Comparative genetic structure reflects underlying life histories of three sympatric small mammal species in continuous forest of south-eastern Australia

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There are strong links between habitat fragmentation, population size and genetic structure. However, to fully understand the long-term effects of fragmentation on population viability, it is necessary to first understand the relationship between life history traits and genetic characteristics in un-fragmented habitats. This is best done through comparing patterns of genetic diversity in sympatric species, since relative data may be maximally informative. We compared genetic structure and diversity among three sympatric small mammals – of which two were marsupial species – over a small spatial scale (<4 km) using microsatellite genotypic data from individuals sampled from four grids spaced in a linear fashion. Expected heterozygosity was high for all three species (average H_e range: 0.781–0.886), but the smallest species had significantly higher genetic diversity (both heterozygosity and allelic diversity) than the two larger species, possibly due to it having; 1) large effective population size and little overlap in generations, and/or 2) high mutation rates in large alleles. Genotypic isolation by distance (measured as relatedness) was detected only at the smallest distance of 750 m, for any species. In the two marsupial species F_{st} , relatedness and percentage of individuals assigned to site of capture were significantly lower in males than in females, corroborating previous reports of male-biased dispersal. However, sex-biased dispersal was not detected for the third species (the native bush rat *Rattus fuscipes*), and presence of significant heterozygous deficits suggested this resulted in inbreeding within kin-structured demes. We speculate that habitat fragmentation will have differing effects on population dynamics, social organisation and mating systems for the two marsupial species compared to the native murid rodent, due to their differing population structure and divergent inbreeding avoidance mechanisms.

Knowledge of the likely effects of habitat fragmentation and climate change on biodiversity relies on understanding the relationship between life history characteristics, dispersal, gene flow and population genetic structure (Lindenmayer and Fischer 2006). Therefore, we need to establish that a predictable relationship exists between life-history traits and genetic characteristics of populations in continuous habitat (Peakall et al. 2003). Given that such characteristics are relative rather than absolute quantities this is best achieved through comparative studies. Such comparative studies are rare and most of our knowledge of life history effects on

population genetics comes from single-species studies (Slatkin 1994, Hedrick 2000). These studies have identified a variety of influential life history traits, such as sex-biased dispersal, population size, mating system, habitat specialisation, body-size and generation time (Slatkin 1994, Hedrick 2000). Sex-biased dispersal results in higher between-population genetic structuring in the non-dispersing sex. For example, in the small Australian marsupial, the agile antechinus *Antechinus agilis*, females usually stay in their natal territory whereas males disperse. Such patterns of behaviour are reflected in both genic (F_{st}) and genotypic (relatedness)

data: females are genetically more differentiated between populations or sampling sites, compared to males (Kraaijeveld-Smit et al. 2002a). Mating system and social organisation also have large impacts on genetic characteristics of populations (Goossens et al. 2001, Sommer et al. 2002, Grapputo et al. 2005). Sexually reproducing species show higher genetic diversity than asexual ones (Grapputo et al. 2005), within vertebrate classes the larger species have less genetic diversity than the smaller ones (Mezhzhherin 2002), monogamous species have less diversity at the major histocompatibility complex than promiscuous ones (Sommer et al. 2002), and animals living in family groups (e.g. Alpine marmots *Marmota marmota*) form distinct genetic clusters (Goossens et al. 2001).

It is rare in studies to date, for different species to be sampled from the same habitat, so that any effect of habitat features on differences in population genetic characteristics among species may not be apparent. One way around this problem is to compare genetic characteristics of sympatric species varying in several life history traits, so that differences in genetic structure may be attributed to the biological properties of the species rather than habitat features. For example, of two sympatric salmonids in the Clark Fork River, Montana, USA, one homed to spawning locations, had low dispersal ability, comprised small population sizes and females chose the most dominant male as a mating partner (Whiteley et al. 2004). The other had high dispersal ability, much larger population sizes, and exhibited a more promiscuous mating behaviour. Given these traits, the former had much lower heterozygosity levels and higher F_{st} values between populations (Whiteley et al. 2004). Comparative studies of this kind are rare, even though this approach has the potential to identify which life history components are most important in shaping population genetic characteristics. Other examples where two sympatric species have been compared come from studies of parasitoids, anurans, bats, fruit flies (*Drosophila* spp.), beetles and lemmings (Fedorov 1999, Newton et al. 2003, Brede and Beebe 2004, Hurtado et al. 2004, Kankare et al. 2005, Schmuki et al. 2006). A general consensus from these studies is that dispersal distances have the largest influence on spatial population genetic structure. However, very few have focused at a fine-scale of spatial resolution (within an individual's dispersal range). Life history differences will tend to operate at the spatial scale of individual home-ranges, and landscape scale and metapopulation processes will be an accumulation of these local processes. Thus, studying fine-scale processes will advance our understanding of how species respond to human impacts like habitat fragmentation. As a consequence, it is important to understand how life history differences impact on the fine-scale genetic structure.

In this paper, we assess the influence of life history attributes on the genetic characteristics of populations by comparing fine-scale genetic structures of three species of sympatric small mammals in southeastern Australia. For each species, five highly variable microsatellites were employed to determine both the genic and genotypic structure among four study grids situated 1–4 km apart in continuous forest habitat. Data for males and females were analysed separately to examine the effects of sex-biased dispersal. Two of the three species-agile antechinus *Antechinus agilis*, and dusky antechinus *A. swainsonii*-share many life history features but differ in body size and usually also in population density (Dickman 1991). The third and largest species, the bush rat *Rattus fuscipes*, differs in that it is not a marsupial, has territorial females (Woodside 1983) and small home-ranges and finally, in lacking an abrupt male annual die-off, thus having overlapping generations of both sexes (Robinson 1987). It is clear from both demographic and genetic data that dispersal is restricted in both sexes of bush rat (Press 1987, Peakall et al. 2003). The effect of limited dispersal in both sexes on the genetic structure will become evident by comparing genic and genotypic data from this species with those for the two marsupial species with known male-biased dispersal in the same habitat. More details on the species' life histories are provided below.

Material and methods

Study site

The study was based at Mt Donna Buang in Victoria, 70 km east of Melbourne, Australia (Cunningham et al. 2005). The forest at the site is dominated by shining gum *Eucalyptus nitens*. This continuous area of forest covers approximately 5000 ha around the Mt Donna Buang area and connects more broadly to over 150 000 ha of native forest in which small mammals are highly abundant (Cunningham et al. 2005).

Study species

The agile antechinus *Antechinus agilis* is a small scansorial (climbing) marsupial that occurs in all forest types and heathland habitats to about 1200 m above sea level, from southern Victoria to southeastern New South Wales. Males weigh up to 40 g and can be twice the size of females (up to 24 g, Kraaijeveld-Smit et al. 2003). All the males die after a two-week mating season in August. Females give birth to a litter of 6–10 young (depending on population-specific teat number) approximately one month after mating. Litters are usually sired by more than one father (Kraaijeveld-Smit et al. 2002b). These young stay attached to the teat for five

weeks, followed by a period in the nest of about two months. Most juvenile males disperse away from the natal territory in January–February (Cockburn et al. 1985). Home ranges of males are around 3 ha, those of females 1.5 ha and there is overlap between and within sexes (Lazenby-Cohen and Cockburn 1991).

The dusky antechinus *A. swainsonii* is a ground-dwelling marsupial found in Tasmania and the eastern part of mainland Australia. It occupies a range of habitat types from subalpine heath and woodland, to wet sclerophyll forest and upland and lowland rainforest. This species is much larger than the *A. agilis*, with males reaching 120 g and females 70 g. Although it is larger, the life history of this species is similar to *A. agilis*, particularly in relation to its synchronised annual breeding season followed by male die-off (Cockburn and Lee 1988). Litter size varies from 6–8 and juvenile males disperse, whereas most juvenile females remain in their natal territory. Little is known about home range sizes and territoriality.

The bush rat *Rattus fuscipes* is an omnivorous mammal that is widely distributed in eastern Australia (Robinson 1987). The species' habitat includes upland and lowland rainforest, wet sclerophyll forest, subalpine scrub or heath, wet scrub or heath, and sedge. Males weigh up to 250 g and are larger than females (up to 150 g). Breeding is usually restricted to a period spanning November to January during which females produce 1–2 litters. Post-breeding mortality among adults appears to be common (Lee and Cockburn 1985). Juveniles of both sexes apparently stay or disperse away from their natal territory in May (Robinson 1987). Home ranges vary between 0.1–1.2 ha and females in particular are highly territorial (Woodside 1983).

Sample collection

Small mammal trapping occurred during ten 3–4 day capture periods at 2–3 month intervals during 1999 and 2000. So that each sampling year started after the initial juvenile dispersal phase for each species, a year went from January to December for *A. agilis* and *A. swainsonii*, and from May to April for *R. fuscipes*. Trapping took place at four grids (A, B, C and D) spaced approximately linearly and approximately 1 km apart (A–B = 950 m, B–C = 1700 m, C–D = 750 m). Each trapping session consisted of four trap-nights with 49 traps at each grid. Elliott traps were baited with oats, honey and peanut butter, and spaced 10 m apart. All newly caught individuals were marked for subsequent identification with a microchip (Cunningham et al. 2005). A small piece of ear tissue (0.5 cm in diameter) was collected from each individual and stored in 96% ethanol for DNA extraction. Female antechinus teats

were counted when visible (i.e. just prior to the winter mating season).

Genotyping

DNA was extracted from the ear tissue using a 'salting out' method (Sunnucks and Hales 1996). Animals were genotyped at five microsatellite loci. For *A. agilis* and *A. swainsonii* we used microsatellite primers developed for *A. agilis* as described in Paetkau (1999), loci: 2H, 4J, 4K, 7D and 7O; primer sequences given in Kraaijeveld-Smit et al. (2002b). Isotopic labelling of PCR product was achieved by end-labelling the forward primers with γ - ^{33}P -dATP (*A. agilis*) (as in Taylor et al. 1994) or by incorporating α - ^{33}P -dATP into the PCR product (*A. swainsonii* and *R. fuscipes*). PCR reactions were similar to those described in Taylor and Cooper (1998), with conditions as follows: initial 2 min denaturing at 95°C, followed by 30 cycles of [30 s at 95°C, 30 s at 56°C (*A. agilis*) or 30 s at 54°C (*A. swainsonii*), 20 s at 72°C], and a final extension at 72°C for 5 min. For *R. fuscipes*, we used microsatellite primers developed for *R. rattus* (Loci: CPB, FGA, TTR, PBPC2, PLANH; Serikawa et al. 1992). For this species PCR reactions with a total volume of 10 μl contained: 7.5 mM Tris-HCl, 2 mM NH_4SO_4 , 0.001% Tween 20, 2.5 (CPB, FGA, PLANH) or 5 (TTR, PBPC2) mM MgCl_2 , 200 μM of dTTP, dCTP, dGTP, 20 μM of ATP, 0.05% BSA, 2 μM of each primer, 0.1 units of Taq polymerase (MBI), 7.0 KBq α - ^{33}P -dATP, and ~ 100 ng DNA. PCR conditions were as follows: initial 2 min denaturing at 95°C, followed by 35 (CPB, FGA and PLANH) or 40 (TTR and PBPC2) cycles of [30 s at 95°C, 30 s at a touch down T_a -range of 55–47°C (CPB, FGA, TTR and PLANH) or 50–45°C (PBPC2), 20 s at 72°C], and a final extension at 72°C for 5 min. Alleles for all species were separated by electrophoresis on 6% denaturing polyacrylamide gels, and visualised by autoradiography.

Basic genetic analyses

Per species/year, Genepop v3.3 was used to calculate allelic diversity as well as observed and expected heterozygosities for each locus and on average (Raymond and Rousset 1995). Tests for heterozygosity deficits, using the default option of 10 000 permutations, and calculating F_{is} values (inbreeding coefficient of an individual (I) relative to the subpopulation (S); Weir and Cockerham 1984) were performed with the same software, per species/year for each microsatellite locus. Tests for deviations from Hardy-Weinberg equilibrium, and for linkage disequilibrium between pairs of loci were also performed in Genepop v3.3 per species/sex/year. F_{st} values (effect of subpopulations (S)

compared to the total population (T); Weir and Cockerham 1984) between sites were calculated per species/sex/year, and Mantel tests with 10 000 permutations were used to test for isolation by distance, again with the aid of Genepop v3.3. Significant F_{st} deviations from zero (bootstrapping with 120 permutations) after Bonferroni corrections to give table-wide significance levels of $p=0.05$ were detected with Fstat 2.9.3 (Goudet 1995). Data for the two years of field sampling were analysed separately, since generations between years are distinct. Only adults were included in the analyses.

Relatedness

For each species, pair-wise relatedness values between all same-sex and opposite-sex individuals, within and between grids, per year were calculated using Kinship 1.1 (Queller and Goodnight 1989). The philopatric sex will be more closely related to individuals caught on the same grid compared to those caught at different grids. This is not the case for the dispersing sex. Thus, sex-specific relatedness values reflect which sex is dispersing. The following steps were undertaken to analyse the relatedness values: 1) data for all three species were combined into one dataset; 2) the relatedness value for each pair of individuals was entered twice into the table: First with individual A as focal individual (ID1) in relation to individual B (ID2), and then vice versa. This ensured that the means we calculated at the next step weren't affected by sample size differences across grids and/or sexes; 3) we then calculated mean relatedness for each of the following comparisons for each individual in column ID1: grid comparison (AA, AB, AC, AD, BB, BC, BD, CC, CD, DD) / sex comparison (female/female, female/male and male/male); 4) from these mean individual values we then calculated mean relatedness for each grid / sex comparison per species and year. By doing this we avoid the problem of dependence that exists between pairwise relatedness data points.

Assignment tests

Assignment tests were calculated separately for each species for each of the two years of field sampling (using grid- and year-specific allele frequencies). Assignment probabilities (the probability that an individual caught at a certain grid originated from that grid) were calculated using Bayesian criteria (Rannala and Mountain 1997) and computed based on a Monte-Carlo resampling method (Paetkau et al. 2004) in GeneClass 2.0 (Piry et al. 2004). A total of 1000 individuals was simulated, incorporating a value for genotyping errors of 0.01. The threshold of 0.1 was used to determine

whether an individual was assigned to the site of capture. Based on this threshold, the proportion of individuals assigned to the site of capture was calculated per sex, species, grid and year.

Statistical analyses

Chi-square tests were used to detect possible differences between species in expected heterozygosities and in the number of alleles in SPSS 12.1. To detect possible differences in F_{st} values between sex and species and a correlation with distance we applied a linear mixed-model fit by REML (restricted maximum likelihood). Sex, species and distance were added as fixed effects, while grid within years was added as a random effect. Relatedness (R) was also analysed using a linear mixed-model fit by REML. We included sex comparison (female/female, female/male and male/male), distance and species as fixed effects, while grid within year was entered as a random effect. To test whether the proportion of individuals assigned to the grid in which it was captured differs between sex and species we also applied a linear mixed-model fit by maximum likelihood. Proportions were arcsin transformed and sex and species were entered as fixed effects. Again grid nested within year was added as a random effect.

All models were built in the statistical program R-the project, ver. 2.3.1. (Venables et al. 2006). These models were reduced to the minimal adequate model (removing all factors which were not significant from the model). In all cases we checked the residual and QQ plots to test the assumptions of normality and constant variance. To correct for the non-normality and heteroscedasticity that was present in the F_{st} and R data, we applied variance strata. The data on the proportion assigned to the population of capture also did not follow normality. Alternative methods to correct for overdispersion did not improve this, thus we applied a General linear model with a quasibinomial distribution to the assignment data. Although this method has the drawback that random effects cannot be analysed, the results of this model were comparable to the linear mixed-model used above. A comparison between measures of genetic diversity is less than straightforward (using different models for each measurement) and we therefore do not discuss this model in the result section.

Results

Population parameters

Grid A had densities of 81.6, 61.1 and 36.7 animals per ha for *R. fuscipes*, *A. agilis* and *A. swainsonii* respectively across the site. The other three grids supported similar densities to those recorded on grid A, for all three

species (Cunningham et al. 2005). Marked seasonal changes in density, sex ratio and age cohort composition were observed in both *Antechinus* spp., as expected given the well known male die-off observed for these small mammal taxa. Of the 26 female *A. agilis* for which teat number was recorded 23 had 8 teats, while 3 had 9 teats. All *A. swainsonii* females for which teat number was recorded had 8 teats ($n = 8$). We observed partial winter die-off for *R. fuscipes* for both sexes.

Genetic diversity and differentiation

Significant global departure from Hardy-Weinberg was found for only one locus (PBPC2) in one species (*R. fuscipes*), suggesting lack of substructure within populations of each species at Mt Donna Buang. Interestingly, 13 out of 40 locus-grid-year combinations exhibited significant heterozygous deficits for *R. fuscipes* (distributed among loci, grids and years), compared with only two of 40 for *A. agilis*, and four of 40 for *A. swainsonii*. Higher F_{is} for *R. fuscipes* (mean ± 1 SE 1999: 0.040 ± 0.042 , 2000: 0.122 ± 0.059) than for the two marsupial species (*A. agilis* 1999: 0.001 ± 0.026 , 2000: 0.025 ± 0.037 , *A. swainsonii* 1999: 0.032 ± 0.023 , 2000: 0.049 ± 0.049) indicated either that null alleles were present or that inbreeding occurs more frequently in the former species. Significant heterozygous deficits using this primer set in this species were also encountered by Lindenmayer and Peakall (2000), but their design and use of species-specific primers did not reveal the additional alleles expected if heterologous priming is causing null alleles (R. Peakall, pers. comm.). Although we cannot completely rule out null alleles, the fact that heterozygous deficits were not restricted to particular loci, grids or years, suggests they may have been caused by a transient Wahlund effect (i.e. inbreeding within local demes). Significant linkage disequilibrium was detected for *A. agilis* between locus pairs: 7D/7O and 7D/4K, but only for females in grid A in the year 1999. Further disequilibria were observed in *A. swainsonii* between loci 2H/7D for males in grid A in the year 1999. Since results regarding Hardy-Weinberg and linkage were not consistent across populations, years or loci, we continued to use all initially selected loci for further genetic analyses.

Expected heterozygosity per locus ranged from 0.84–0.93, 0.61–0.87 and 0.69–0.87 for *A. agilis*, *A. swainsonii* and *R. fuscipes*, respectively (Table 1). The number of alleles per locus ranged from 14–26, 7–15 and 8–14 for *A. agilis*, *A. swainsonii* and *R. fuscipes*, respectively (Table 1). Measures of genetic diversity differed significantly among species (heterozygosity expected: $\chi^2 = 6.82$, $p = 0.033$, alleles: $\chi^2 = 7.75$, $p = 0.0021$). Post-hoc analyses showed that this difference

was caused by higher values in *A. agilis* than in *A. swainsonii* and *R. fuscipes* (Table 1).

Low (< 0.05) to medium (0.05–0.15) among-grid F_{st} values (Nei 1978) were observed for all three species, and values differed between years (Table 2). Among pair-wise combinations, a significant deviation from zero was found between grid A and B, and A and D in female *A. agilis*, but only in one year (Table 2). Deviations from zero in *A. swainsonii* were also only found between female sample pairs, namely between grids B and C, and B and D (Table 2). In *R. fuscipes* deviations from zero were found only between males from grids A and B, and C and D (Table 2). Thus, genetic sub-structuring occurred between some female groups of *A. agilis* and *A. swainsonii*, while genetic sub-structuring in *R. fuscipes* occasionally occurred in males.

Applying a mixed-linear model, adding grid nested within year as a random effect and sex, species and distance between grids as fixed effects provided further evidence for differences in F_{st} values between the sexes ($F_{1,55} = 43.5$, $p = < 0.0001$) and between the three species ($F_{2,55} = 7.4$, $p = 0.0014$). There was a significant interaction between sex and species ($F_{2,55} = 5.2$, $p = 0.0087$) indicating that although sexes differ in F_{st} , the way in which F_{st} differs between the sexes is not the same for each species. Males of *A. agilis* and *A. swainsonii* had lower F_{st} values compared to all other groups, female *A. swainsonii* had significantly higher F_{st} values compared to all other groups and in *R. fuscipes* there was no significant difference in F_{st} values between the sexes (Fig. 1). Distance between grids did not have an effect on the F_{st} values, indicating that there was no pattern of isolation by distance over the spatial scale of the study. Mantel tests for each species/sex group further supported the lack of isolation by distance (p – range = 0.045–0.963, none significant after Bonferroni correction).

Recent dispersal: relatedness

Relatedness values were used to investigate recent dispersal events. We tested for isolation by distance and differences in dispersal behaviour between sex and species by applying a mixed-linear model to the data. Sex comparison (female/female, female/male and male/male), distance and species were entered as fixed effects and grid within year as a random effect. A significant correlation between R and distance was observed ($F_{1,17} = 5.7$, $p = 0.0287$), thus individuals in close proximity are more related than they are to those caught further away. Furthermore, a significant effect for sex comparison ($F_{2,155} = 9.6$, $p = 0.0001$) and sex comparison $\times$ distance ($F_{2,155} = 4.2$, $p = 0.0106$) was found. Relatedness values between males and between females/males are lower than those between females.

Table 1. Characteristics of the microsatellites used in this study. n = number of individuals, H_o = observed heterozygosity, H_e = expected heterozygosity. The same loci were used for *A. swainsonii* and *A. agilis*. Samples for years, grids and sexes are pooled. Means of the characteristics with the same superscript were not significantly different (Mann-Whitney test).

<i>A. agilis</i> (n =206)					<i>A. swainsonii</i> (n =166)					<i>R. fuscipes</i> (n =230)				
Locus	No. of alleles	Size range	H _o	H _e	No. of alleles	Size range	H _o	H _e	Locus	No. of alleles	Size range	H _o	H _e	
2H	17	141–177	0.907	0.871	15	132–168	0.802	0.823	CPB	11	90–126	0.729	0.792	
4J	26	165–249	0.883	0.931	13	165–197	0.838	0.853	FGA	13	86–120	0.692	0.694	
4K	14	132–166	0.908	0.899	7	147–165	0.498	0.610	TTR	14	105–161	0.790	0.839	
7D	15	201–229	0.782	0.839	10	186–206	0.856	0.821	PBPC2	14	119–254	0.637	0.865	
7O	19	109–155	0.869	0.890	13	103–129	0.835	0.871	PLANH	8	124–138	0.668	0.716	
Mean (±SE)	18.2 ^a	–	0.870 ^A ±0.02	0.886 ¹ ±0.01	11.6 ^b	–	0.675 ^B ±0.06	0.796 ² ±0.04		12.0 ^b	–	0.703 ^B ±0.02	0.781 ² ±0.03	

The significant interaction describes the lower within grid relatedness of males compared to females, resulting in slopes of R over distance that are less steep for males than for females (Fig. 2). A similar isolation by distance model excluding within grid comparisons did not show a significant correlation between R and distance (data not shown). This indicated that isolation by distance occurred over the smallest distance sampled (750 m). No species effect was found. However, if one very high outlier (relatedness within grid A for 2000 *R. fuscipes* females) was excluded from the data set, a significant species effect was found (data not shown). Given the F_{st} and assignment results (see below) it is likely that this species effect is real. This is further supported by the fact that within grid R values of both male and female *R. fuscipes* are high, indicating low dispersal in both sexes, while those values are much lower in male *A. agilis* and *A. swainsonii*, consistent with male-biased dispersal in these species (Fig. 2).

Recent dispersal: assignment test

A general linear model (with a quasibinomial error distribution) including grid nested within year as a random effect and sex and species as fixed effects on the proportion of individuals assigned to their site of capture revealed significant differences between species ($F_{2,37} = 19.9$, $p < 0.0001$) and sexes ($F_{1,37} = 10.2$, $p = 0.0029$). *R. fuscipes* had a significantly higher proportion of individuals assigned to their site of capture compared to *A. agilis* and *A. swainsonii* (Fig. 3). A lower proportion of males were assigned to the population of capture compared to females in the two marsupial species, but not in *R. fuscipes* (Fig. 3).

Discussion

By analysing the genetic structure (F_{st} , relatedness and assignment tests) of males and females separately we confirmed that in both marsupial species male-biased dispersal occurred and that sex-biased dispersal was absent in *R. fuscipes*. Relatedness declined over distance, at least in female *A. agilis* and *A. swainsonii*, and this correlation was present only at the smallest scale (< 750 m). These results suggest that on an ecological timescale (i.e. a generation), there are substantial differences among species in the proximity of relatives, as a result of differential dispersal distances/capabilities. The standard measures of genetic diversity-heterozygosity and number of alleles-were highest in the smallest species, *A. agilis*. The two larger species did not differ in those respects.

Table 2. F_{st} values between grids below the diagonal. Whether F_{st} values differ significantly from zero is indicated above the diagonal. ns = not significant, - = not available (too few data points).

Species	Year	Grid	Females				Males			
			A	B	C	D	A	B	C	D
<i>A. agilis</i>	1999	A	—	0.008	ns	0.008	—	ns	ns	ns
		B	0.032	—	ns	ns	-0.010	—	ns	ns
		C	0.031	0.029	—	ns	0.000	-0.003	—	ns
		D	0.035	0.002	0.021	—	-0.008	-0.005	-0.001	—
	2000	A	—	ns	ns	ns	—	ns	ns	ns
		B	0.071	—	ns	ns	0.038	—	ns	ns
		C	0.039	0.102	—	ns	-0.014	-0.004	—	ns
		D	0.025	0.057	0.036	—	0.006	0.030	-0.004	—
<i>A. swainsonii</i>	1999	A	—	ns	ns	ns	—	ns	ns	ns
		B	0.083	—	0.008	ns	0.003	—	ns	ns
		C	0.060	0.098	—	ns	0.003	-0.013	—	ns
		D	0.069	0.099	0.029	—	0.015	-0.012	-0.012	—
	2000	A	—	ns	ns	ns	—	ns	ns	ns
		B	0.153	—	ns	0.008	0.013	—	ns	ns
		C	-0.007	0.145	—	ns	-0.015	0.015	—	ns
		D	0.032	0.115	0.003	—	-0.049	-0.024	-0.066	—
<i>R. fuscipes</i>	1999	A	—	ns	ns	—	—	0.008	ns	—
		B	0.043	—	ns	—	0.033	—	ns	—
		C	0.035	0.015	—	—	0.018	0.028	—	—
		D	-0.024	0.014	0.034	—	0.014	0.064	0.015	—
	2000	A	—	ns	ns	—	—	ns	ns	ns
		B	0.090	—	ns	—	0.023	—	ns	ns
		C	0.082	0.048	—	—	0.006	0.022	—	0.008
		D	0.159	0.044	0.027	—	-0.004	0.041	0.018	—

Broadscale dispersal

When using the three different genetic measures to investigate genetic differentiation between grids some interesting observations for the three species can be made. First of all, the classical measure to detect population differentiation, F_{st} can also detect sex difference. Since only a small proportion of between-

grid values were significantly higher than zero and isolation by distance was not apparent, we conclude that the overall effect of dispersal is high, leading to low genetic structuring between grids at the spatial scale of this study, but does result in different values for males and females and between species. Second, our genic and genotypic analyses clearly confirm the known

Fig. 1. Boxplot of average F_{st} values per sex and species, showing median and interquartile range (50% of all data points). Whiskers (bars) represent either the maximum/minimum or 1.5 times the interquartile range of the data (whichever is the smaller).

Fig. 2. Mean relatedness within and between grids for each species and sex comparison (female/female, female/male and male/male). See Fig. 1 for details on the boxplot.

Fig. 3. Proportion of individuals assigned to grid of capture. Each dot represents the proportion for one grid in one year. Proportions sometimes overlap with other grids, resulting in less than eight data points.

male-biased dispersal in the two marsupial species (Cockburn et al. 1985, Fisher et al. 2006). Not only is the genetic differentiation (F_{st}) between samples of females on different grids higher than that for males, but within-grid relatedness and assignments are also higher for females than for males. These three components indicate high female site fidelity and male-biased dispersal for the *Antechinus* spp. Modelling has shown that F_{st} values, particularly when measured after juvenile dispersal has occurred, are indeed expected to be higher in the non-dispersing sex (Vitalis 2002). Male-biased dispersal is the rule rather than the exception in mammals (Greenwood 1980) and can easily be detected by analysing the genetic structure of populations. In rufous bettong *Aepyprymnus rufescens* for example, females within one site were more genetically structured compared to males, indicating females are the philopatric sex (Pope et al. 2005). By contrast, our genetic data did not give clear evidence of male-biased dispersal in *R. fuscipes*. This is in concordance with previous demographic and genetic data on dispersal (Press 1987, Peakall et al. 2003). The fact that both same-sex and opposite-sex relatedness values between individuals caught on the same grid are higher than those between individuals from different grids supports the idea that dispersal is over short distances, and not sex-biased in *R. fuscipes*. Removal experiments showed that *R. fuscipes* populations recovering in native vegetation patches within an inhospitable pine plantation were genetically more similar to the individuals caught prior to removal than to animals from neighbouring patches (Lindenmayer et al. 2005). Thus, dispersal in this species is evidently low, but still high enough to maintain gene flow between grids.

Finescale genetic patterns and social organisation

Antechinus agilis is social, with many animals sharing nests and overlapping home-ranges. Thus, animals tolerate individuals in their close proximity, even when these individuals are not closely related (Lazenby-Cohen and Cockburn 1991, Banks et al. 2005c). *Rattus fuscipes* also appears to be social, and may even exhibit paternal care behaviour (Horner and Taylor 1969, Sanecki et al. 2006). Female bush rat home-ranges never overlap, but during the mating season, males overlap territories both with each other and with females (Robinson 1987). In contrast, *A. swainsonii* does not demonstrate social behaviour, and females in particular have strongly delineated home-ranges that do not overlap with other individuals (Sanecki et al. 2006). Pairs of *R. fuscipes* with neighbouring territories are probably often closely related since they seem to consist largely of non-dispersed juveniles of both sexes from the previous season (Robinson 1987). This would be expected to result in higher within-grid relatedness values in both sexes for this species.

Sex-biased dispersal is usually seen as a mechanism to avoid matings between close kin (Greenwood 1980). Thus, when sex-biased dispersal is absent and dispersal distances are generally low, pairs of opposite-sex animals in close proximity may be related, increasing the risk of consanguineous matings. Which sex is the dispersing one depends on which sex benefits most from staying in the natal area. Birds for example usually employ a resource-defence strategy. Males, which are the philopatric sex, defend a resource which is of importance to females, and benefit most from staying at home. In mammals however, a mate-defence strategy is usually in place, where males defend females rather than another resource. This defence can be just as efficient away from the natal area as it can be at home. However, lactating females have high energy demands so benefit more from staying in their natal area where the quality and location of resources is known. Hence in mammals, males are typically the dispersing sex (Greenwood 1980). Clearly both *Antechinus* spp. follow this rule, and this is reflected in the genetic structure. Our data on *R. fuscipes* suggest that this species forms an exception to this general dispersal pattern across species. This implies either that mating between relatives may be reasonably common, or deliberately avoided with the aid of kin recognition mechanisms, for example via chemical signals such as odours (Woodside 1983) as occurs in mice, *Mus musculus* (Penn and Potts 1998). The relatively high degree of heterozygous deficits observed within grids for *R. fuscipes* is consistent with the expectation that lack of a sex-bias in dispersal may indeed result in inbreeding within 'demes' (Chesser 1983). However this is unlikely to be detrimental to fitness in continuous habitat where turnover of

dominant males will be high and gene flow may be sufficient to prevent spatial accumulation of deleterious alleles or unfavourable genetic combinations. Under such circumstances active avoidance of kin as mates may not be necessary.

Genetic diversity

Genetic diversity observed in this study was higher than that reported for the same markers in other populations of these species where comparative data are available, for *A. agilis* and *R. fuscipes* (Kraaijeveld-Smit et al. 2002a, Peakall et al. 2003, Banks et al. 2005a, Beckman et al. 2007). Such high values are to be expected for abundant species in relatively undisturbed, continuous forest, and suggest that at least these two species have not previously suffered loss of diversity through genetic bottlenecks at our site. We observed higher genetic diversity in *A. agilis* than in the two larger species. Perhaps the inter-species difference resulted from a measurement or ascertainment bias, related to the source of microsatellite markers. The microsatellites used for *A. agilis* were developed for that species, while those used for both *A. swainsonii* and *R. fuscipes* involved cross-species amplification, albeit to close congeners. There is evidence that with increasing evolutionary divergence between the species from which the loci were isolated (target) and the non-target study species, the proportion of polymorphic loci in the latter decreases (Primmer and Merilä 2002). However, even if the number of polymorphic loci is lower in non-target species, allelic diversity does not necessarily differ between target and non-target species (Arigoni and Largiader 2000). In relation to the current study species, Peakall et al. (2003) observed little difference in expected heterozygosity in *R. fuscipes* whether they used species-specific markers ($H_E = 0.80 \pm 0.09$) or those from *R. rattus* ($H_E = 0.77 \pm 0.02$). Furthermore, the mitochondrial DNA divergence between *A. agilis* and *A. swainsonii* is estimated at 3–4% (Kirsch et al. 1990), the same low level as that seen between two wombat species for which ascertainment bias was not observed (Taylor et al. 1994). Thus overall, it is unlikely that the lower genetic diversity observed in the two larger species, *A. swainsonii* and *R. fuscipes*, was solely due to ascertainment bias.

Higher genetic diversity in animals with smaller body size has been previously reported for mammals, and is expected on the basis that smaller animals generally live at higher densities, produce a larger number of offspring and have shorter and less overlapping generation times (Mezhzherin 2002). These factors will mediate retention of higher rates of genetic diversity via maximisation of the effective population size (N_e) (Frankham 1996). At Mt Donna Buang, the

largest species, *R. fuscipes*, was numerically dominant, exhibiting almost the same population density as the two *Antechinus* spp. combined. While *A. agilis* was more common than *A. swainsonii*, it was only marginally so (Cunningham et al. 2005, MacGregor unpubl.). Furthermore, these two species had very similar test numbers and thus potential for reproductive output in the Mt Donna Buang population (MacGregor unpubl.). *R. fuscipes* may have a higher reproductive output than both the *Antechinus* spp. because they produce up to seven offspring per litter, and often produce more than one litter during each breeding season (Cockburn et al. 1985, Robinson 1987). Thus, although the higher genetic diversity in *A. agilis* than the other two species does follow the trend expected for smaller animals, it cannot be explained by census population size and reproductive output in the study population. It is possible however, that mutation rates differ among taxa based on body size: Martin and Palumbi (1993) reported a strong relationship between nucleotide substitution rate and body size, with both generation time and metabolic rate explaining substantial amounts of the variation in substitution rate among taxa. Microsatellite allele mutations entail the deletion or acquisition of a repeat sequence. Theoretical and empirical work has shown that mutation rate increases with increased number of repeats (Whittaker et al. 2003). We do not know the exact number of repeats in the alleles, but if we compare the allele sizes of the two marsupial species at the same microsatellite markers, we do see that the larger species (*A. swainsonii*) has shorter allele sizes. This may suggest that the number of repeats, and thus mutation rate, is lower in *A. swainsonii* compared to *A. agilis*, leading to higher genetic diversity in the smaller *A. agilis*.

Four further life history characteristics that may affect genetic diversity via their impacts on N_e are adult sex ratio, the degree of overlap in generations, mating system and level of multiple paternity in litters (Sugg and Chesser 1994). The adult sex ratio for all three species varied among grids and in some cases years, but was in the range of 50–100% males in all species (MacGregor unpubl.). Thus the sex ratios for the three species did not differ in a consistent way that might contribute to differences in N_e . However, differences in degree of overlap of generations may contribute to the observed differences in genetic diversity. Although in both *Antechinus* spp. all males die after the mating season, approximately 42% of female *A. swainsonii* breed in two consecutive seasons, compared to only 16% in *A. agilis* (Cockburn et al. 1985), such that the degree of overlap in generations is lower, and thus N_e higher, in the latter (Frankham et al. 2002). Multiple paternity within litters also increases N_e (Sugg and Chesser 1994) and it is known that *A. agilis* litters are sired by multiple males (Kraaijeveld-Smit et al. 2002b).

However, if, and to what extent multiple paternity occurs and what kind of mating system is in place in the other two species is not known and warrants further research. Thus, the possibility that differences in mating system and levels of multiple paternity within litters can explain the differences in genetic diversity between species remains open.

Conclusions

The comparative approach employed in this study shows clearly that population genetic characteristics of three sympatric small mammals are correlated with both body size and dispersal patterns. These factors were found to have a large effect on the population genetic variability and structure, especially at this fine scale, in agreement with previous studies (Fedorov 1999, Newton et al. 2003, Brede and Beebee 2004, Hurtado et al. 2004, Kankare et al. 2005). That close kin mating is avoided through sex-biased dispersal in two of the three study species but not the third, suggests that habitat fragmentation will have different genetic effects on them. Evidence is accumulating that especially male dispersal in *A. agilis* is limited when habitat is fragmented, thus increasing the risk of encountering kin during the mating season (Kraaijeveld-Smit et al. 2002a, Banks et al. 2005b). Apparently in response, *A. agilis* significantly avoided as nest-mates relatives of the opposite sex, in fragmented but not un-fragmented habitat (Banks et al. 2005c). This field observation is supported by laboratory experiments, in which females spend more time near odours of genetically dissimilar males compared to those that are more closely related, and in which less-related males sire more offspring (Kraaijeveld-Smit et al. 2002c, Parrott et al. 2007). Furthermore, in fragmented areas, the level of multiple paternity within litters was reduced compared to that in continuous habitats (Banks et al. 2005c). Recent research has shown that polyandrous *A. agilis* females have higher offspring fitness than monandrous females (Fisher et al. 2006). Thus, habitat fragmentation may have substantial genetic and demographic effects on these species. In the case of *R. fuscipes*, which in this study exhibited genetic signatures consistent with inbreeding within demes, restricted gene flow due to habitat fragmentation (Lindenmayer and Peakall 2000) may allow deleterious alleles to drift to high frequencies within demes. Continued inbreeding may thus promote expression of deleterious traits and inbreeding depression. A comparative study similar to the one we conducted but in fragmented habitat (Peakall et al. 2003, Banks et al. 2005b,c) would be useful for unravelling the effects of fragmentation on genetic structures via life history trait responses.

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