

# Land clearing reduces gene flow in the granite outcrop-dwelling lizard, *Ctenophorus ornatus*

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## Abstract

An important question for the conservation of species dwelling in fragmented habitats is whether changes to the intervening landscape create a barrier to gene flow. Here, we make use of the spatial distribution of the granite outcrop-dwelling lizard, *Ctenophorus ornatus*, to compare inferred levels of gene flow between outcrops in a nature reserve with that between outcrops in the adjacent agricultural land. Genetic variation, relatedness and subdivision were compared within groups of individuals from different outcrops similar in size and distance apart at each site. In the agricultural land, we found significantly lower genetic variation within outcrops and greater genetic differentiation between outcrops than in the reserve. Further, the rate at which genetic divergence between outcrops increased over geographical distance was significantly greater in the agricultural land than in the reserve. We also found that individuals were more closely related within outcrops but more distantly related between outcrops in the cleared land. These effects occur over a small spatial scale with an average distance between outcrops of less than five kilometres. Thus, even though land clearing around the outcrops leaves outcrop size unchanged, it restricts gene flow, reducing genetic variation and increasing population structure, with potentially negative consequences for the long-term persistence of the lizards on these outcrops.

*Keywords:* fragmented habitats, gene flow, genetic variation, isolation by distance, land clearing, ornate dragon lizard

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## Introduction

Land clearing is a common feature of most agricultural and urban environments and is largely responsible for the fragmentation of continuous habitats (Bender *et al.* 1998). While small habitat patches are frequently retained, they can be disconnected by vast tracts of cleared and agricultural landscapes (Saunders *et al.* 1991; Bender *et al.* 1998). In these altered environments, the replacement vegetation may not provide adequate resources or protection from predation (Stow *et al.* 2001; Berry *et al.* 2005; Hoehn *et al.* 2007). This may pose a significant obstruction to dispersal and subsequent interbreeding (gene flow) between remnant popu-

lations (Bender *et al.* 1998). Gene flow is important for maintaining genetic variation within populations and reducing the level of genetic differentiation among populations (Madsen *et al.* 1999; Hinten *et al.* 2003; Bollmer *et al.* 2005; Ditto & Frey 2007; Howeth *et al.* 2008). However, land clearing also causes extensive habitat loss, with remnant habitat patches only able to support a limited number of individuals (Bender *et al.* 1998). As small populations are more susceptible to the effects of genetic drift and inbreeding, habitat loss can also be responsible for significant reductions in genetic variation (Keyghobadi 2007).

Habitat fragmentation and loss tend to be inseparable processes, and identifying the impact of each on population genetic variation is difficult. Indeed, most studies use genetic variation as an indicator of levels of gene flow (e.g. Driscoll & Hardy 2005; Hoehn *et al.* 2007;

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Taylor *et al.* 2007), but with the concurrent impact of habitat loss, the extent of disruption to gene flow can be overestimated. Alternatively, the effect of land clearing on gene flow may be underestimated. Driscoll & Hardy (2005) found similar levels of genetic variation within populations of the lizard *Amphibolurus nobbi* in cleared areas and nature reserves. They also identified a pattern of isolation by distance in the reserves, suggestive of limited dispersal, but not in the cleared land. While this would seem to indicate dispersal is enhanced in agricultural areas, it may also have resulted from individuals relocating to other populations following habitat loss, thereby increasing overall genetic variation. Hence, teasing apart the effects of habitat fragmentation and loss on genetic variation is necessary to identify the true impacts of isolation, through intervening habitat alteration, on dispersal and gene flow. Here, we examine the levels of genetic variation and population structure of the ornate dragon lizard, *Ctenophorus ornatus*, an ideal species for addressing the aforementioned issues, because they persist in discrete, replicate groups separated by both remnant vegetation and cleared landscapes.

The ornate dragon is a small (20 g) agamid lizard endemic to the southwest of Western Australia, an area that has undergone extensive clearing for agricultural purposes over the past 150 years (Saunders 1989). This region is made up of approximately 140 000 km<sup>2</sup> of exotic grasses and cereal crops with only small isolated pockets of native woodlands, scrub, heath and thicket persisting throughout the region (Saunders 1989). The ornate dragon is found in both of these environments (remnant fragments and cleared agricultural land) and exclusively inhabits granite outcrops (Storr *et al.* 1983) that are unsuitable for agriculture and therefore have been spared direct clearing and reductions in size (Yates *et al.* 2007). These habitat islands in a 'sea' of either agricultural land or native bush provide an ideal opportunity to focus on the effect of land clearing on gene flow among populations with habitats that are unchanged in size and therefore have experienced minimal, if any, habitat loss. By their nature, the granite outcrops also enable the examination of numerous discrete groups of individuals, similar in size and distribution in both environments. In this study, we compare the population genetic variation, structure and inferred dispersal success of *C. ornatus* from a set of outcrops in a nature reserve and a set of outcrops in the adjacent cleared land. If land clearing reduces gene flow among outcrops, we expect that outcrops in the cleared land will have reduced genetic variation and more pronounced genetic structure than outcrops in the reserve.

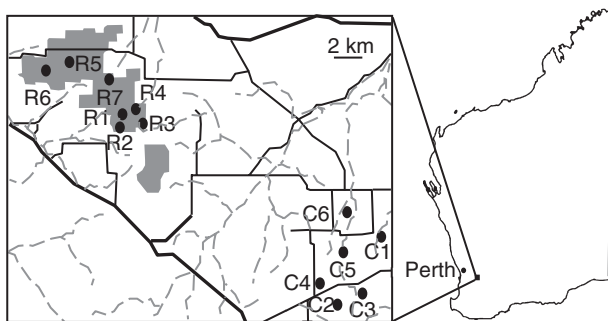
## Materials and methods

### *Study species*

The ornate dragon, a diurnal, polygynous agamid lizard with an average lifespan of 3 years (Bradshaw 1965), is highly adapted to life in the stark and often harsh environment of the granite outcrop (Bradshaw 1965; Whithers 2000). These lizards are extremely flat with severely depressed skulls, pectoral and pelvic girdles (Bradshaw 1965; Wilson & Knowles 1988). This enables them to make use of thin cavities under exfoliating slabs of granite as shelter from temperature extremes and predation (Bradshaw 1965). As exfoliating granite is generally absent from the surrounding bush, the home range of these lizards is believed to be restricted to the granite outcrop. They are almost entirely insectivorous (Bradshaw 1971) with a diet largely consisting of flying insects (Bradshaw 1971; LeBas & Marshall 2000). *Ctenophorus ornatus* is sexually dimorphic and dichromatic for a range of traits, most noticeably the black chest patch that becomes apparent in males at the onset of the breeding season (LeBas & Marshall 2000, 2001). The breeding season extends from October to April with males exhibiting courting behaviour similar to other *Ctenophorus* species (Bradshaw 1965; Carpenter *et al.* 1970). Both sexes are territorial, establishing new territories every year after emergence from winter hibernation. For the duration of the breeding season, males defend large territories that often encompass several female territories (Bradshaw 1971; LeBas & Marshall 2001), yet females do not exclusively mate with the male in whose territory they reside (LeBas 2001). The ornate dragon is oviparous, laying an average of two to three eggs in egg chambers dug by females in the bush at the edge of the outcrop (Bradshaw 1971; Wilson & Knowles 1988). Hatchlings and juveniles seem to be confined to particular areas on the outcrop, close to egg-laying areas, where they are exempt from aggressive territorial male behaviour (Bradshaw 1971). Dispersal occurs after the breeding season with large numbers of juveniles migrating to surrounding patches of granite. During this time, movements of up to 500 m per day are common (Bradshaw 1971). Some of these juveniles return to the outcrop in the following breeding season as sexually mature adults and compete with resident adults for territories (Bradshaw 1971). Evidence from mark-recapture studies over a period of 5 years suggests that migration between nearby outcrops does occur (LeBas unpublished data), presumably at the juvenile stage with an intermediate stay on smaller granite patches.

### Study site

This study took place at Tutanning nature reserve (32.541°S, 117.325°E) and an area in the adjacent agricultural land (32.626°S, 117.486°E), located approximately 180 km southeast of Perth, Western Australia (Fig. 1). Lizards from 13 outcrops were sampled: seven outcrops from within Tutanning reserve and six from outcrops in the adjacent agricultural land (Fig. 1). In the cleared agricultural land, only outcrops that were fenced off from the surrounding paddocks or showed minimal degradation were sampled. To control for the potential effects of outcrop size and distance between outcrops on genetic structure, only outcrops as similar as possible with respect to size and distance apart were selected for sampling (average outcrop size: reserve =  $26\ 034\ \text{m}^2 \pm 9264\ \text{m}^2$ , cleared =  $37\ 032\ \text{m}^2 \pm 5592\ \text{m}^2$ ; average distance apart: reserve =  $3.68\ \text{km} \pm 0.48\ \text{km}$ , cleared =  $3.09\ \text{km} \pm 0.27\ \text{km}$ ). Another factor that may affect population genetic structure at each site is the presence of outcrops between those that were sampled. Even if these outcrops are too small to support a stable population of *C. ornatus*, they may provide temporary refuges during dispersal events. In this way, gene flow can be facilitated between distant populations if small granite patches are present between them. To account for this, aerial photographs of both sites were examined and all granite patches between sampled outcrops were identified. The pairwise distances between all patches and outcrops and the total area encompassed by the outcrops at each site were measured. The number of granite patches was slightly greater in the cleared land (reserve = 2.31 granite patches/ $\text{km}^2$  and cleared = 2.57 granite patches/ $\text{km}^2$ ), and the average pairwise distance between all granite was greater in the reserve site (reserve = 2.5 km and cleared = 1.8 km).



**Fig. 1** Location of reserve and cleared land sites in Western Australia and outcrops sampled within these sites. Tutanning Reserve is shaded in grey; all other areas are agricultural land. Main roads are represented by solid bold lines and minor roads by solid lines. Grey dashed lines are the locations of seasonal creeks.

### Sampling

Lizards were captured by hand at first light when they were still relatively inactive by lifting exfoliated granite slabs. If less than 15 lizards on a single outcrop were captured by this method, the outcrop was resampled during the afternoon until at least 20 lizards had been caught. At this time, lizards are very active, so nets were used to catch the additional lizards. Each lizard was toe-clipped to provide a tissue sample for genetic analysis and then returned to their outcrop. Toe clips were stored in 100% ethanol prior to DNA extraction. In lizards, toe and tail clipping is commonly used for identification and DNA analysis (Olsson 1994; LeBas 2001; Berry *et al.* 2005), and *C. ornatus* are known to lose toes as a result of predation and rock-associated injuries (LeBas pers. obs.). As juveniles are likely to aggregate in groups of closely related individuals (Bradshaw 1965), potentially creating a sampling bias, only adult lizards were retained for use in the proceeding analyses. The minimum number of adult samples from any outcrop was 12. Final sample sizes for each outcrop can be found in Table 1.

### Genetic analysis

DNA was extracted for PCR using the standard salting out method described in Sunnucks & Hales (1996) with the exception of the incubation stage that was carried out overnight at 56 °C. PCRs of 13  $\mu\text{L}$  contained: 10 ng DNA, 11  $\mu\text{L}$  of Platinum PCR supermix (Invitrogen: 22 U/mL complexed recombinant *Taq* DNA polymerase with Platinum *Taq* Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM  $\text{MgCl}_2$ , 220  $\mu\text{M}$  dGTP, 220  $\mu\text{M}$  dATP, 220  $\mu\text{M}$  dTTP, 220  $\mu\text{M}$  dCTP) and 3.3  $\mu\text{mol}$  fluorescent-tagged forward primer. PCR amplifications were carried out in an Eppendorf Mastercycler and consisted of an initial denaturation at 94 °C for 2 mins followed by 30 cycles of 45 s at 94 °C; 45 s at annealing temperature (56 °C or 57 °C) and 45 s at 72 °C and finally 72 °C for 5 mins. Each individual was genotyped for 22 microsatellite loci using seven multiplex reactions. Two loci (Co6A6 and Co9C11) are described in LeBas & Spencer (2000) and the remainder in Levy *et al.* (2010). An Applied Biosystems 3730 capillary sequencer and Genemapper 3.7 software (Applied Biosystems, Foster City, California, USA) were used to score alleles.

### Data analysis

**Genetic variation within outcrops.** The program FreeNa (Chapuis & Estoup 2007) was used to estimate null allele frequencies for each locus and outcrop, based on the expectation maximization algorithm (Dempster

Outcrop	Sample size	Allelic richness	$H_E$	$H_O$	$F_{IS}$
Reserve outcrops					
R1	41	7.8 ± 0.4	0.845 ± 0.023	0.800 ± 0.016	0.056 ± 0.026**
R2	13	6.9 ± 0.5	0.832 ± 0.019	0.803 ± 0.037	0.034 ± 0.032
R3	14	6.8 ± 0.5	0.825 ± 0.023	0.788 ± 0.023	0.049 ± 0.049
R4	12	7.6 ± 0.5	0.847 ± 0.025	0.747 ± 0.020	0.117 ± 0.043**
R5	29	6.6 ± 0.5	0.802 ± 0.027	0.775 ± 0.015	0.036 ± 0.037
R6	19	7.1 ± 0.4	0.839 ± 0.018	0.791 ± 0.026	0.064 ± 0.032*
R7	20	6.8 ± 0.4	0.809 ± 0.025	0.792 ± 0.025	0.021 ± 0.029
Mean	21.1 ± 4.0	7.1 ± 0.2	0.828 ± 0.007	0.785 ± 0.007	0.054 ± 0.012
Cleared land outcrops					
C1	16	5.1 ± 0.4	0.699 ± 0.041	0.695 ± 0.022	0.009 ± 0.042
C2	24	5.1 ± 0.3	0.754 ± 0.022	0.743 ± 0.022	0.022 ± 0.040
C3	15	4.3 ± 0.3	0.639 ± 0.042	0.627 ± 0.014	0.020 ± 0.057
C4	19	5.6 ± 0.3	0.762 ± 0.027	0.711 ± 0.020	0.075 ± 0.041*
C5	12	5.6 ± 0.4	0.741 ± 0.035	0.698 ± 0.020	0.067 ± 0.063
C6	19	5.0 ± 0.3	0.737 ± 0.026	0.719 ± 0.026	0.025 ± 0.025
Mean	17.5 ± 1.7	5.1 ± 0.2	0.722 ± 0.019	0.699 ± 0.016	0.036 ± 0.011

**Table 1** Means and standard errors for estimates of allelic richness, gene diversity ( $H_E$ ), observed heterozygosity ( $H_O$ ) and the inbreeding coefficient ( $F_{IS}$ ) within reserve (R1–R7) and cleared land (C1–C6) outcrops. Significant deviations from Hardy–Weinberg Equilibrium are indicated by \*\*( $\alpha = 0.01$ ) and \*( $\alpha = 0.05$ ) with Bonferroni correction

*et al.* 1977). This program creates a data set corrected for null alleles and uses it to calculate global and pairwise  $F_{ST}$  values across all loci and for each locus. As there was no difference (corrected  $F_{ST} = 0.097$  95% CI 0.086–0.108, uncorrected  $F_{ST} = 0.100$  95% CI 0.088–0.113) between these corrected  $F_{ST}$  values and the uncorrected values, the original data set was used for all remaining analyses.

The presence of linkage disequilibrium (LD) was tested for using FSTAT 2.93 software package (Goudet 2001). Seven pairs of loci (Co9C11 × CoC120, CoB11 × CoB108, CoD111 × CoA1, CoA107 × CoC11, CoD115 × CoB108, CoD115 × CoD114 and CoB12 × CoD10) were found to be in LD across all outcrops ( $\alpha = 0.01$ , Bonferroni-corrected). However, when each outcrop was examined separately, only two pairs of loci remained in LD [Co9C11 × CoC120 was in LD in three cleared land outcrops (C2, C4 and C6) and CoD115 × CoD10 in one reserve outcrop (R1)]. Detailed results of these tests can be found in Table S2. In response, two loci (CoC120 and CoD115) were conservatively removed from all further analyses.

Genetic variation within each outcrop was measured using allele frequency data, from which average allelic richness, inbreeding coefficient ( $F_{IS}$ ) and gene diversity ( $H_E$ ) were calculated with FSTAT 2.93 (Goudet 2001). Observed heterozygosity ( $H_O$ ) was calculated as the mean proportion of heterozygous loci for each outcrop. Departures from Hardy–Weinberg Equilibrium (HWE) within outcrops were tested using FSTAT 2.93 (Goudet 2001), and sequential Bonferroni correction was applied to all critical significance levels used (Rice 1989). Tests for differences in genetic variation between the reserve and cleared land outcrops were carried out using Kolmogorov–Smirnov tests.

*Relatedness analyses.* To compare the patterns of genetic similarity within and across outcrops, Queller and Goodnight’s asymmetric relatedness estimator ( $R$ ) was calculated for each possible individual pairwise comparison at each site using the excel add-in GENALEX 6.2 (Peakall & Smouse 2006). The means and standard deviations were determined for all pairwise comparisons for each sex, both within and between outcrops for each site. The 95% confidence intervals for these comparisons were calculated by bootstrapping 1000 times.

*Population structure.* The software program 2MOD 0.2 (Ciofi *et al.* 1999; Beaumont 2000) was used to examine the demographic history of the outcrops at each site. Here, a Markov Chain Monte Carlo (MCMC) simulation approach is used in conjunction with Metropolis–Hastings sampling to explore whether a gene-flow or pure drift model is most likely to have generated the observed allele frequencies. A total of 100 000 iterations of the MCMC search were carried out, and the first 10% of runs were discarded to remove potential effects of the initial starting parameters. The Bayes factor was calculated as the ratio of the likelihood probabilities of both models. For each outcrop,  $M$  (the number of migrants per generation) was calculated according to the formula  $M = (1-F)/4F$  for the gene-flow model, and for the drift model,  $M = -\log(1-F)$  where  $F$  is the probability that two genes share a common ancestor within a population (Ciofi *et al.* 1999). The average  $M$  was determined across all outcrops at each site.

To estimate genetic divergence between outcrops, pairwise  $\theta$ -values, an unbiased estimate of  $F_{ST}$  (Weir & Cockerham 1984), were calculated over all loci with FSTAT 2.93 (Goudet 2001). We assessed the spatial genetic structure of outcrops at each site by analysing

the correlation between genetic divergence and geographical distance. We compared an  $F_{ST}/(1-F_{ST})$  matrix with a geographical distance matrix (log km) (Rousset 1997), using a Mantel test (10 000 permutations) calculated with the software package IBDWS (Jensen *et al.* 2005). Confidence levels for the mean pairwise  $F_{ST}$ , the slope and the coefficient of determination ( $R^2$ ) of the linear regression between genetic and geographical distance were determined by bootstrapping over loci 1000 times.

Another way to assess spatial genetic structure is to test for spatial autocorrelation (SA). SA examines the correlation between individual genotypes at each location and those at neighbouring localities and then evaluates the correlation as a function of distance (Manel *et al.* 2003). The software package SPAGeDI 1.3 (Hardy & Vekemans 2002) was used to calculate Moran's I for all pairwise comparisons of individuals within a given distance class. The same distance classes (a total of eight classes at intervals of 1 km) were used in both analyses to enable direct comparisons between the two sites. Permutation tests (1000 permutations) and jackknifing across loci were conducted to establish confidence intervals and standard error margins for each distance class.

Population structure was also analysed with two Bayesian assignment approaches implemented using the software programs STRUCTURE 2.3.1 (Pritchard *et al.* 2000) and GENELAND 3.1.5 (Guillot *et al.* 2005). Both these programs group individuals into the most likely number of clusters ( $K$ ) that maximizes the within-cluster Hardy-Weinberg and linkage equilibria. GENELAND differs from STRUCTURE in that geographical information can be incorporated to produce more accurate inferences of population structure based on the spatial distribution of individuals.

The STRUCTURE and GENELAND analyses involved two steps. First, all outcrops from both the reserve and cleared land sites were analysed together. Second, outcrops from each site were analysed separately. Analyses involving STRUCTURE were based on a model that assumed admixture of ancestry and independent allele frequencies. The assumption of the independent allele frequencies in the model reduces the risk of overestimating  $K$  when allele frequencies are likely to vary significantly between samples (Pritchard *et al.* 2009). Twenty independent runs were performed for each value of  $K$  (1–17 for the combined outcrops and 1–10 when reserve and cleared land outcrops were analysed separately) with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number of clusters ( $K$ ) for each analysis was determined using the method described by Evanno *et al.* (2005).

For the GENELAND analysis, the coordinates (latitude and longitude) of each outcrop were used to run the spatial model. The uncertainty of coordinates was set at 0.53 km as this is the maximum diameter of sampled outcrops. Thus, any individual could have been collected within 0.53 km of the given coordinates. The uncorrelated and null allele models were adopted for twenty independent runs of  $K = 1-17$  for the combined outcrops and  $K = 1-10$  when reserve and cleared land outcrops were analysed separately. Each run consisted of 100 000 MCMC iterations with a thinning of 100 and a burnin of 1000. The most likely number of clusters was chosen as the model  $K$  (from each independent run) with the highest posterior probability.

*Historic gene flow.* Simulations were carried out to determine whether the disruption of gene flow would affect genetic variation and population structure over the time spans relating to this study. Following the method outlined in Lada *et al.* (2008), data sets were created using EASYPOP 2.0.1 software (Balloux 2001) and the outputs run through FSTAT and STRUCTURE. The simulations were based on models where there was constant gene flow among outcrops for 1000 generations after which gene flow was continued, stopped or reduced to one migrant per generation. The initial level of gene flow in all models was the average number of migrants per generation in the reserve site estimated from the actual data using the 2MOD software program.

After the first 1000 generations, simulated genotypes were sampled at 30 generation intervals for a total of 90 generations. Ninety generations reflects the time since large-scale land clearing around the study area is reported to have begun (Jarvis 1986). Population sizes for the simulated data were set to 60 (30 males and 30 females) for all outcrops because this was the average effective population size estimated by the program LDNE (Waples & Do 2008). The simulations were based on 20 loci with 20 alleles (the average number of alleles at each locus), free recombination and maximum initial variability. Microsatellites typically have mutation rates around 0.001 (Ellegren 2000; Goudet *et al.* 2002) and are generally believed to follow the SSM model of mutation (Valdes *et al.* 1993). However, it has been suggested that microsatellites may be more likely to adhere to two-phased model of mutation (Di Rienzo *et al.* 1994). Therefore, simulations were run using both mutation models with a mutation rate of 0.001 for each gene-flow model. The spatial migration model used had six populations with geographical coordinates the same as the actual cleared land outcrops. Five simulated data sets were created for each model at each time interval, and a random subset of genotypes was selected for each population, directly corresponding to the number of

individuals sampled from that outcrop. Levels of within-population genetic variation were estimated with FSTAT 2.93, and population structure was examined with STRUCTURE using the same parameters employed for the observed data, except that only five STRUCTURE runs were carried out for each value of  $K$ .

## Results

### Genetic variation within outcrops

Estimates of genetic variation within each outcrop are given in Table 1 and for each locus in Table S1. In all cases, the mean values of genetic variation were higher in the reserve than in the cleared land. Kolmogorov–Smirnov tests revealed significant differences between the reserve and cleared land outcrops in the allelic richness ( $Z = 1.797$ ,  $P = 0.003$ ),  $H_E$  ( $Z = 1.797$ ,  $P = 0.003$ ) and  $H_O$  ( $Z = 1.797$ ,  $P = 0.003$ ). All but four outcrops, three in the reserve and one in the cleared land, were found to be in HWE after correction for multiple comparisons. All outcrops that deviated from HWE showed a deficit of heterozygotes (Table 1). These deviations may have been caused by high frequencies of null alleles or the presence of fine-scale population structure within these outcrops.

### Relatedness analyses

In both the cleared land and reserve, individuals were more related within outcrops than they were between outcrops (95% confidence intervals of pairwise  $R$  did

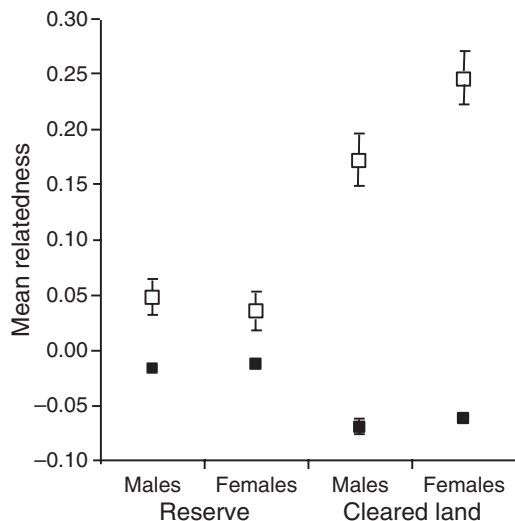


Fig. 2 Mean pairwise relatedness and 95% confidence intervals (as determined by bootstrapping) between individuals within outcrops (open squares) and between outcrops (closed squares).

not overlap, Fig. 2). In addition, cleared land individuals were significantly more related within outcrops and less related between outcrops than those in the reserve (Fig. 2). Males were significantly less related to each other than females within outcrops in the cleared land, but there was no significant difference in relatedness between the sexes in the reserve (Fig. 2). Relatedness between outcrops did not differ between the sexes in both the cleared land and reserve (Fig. 2).

### Population structure

The 2MOD analysis revealed that allele frequencies were most likely to have been generated by a migration–drift model in the reserve (gene-flow model:  $P = 0.942$ , Bayes factor = 16.0) and by a mutation–drift model in the cleared land (drift model:  $P = 0.999$ , Bayes factor = 18000). A Bayes factor greater than 3 is considered substantial support for a model (Kass & Raftery 1995). Further, the average number of migrants was considerably higher in the reserve outcrops ( $M = 7.090$ ) than in the cleared land ( $M = 0.062$ ).

Significant subdivision among outcrops was evident in both reserve ( $F_{ST} = 0.034$ ,  $P = 0.001$ ) and cleared land ( $F_{ST} = 0.150$ ,  $P = 0.001$ ). There was also evidence of an isolation-by-distance (IBD) relationship between genetic divergence and geographical distance at both sites (Mantel tests,  $P = 0.012$  and  $P = 0.005$  for the reserve and cleared land, respectively). These relationships are shown in Fig. 3. While there was no significant difference in the variance explained by each relationship ( $R^2$ : reserve = 0.343, 95% CI = 0.173–0.462; cleared = 0.303, 95% CI = 0.063–0.482), nonoverlapping confidence intervals revealed there were significant differences in

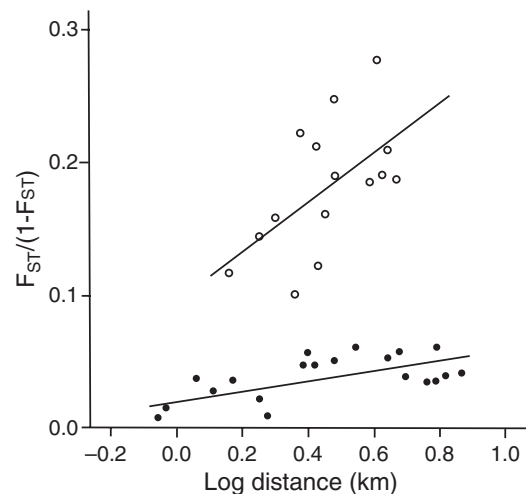


Fig. 3 Relationships between genetic divergence and geographical distance for reserve (closed circles) and cleared land (open circles) outcrops (a log of 0.1 = 100 m and log of 1 = 10 km).

the rate at which genetic divergence increased over distance and the average level of divergence between outcrops, with cleared land outcrops having a steeper slope (reserve = 0.030, 95% CI = 0.023–0.038; cleared = 0.167, 95% CI = 0.085–0.256) and higher average pairwise  $F_{ST}$  values (reserve = 0.033, 95% CI = 0.031 – 0.036; cleared = 0.145, 95% CI = 0.136 – 0.154) than outcrops in the reserve.

The SA analysis revealed patterns of IBD similar to the Mantel test (Table 2). Over short distances, there was greater structure between cleared land outcrops than between reserve outcrops. Reserve outcrops less than three km apart had significant population structure, while cleared land outcrops lost population structure over distances between two and four km. At greater distances (4–8 km), both sets of outcrops have significantly negative structure (Table 2). For each distance class, Moran's I is significantly different between the two sites except at the intermediate distance classes (2–4 km) (Table 2).

Analyses of population structure using STRUCTURE revealed  $K = 9$  when all outcrops were considered simultaneously. An analysis of the same data set using GENELAND gave a clear mode at  $K = 9$  (range  $K = 7–9$ ). The reserve outcrops were grouped into three clusters, and each outcrop in the cleared land formed an independent cluster. This clustering pattern was repeated when the reserve and cleared land outcrops were analysed separately. The STRUCTURE analysis yielded  $K = 3$  in the reserve and  $K = 6$  in the cleared land. Similarly, the GENELAND analysis revealed the most likely number of clusters to be three (range  $K = 2–5$ ) in the reserve and six (range  $K = 4–6$ ) in the cleared land. The lower number of clusters compared to outcrops in the reserve was attributable to the grouping of outcrops less than 2 km apart into single clusters, namely R1–R4 formed a single cluster, R5 and R6 another cluster and R7 the final cluster. The level of

admixture among outcrops was examined using STRUCTURE by setting  $K$  as the number of outcrops (seven for the reserve and six for the cleared land). There was minimal evidence of admixture between the reserve and cleared land outcrops (Fig. 4a), nor within the cleared land. Two exceptions were a single individual in C5 that clusters with C1 and an individual in C3 that clusters with C5 (Fig. 4c). However, individuals with mixed ancestry, which are indicative of admixture between outcrops, were clearly evident in the reserve (Fig. 4b).

### Historic gene flow

The simulations show that reduced gene flow leads to significant declines in genetic variation within outcrops over time spans relevant to this study. When gene flow was maintained at seven migrants per generation, mean allelic richness and genetic diversity ( $H_E$ ) were relatively constant (Fig. 5). However, when gene flow was reduced to one migrant per generation, both mean  $H_E$  and allelic richness decreased after 30 generations, although they returned to previous levels by generation 60. When gene flow was reduced to zero, there was a marked decline in genetic variation after 30 generations and 60 generations, after which mean  $H_E$  and allelic richness remained constant (Fig. 5).

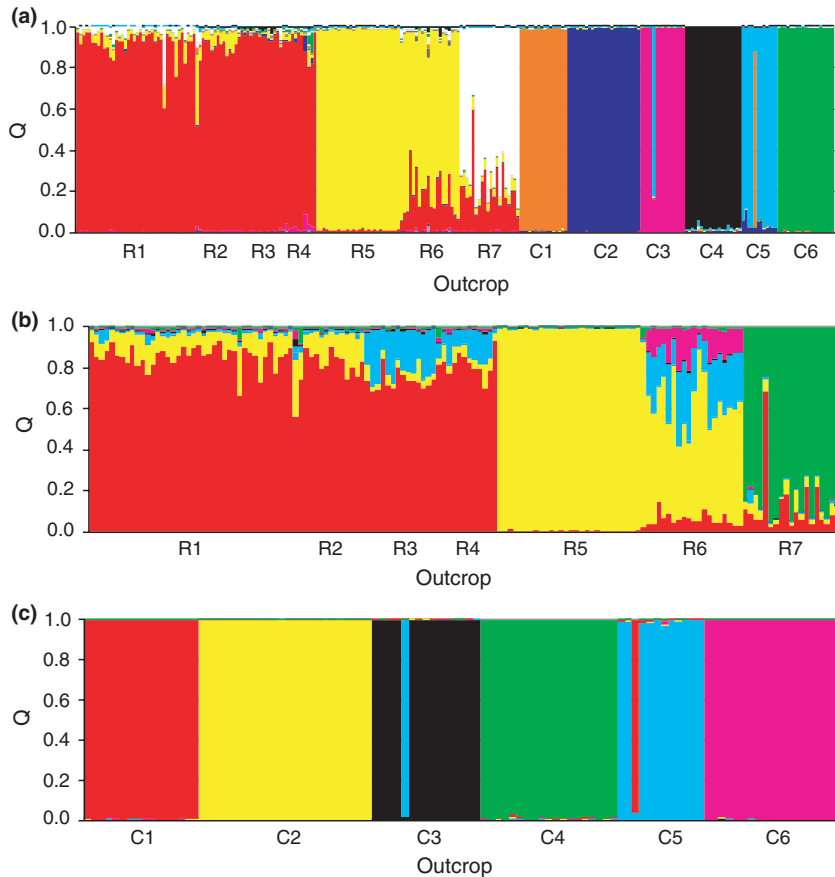
The simulations also show that reductions in gene flow have drastic effects on population structure after relatively few generations. STRUCTURE analyses of the simulated data sets revealed that when gene flow was stopped,  $K$  rapidly increased from an average  $K = 1.2$  (range  $K = 1–2$ ) to 4.4 (range  $K = 4–6$ ) in just 30 generations. From then onwards, average  $K$  increased by one every 30 generations (Fig. 5). A similar rapid increase in  $K$  was evident when gene flow was reduced to one migrant per generation. The average  $K$  increased from 1.2 (range  $K = 1–2$ ) to 3.6 (range  $K = 1–5$ ) in 30 generations. Thereafter, average  $K$  did not change significantly (Fig. 5). In all cases,  $K$  was much greater in models where gene flow had been reduced or stopped than when it remained unchanged (Fig. 5). Note that both mutation models produced similar results for both genetic variation within populations and population structure, so we have reported only the results for the SSM model of mutation.

### Discussion

A major consequence of land clearing is believed to be a reduction in gene flow between remnant populations (Keyghobadi 2007). Gene flow acts to overcome the effects of random genetic drift, namely reduced genetic variation and enhanced population divergence (Lacy 1987; Templeton *et al.* 1990; Allendorf & Luikart 2007).

**Table 2** Moran's I for spatial autocorrelation. Significant spatial structure is denoted by\*. Two standard errors calculated by jackknifing are also given, and distance classes where there is no overlap between sites are in bold

Distance Class (km)	Reserve	Cleared land
<b>0</b>	<b>*0.048 ± 0.006</b>	<b>0.218 ± 0.028*</b>
0.1–1	*0.016 ± 0.007	–
1.1–2	*0.007 ± 0.006	0.006 ± 0.022
2.1–3	*–0.020 ± 0.008	–0.015 ± 0.013
<b>3.1–4</b>	<b>*–0.007 ± 0.013</b>	<b>–0.059 ± 0.018*</b>
<b>4.1–5</b>	<b>*–0.014 ± 0.011</b>	<b>–0.088 ± 0.027*</b>
5.1–6	*–0.025 ± 0.009	–
6.1–7	*–0.019 ± 0.005	–
7.1–8	–0.006 ± 0.018	–



**Fig. 4** Bayesian population assignment from the software program STRUC-TURE when  $K$  was set to the number of sampled outcrops. Each column represents a single individual, and the proportion of each individual genotype that assigns to a particular cluster is shown by a different colour. Individuals are ordered according to their outcrop of origin. a) Combined *C. ornatus* samples from the reserve and cleared land. b) *C. ornatus* samples from the reserve. c) *C. ornatus* samples from the cleared land.

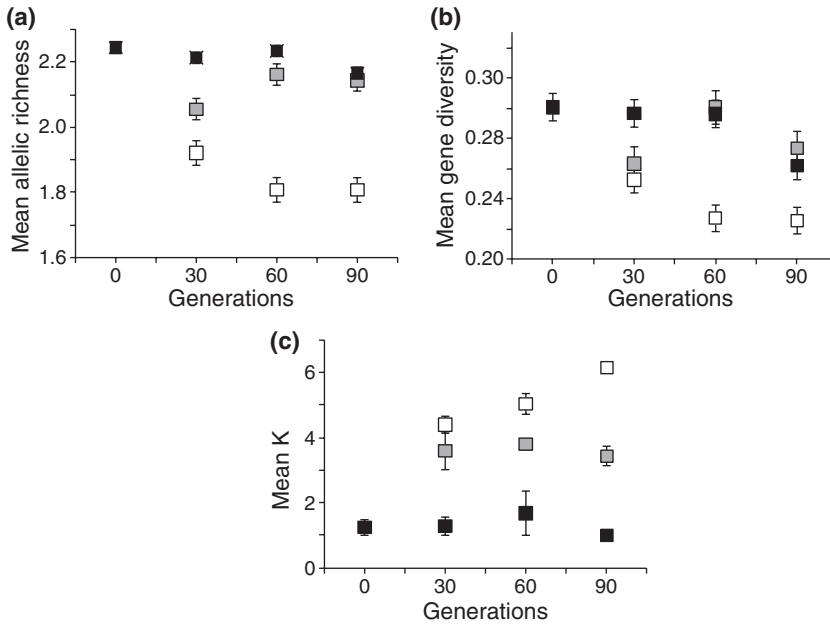
Therefore, if land clearing has obstructed gene flow in *C. ornatus*, genetic variation within outcrops is expected to be lower and population structure is more pronounced in the cleared land compared to the reserve. The effects of genetic drift can also be enhanced by diminished population size (Allendorf & Luikart 2007), resulting in an overestimation of the impacts of reduced gene flow on genetic variation. However, because *C. ornatus* has experienced minimal habitat loss as a result of land clearing, any differences in genetic variation and population structure between the cleared land and reserve are likely to be due to reduced gene flow between outcrops.

Consistent with our predictions, significantly lower levels of genetic variation were identified within cleared land outcrops compared to those in the reserve. Such a loss of genetic variation suggests that gene flow between the cleared land outcrops has not been sufficient to counter the effects of genetic drift. This was supported by the Bayesian likelihood analyses that show that genetic drift is the most influential evolutionary process in the cleared land and that gene flow predominates in the reserve.

In the cleared land, the lizards inhabiting each outcrop were more closely related to each other and less

related to individuals from other outcrops than in the reserve. These results provide further evidence that dispersal between cleared land outcrops is less frequent than in the reserve. Interestingly, females were more closely related than males in the cleared land, but the same pattern was not found in the reserve. Such a disparity in intra-sex relatedness could be caused by sex-biased dispersal in the cleared land but not the reserve, with males more likely to embark on dispersal attempts in the cleared land. Altered dispersal patterns of one sex in fragmented landscapes have rarely been documented (but see Stow *et al.* 2001; Berry *et al.* 2005; Sumner 2005). In line with our findings in *C. ornatus*, the rock-dwelling skink *Egernia cunninghami* was shown to experience male-biased dispersal following habitat fragmentation (Stow *et al.* 2001). The authors suggest this may be because of inbreeding avoidance within highly related fragmented populations.

The mantel and spatial autocorrelation tests revealed patterns of isolation by distance (IBD) in both the reserve and cleared land. IBD occurs when gene flow is restricted by distance, suggesting that some level of gene flow is apparent at both sites. The rate of increase in genetic divergence over distance and average genetic divergence were both greater in the cleared



**Fig. 5** Mean allelic richness (a), gene diversity (b) and  $K$  from STRUCTURE (c), with standard errors, on simulated data generated using various gene-flow scenarios. Closed squares denote uninterrupted gene flow, grey squares indicate when gene flow is reduced to one migrant per generation, and open squares show when gene flow is stopped. In all models, there were 1000 generations of continuous gene flow at 7 migrants per generation prior to the altered gene-flow regimes. Details of other parameters used in the simulations are given in the Methods.

land, implying that gene flow has been reduced among these outcrops. This was supported by the Bayesian clustering analyses that indicate the level of gene flow in the cleared land was lower with only rare dispersal events taking place between outcrops.

In the reserve, outcrops <2 km apart were grouped into single clusters by the Bayesian clustering analyses, whereas those in the cleared land formed discrete clusters. Further, there was a distinct lack of admixture among clusters in the cleared land, suggesting that gene flow is nearly absent between outcrops at this site. However, two individuals sampled from the cleared land had genotypes assigned to clusters different to those assigned to the outcrops from which they were taken, suggesting that migration between outcrops still occurs. Evidence of individuals assigned to clusters other than those they were sampled from was not apparent in the reserve, where the inferred ancestry of most individuals was a mixture of clusters. This implies a high level of admixture between outcrops in the reserve and a history of relatively uninterrupted, consistent gene flow, making the detection of recently dispersing individuals more difficult than in the cleared land. Together, these results suggest that while dispersal is still possible through the cleared landscapes, it is likely to occur infrequently.

Previous studies on lizards have identified similar patterns of reduced population genetic variation as well as enhanced IBD and population structure in fragmented habitats (Stow *et al.* 2001; Berry *et al.* 2005). Yet, studies on other species present inconsistent results. An increase in genetic variation may be attributable to recent fragmentation events and the redistribution of

individuals among remaining habitat fragments (Driscoll & Hardy 2005; Sumner 2005). Alternatively, species-specific habitat requirements and/or dispersal abilities can play an important role in enabling or restricting gene flow in altered environments (Ricketts 2001). This is exemplified in a study of two coexisting arboreal geckos, *Oedura reticulata* and *Gehyra variegata* by Hoehn *et al.* (2007). Here, fragmented populations of *O. reticulata* were found to have much greater genetic structure and diversity between populations than *G. variegata*, but in continuous populations, both species exhibited similar levels of structure and diversity. The authors attribute this to differences in dispersal abilities and habitat requirements.

Reduced gene flow in the cleared site may be attributable to less successful dispersal attempts or a lower rate of dispersal through the cleared land. Ornate dragons are highly adapted to an existence on granite outcrops, making use of exfoliated slabs of granite as shelter to escape predation and the heat of the day (Bradshaw 1965). While neither natural bush nor cleared land provides the same resources, shelter might be found in fallen logs, leaf litter and other debris in natural bush, but not in sparse agricultural landscapes. Cleared agricultural land in Western Australia is usually cropped on a cyclic basis with several years of sheep grazing in between years with arable crops. Crops and grass grow over the winter and spring when lizards are least active, and by summer, the land is characterized by expanses of stubble or very short-grazed dry grass. The lack of shelter may therefore have contributed to reduced dispersal and gene flow in *C. ornatus* between the cleared land outcrops.

The simulations revealed that the disruption of gene flow could significantly affect genetic variation and population structure over the time spans relating to this study. However, while some genetic variation was initially lost and outcrops became more isolated as gene flow between outcrops was reduced to one migrant per generation, it was only when gene flow was completely stopped that genetic variation declined and population structure resembled that seen in the observed data. Therefore, it would seem that in the cleared land, gene flow has been negligible since fragmentation. While rare migration events may still take place, it is unlikely they would occur as frequently as one migrant per generation and hence may have been insufficient to counter the effects of genetic drift. This suggestion is supported by the Bayesian likelihood analyses that find genetic drift the most likely process to have generated the observed allele frequencies in the cleared land outcrops.

In the agamid lizard *C. ornatus*, it appears that land clearing has caused a decline in genetic variation within outcrops and increased between-population divergence by reducing gene flow. While the period of isolation may not have been long enough to have had devastating effects on persistence, a continued lack of connectivity between outcrops may eventually cause widespread extinctions with perhaps only the largest and healthiest populations surviving. Indeed, there appear to have been local extinctions around the town of York (W. Gibb & LeBas pers. obs.), one of the earliest cleared regions in Western Australia. To prevent such extinctions, it might be necessary to re-establish gene flow between outcrops in cleared land. More generally, these results are concerning, because they suggest that in other species with similar biology, seemingly healthy populations may be at risk and that the problem of habitat fragmentation and loss may be more extensive than previously thought.

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## References

Allendorf FW, Luikart G (2007) *Conservation and the Genetics of Populations*. Blackwell Publishing, Victoria, Australia.

- Balloux F (2001) EASYPOP (version 1.7): a computer program for population genetics simulations. *Journal of Heredity*, **92**, 301–302.
- Beaumont MA (2000). 2Mod 0.2. Available from <http://www.rubic.rdg.ac.uk/~mab/software.html>.
- Bender DJ, Contreras TA, Fahrig L (1998) Habitat loss and population decline: a meta-analysis of the patch size effect. *Ecology*, **79**, 517–533.
- Berry O, Tocher MD, Gleeson DM, Sarre SD (2005) Effect of vegetation matrix on animal dispersal: genetic evidence from a study of endangered skinks. *Conservation Biology*, **19**, 855–864.
- Bollmer JL, Whiteman NK, Cannon MD, Bednarz JC, De Vries T, Parker PG (2005) Population genetics of the Galapagos hawk (*Buteo galapagoensis*): genetic monomorphism within isolated populations. *Auk*, **122**, 1210–1224.
- Bradshaw SD (1965). *The Comparative Ecology of Lizards of the Genus Amphibolurus*. PhD thesis, University of Western Australia, Nedlands, Perth, Western Australia.
- Bradshaw SD (1971) Growth and Mortality in a Field Population of Amphibolurus Lizards Exposed to Seasonal Cold and Aridity. *Journal of Zoology*, **165**, 1–25.
- Carpenter CC, Badham JA, Kimble B (1970) Behavior patterns of three species of *Amphibolurus* (Agamidae). *Copeia*, **1970**, 497–505.
- Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, **24**, 621–631.
- Ciofi C, Beaumont MA, Swingland IR, Bruford MW (1999) Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266**, 2269–2274.
- Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via EM algorithm. *Journal of the Royal Statistical Society B-Methodological*, **39**, 1–38.
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Science*, **91**, 3166–3170.
- Ditto AM, Frey JK (2007) Effects of ecogeographic variables on genetic variation in montane mammals: implications for conservation in a global warming scenario. *Journal of Biogeography*, **34**, 1136–1149.
- Driscoll DA, Hardy CM (2005) Dispersal and phylogeography of the agamid lizard *Amphibolurus nobbi* in fragmented and continuous habitat. *Molecular Ecology*, **14**, 1613–1629.
- Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics*, **16**, 551–558.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (vers. 2.9.3). Available from <http://www.unil.ch/izea/software/fstat.html>. Updated from Goudet (1995).
- Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, **11**, 1103–1114.
- Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

- Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–320.
- Hinten G, Harriss F, Rossetto M, Braverstock PR (2003) Genetic variation and island biogeography: microsatellite and mitochondrial DNA variation in island populations of the Australian bush rat, *Rattus fuscipes greyii*. *Conservation Genetics*, **4**, 759–778.
- Hoehn M, Sarre SD, Henle K (2007) The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? *Molecular Ecology*, **16**, 3299–3312.
- Howeth JG, McGaugh SE, Hendrickson DA (2008) Contrasting demographic and genetic estimates of dispersal in the endangered Coahuilan box turtle: a contemporary approach to conservation. *Molecular Ecology*, **17**, 4209–4221.
- Jarvis N (1986) *Western Australia: An Atlas of Human Endeavour*. Department of Lands and Surveys, Perth.
- Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by Distance, Web Service BMC Genetics 6: 13 (vers. 3.16).
- Kass RE, Raftery AE (1995) Bayes Factors. *Journal of the American Statistical Association*, **90**, 773–795.
- Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology*, **85**, 1049–1064.
- Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology*, **1**, 143–158.
- Lada H, Mac Nally R, Taylor AC (2008) Distinguishing past from present gene flow along and across a river: the case of the carnivorous marsupial (*Antechinus flavipes*) on southern Australian floodplains. *Conservation Genetics*, **9**, 569–580.
- LeBas NR (2001) Microsatellite determination of male reproductive success in a natural population of the territorial ornate dragon lizard, *Ctenophorus ornatus*. *Molecular Ecology*, **10**, 193–203.
- LeBas NR, Marshall NJ (2000) The role of colour in signalling and male choice in the agamid lizard *Ctenophorus ornatus*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267**, 445–452.
- LeBas NR, Marshall NJ (2001) No evidence of female choice for a condition-dependent trait in the agamid lizard, *Ctenophorus ornatus*. *Behaviour*, **138**, 965–980.
- LeBas NR, Spencer PB (2000) Polymorphic microsatellite markers in the ornate dragon lizard, *Ctenophorus ornatus*. *Molecular Ecology*, **9**, 365–378.
- Levy E, Kennington WJ, LeBas NR (2010) Characterization of microsatellite loci for the ornate dragon lizard *Ctenophorus ornatus*. *Conservation Genetics Resources*, **2**, 313–315.
- Madsen T, Shine R, Olsson M, Wittzell H (1999) Restoration of an inbred adder population. *Nature*, **402**, 34–35.
- Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.
- Olsson M (1994) Nuptial coloration in the sand lizard, *Lacerta Agilis* - an intra-sexually selected cue to fighting ability. *Animal Behaviour*, **48**, 607–613.
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Pritchard JK, Wen X, Falush D (2009) Documentation for STRUCTURE software (Vers 2.3). Available from <http://pritch.bsd.uchicago.edu/structure.html>.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Ricketts TH (2001) The matrix matters: effective isolation in fragmented landscapes. *American Naturalist*, **158**, 87–99.
- Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.
- Saunders DA (1989) Changes in the avifauna of a region, district and remnant as a result of fragmentation of native vegetation - the wheatbelt of Western Australia - a case-study. *Biological Conservation*, **50**, 99–135.
- Saunders DA, Hobbs RJ, Margules CR (1991) Biological Consequences of Ecosystem Fragmentation - a Review. *Conservation Biology*, **5**, 18–32.
- Storr GM, Smith LA, Johnstone RE (1983) *Lizards of Western Australia II. Dragons and Monitors* Western Australian Museum, Western Australia.
- 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 (2005) Decreased relatedness between male prickly forest skinks (*Gnypetoscincus queenslandiae*) in habitat fragments. *Conservation Genetics*, **6**, 333–340.
- Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510–524.
- Taylor AC, Tyndale-Biscoe H, Lindenmayer DB (2007) Unexpected persistence on habitat islands: genetic signatures reveal dispersal of a eucalypt-dependent marsupial through a hostile pine matrix. *Molecular Ecology*, **16**, 2655–2666.
- Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*, **77**, 13–27.
- Valdes AM, Slatkin M, Freimer NB (1993) Allele frequencies at microsatellite loci - the stepwise mutation model revisited. *Genetics*, **133**, 737–749.
- Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, **8**, 753–756.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Whithers PC (2000) Overview of granite outcrops in Western Australia. *Journal of the Royal Society of Western Australia*, **83**, 103–108.
- Wilson SK, Knowles DG (1988) *Australia's Reptiles: A Photographic Reference to the Terrestrial Reptiles of Australia*. William Collins, Sydney.
- Yates CJ, Ladd PG, Coates DJ, McArthur S (2007) Hierarchies of cause: understanding rarity in an endemic shrub *Verticordia staminosa* (Myrtaceae) with a highly restricted distribution. *Australian Journal of Botany*, **55**, 194–205.

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### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Results of individual locus tests for adherence to Hardy–Weinberg Equilibrium (HWE) for each outcrop

**Table S2** Results of tests for linkage disequilibrium in each outcrop

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