The effects of habitat fragmentation via forestry plantation establishment on spatial genotypic structure in the small marsupial carnivore, *Antechinus agilis*

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Abstract

Dispersal is an important influence on species' distributions, patch colonization and population persistence in fragmented habitat. We studied the impacts of habitat fragmentation resulting from establishment of an exotic pine plantation on dispersal of the marsupial carnivore, *Antechinus agilis*. We applied spatial analyses of individual multilocus microsatellite genotypes and mitochondrial haplotypes to study patterns of gene flow in fragmented habitat and natural habitat 'control' areas, and how this is affected by the spatial dispersion of habitat patches, the presence of corridors and a 'mainland' source of migrants. Spatial analysis of molecular variance and partial Mantel tests confirmed the absence of cryptic barriers to gene flow in continuous habitat, which if present would confound the comparison of genetic structures in fragmented vs. unfragmented habitats. Spatial genotypic structure suggested that although dispersal was male-biased in both habitat types, fragmentation restricted dispersal of males more than that of females and the degree of restriction of male dispersal was dependent on the geographical isolation of the patch. The scale of positive genotypic structure in fragmented habitat was restricted to the two closest patches for females and the three closest patches for males. Our results provide evidence for significantly increased gene flow through habitat corridors relative to that across the matrix and for significantly lower gene flow between 'mainland' unfragmented habitat and habitat patches relative to that within either habitat type, suggesting a behavioural barrier to crossing habitat interfaces.

Keywords: *Antechinus agilis*, corridor, dispersal, habitat fragmentation, microsatellite, spatial autocorrelation

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Introduction

The destruction and subsequent fragmentation of habitat is recognized as one of the greatest contributors to recent and potential future extinctions (Tilman *et al.* 1994; Henle *et al.* 2004). Together with reduction in habitat area and quality, reduction in habitat connectivity is one of the major factors influencing species' persistence in fragmented landscapes. Where the rate of successful migration is reduced, remnant populations may become isolated and increasingly susceptible to extinction through environmental,

demographic and genetic influences (Stacey & Taper 1992; Mills & Smouse 1994). Dispersal patterns in relation to the spatial configuration of habitat patches also influence the probability of patch colonization under a metapopulation model and are therefore important determinants of the distributions of species that are able to persist in fragmented habitats (Hanski 1994a; King & With 2002).

Given the difficulty in monitoring dispersal using field-based means (Koenig *et al.* 1996), genetic studies have provided the most accessible approach to investigating the effects of habitat fragmentation on dispersal. Loss of genetic variation in fragmented systems has been used to infer population isolation, and contrasting patterns of mitochondrial and autosomal DNA variation have revealed

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differing effects of fragmentation on the sexes (Tallmon *et al.* 2002; Johnson *et al.* 2003). However, the power to detect specific effects of fragmentation on dispersal is limited as loss of genetic variation in fragmented populations is influenced not only by degree of isolation, but by effective population size and the number of generations since fragmentation (Steinberg & Jordan 1997). Analyses based on individual multilocus genotypic arrays, such as spatial autocorrelation (Smouse & Peakall 1999; Peakall *et al.* 2003) may be more sensitive to contemporary population structure than those based solely on genetic diversity or differentiation (Sunnucks 2000).

Although most habitat loss and fragmentation in Australia are caused by clearing for agriculture or urbanization (Burgman & Lindenmayer 1998), the area covered by plantations of exotic softwood species is planned to increase from approximately 1.5 million hectares to over 3 million hectares by 2020 (Department of Primary Industries and Energy 1997). Softwood plantations provide poor quality habitat for many native species, but populations can persist in remnant patches of eucalypt forest within the plantation matrix (Lindenmayer *et al.* 1997, 1999a). The retention of eucalypt patches therefore provides an opportunity to conserve native wildlife in softwood plantation landscapes. However, the remnant eucalypt forest is generally highly fragmented and to understand the impacts of habitat fragmentation on species surviving in remnant native habitat within plantation landscapes, ecological and genetic studies are being conducted on a range of native taxa in and adjacent to a large (approximately 50 000 ha) plantation of exotic Monterey pine (*Pinus radiata*) near Tumut in southeastern Australia (Lindenmayer *et al.* 1999a, b; Lindenmayer & Peakall 2000; Peakall *et al.* 2003; Schmuki 2003; Banks *et al.* 2005). In this study, we investigated the effects of habitat fragmentation due to the establishment of this pine plantation on patterns of gene flow in the agile antechinus (*Antechinus agilis*), a small (males: 20–45 g, females: 16–25 g), semelparous carnivorous dasyurid marsupial. Previous work reported that *A. agilis* populations persist in 70% of eucalypt forest remnants (< 1 ha to approximately 100 ha) within the pine plantation, although the available habitat comprised only 2% of the area studied (Banks *et al.* 2005). The presence of *A. agilis* in a patch was influenced predominantly by habitat quality and proximity to another occupied site. Estimated population sizes in occupied sites were, in turn, influenced predominantly by habitat quality variables and patch area. The finding of no significant reduction in genetic diversity (at both microsatellite and mitochondrial loci) in populations in the fragmented habitat relative to unfragmented controls was inconsistent with simulated expectations assuming isolation of the patch populations (Banks *et al.* 2005). However, the apparent importance of proximity to another occupied site in determining the presence of *A. agilis* in a patch

implies that although the patch populations are not isolated, the species' distribution in the fragmented habitat is limited by its dispersal capability. While highlighting the importance of dispersal for the distribution of this species in fragmented habitat, the previous study provided no specific information on how dispersal is affected by habitat fragmentation.

To explore the effects of habitat fragmentation on dispersal of *A. agilis*, we compared the spatial patterns of genotypic structure in fragmented and unfragmented habitat. We first used spatial analysis of molecular variance (Dupanloup *et al.* 2002) and partial Mantel tests to determine whether there are any underlying patterns of genetic structure in the fragmented or unfragmented areas that may be caused by historical barriers to gene flow. If so, this would mean that any differences between the two habitat types would not be solely attributable to habitat fragmentation. We then used specific hypotheses relating to expected patterns of spatial genotypic structure to address the following questions about the impact of habitat fragmentation on dispersal:

- 1 Is dispersal sex-biased in unfragmented and fragmented habitat?
- 2 Is dispersal of either sex restricted by habitat fragmentation?
- 3 Is dispersal influenced by the geographical isolation of habitat patches?
- 4 Do riparian strips of habitat facilitate dispersal in fragmented habitat?
- 5 Is dispersal restricted to the most proximal sites in fragmented habitat?
- 6 Is there a 'mainland effect', whereby adjacent unfragmented habitat acts as a major source of immigrants to the patches?

Methods

Study area and sampling

The study was conducted at Buccleuch State Forest (SF) between Canberra and Tumut in southeastern New South Wales, Australia (148°40'E, 35°10'S). Buccleuch SF is a large (50 000 ha) plantation of exotic *Pinus radiata*, within which there are 192 patches of remnant *Eucalyptus* forest (Lindenmayer *et al.* 1999a). Adjacent to the southern and eastern borders of the plantation is a large area of unfragmented native eucalypt forest that is contiguous with Namadgi and Kosciuszko National Parks (Fig. 1). *Antechinus agilis* is common in the unfragmented eucalypt forest and is found in more than 50% of eucalypt patches within the pine plantation, but not in the pine plantation itself (Lindenmayer *et al.* 1999a; Banks *et al.* 2005).

Sampling of *A. agilis* was conducted after the period of postweaning dispersal (January–March) but prior to the

Fig. 1 Map of Buccleuch State Forest pine plantation and surrounding areas showing fragmented and unfragmented study areas.

Fig. 2 Diagram of the unfragmented control area showing the spatial location of the different trapping grids. The number of *Antechinus agilis* sampled at each site is presented (no. of females, no. of males). A total of 21 females and 53 males was sampled in the 36 10-trap sites.

mating season (August), so that we could study patterns of spatial genetic structure resulting from postweaning dispersal patterns. Trapping in the unfragmented 'control' area (Figs 1 and 2) was conducted in 2001 using grids of 100 aluminium box traps (Elliott Scientific), either arranged in four rows of 25 traps spaced at 15-m intervals or two rows of 50 traps along either side of a gully. A series of 36 smaller trapping sites (with 10 randomly placed traps in each) were set up at 300-m intervals on a 1.5×1.5 km grid. Trapping was conducted for four nights at the 10 trap sites and gully sites, and for four to seven nights at the 4×25 trap grids depending on the capture rate (until at least 20 *A. agilis* had been sampled at each site). We also trapped at four

Fig. 3 Map of fragmented study area showing the location of remnant patches of eucalypt forest within the pine plantation. The number of *Antechinus agilis* sampled in each patch (no. of females, no. of males) is presented next to each site. The four sites in the large area of eucalypt forest to the west of the plantation (labelled 'M') were not included among the fragmented sites but used as the 'mainland' area for some of the analyses. Those labelled 'C' were riparian strips (corridors) of eucalypt forest used to compare dispersal patterns with those across the pine matrix.

sites in eucalypt forest immediately adjacent to the western boundary of the region of pine plantation containing our patch sites (Fig. 3) for three nights using four lines of 25 traps spaced at 15-m intervals. These were not used as unfragmented control sites for comparison to the fragmented study area, but were used to compare patterns of spatial genetic structure in different areas of unfragmented habitat.

Trapping in the habitat patches within the pine plantation was conducted in 2002 as described in Banks *et al.* (2005). The location of the remnant patches and sample sizes are shown in Fig. 3. Two patches were riparian strips of eucalypt forest approximately 3 km long and 25–200 m wide, which provided suitable habitat for *A. agilis*. Traps were spaced at 10–15 m intervals through the corridors.

Captured individuals were sampled by ear notching and the resulting tissue was stored in 99% ethanol. The location of each capture was recorded with a Garmin GPS unit and each individual was assigned a geographical location for the spatial genotypic analyses corresponding to the average UTM coordinate of its capture locations within a site.

Laboratory analysis

A total of 737 *A. agilis* was genotyped for this study, including 337 from the unfragmented 'control' area (191 males and 146 females), 282 from the remnant patches within the pine plantation (111 males and 171 females) and 118 from the eucalypt forest immediately adjacent to the pine plantation (65 males and 53 females). We genotyped all samples at 15 microsatellite loci and used single-stranded

conformation polymorphism (SSCP) analysis and sequencing to score mitochondrial DNA (mtDNA) haplotypes for 492 base pairs of control region (D-loop) sequence as described in Banks *et al.* (2005).

Assessment of the suitability of the unfragmented sampling areas as controls for fragmentation effects

In using adjacent unfragmented habitat as a control to study the effects of habitat fragmentation, we make the assumption that there were no pre-existing differences in patterns of gene flow within each sampling area prior to fragmentation. Such differences could be due to (i) the presence of geographical barriers to gene flow and/or (ii) variation in isolation-by-distance (IBD) patterns due to differences in habitat quality and population density.

Identification of barriers to gene flow

To identify putative barriers to gene flow, we used the simulated annealing approach described by Dupanloup *et al.* (2002) to identify groups of adjacent sampling sites amongst which the proportion of genetic variance (F_{CT}) was maximized. We conducted this analysis for both fragmented and unfragmented areas with data from multilocus microsatellite genotypes and mtDNA haplotypes, using the program SAMOVA 1.0 (Dupanloup *et al.* 2002). We used 100 starting configurations and identified two, three and four maximally differentiated groups for each sampling area and genetic marker type.

Use of the F_{CT} index to partition sampling sites into differentiated groups may reveal significant differentiation between groups in the absence of any barriers, simply due to IBD (Dupanloup de Ceuninck *et al.* 2000; Dupanloup *et al.* 2002). We used partial Mantel tests to determine if the putative barriers identified by SAMOVA explained a significant component of the variance in F_{ST} between sampling sites that was not explained by IBD. Pairwise F_{ST} between sites was calculated in ARLEQUIN version 2.000 (Schneider *et al.* 2000) and the program IBD 1.52 (Bohonak 2002) was used to estimate the partial correlation of $F_{ST}/(1 - F_{ST})$ and the matrix based on putative barriers (0 = both populations in same SAMOVA group, 1 = populations in different SAMOVA groups) and controlling for the effect of log-transformed geographical distance. If any of the partial correlations were significant, it would suggest the presence of a barrier to gene flow.

Testing for variation in spatial genetic structure in unfragmented habitat

We tested for differences in spatial genetic structure in unfragmented habitat on either side of the fragmented study area (the unfragmented control area in Fig. 2 and the

unfragmented sites to the west of the plantation in Fig. 3). If the patterns were similar between the unfragmented areas, it would suggest that spatial genotypic structure is reasonably consistent in unfragmented habitat and would support our conclusions that any differences between the fragmented and unfragmented areas were caused by fragmentation. We measured spatial genetic structure by calculating the spatial autocorrelation of multilocus microsatellite genotypes of individuals separated by up to 3000 m, according to the method of Smouse & Peakall (1999) using the software GENALEX 5.1 (Peakall & Smouse 2001). Queller & Goodnight's (1989) r has also been used to study dispersal (Taylor *et al.* 1997; Knight *et al.* 1999; Stow *et al.* 2001; Banks *et al.* 2002) and we found that the two measures were highly correlated over the 15 microsatellite loci used here (Pearson correlation coefficient = 0.988 based on pairwise values among all 644 sampled individuals). We used the former as the GENALEX software incorporates a flexible permutation and bootstrap testing procedure. For each of the unfragmented habitat zones, we calculated the spatial autocorrelation coefficient r among individuals separated by 0–500 m and by 0–1000 m. If the r values differed significantly between habitat zones, this would indicate variation in spatial genetic structure in unfragmented habitat that may confound any conclusions about the impacts of fragmentation.

Is dispersal sex-biased in unfragmented and fragmented habitat?

Site-level comparisons. Dispersal of *A. agilis* in a continuous habitat is strongly male-biased (Cockburn *et al.* 1985). This results in higher relatedness among females in a given area than among males (Kraaijeveld-Smit *et al.* 2002). We used multilocus microsatellite genotypes to calculate r values (Smouse & Peakall 1999) among all individuals, males, females and opposite-sex pairs within each site and combined the data for all sites in each habitat type using the 'Multiple Pops' option for the spatial autocorrelation analysis in GENALEX 5.1. So that the magnitude of the r values was not biased between sites or habitat types, for each site we calculated r values among genotypes in a database from *Antechinus* sampled over a scale of up to 6000 m from that site (calculation of within-group r values will be implemented in GENALEX 6). For each category, we determined if the r value was significantly positive (indicating spatial clustering of genetically similar individuals on the scale of the sampling site) or negative using the permutation procedure implemented in GENALEX 5.1. To determine whether dispersal was sex-biased in each habitat type, we performed 5000 random reassignments of individuals within each site as male or female, using a permutation procedure in POPTOOLS 2.6.2 (Hood 2004). If the observed male–female difference in mean within-site

r values was greater than 95% of the mean differences from the permuted data set, it was deemed significant.

To determine whether dispersal of either sex was restricted by habitat fragmentation, we compared the r values for the four categories (males, females, opposite-sex pairs and all individuals). We used a randomization procedure whereby n (the maximum possible number of independent data points in the smaller data set) independent data points (pairwise r values between individuals) were sampled from the fragmented and unfragmented habitat and the data points were randomly distributed between the two habitats. This was repeated 5000 times using PORTTOOLS 2.6.2 (Hood 2004) and the observed means of the two groups were deemed significantly different if the observed mean exceeded the permuted mean on more than 95% of occasions.

Individual-level spatial analyses. Spatial autocorrelation analyses of pairwise multilocus genetic distances (and single-locus mtDNA distances) between individuals were conducted to investigate small-scale (6×200 m distance intervals) and larger-scale (6×1000 m) patterns of spatial genotypic structure. If gene flow is restricted relative to the scale of the analysis, we expect significant positive autocorrelation over the spatial scale to which gene flow is limited. Spatial autocorrelations were conducted using GENALEX 5.1, and the bootstrap 95% confidence intervals (CIs) around each estimate of r (Peakall *et al.* 2003) were used to determine if there was significant positive or negative autocorrelation at that interval and whether the r values for males and females differed significantly. We compared the correlograms based on multilocus microsatellite genotypes between males and females in each habitat type. Given that dispersal in *A. agilis* is male-biased (Cockburn *et al.* 1985; Kraaijeveld-Smit *et al.* 2002), it is possible that patterns of spatial structure of nuclear marker genotypes among females could be influenced by male dispersal. We therefore carried out spatial autocorrelations for females only using genetic distances based on mtDNA haplotypes (0 or 1 depending on whether individuals shared the same haplotype or not) and compared these to the correlograms based on the microsatellite genotypes (results are presented only for the large-scale correlograms).

Is dispersal influenced by the geographical isolation of habitat patches?

We hypothesized that the geographical isolation of a habitat patch in the fragmented system influences the degree of migration to and from that site. If this is the case, there should be a positive relationship between r values among individuals within a site and the distance to the nearest patch of suitable habitat. We classed all occupied

patches as suitable habitat and calculated r values among males and females within a site, using the 'Multiple Pops' option in GENALEX 5.1 to combine over sites that were isolated by the following distances: 0–250 m, 250–500 m, 500–750 m, 750–1000 m and greater than 1000 m.

Do riparian strips of natural habitat facilitate dispersal in fragmented habitat?

Dispersal in the fragmented habitat may occur either through the pine matrix or along riparian strips of eucalypt forest (marked 'C' in Fig. 3). Although these are not true 'corridors' linking nonlinear patches, increased dispersal through these riparian strips relative to the plantation matrix would support the utility of corridors in promoting dispersal in fragmented landscapes. We compared r values between individuals separated by equivalent distances of pine plantation matrix and the riparian strips of habitat. Because of the relatively small sample size from the riparian strips (96 individuals), we pooled the data for males and females and categorized the geographical distances according to whether the habitat between a pair of individuals was pine plantation or riparian eucalypt habitat. We calculated r values for individuals in each category over distance bins of 250–1000 m, 1000–2000 m and 2000–3000 m. The first 250 m were omitted as there were relatively few interindividual distances smaller than this across the pine plantation. The r values were compared between habitat types using the bootstrap 95% CIs. This analysis differs from spatial autocorrelation in that the latter is concerned with detecting the spatial scale and strength of clustering of genetically similar individuals within a group, whereas this analysis involves a comparison between two categories of the level of genetic similarity of individuals separated by a given spatial scale. If the rate of dispersal over a given distance (say, 1000–2000 m) is higher through the riparian strips of eucalypt forest than the pine matrix, we would expect higher r values over that scale in that category than the 'pine' category for that distance class.

Is dispersal restricted to the most proximal sites in fragmented habitat?

If the geographical scale of dispersal in fragmented habitat is restricted, migrant exchange will occur primarily between neighbouring sites (i.e. those within dispersal range). If dispersal of *A. agilis* in the pine plantation follows a stepping-stone model, we would expect to find positive autocorrelation between genotypes in only the most proximal sites and negative or nonsignificant autocorrelation between non-neighbouring sites. We transformed the distances between patches in the fragmented habitat to ranks such that 0 represented the distance between

individuals in the same site, 1 represented the distance to the most proximal site, 2 represented the distance to the second closest site, etc. Spatial autocorrelation analyses were conducted using these distance classes using the microsatellite data for both sexes and mtDNA data for females.

Is there a 'mainland effect', whereby adjacent unfragmented habitat acts as a major source of immigrants to the patches?

Unfragmented eucalypt forest adjacent to the pine plantation (the 'mainland', see Fig. 3) is potentially an important source of immigrants for the patch populations. However, individuals may be more likely to remain within unfragmented habitat rather than venture into the fragmented system where less than 5% of the landscape provides suitable habitat and dispersal mortality is probably higher (Banks *et al.* 2005). We compared the r values to determine whether the correlation between multilocus genotypes across the fragmentation boundary (i.e. between individuals in the mainland and the patches) differed from that over the same spatial scale in one habitat or the other. If there is a behavioural barrier to migration from the 'mainland' into the fragmented habitat, we would expect to observe lower r values between individuals across the habitat boundary than over the same scale within the unfragmented habitat and possibly even in the fragmented habitat. We divided the geographical distances into those among individuals within the 'mainland' unfragmented habitat adjacent to the plantation, those among individuals within the fragmented habitat, and those between pairs of individuals separated by the border of the fragmented habitat. We calculated r values and their 95% CIs for the pooled male and female genotypes and compared them among the three categories over a 0–1000 m distance class.

Results

Assessment of the suitability of the unfragmented sampling areas as controls for fragmentation effects

Identification of barriers to gene flow. The F_{CT} indices among the maximally differentiated groups of sites in the fragmented and unfragmented sampling areas are presented in Table 1. F_{CT} was generally higher (and more significant) as the number of groups increased, which is expected as F_{CT} and F_{SC} (variance among subpopulations within the same group) are components of F_{ST} (variance among subpopulations), and F_{SC} is expected to decrease as the average number of subpopulations within a group decreases (Dupanloup *et al.* 2002). However, the partitioning of sampling sites into groups was not consistent between the microsatellite and mitochondrial DNA analyses for either habitat type and the inferred group structure did not explain a significant component of genetic differentiation not explained by IBD. Therefore, we concluded there was no evidence for any geographical barriers influencing genetic population structure in either habitat type and therefore it was valid to attribute any differences in genetic structure between the habitat types to fragmentation. Scatterplots of pairwise F_{ST} and geographical distance between sites in the fragmented and unfragmented habitat are shown for microsatellite and mtDNA in Fig. 4a and b, respectively. The results suggest no major effect of habitat fragmentation on the pattern of IBD of mtDNA, but an effect of fragmentation in the microsatellite IBD pattern (a higher intercept but similar slope).

Testing for variation in spatial genetic structure in unfragmented habitat

There were no significant differences in the first-interval spatial autocorrelation coefficients between the two

Table 1 Summary of results of spatial analysis of molecular variance to identify putative barriers to gene flow between maximally differentiated groups of sampling sites. The proportion of variance in genetic diversity explained by the group structure (F_{CT}) is presented and its P value is the proportion of 1023 randomized group structures resulting in an equivalent or higher F_{CT} . The r_{xy} values from the partial Mantel test denote the partial correlation of pairwise $F_{ST}/(1 - F_{ST})$ with the putative barriers based on the identified group structure, controlling for the effect of log-transformed geographical distance on the variance in $F_{ST}/(1 - F_{ST})$. P values for the partial correlation coefficients are the proportion of r values based on permutation of the $F_{ST}/(1 - F_{ST})$ matrix that are not less than the observed value (1000 permutations)

Marker type	No. of groups	Unfragmented		Fragmented	
		F_{CT} (P value)	r_{xy} (P value)	F_{CT} (P value)	r_{xy} (P value)
Microsatellites	2	0.015 (0.006)	0.145 (0.224)	0.011 (0.003)	0.143 (0.160)
	3	0.015 (0.001)	0.145 (0.208)	0.013 (0.001)	0.149 (0.164)
	4	0.014 (0.000)	0.147 (0.223)	0.014 (0.000)	0.159 (0.142)
mtDNA	2	0.284 (0.145)	−0.101 (0.685)	0.154 (0.075)	0.062 (0.334)
	3	0.236 (0.049)	−0.078 (0.630)	0.155 (0.001)	0.055 (0.360)
	4	0.202 (0.009)	0.012 (0.491)	0.163 (0.001)	0.030 (0.411)

Table 2 Spatial autocorrelation coefficients (r) for the first distance interval (0–500 m and 0–1000 m) for multilocus microsatellite genotypes of *Antechinus agilis* sampled from two unfragmented areas on either side of the fragmented habitat: the control sampling area (1) and the 'mainland' unfragmented habitat (2) shown in Figs 1 and 2. The P value is the proportion of r values based on random permutations of the geographical locations of individuals separated by up to 3000 m that would result in an r value higher than observed and the confidence limits are based on a 95% bootstrap confidence interval

	Interval size (m)	r	P ($r >$ permuted r)	2.5% CL	97.5% CL
Unfragmented-1	500	0.012	0.001	0.007	0.017
	1000	0.008	0.001	0.004	0.012
Unfragmented-2	500	0.013	0.001	0.009	0.017
	1000	0.008	0.001	0.006	0.011

Fig. 4 Scatterplots showing the relationship between pairwise F_{ST} and distance between large ($n > 10$) populations in fragmented habitat and sites in unfragmented habitat for microsatellite (a) and mitochondrial DNA (b) markers.

unfragmented habitat zones on either side of the fragmented study area (Table 2). Therefore, there was no reason to assume that there were pre-existing differences in spatial genetic structure between the currently fragmented habitat and the unfragmented control area.

Is dispersal sex-biased in unfragmented and fragmented habitat?

Site-level comparisons. The r values among individuals within a site in the fragmented and unfragmented habitat are presented in Table 3. Average r was significantly higher than that based on random selections of individuals separated by up to 6000 m for all categories except males in unfragmented habitat, suggesting a high level of dispersal for this

group. Within each habitat context, mean r values among females within a site were significantly higher than among males (unfragmented: $P = 0.0002$, fragmented: $P = 0.0002$).

Mean r values among all individuals, males and opposite-sex pairs were significantly higher in sites in the fragmented habitat than in the unfragmented habitat (all: $P = 0.0018$; males: $P = 0.0016$; opposite sex: $P = 0.0008$), but the same comparison for females was not significant ($P = 0.635$). This suggests that the effects of fragmentation on dispersal are largely limited to males.

Individual-level spatial analyses. The 6000 m scale microsatellite spatial correlograms for males and females in unfragmented habitat reveal significant positive spatial structure only to 1000 m for females but to 2000 m for males (Fig. 5a). The r value for females was significantly higher than that for males in the first distance class, which is consistent with reduced dispersal of females. The smaller-scale correlograms (Fig. 5b) suggest that the spatial scale of significant positive genotypic structure extends only to 400 m for females, whereas there is no detectable structure on this scale for males. The results based on mtDNA markers (Fig. 5e) reveal the same scale of positive spatial genotypic structure among females as the microsatellite genotypes.

In the fragmented habitat, there was significant positive spatial structure for both sexes up to 3000 m (Fig. 5c), but the r values were significantly higher for females in the first two intervals (0–2000 m) and significantly lower at the 5000–6000 m interval. The scale of significant positive structure among females according to the mtDNA data was 2000 m. The small-scale correlograms (Fig. 5d) show that the r values for females are significantly higher than for males for the first interval (0–200 m). These results suggest that dispersal is male-biased in fragmented habitat but the pattern of spatial genotypic structure differs from that in unfragmented habitat.

Is dispersal influenced by the geographical isolation of habitat patches?

We investigated the influence of the degree of patch isolation on r values among males and females within a site

Table 3 Combined r values from multilocus microsatellite genotypes of individuals sampled in the same site for unfragmented and fragmented habitat. FF, MM and MF represent r values among females, males and opposite-sex pairs, respectively. The number of pairwise r values in each category is presented, along with the bootstrap 95% confidence interval around each estimate of r . The P values presented in brackets are the proportion of r values based on random permutations of individuals separated by up to 6000 m that were higher than the observed within-site r values. For the sites in the fragmented area, r values and their associated P values are presented for males and females in habitat patches isolated from the nearest occupied site by increasing geographical distances across the pine plantation matrix

Category		Unfragmented	Fragmented					
			All	< 250	250–500	500–750	750–1000	> 1000
FF	n	467	1794	185	1130	276	175	28
	r	0.055	0.071	0.122	0.058	0.083	0.082	0.066
	97.5% CL	0.07	0.078	0.145	0.067	0.100	0.103	0.116
	2.5% CL	0.039	0.064	0.096	0.050	0.065	0.059	0.019
	P	0.001	0.001	0.001	0.001	0.001	0.001	0.006
MM	n	813	666	18	331	191	33	93
	r	0.006	0.031	0.001	0.018	0.046	0.035	0.049
	97.5% CL	0.013	0.04	0.040	0.031	0.061	0.078	0.075
	2.5% CL	–0.003	0.022	–0.039	0.005	0.031	–0.004	0.023
	P	0.059	0.001	0.438	0.001	0.001	0.023	0.001
MF	n	1232	2186					
	r	0.011	0.037					
	97.5% CL	0.016	0.042					
	2.5% CL	0.005	0.031					
	P	0.001	0.001					
All	n	2512	4646					
	r	0.017	0.046					
	97.5% CL	0.022	0.050					
	2.5% CL	0.012	0.042					
	P	0.001	0.001					

in the fragmented habitat (Table 3). For sites less than 250 m from another occupied site, r values among males were not significantly positive (a similar pattern to that observed in unfragmented habitat). However, r values among males increased with increasing patch isolation and the confidence intervals of the r estimates overlapped for males and females overlapped for sites isolated by over 750 m. There was no trend towards increasing r values among females with increasing geographical isolation of a patch. These results are consistent with male dispersal being restricted by the geographical isolation of habitat patches.

Do riparian strips of natural habitat facilitate dispersal in fragmented habitat?

The correlograms in Fig. 6 show significantly higher r values among individuals linked by riparian strips of eucalypt forest than among individuals separated by equivalent distances of pine plantation over all intervals up to 3000 m. Significant positive autocorrelation was detected up to 3000 m among individuals separated by pine plantation. These findings are consistent with greater gene flow between localities in the fragmented system linked by native forest retained along gullies.

Is dispersal restricted to the most proximal sites in fragmented habitat?

The correlograms presented in Fig. 7a show that for the microsatellite genotypes there was significant positive autocorrelation among males sampled in the same site and in the three most proximal occupied sites. For females, there was significant positive autocorrelation among individuals sampled in the same site and the two most proximal occupied sites. The r values for females in the same site and the most proximal site were significantly higher than those for males. The pattern of spatial autocorrelation of mtDNA haplotypes among females was similar to that observed for microsatellites (Fig. 7b). These findings match the expectations if gene flow were restricted to the most proximal sites in the fragmented habitat.

Is there a 'mainland effect', whereby adjacent unfragmented habitat acts as a major source of immigrants to the patches?

There was no significant correlation between genotypes in the 'mainland' unfragmented habitat adjacent to the pine plantation and the genotypes of individuals in the habitat patches over the 1000 m spatial scale compared ($r = 0.001$,

Fig. 5 Correlograms showing the spatial correlation (r) for microsatellite and mitochondrial genetic marker data for male and female *Antechinus agilis* over a range geographical distance classes (Dclass) in unfragmented and fragmented habitat. (a) and (b) are spatial correlograms based on microsatellite genotypes for males and females in unfragmented habitat. (c) and (d) are spatial correlograms based on microsatellite genotypes for males and females in fragmented habitat. (e) shows spatial correlograms for females only based on mitochondrial DNA haplotype-sharing in fragmented and unfragmented habitat. The error bars represent the bootstrap 95% confidence interval for each estimate of r , which is considered significant if the bootstrap CI does not include 0. The values above the correlograms are the number of pairwise values for males (top) and females (bottom) in each distance class.

$P = 0.435$). In contrast, there was significant positive spatial autocorrelation between the genotypes of individuals sampled within one habitat type or the other (mainland: $r = 0.022$, $P = 0.001$; unfragmented: $r = 0.039$, $P = 0.001$).

Discussion

Assessing the validity of unfragmented areas as 'controls' for fragmentation studies

Genetic studies of the effects of anthropogenic habitat fragmentation on population structure usually involve the comparison of patterns detected in fragmented and unfragmented habitat (Gerlach & Musolf 2000; Knutsen *et al.* 2000; Mossman & Waser 2001; Stow *et al.* 2001;

Tallmon *et al.* 2002; Sumner *et al.* 2004). Although this is probably a valid approach in most cases, it assumes a lack of pre-existing differences in genetic structure between the habitat types. Such pre-existing differences could be due to differences in habitat quality, which may affect population density, dispersal distances and genetic structure (Rousset 2000), or to barriers to dispersal, such as mountain ridges or rivers (Lugon-Moulin & Hausser 2002). In the present study, there was no variation in spatial genotypic structure in unfragmented habitat on either side of the fragmented area. This result, and the fact that the forest type present prior to fragmentation was similar to that in the currently unfragmented areas (Tyndale-Biscoe & Smith 1969), suggests there were no pre-existing differences in spatial genotypic structure between 'control' and fragmented

Fig. 6 Correlograms showing r values among multilocus microsatellite genotypes of individuals in the fragmented habitat. Geographic distances are partitioned into those corresponding to riparian strips of eucalypt forest and the pine plantation matrix. The error bars show the bootstrap 95% CI for each estimate of r and the number of pairwise data points in each distance class is presented above the correlogram (top: riparian; bottom: pine).

Fig. 7 Correlograms of microsatellite spatial autocorrelation of microsatellite genotypes among male and female *Antechinus agilis* (a) and of mitochondrial DNA haplotypes among female *A. agilis* (b) in the fragmented habitat. Interval 0 refers to the autocorrelation coefficient (r) among individuals sampled at the same site, interval 1 refers to the r value between individuals in the most proximal site, interval 2 refers to the r value between individuals in the second most proximal site, etc.

areas. Similarly, although there were no obvious potential barriers to gene flow in either habitat type (other than fragmentation), we used a two-step approach to first identify putative barriers to gene flow and then determine whether or not the structure could be explained by isolation by distance (IBD) alone. Using a similar approach, Lugon-Moulin & Hausser (2002) demonstrated that mountain passes did not present significant barriers to gene flow for the shrew *Sorex araneus*. In general, partial Mantel test results should be treated with caution as there has been some recent debate about the reliability of their significance values (Raufaste & Rousset 2001; Castellano & Balleto 2002; Rousset 2002). However, because the significance values for the partial correlation of genetic distance and putative

barriers were all much greater than 0.05 in our study, we are confident that putative barriers had no significant effect on the partitioning of genetic variance among sites. We therefore considered it valid to attribute any observed differences between the habitat types to fragmentation.

Sex-biased dispersal and spatial genotypic structure in unfragmented habitat

Male-biased dispersal in unfragmented habitat was indicated by significantly stronger genotypic correlation among females than males within a sampling site. Further, significant positive spatial autocorrelation was restricted to a smaller spatial scale for females than males. The pattern observed among females was consistent between the mtDNA and microsatellite markers, suggesting that the estimate of the scale of positive genetic structure was accurate. This also suggests that the signal of spatial autocorrelation of genotypes reflects dispersal patterns in only very recent generations, because if the pattern of spatial autocorrelation was driven by numerous generations of dispersal, we would expect the spatial genotypic structure of females to be similar to that of males because of effective gene flow by males in preceding generations. Given that female home ranges could averaged up to 3 ha in a multiyear study (Lazenby-Cohen & Cockburn 1991), the observed scale of significant positive spatial structure among the genotypes of females is consistent with observations that females rarely disperse out of their natal territory (Cockburn *et al.* 1985). A pattern of significantly higher than average genetic relatedness was also detected over the scale on which individuals are expected to have overlapping home-ranges for the less-dispersing sex of the common wombat, *Vombatus ursinus* (Banks *et al.* 2002), suggesting that such a pattern may be common when one sex is philopatric. Interestingly, the scale of positive spatial structure for both sexes of *Antechinus agilis* was higher than that detected in another small mammal studied in the same area, the native bush rat (*Rattus fuscipes*) (Peakall *et al.* 2003). A comparison of breeding and dispersal strategies of the two species of the same habitat may shed new light on the influence of these life history traits on spatial genetic structure.

Fragmentation effects on sex-biased dispersal

Previous studies have revealed that fragmentation may affect the dispersal of one sex to a greater degree than the other, thereby affecting patterns of sex-biased dispersal (Stow *et al.* 2001; Tallmon *et al.* 2002). In *A. agilis*, habitat disjunction due to the presence of a pine matrix appeared to cause restricted dispersal of males but not of females. Since most females do not disperse (even in unfragmented habitat), there may be little scope for further restriction of dispersal

because of fragmentation, whereas males disperse more and are therefore more affected. The degree to which male dispersal was restricted by fragmentation was dependent on the geographical isolation of the habitat patch. Fragmentation appeared to have little impact on male dispersal in patches isolated by less than 250 m, but in patches further than 750 m from the nearest occupied site, male dispersal appeared to be restricted to such an extent that r values among males were not significantly lower than females.

Reduced gene flow by males across large distances of pine plantation could be due to increased dispersal-associated mortality and/or behavioural factors. A comparison of the sex ratios between the habitat types revealed a significant deficiency of males in the fragmented habitat relative to the unfragmented habitat, suggesting that dispersal mortality is higher in the fragmented system (Banks *et al.* 2005). This would result in elevated genotypic similarity among males in a patch resulting from less immigration by unrelated males. Males also may simply be more reluctant to disperse where this involves crossing a large area of unsuitable habitat, causing increased genotypic similarity among males within the patch due to the presence of close relatives. Forest specialist birds are less likely to cross clear-cut gaps than larger distances of undisturbed habitat (Rail *et al.* 1997) and red squirrels (*Tamiasciurus hudsonicus*) are less likely to cross gaps than take a longer detour through unfragmented habitat, depending on the efficiency of the detour (Bakker & Van Vuren 2004).

Landscape influences on dispersal in fragmented habitat

Our finding of significantly higher r values between individuals separated by riparian strips of native eucalypt forest than by pine matrix is consistent with a higher rate of dispersal through eucalypt 'corridors' than pines in fragmented habitat. This could be due to preferential dispersal of *A. agilis* through strips of eucalypt forest within the plantation area, as well as reduced dispersal-associated mortality in such habitat compared to the pine matrix. Although the riparian strips of eucalypt forest were not true corridors linking larger areas of habitat, these findings provide evidence for the utility of corridors for enhancing dispersal in fragmented landscapes because they show that dispersal is greater through native habitat retained along gullies than through the matrix. The value of habitat corridors for conservation has been hotly debated. It has been argued that empirical evidence is lacking to support their utility and that they may actually be detrimental in some situations (Simberloff & Cox 1987; Hobbs 1992; Simberloff *et al.* 1992; Beier & Noss 1998). The present study joins other recent empirical studies in highlighting the utility of corridors as habitat (Downes *et al.* 1997) and for connectivity (Haddad 1999; Mech & Hallett 2001).

Although significant positive spatial autocorrelation was detected among genotypes separated by the pine matrix up to the 2000–3000 m interval, this corresponded to only the two most proximal sites for females and the three most proximal sites for males. This suggests that where it is not facilitated by habitat corridors, interpatch dispersal follows, to a certain extent, a stepping-stone model (Kimura 1953). Given that females appear to disperse less often and over shorter distances than males, the scale over which demographic processes such as patch recolonization occur is likely to be limited by female dispersal capacity. Although we cannot infer precise dispersal distances from the spatial genotypic analyses, the scale of positive genotypic autocorrelation among females (3000 m for microsatellites and 2000 m for mtDNA) is similar to the maximum scale of geographical isolation (distance to the nearest occupied site) of occupied patches.

Evidence for a behavioural barrier to dispersal between the 'mainland' unfragmented habitat and the fragmented system

Studies of patch occupancy in fragmented habitat often incorporate a 'mainland' (a large area of habitat with a population invulnerable to extinction) in models to predict or explain observed species' distributions (Hanski 1994a, b; Lindenmayer *et al.* 1999a, b; Lawes *et al.* 2000; Banks *et al.* 2005). Proximity of a patch to the mainland may increase colonization probability and thus decrease extinction probability (Hanski 1994a). Large areas of unfragmented eucalypt forest adjacent to the pine plantation at Tumut may provide a source of migrants and increase the likelihood of the long-term persistence of the patch populations. Lindenmayer *et al.* (1999a) found that *A. agilis* were less likely to be present in patches far from large areas of unfragmented eucalypt forest and Banks *et al.* (2005) found that the distance to unfragmented habitat was a component of the best model identified to explain the variation in estimated population size in habitat patches. Our results revealed no significant positive autocorrelation between genotypes in the 'mainland' and the fragmented system, whereas positive genotypic spatial autocorrelation was detected among individuals within each of the 'mainland' and unfragmented areas. This suggests that the level of gene flow between the unfragmented habitat (the mainland) and the patches is low relative to that within the unfragmented habitat and even within the fragmented habitat. It is possible that individuals in unfragmented habitat are reluctant to disperse through an inhospitable matrix when dispersal through unfragmented habitat is possible. These results do not mean that the 'mainland' is not a source of migrants into the patches. Rather, dispersal between the habitat types may be rarer than expected.

Comparisons of genotypic and allelic analyses

The contrasting impact of habitat fragmentation on dispersal of males and females was detected by analyses of individual-level spatial genotypic patterns (comparisons between male and female multilocus genotypes) and population-level genetic differentiation (comparisons between mtDNA and nuclear markers). However, the comparison of genetic variation between the fragmented and unfragmented sites presented in Banks *et al.* (2005) did not detect any major differences between the two marker types or habitat types, suggesting that analyses of genetic variability have relatively little power to elucidate the effects of recent habitat fragmentation on dispersal (Sunnucks 2000). In another example, Keller & Largiader (2003) observed a significant reduction in microsatellite allelic richness in only the smallest of several ground beetle populations that were apparently isolated by road building: time since isolation may have been insufficient to have yet had an effect on diversity in the larger ones.

Conclusions and implications for conservation

An earlier study of the demography and genetic variation of *A. agilis* populations at Tumut concluded that fragmentation has not caused the total isolation of the extant *A. agilis* populations in the habitat patches in the pine plantation (Banks *et al.* 2005). However, the present study revealed that habitat fragmentation due to pine-plantation establishment does restrict dispersal. Dispersal of male *A. agilis* was particularly affected and was dependent upon the degree of geographical isolation of the habitat patch. This finding, and the observation that gene flow occurs primarily between the most proximal patches, is consistent with the fact that patch occupancy is dependent on proximity to another occupied site (Banks *et al.* 2005). Understanding the factors influencing population connectivity is therefore very important to our ability to conserve species such as *A. agilis* in fragmented landscapes. In a simulation study of the importance of landscape structure for dispersal success in fragmented landscapes, King & With (2002) found that the spatial configuration of habitat had more impact on dispersal success when dispersal was limited and the proportion of suitable habitat in the landscape was low. In our fragmented study area, dispersal is limited and is an important process influencing the distribution of *A. agilis* among patches with suitable habitat, which comprise only around 2% of the total area encompassed within the pine plantation boundaries. Landscape design to optimize the occupancy rate of suitable habitat patches should therefore aim to maximize the connectivity of patch populations through spatial proximity or habitat corridors (possibly along gullies). Proximity to a 'mainland' source of migrants may

be of benefit for the long-term persistence of patch populations. However, the apparently lower level of gene flow across the unfragmented–fragmented habitat interface suggests that it would be preferable to link mainland source populations to patches by corridors.

The lack of a major reduction in genetic diversity in the fragmented habitat relative to the unfragmented sites (Banks *et al.* 2005) suggests that these populations are not at risk from factors associated with loss of genetic variation. However, the increase in the genetic similarity among opposite-sex individuals within a patch resulting from restriction of male dispersal may have implications for mate choice and reproductive success in patch populations. Cunningham's skinks (*Egernia cunninghami*) also exhibited restricted dispersal in fragmented habitat, yet adult skinks were able to avoid inbreeding by selectively mating with genetically unrelated partners (Stow & Sunnucks 2004). Whether *A. agilis*, in which dispersal is thought to be an inbreeding avoidance strategy (Cockburn *et al.* 1985), can similarly avoid inbreeding in fragmented habitat will be the subject of a future study.

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References

- Bakker VJ, Van Vuren DH (2004) Gap-crossing decisions by the red squirrel, a forest-dependent small mammal. *Conservation Biology*, **18**, 689–697.
- Banks SC, Finlayson GR, Lawson SJ *et al.* (2005) The effects of habitat fragmentation due to forestry plantation establishment on demography and genetic variation in a marsupial carnivore, *Antechinus agilis*. *Biological Conservation*, **122**, 581–597.
- Banks SC, Skerratt LF, Taylor AC (2002) Female dispersal and relatedness structure in common wombats (*Vombatus ursinus*). *Journal of Zoology*, **256**, 389–399.
- Beier P, Noss RF (1998) Do habitat corridors provide connectivity? *Conservation Biology*, **12**, 1241–1252.
- Bohonak AJ (2002) IBID (ISOLATION BY DISTANCE): a program for analyses of isolation by distance. *Journal of Heredity*, **92**, 153–154.
- Burgman MA, Lindenmayer DB (1998) *Conservation Biology for the Australian Environment*. Surrey Beatty and Sons, Chipping Norton.
- Castellano S, Balletto E (2002) Is the partial Mantel test inadequate? *Evolution*, **56**, 1871–1873.
- Cockburn A, Scott MP, Scotts DJ (1985) Inbreeding avoidance and male-biased natal dispersal in *Antechinus* spp. (Marsupialia, Dasyuridae). *Animal Behaviour*, **33**, 908–915.

- Department of Primary Industries and Energy (1997) *Plantations for Australia: The 2020 Vision. Joint Report from Ministerial Council on Forestry, Fisheries and Aquaculture, Standing Committee on Forestry, Plantations Australia, Australian Forest Growers, National Association of Forest Industries*. Department of Primary Industries and Energy, Canberra.
- Downes SJ, Handasyde KA, Elgar MA (1997) The use of corridors by mammals in fragmented Australian eucalypt forests. *Conservation Biology*, **11**, 718–726.
- Dupanloup de Ceuninck I, Schneider S, Langaney A, Excoffier L (2000) Inferring the impact of linguistic boundaries on population differentiation: application to the Afro-Asiatic-Indo-European case. *European Journal of Human Genetics*, **8**, 750–756.
- Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.
- Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology*, **14**, 1066–1074.
- Haddad NM (1999) Corridor and distance effects on interpatch movements: a landscape experiment with butterflies. *Ecological Applications*, **9**, 612–622.
- Hanski I (1994a) Patch-occupancy dynamics in fragmented landscapes. *Trends in Ecology & Evolution*, **9**, 131–135.
- Hanski I (1994b) A practical model of metapopulation dynamics. *Journal of Animal Ecology*, **63**, 151–162.
- Henle K, Lindenmayer DB, Margules CR, Saunders DA, Wissel C (2004) Species survival in fragmented landscapes: where are we now? *Biodiversity and Conservation*, **13**, 1–8.
- Hobbs RJ (1992) The role of corridors in conservation: solution or bandwagon? *Trends in Ecology and Evolution*, **7**, 389–392.
- Hood GM (2004) *POPTOOLS Version 2.6.2*. Available in the internet: <http://www.cse.csiro.au/poptools>.
- Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology*, **12**, 3335–3347.
- Keller I & Largiadere CR (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 417–423.
- Kimura M (1953) 'Stepping stone' model of population structure. *Annual Report of the National Institute of Genetics, Japan*, **3**, 62–63.
- King AW, With KA (2002) Dispersal success on spatially structured landscapes: when do spatial pattern and dispersal behaviour really matter? *Ecological Modelling*, **147**, 23–29.
- Knight ME, Van Oppen MJH, Smith HL, Rico C, Hewitt GM, Turner GF (1999) Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology*, **8**, 1521–1527.
- Knutsen H, Rukke BA, Jorde PE, Ims RA (2000) Genetic differentiation among populations of the beetle *Bolitophagus reticulatus* (Coleoptera: Tenebrionidae) in a fragmented and a continuous landscape. *Heredity*, **84**, 667–676.
- Koenig WD, Van Vuren D, Hooze PN (1996) Detectability, philopatry and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution*, **11**, 514–517.
- Kraaijeveld-Smit FJL, Lindenmayer DB, Taylor AC (2002) Dispersal patterns and population structure in a small marsupial, *Antechinus agilis*, from two forests analysed using microsatellite markers. *Australian Journal of Zoology*, **50**, 325–338.
- Lawes MJ, Mealin PE, Piper SE (2000) Patch occupancy and potential metapopulation dynamics of three forest mammals in fragmented afro-montane forest in South Africa. *Conservation Biology*, **14**, 1088–1098.
- Lazenby-Cohen KA, Cockburn A (1991) Social and foraging components of the home range in *Antechinus stuartii* (Dasyuridae: Marsupialia). *Australian Journal of Ecology*, **16**, 301–307.
- Lindenmayer DB, Peakall R (2000) The Tumut experiment – integrating demographic and genetic studies to unravel fragmentation effects. In: *Genetics, Demography and Population Viability* (eds Clarke G, Young A), pp. 173–201. Cambridge University Press, Cambridge.
- Lindenmayer DB, Cunningham RB, Pope ML (1999a) A large-scale 'experiment' to examine the effects of landscape context and habitat fragmentation on mammals. *Biological Conservation*, **88**, 387–403.
- Lindenmayer DB, McCarthy MA, Pope ML (1999b) Arboreal marsupial incidence in eucalypt patches in southeastern Australia: a test of Hanski's incidence function metapopulation model for patch occupancy. *Oikos*, **84**, 99–109.
- Lindenmayer DB, Cunningham RB, Pope ML, Donnelly CF (1997) The response of arboreal marsupials to landscape context: a large scale fragmentation study. *Ecological Applications*, **9**, 594–611.
- Lugon-Moulin N, Hausser J (2002) Phylogeographical structure, postglacial recolonization and barriers to gene flow in the distinctive Valais chromosome race of the common shrew (*Sorex araneus*). *Molecular Ecology*, **11**, 785–794.
- Mech SH, Hallett JG (2001) Evaluating the effectiveness of corridors: a genetic approach. *Conservation Biology*, **15**, 467–474.
- Mills LS, Smouse PE (1994) Demographic consequences of inbreeding in remnant populations. *American Naturalist*, **144**, 412–431.
- Mossman CA, Waser PM (2001) Effects of habitat fragmentation on population genetic structure in the white-footed mouse (*Peromyscus leucopus*). *Canadian Journal of Zoology*, **79**, 285–295.
- Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.
- Peakall R, Smouse PE (2001) *GENALEX version 5 (GENETIC ANALYSIS IN EXCEL): population genetic software for teaching and research*. Australian National University, Canberra, Australia. Available at <http://www.anu.edu.au/BoZo/GenALEX/>.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.
- Rail JF, Darveau M, Desrochers A, Huot J (1997) Territorial responses of boreal forest birds to habitat gaps. *Condor*, **99**, 976–980.
- Raufaste N, Rousset F (2001) Are partial Mantel tests adequate? *Evolution*, **55**, 1703–1705.
- Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, **13**, 58–62.
- Rousset F (2002) Partial Mantel tests: reply to Castellano and Balletto. *Evolution*, **56**, 1874–1875.
- Schmuki C (2003) *Impacts of habitat fragmentation on two species of Adeliini beetle at Tumut*. NSW Honours Thesis, Latrobe University.
- Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: a software for population genetics data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.
- Simberloff D, Cox J (1987) Consequences and costs of conservation corridors. *Conservation Biology*, **1**, 63–71.

- Simberloff D, Farr JA, Cox J, Mehlman DW (1992) Conservation corridors: conservation bargains or poor investments? *Conservation Biology*, **6**, 493–504.
- Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.
- Stacey PB, Taper R (1992) Environmental variation and the persistence of small populations. *Ecological Applications*, **2**, 18–29.
- Steinberg EK, Jordan CE (1997) Using molecular genetics to learn about the ecology of threatened species: the allure and illusion of measuring genetic structure in natural populations. In: *Conservation Biology for the Coming Decade* (eds Fiedler PL, Kareiva PM), pp. 440–460. Chapman & Hall, New York.
- Stow AJ, Sunnucks P (2004) Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology*, **13**, 443–447.
- Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology*, **10**, 867–878.
- Sumner J, Jessop T, Paetkau D, Moritz C (2004) Limited effect of anthropogenic habitat fragmentation on molecular diversity in a rainforest skink, *Gnypetoscincus queenslandiae*. *Molecular Ecology*, **13**, 259–269.
- Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology & Evolution*, **15**, 199–203.
- Tallmon DA, Draheim HM, Mills LS, Allendorf FW (2002) Insights into recently fragmented vole populations from combined genetic and demographic data. *Molecular Ecology*, **11**, 699–709.
- Taylor AC, Horsup A, Johnson CN, Sunnucks P, Sherwin WB (1997) Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **6**, 9–19.
- Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. *Nature*, **371**, 65–66.
- Tyndale-Biscoe CH, Smith RFC (1969) Studies on the marsupial glider, *Schoinobates volans* (Kerr): II. Population structure and regulatory mechanisms. *Journal of Animal Ecology*, **38**, 637–650.

The work presented here forms part of Sam Banks' PhD thesis on the effects of habitat fragmentation on the population biology of *Antechinus agilis*. David Lindenmayer's research interests include large-scale landscape ecology and conservation biology, responses of vertebrates to disturbance and ways to mitigate human impacts on biodiversity through improving the integration of conservation and production. Simon Ward is interested in the natural history and behavioural ecology of small mammals, particularly marsupials, and seabirds. Andrea Taylor has diverse interests in the application of genetic markers in conservation biology of Australian native fauna, most recently in aiding understanding of population processes accompanying habitat fragmentation.