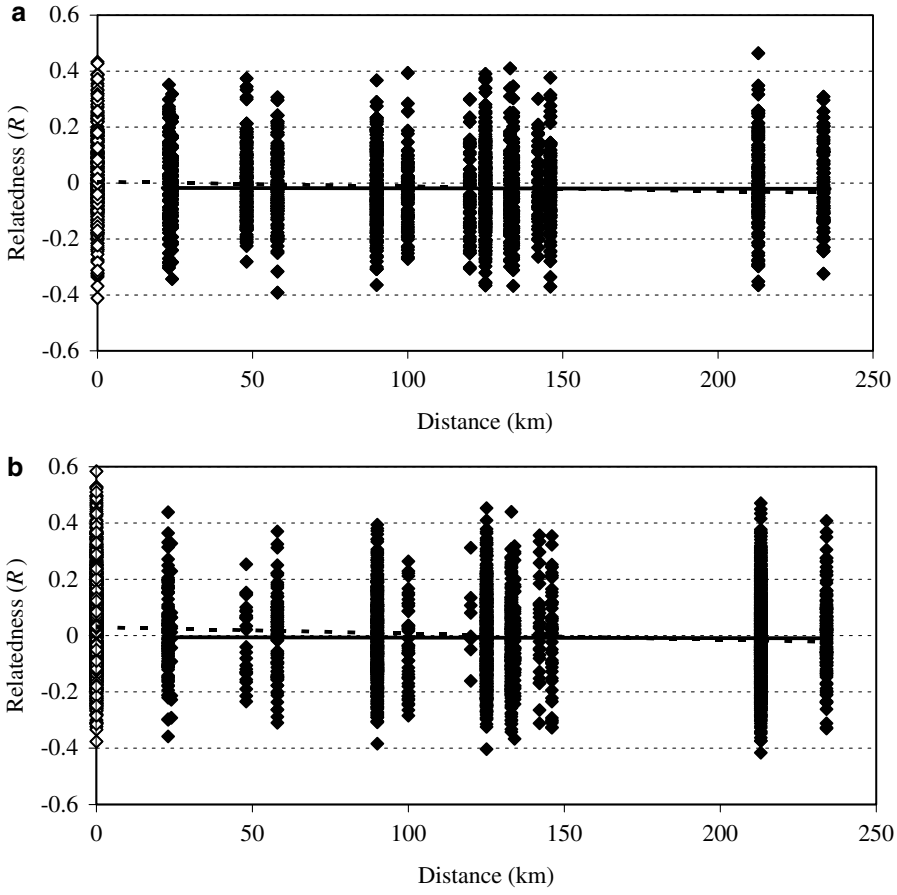

Figure 3 Relation between pairwise *R* and corresponding distance values for male (a) and female (b) eastern grey kangaroos. Within-population values indicated by open diamonds, while among-population values are denoted by filled diamonds. Mean pairwise relatedness (±SE) within populations and among populations are 0.026±0.0005, −0.019±0.0004 for males and 0.042±0.0005, −0.007±0.0004 for females. Solid line represents isolation by distance correlation. Broken line indicates average within- versus among-population differences.

values separately between the sexes, there was again no detectable association (males: $P=0.75$, females: $P=0.74$). In fact, populations separated by large distances (~230 km) demonstrated equivalent numbers of close relatives when compared to populations separated by small distances (~20 km; Figure 3).

Phylogeographical analysis

The reconstruction of relations between *M. giganteus* haplotypes using parsimony and NJ methods produced trees with corresponding topologies in relation to both the positioning and support of the groups (Figure 4). Phylogenetic relations between the subspecies revealed that *M. g. tasmaniensis* haplotypes were closely related to some *M. g. giganteus* haplotypes and clustered within one of the four major southern mainland lineages. Within *M. g. giganteus*, northern Australian (QLD) haplotypes were monophyletic (98–99% support), while there were four main lineages with significant bootstrap support (>80%) for southern Australian haplotypes (NSW and VIC). Two of these lineages, however, cluster with and are basal to the northern lineage (83–94% support), making the mtDNA of southern *M. giganteus* paraphyletic. There was no obvious structuring within both the northern and southern populations, as haplotypes from different lineages were spread throughout the different populations (Figure 4; Table 2). Furthermore, northern haplotypes exhibit reduced divergence compared to southern lineages, suggesting a recent derivation from southern stock. This claim is also supported by the mismatch analysis which indicates a historical population expansion (SSD P-value = 0.49).

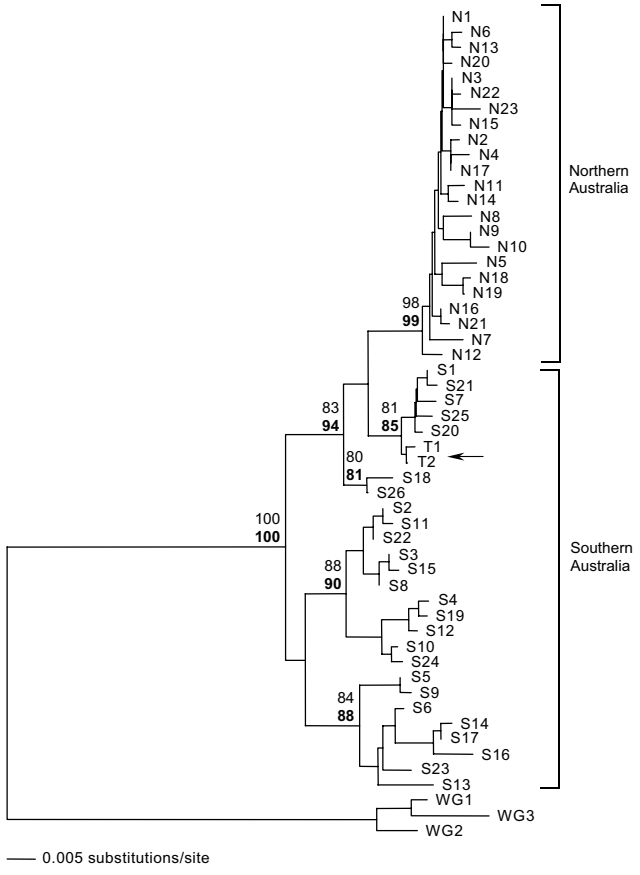

Figure 4 NJ tree displaying relation between eastern grey kangaroo mtDNA haplotypes and geographic region. *M. g. tasmaniensis* haplotypes identified by the arrow, while all other haplotypes are *M. g. giganteus* except for the outgroup which are *M. fuliginosus* (western grey eg, WG1) individuals. Values at nodes indicate the statistical support as obtained from 1000 bootstrap replicates. Numbers in plain text are from NJ analysis, while values in bold are derived from the MP method.

Discussion

Genetic diversity

The eastern grey kangaroo has extensive genetic diversity in both microsatellite and mtDNA variability. The mean number (±SD) of observed alleles across the 10 microsatellite loci was 15.8±5.1. Unfortunately, two of the heterologous loci (Me14 and Y151) displayed significant frequencies of null alleles, and were subsequently removed. Recently, species-specific microsatellite loci have been developed for *M. giganteus* to alleviate this problem (Zenger and Cooper, 2001b). Utilizing the remaining eight microsatellite loci, the average expected heterozygosity (H_E±SD) across all populations was high at 0.82±0.02 (Table 1). This level is one of the highest recorded for marsupial taxa (H_E range 0.05–0.86, $n = 24$; see Bowyer *et al*, 2002). Haplotypic diversity (h±SD) across all *M. giganteus* individuals was also high (94.83±0.66%), and again greater than most other marsupial taxa (h range 12–99%, $n = 14$; see Bowyer *et al*, 2002). Based on these data, it appears that *M. giganteus* has had a relatively large long-term effective population size (Frankham, 1996).

The sequence divergence between *M. giganteus* mtDNA haplotypes varied between 0.16 and 6.88% and is within the range observed from other macropods including the red kangaroo, *M. rufus* (0.1–6.2%; Clegg *et al*, 1998) and *P. xanthopus* (0.3–7.3%; Pope *et al*, 1996). Surprisingly, there was greater average sequence divergence within *M. g. giganteus* than between the subspecies, indicating that *M. g. giganteus* and *M. g. tasmaniensis* are not well differentiated genetically. In fact, the minimum sequence divergence between the taxa was only 0.47% (three substitutions; Figure 2). This value is substantially less than the minimum differences observed between *P. xanthopus* subspecies (5.20%; Pope *et al*, 1996), and between mainland and Tasmanian tiger quolls, *Dasyurus maculatus* (2.71%; Firestone *et al*, 1999).

When comparing geographical regions, haplotypes from southern Australia (NSW and VIC) demonstrated greater mean sequence divergence (3.23%) than those from northern Australia (QLD; 0.86%), suggesting a recent common origin for the QLD lineage of *M. g. giganteus* (see Phylogeography). Although *M. g. giganteus* is characterized by high mtDNA genetic diversity, the variation within each geographic region (NSW and QLD) is mostly distributed within ($\pi = 1.76\%$) rather than among populations ($d_A = 0.46\%$). These values are indicative of weak genetic structure (see below), which has also been demonstrated in *M. rufus* ($\pi = 2.90\%$, $d_A = 0.70\%$; Clegg *et al*, 1998).

Population structure and dispersal

Although there are limited ecological data for dispersal patterns in *M. giganteus* (eg, Kaufmann, 1975; Jarman and Taylor, 1983; Jaremovic and Croft, 1991), these studies suggest strong site fidelity, whereby individuals only disperse small distances (<20 km). Unfortunately, these conclusions may be limited by their methodology (see Introduction), resulting in an incomplete representation of dispersal for this species.

Contrary to these ecological studies, our results reveal weak genetic structure of populations both on a local (<50 km) and regional scale (50–230 km), from which

high levels of dispersal may be inferred. Although several pairwise θ_{ST} and Φ_{ST} values were significant (Table 2), both distance indices only revealed relatively small amounts of differentiation between the populations at these spatial scales (mean = 0.015, 0.183, respectively). Similarly, only 21% of total mtDNA variation was distributed among NSW populations when analyzed using AMOVA (Excoffier *et al*, 1992). Further analysis using the multilocus clustering method of Pritchard *et al* (2000) revealed comparable results, whereby no genetic subdivision could be resolved within this group and the six NSW populations were considered to function as a single population.

Population processes, such as individual movements and relatedness of interacting individuals, were investigated using genotypic approaches to identify fine-scale dispersal of individuals within NSW. Pairwise relatedness analysis indicated weak genetic structuring, whereby individuals are dispersing and breeding across all populations. In particular, females demonstrated only slightly higher levels of site philopatry than males. This sex-biased dispersal is also evident when comparing the average number of migrants ($N_em = 22.61$) to the average number of female migrants ($N_fm = 2.73$) among NSW populations. These results are consistent with numerous *M. giganteus* ecological studies that suggest greater male dispersal associated with breeding and social structure constraints (eg, Johnson, 1989; Jaremovic and Croft, 1991). Although there are fewer females dispersing than males, neither show significant isolation by distance when using pairwise relatedness values (Figure 3). In fact, when comparing all pairwise genetic distances, none was significant ($P > 0.05$). This lack of differentiation over large distances ($\sim$230 km) typifies the high levels of dispersal within these populations.

When examining genetic differentiation on a continental scale, there was strong evidence of genetic isolation between NSW and QLD regions. This result is clearly illustrated using both mtDNA and microsatellite data. For example, hierarchical analysis of mtDNA variance using AMOVA (Excoffier *et al*, 1992) partitioned a significant proportion of genetic variation (49%) between the two areas. In addition, average sequence divergence between the two regions was high (4.5%), with haplotypes being phylogenetically distinct (see Phylogeography). Furthermore, the multilocus clustering method of Pritchard *et al* (2000) indicated that NSW and QLD were almost certainly ($P = 0.999$) two separate populations. These data suggest that both regions are, or have been, isolated for a significant period of time, at least with respect to maternal gene flow. However, this does not exclude the possibility of admixture or gene flow occurring at a contact zone between our sampled NSW and QLD populations. Consequently, further sampling is needed to clarify the extent of this genetic subdivision.

Phylogeography

Subspecies are largely regional variants of a species, and hence can be related to an evolutionary significant unit (ESU) (Moritz, 1994). Under this definition, the lack of genetic distinction (reciprocal monophyly) between *M. g. giganteus* and *M. g. tasmaniensis* does not reflect current taxonomic classification. Although morphological differences exist (Kirsch and Poole, 1972), they probably reflect clinal adaptation to environmental differences and/or stochastic effects associated with small founder populations, rather than taxonomic separation. Consequently, our data indicate that only one ESU exists for *M. giganteus*, and that the use of the present taxonomic subdivision within Australia should be reassessed.

Within mainland Australia, there is clear phylogeographic distinction between northern and southern populations. Although there is no reciprocal monophyly between these groups, the results suggest significant isolation for an extensive period of time. Owing to the lack of samples connecting these two areas, the cause of these differences is unknown, although there are relatively few physical barriers to dispersal. Within each of these groups, however, variant haplotypes are spread throughout all sampled populations (especially in southern Australia). This pattern implies that regional populations have been connected by large amounts of historical and/or current mtDNA geneflow. The limited divergence among northern haplotypes compared to southern, suggests a relatively recent colonization of QLD by *M. giganteus*. As two mtDNA lineages from southern Australian are basal to the QLD lineage, it seems likely that a small number of southern *M. giganteus* originally colonized northeastern Australia and that control region variation has since been generated via mutation.

Implications for management

Management of kangaroo populations whether for conservation, or as a renewable resource, or for pest control, requires effective population monitoring and understanding of the biology of the species. The findings of this study challenge previous ecological studies by providing a perspective on parameters such as historical and contemporary population structure and dispersal, difficult to obtain through direct observations. These results will aid in the future management of this species, by providing knowledge of the geographical scale at which *M. giganteus* populations are genetically distinct. Several important observations emerge from our findings. First, patterns of weak population differentiation within NSW are almost certainly a consequence of high gene flow among these geographically distinct populations (up to 230 km apart). Consequently, management regimes will need to focus on a regional scale rather than a local scale to effectively manage these populations. In addition, females should be targeted, as these appear to have higher site fidelity and ultimately will preferentially control the rate of net recruitment within specific populations. Second, the lack of genetic differentiation within regions suggests that gene flow may over-ride local selection. This has implications for translocation of animals and/or reintroductions. Currently, in the interests of genetic integrity of kangaroo populations, NSW National Park and Wildlife Service policy states that the release of large macropods is constrained within 50 km of the encounter point (Hardy, 2000). Obviously, this geographical range may be increased regarding *M. giganteus* given the lack of substantial genetic differentiation among NSW populations. Thirdly, the current taxonomic status of mainland and Tasmanian *M. giganteus* populations should be revised to reflect separate populations, rather than separate subspecies. Lastly, the extent of phylogenetic separation between southern (NSW/VIC) and northern (QLD) *M. giganteus*

populations and the apparent reduction in mtDNA diversity within northern populations are sufficient for these regions to be recognized as separate entities for management regimes on a continental scale.

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