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Restricted dispersal and genetic diversity in populations of an endangered montane lizard (*Eulamprus leuraensis*, Scincidae)

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Abstract

Many alpine species are under threat from global climate change, as their geographic ranges become increasingly fragmented and unsuitable. Understanding rates and determinants of gene flow among such fragmented populations, over historical as well as recent timescales, can help to identify populations under threat. It is also important to clarify the degree to which loss of local populations reduces overall genetic diversity within the taxon. The endangered Blue Mountains Water Skink (*Eulamprus leuraensis*) is restricted to <40 small swamps in montane south-eastern Australia. Our analyses of seven microsatellite loci of 241 animals from 13 populations show strong geographic structure, with major genetic divergence even between populations separated by <0.5 km. Dispersal between populations is scarce, and appears to involve mostly males. Our analyses suggest potential recent bottleneck events in all the identified populations, and lower genetic diversity and population size parameter at lower-elevation sites than at higher-elevation sites. Management of this endangered taxon thus needs to treat most populations separately, because of their genetic distinctiveness and low rates of genetic exchange.

Keywords: altitudinal gradient, dispersal, microsatellites, reptiles, spatial structure

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Introduction

Processes such as climate change and habitat degradation can result in fragmentation of previously continuous populations. Fragmentation poses a major threat to population viability, because it not only reduces population size within any given habitat fragment, but also decreases rates of migration (and hence, gene flow) among previously connected populations (e.g. Frankham *et al.* 2002; Cushman 2006), leading to a loss of genetic diversity within fragments (e.g. Hitchings & Beebee 1997). Climate change may well induce fragmentation of currently continuous populations, via shifts in both precipitation and thermal regimes. Modelling of future climates in Europe has predicted that populations of reptiles and amphibians will be more affected by the reductions in precipitation than by increases in temperature; the impact of reduced water availability will be particularly important in areas that are already under hydrological stress (Araújo *et al.* 2006). Already rare organisms restricted to montane habitat types, as well as those confined to the tops of low mountains, are under imminent threat from global warming (for studies on amphibians and reptiles, see Raxworthy *et al.* 2008; Pounds *et al.* 1999; plants, Theurillat & Guisan 2001; insects, Scalercio 2009).

Moreover, we need to identify genetic units that warrant separate conservation and management, due to their independent evolution and resulting local adaptation (see e.g. King 2009). Understanding dispersal and population fragmentation can also allow us to estimate the potential of a species to colonize new habitat, a critical determinant of its chance of long-term survival in a context of global climate change (e.g. Almany *et al.* 2009; Gaston 2009; Wilson *et al.* 2009). The ability of reptiles and amphibians to perform rapid large-scale

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migrations remains controversial (e.g. Araújo & Pearson 2005; Smith & Green 2005; Araújo *et al.* 2006).

The spatial distribution of gene frequencies within a taxon has important consequences not only for issues such as inbreeding rates and opportunities for local adaptation, but also can provide insights into mating systems and movement patterns (e.g. DeWoody & Avise 2001; Dalerum *et al.* 2007; Keogh *et al.* 2007; Dubey *et al.* 2008). If individuals are highly sedentary, or return to natal sites to breed, strong spatial structure in gene frequencies is expected; that is, local populations will diverge, and inbreeding will be common. In contrast, little spatial genetic structure is expected in highly mobile, widely dispersing animals. The sexes often differ in their degree of dispersal and, hence, in their contribution to spatial genetic structure both within and among populations (Handley & Perrin 2007).

The Blue Mountains water skink (*Eulamprus leuraensis*) is a medium-sized (to 200 mm total length) viviparous scincid lizard. It is restricted to montane areas west of Sydney in south-eastern Australia. The species is known from fewer than 40 isolated small swamps at 560–1060 m elevation, distributed in two distinct patches separated by about 20 km (in the Blue Mountains and Newnes Plateau). This species is classified as 'endangered' under the Threatened Species Conservation Act (1995) and the Environmental Protection and Biodiversity Conservation Act (1999; see http://www.environment.nsw.gov.au/threatenedspecies/ for both Acts), on the basis that it is an ecological specialist, with severely reduced populations subject to substantial ongoing threats. Because the distribution of the species is strictly limited to montane areas, it may be under significant risk from global climate change. Models of climatic variation predict higher temperatures and lower rainfall in the Blue Mountains (http://www.climatechangeinaustralia.gov.au). Such changes might affect both the skink's habitat (e.g. reduced rainfall and thus seepage might dry out the hanging swamps) and the lizard itself. Despite these major threats and its current classification as 'endangered', the ecology of *E. leuraensis* is poorly known (Shea & Peterson 1985; LeBreton 1996). The level of genetic differentiation of the populations, and the capacity for individuals of this species to disperse among populations, remain unknown. An understanding of these topics can facilitate the effective management of these few remaining populations.

Materials and methods

Tissue sampling and DNA extraction

Tissue samples from 241 *Eulamprus leuraensis* were collected from November 2008 to April 2009 across the entire distribution of the species. Our sampling included ten populations in the Blue Mountains (BH3, BH4, BH5, KT1, MH4, MRP1, WF1, WF5, WFL) and three in the Newnes Plateau (NP4, PNP1, XFC1; Fig. 1).

The animals were captured with funnel traps and pitfall traps, sexed by manual eversion of hemipenis (as in Harlow 1996), and measured with callipers. Individuals of all size classes and both sexes were caught, but the sex ratio was slightly skewed towards females (126F/106M/9 undetermined).

Total cellular DNA was isolated from small tail clips. Tissues were placed in 200 μL of 5% Chelex containing 0.2 mg/mL of proteinase K, incubated overnight at 56°C, boiled at 100°C for 10 min and centrifuged at 13 300 *g* for 10 min. Then, the supernatant, containing purified DNA, was removed and stored at −20°C.

Microsatellite analysis

Seven microsatellite loci isolated and characterized from *Eulamprus kosciuskoi* (Scott *et al.* 2001; EK8, EK23, EK37, EK39, EK100, EK107) and *Gnypetoscincus queenslandiae* (Sumner *et al.* 2001; GQ20/21) were amplified and scored. PCR amplifications were performed in a 9800 Fast thermal cycler (Applied Biosystems) as 5 μL reactions containing 0.075 U *Taq Ti* DNA polymerase (Biotech), 0.1 mM dNTPs, 0.4 mM of each primer, 20 mM Tris–HCl, pH 8.5, 50 mM KCl and 1.25 mM $MgCl_2$. Cycling conditions included a hot start denaturation of 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 60°C (55°C for EK23, GQ20/21 and EK37) annealing temperature for 30 s, 72°C for 30 s (1 min for EK23, GQ20/21 and EK37) and a final extension of 72°C for 30 min. Amplified products were genotyped with a 3130-xl genetic analyzer (Applied Biosystems) using GeneMapper software V3.7 (Applied Biosystems).

F-statistics and genetic diversity parameters

Gene diversities comprising observed (H_o), expected within-subpopulation (H_s) and expected overall heterozygosities (H_T) were estimated following Nei & Chesser (1983). Genotypic disequilibria between loci in each sample and deviations from Hardy–Weinberg equilibrium (HWE) within samples were tested based on 5460 permutations and 10 000 randomizations respectively. Wright's fixation indices for within-subpopulation deviation from random mating (F_{IS}), as well as pair-wise subpopulation differentiation (F_{ST}), were estimated following Weir & Cockerham (1984). The deviation from random mating within populations (F_{IS}) per locus and sample was computed with a bootstrap procedure (1820 randomizations). Statistical support for pair-wise population differentiation was obtained through exact *G*-tests

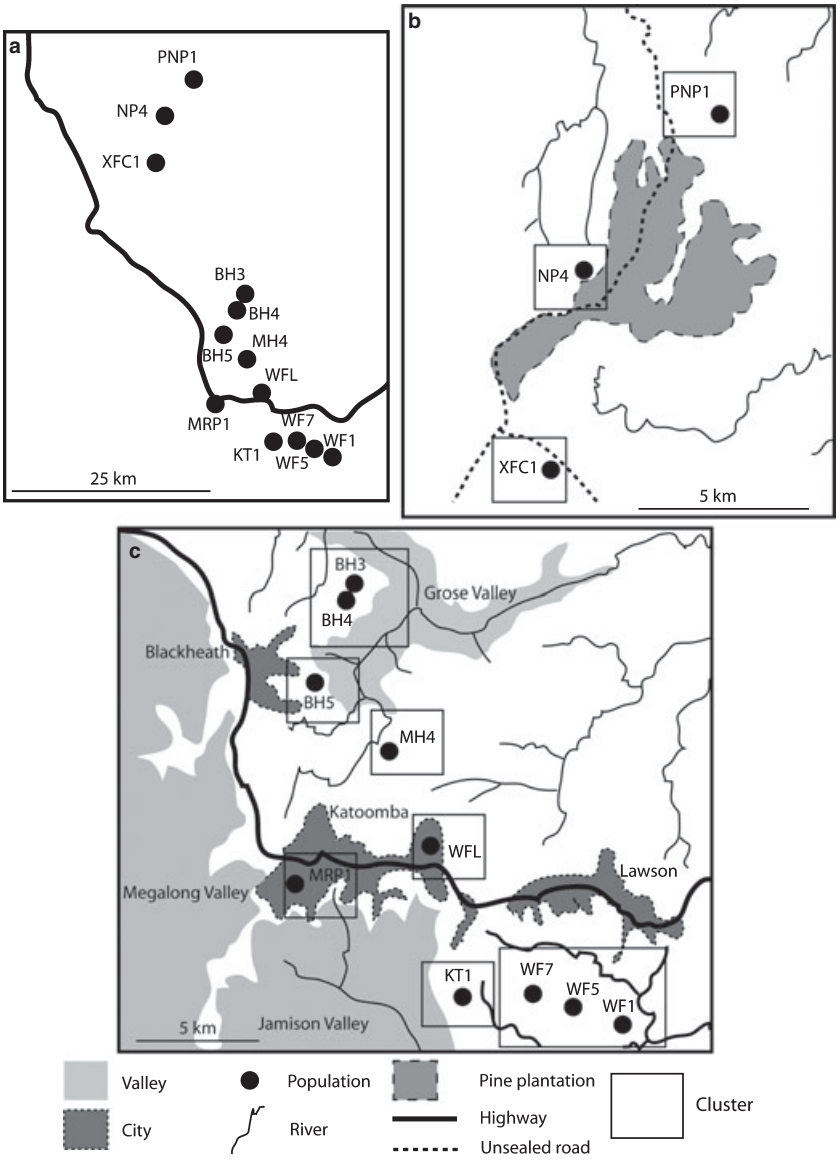

Fig. 1 Distribution of sampled populations (a), in the Newnes Plateau (b) and Blue Mountains (c), and genetic clusters identified by STRUCTURE analysis (K = 10).

on allelic frequencies as described by Goudet *et al.* (1996) with 10 000 randomizations. Allelic richness was estimated using the rarefaction method of El Mousadik & Petit (1996), in which genotype data are resampled to give sample sizes equal to the smallest population (in this case WF1, n = 5). AR (allelic richness) provides additional information on the level of genetic diversity present in population (compared with H_s), as it is expected to decline more rapidly than H_s when the effective population size is small and therefore is a sensitive indicator of population bottlenecks (Leberg 2002; Epps *et al.* 2006).

To compute these summary statistics and tests, FSTAT Version 2.9.3.2 was used (Goudet 2002; FSTAT, a program to estimate and test gene diversities and fixation indices, http://www.unil.ch/Jahia/site/dee/op/edit/pid/36921). Significance values were corrected for multiple tests using the sequential Bonferroni procedure (Rice 1989). The detection of null alleles was tested according to Chakraborty & Jin (1992).

Mantel and partial Mantel tests (Mantel 1967) were performed with genetic distance as the dependent variable and the distance between sites and difference of elevation between sites as explanatory variables; P-values were calculated after 10 000 randomizations. The tests were run on combined data, and within the Blue Mountains only.

Sex-biased dispersal

Three tests for sex-biased dispersal were performed using FSTAT Version 2.9.3.2 (Goudet 2002) based on the

entire data set and on 10 000 randomizations: (i) H_s of both sexes were compared (this descriptor should be higher for males under male-biased dispersal). (ii) Similarly the mean values of the corrected assignment index were compared (mAIc; Favre *et al.* 1997; immigrants will tend to have lower AIc values than residents). Under sex-biased dispersal, the average index for the sex that disperses most is expected to be lower than that for the more philopatric sex. A two-sided *t*-statistic is used for the test. (iii) We also compared the variance in AIc (vAIc) in males vs. females. Because members of the dispersing sex will include both residents (with common genotypes) and immigrants (with rare genotypes), vAIc should be larger for the sex dispersing most. Three different data sets were used to test for sex-biased dispersal, in order to estimate what stage of the life history of skinks disperses: (i) one including all the animals with snout–vent length (SVL) >40 mm [i.e. excluding neonates (including only potential dispersers)]; (ii) all the animals >50 mm (i.e. both subadult and adult individuals; size criterion based on unpublished data); and (iii) all the animals >60 mm (i.e. sexually mature individuals; S Dubey & R Shine, unpublished data). Thus, individuals >50 mm and >60 mm were included within the data set of 'all animals >40 mm', and individuals >60 mm were included within the data set of 'all animals >50 mm'. Tests on these three categories were performed separately, in order to see which components of the population included migrating individuals.

The number of males and females was not equal within each analysed populations, which could skew the results of our analyses (see Goudet *et al.* 2002). Thus, samples were removed randomly from these populations to obtain an equal sex-ratio, and we repeated the analyses with five different randomly selected data sets, including respectively 166 (88 males, 88 females), 166 (88 males, 88 females) and 156 (78 males, 78 females) individuals for each of the three body-size categories (>40, >50 and >60 mm).

In a separate analysis, first-generation migrant tests in GENECLASS2 (Piry *et al.* 2004) were performed, to estimate contemporary migration events in our 13 populations and identify the sex and size of the migrants. This test identifies migrants as individuals that were born at a breeding site other than the one in which they were collected. Because not all potential source sites were sampled, Lh was used (Lhome, the likelihood for the site where an individual was sampled; Paetkau *et al.* 2004) as our statistical criterion for the likelihood computation. The Bayesian method of Rannala & Mountain (1997) was used in combination with the Monte-Carlo resampling algorithm of Paetkau *et al.* (2004) to determine the critical value of the test statistic at $\alpha = 0.01$.

Unidirectional gene flow among populations

The software Migrate 2.0.6 (Beerli & Felsenstein 2001; Beerli 2004) was used to estimate the scaled migration rate (M) and the population size parameter (θ). This software is based on a coalescence model with mutation and migration, and estimates a measure of effective population size, θ, defined as $4N_e\mu$, where μ denotes the mutation rate and N_e the effective population size, and migration M, defined as m/μ, where m denotes migration rate. A stepwise mutation model (SMM) was assumed and estimates were based on 15 short [10^4 Markov Chain Monte Carlo (MCMC) steps] and five long (10^5MCMC steps) chains. To ensure convergence, the 'adaptive heating' option with one 'cold' and three 'hot' chains was used.

Structure of populations

A Bayesian model-based clustering method (Pritchard *et al.* 2000) for inferring population structure and assigning individuals to populations was implemented in STRUCTURE version 2.1 (Falush *et al.* 2003). Based on allele frequencies, a MCMC simulation is used to assign each individual a membership coefficient for each of K populations. Ten runs of 6×10^5 iterations (the first 10^5 considered as burn-in) for $K = 1$–13, including all the populations were performed. The number of populations best fitting our data set was defined as described in Evanno *et al.* (2005). The latter statistic compares the rate of change in the log probability of data between successive K and the corresponding variance of log probabilities.

Bottleneck

The existence of recent genetic bottlenecks was investigated using Bottleneck version 1.2.02 (Cornuet & Luikart 1996). According to Cornuet & Luikart (1996) and Lowe *et al.* (2004), populations that have experienced a recent reduction in effective population size exhibit both reduced allelic diversity and reduced heterozygosity at polymorphic loci. Computer simulations suggest that allelic diversity is reduced faster than heterozygosity, so that a comparison between these two parameters is the basis of the tests involved in Bottleneck. This comparison (heterozygosity relative to allelic diversity) thus examines a different genetics parameter than do analyses of heterozygosity relative to null models (i.e. to detect departures from HWE), as conducted by programs such as FSTAT.

A generalized stepwise mutation (GSM) model was used in which the change in the number of repeat units forms a geometric random variable, with a variance of

the geometric distribution fixed to 0.36, which seems to be the most appropriate for microsatellites (Estoup *et al.* 2001). The significance of deviation from the expected heterozygosity under mutation–drift equilibrium was determined with the Wilcoxon signed rank test (Luikart & Cornuet 1998).

Results

F-statistics

No linkage disequilibria or null alleles were detected, and all loci were in HWE. For the seven microsatellite loci, the number of alleles per locus ranged from 5 to 23 (average = 17.86), with a total of 125 alleles across seven loci (Table 1). AR within populations ranged from 2.85 to 4.87, with an overall value of 5.31 (Table 2). Populations from the Blue Mountains (BM; 10 populations: BH3, BH4, BH5, KT1, MH4, MRP1, WF1, WF5, WF7, WFL) vs. Newnes Plateau (NP; 3 populations: NP4, PNP1, XFC1) differed significantly in AR (BM: 3.79; NP: 4.70; $P = 0.03$).

Expected heterozygosities per locus (H_s) ranged from 0.32 to 0.81, with an average of 0.70, whereas expected overall heterozygosity (H_t) averaged 0.82 (range per locus: 0.37–0.91; Tables 1 and 2). Values for observed heterozygosity within populations (H_o) varied from 0.43 to 0.83, with an average of 0.68 (Table 1). There was no significant deviation from random mating in the analysed demes (overall $F_{IS} = 0.02$), suggesting no significant within-sample substructure. Populations from the Blue Mountains vs. Newnes Plateau did not differ significantly in H_o (BM: 0.673; NP: 0.796; $P = 0.06$), H_s (BM: 0.697; NP: 0.797; $P = 0.07$) and F_{IS} (BM: 0.034; NP: 0.000; $P = 0.35$).

Genetic distances among populations (pair-wise F_{ST}) were high (0.028–0.32; Table 3), as was the mean F_{ST} per population (0.113–0.238; Table 3) and the overall F_{ST} (0.128; $P < 0.001$; Table 3). All pair-wise F_{ST} were significant, except five comparisons involving small numbers of sampled individuals (WF1 and MH4; even in these cases, all the F_{ST} values were high). The mean F_{ST} between the Newnes Plateau and the Blue Mountains was 0.137. The mean F_{ST} of populations within the Blue Mountains did not differ significantly from those in the Newnes Plateau (BM: 0.138; NP: 0.060; $P = 0.08$).

No significant isolation by distance was detected for the 13 populations overall ($R^2 = 0.0$, correlation coefficient = 0.002, $P = 0.98$ NS; Table 4). However, significant isolation by distance was detected ($R^2 = 0.12$, correlation coefficient = 0.34, $P = 0.018$*) when we restricted comparisons to the 10 populations from the Blue Mountains.

In addition, the partial Mantel test with elevation and distance between sites as explanatory variables revealed significant isolation by elevation but no significant isolation by distance. These results were seen overall and also within the Blue Mountains (overall: $R^2 = 0.27$, elevation – partial correlation = 0.37, $P = 0.0006$***, distance – partial correlation = −0.18, $P = 0.12$ NS; BM: $R^2 = 0.21$, elevation – partial correlation = 0.44, $P = 0.0016$**, distance – partial correlation = 0.10, $P = 0.50$ NS).

Sex-biased dispersal

Mean assignment indices (mAIc) differed significantly between males and females in the five randomized data sets for the categories with individuals >40 and >50 mm (mean for the five data sets; >40 mm – *F*: 0.44, *M*: −0.44; $P = 0.0092$**; >50 mm – *F*: 0.44, *M*: −0.44;

Table 1 Genetic diversities of Blue Mountains water skinks at seven microsatellite loci

Locus	H_o	H_s	H_t	N_a
GQ20/211	0.74	0.78	0.91	18
EK8	0.75	0.72	0.86	23
EK23	0.79	0.81	0.91	21
EK37	0.64	0.69	0.85	5
EK39	0.72	0.79	0.90	23
EK100	0.77	0.81	0.91	16
EK107	0.34	0.32	0.37	19
Overall	0.68	0.70	0.82	125

H_o, observed heterozygosity; H_s, expected heterozygosity; N_a, number of alleles.

Table 2 Elevation of sampled sites and number of samples of lizard tissue collected (*N*) with sex (M, male; F, female; U, unidentified), observed heterozygosity (H_o), expected heterozygosity (H_s) and allelic richness (AR)

Population	Elevation	*N* (M/F/U)	H_o	H_s	AR
BH3	965	16 (5/11/0)	0.66	0.74	4.22
BH4	960	22 (5/17/0)	0.64	0.68	3.95
BH5	980	16 (7/8/1)	0.74	0.78	4.46
KT1	805	19 (7/12/0)	0.67	0.71	3.96
MH4	922	6 (3/3/0)	0.43	0.52	2.88
MRP1	963	20 (9/11/0)	0.76	0.74	4.21
WF1	560	5 (3/2/0)	0.50	0.52	2.85
WF5	620	14 (9/5/0)	0.55	0.56	3.14
WF7	676	19 (10/8/1)	0.65	0.67	3.79
WFL	885	19 (10/8/1)	0.80	0.78	4.40
NP4	1056	22 (9/12/1)	0.77	0.82	4.87
PNP1	1012	23 (7/15/1)	0.83	0.79	4.51
XFC1	1071	40 (22/14/4)	0.80	0.79	4.71
Overall		241 (106/126/9)	0.68	0.70	5.31

Table 3 Pair-wise F_{ST} (upper matrix; in bold: nonsignificant values), mean F_{ST} per population (diagonal; in italic) and geographic distances (in metres; lower matrix) among populations of Blue Mountains water skinks

	BH3	BH4	BH5	KT1	MH4	MRP1	WF1	WF5	WF7	WFL	NP4	PNP1	XFC1
BH3	*0.142*	0.0281	0.0771	0.1287	0.2264	0.142	**0.2038**	0.226	0.1526	0.1277	0.0987	0.1349	0.1603
BH4	455	*0.153*	0.0787	0.1493	0.2179	0.1607	0.2251	0.2301	0.1593	0.14	0.1199	0.1464	0.1829
BH5	4015	3568	*0.113*	0.0959	0.1976	0.0947	0.1697	0.1787	0.1072	0.0681	0.0679	0.0913	0.1236
KT1	18 099	17 697	15 140	*0.124*	0.2070	0.1058	0.1225	0.1431	0.081	0.0982	0.1068	0.1114	0.1395
MH4	12 628	12 198	9352	5901	*0.238*	0.1663	**0.3137**	0.3344	0.2665	0.2162	0.2252	0.2357	0.2474
MRP1	12 696	12 241	8802	8767	3950	*0.124*	0.1863	0.1157	0.1197	0.1061	0.096	0.0893	0.1022
WF1	22 018	21 701	20 047	7346	12 070	15 665	*0.185*	**0.1873**	**0.1191**	**0.181**	0.1577	**0.152**	0.198
WF5	20 235	19 884	17 899	4423	9417	12 862	2923	*0.177*	0.0851	0.1777	0.1716	0.1147	0.1601
WF7	19 451	19 090	17 008	3428	8405	11 822	3937	1042	*0.131*	0.1134	0.1123	0.1036	0.1476
WFL	11 557	11 160	8814	6547	2264	5853	11 453	9119	8203	*0.128*	0.0873	0.0868	0.13
NP4	26 534	26 917	29 572	44 583	38 909	37 912	48 274	46 706	45 958	38 068	*0.114*	0.0589	0.0689
PNP1	30 047	30 467	33 482	48 105	42 659	42 124	51 059	49 778	49 117	41 558	6250	*0.115*	0.0532
XFC1	21 582	21 933	24 329	39 446	33 679	32 458	43 553	41 816	41 021	32 989	5750	11 477	*0.143*

Table 4 Results of mantel and partial mantel tests to investigate the relationship between geographic distance and elevation, overall and within the Blue Mountains populations (BM) of the lizard *Eulamprus leuraensis*

Isolation by distance	R^2	Correlation coefficient
Overall (13 populations)	0.0	0.002; $P = 0.98$ (NS)
Within BM (10 populations)	0.12	0.34; $P = 0.018$*
Isolation by elevation and distance	R^2	Partial correlation
Overall (13 populations)	0.27	0.37; $P = 0.0006$***
		0.18; $P = 0.12$ NS
Within BM (10 populations)	0.21	0.44; $P = 0.0016$**
		0.10; $P = 0.50$ NS

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

$P = 0.0125$*; Table 5), and in three of five data sets for the analyses based only on individuals >60 mm (mean for the five data sets – *F*: 0.34, *M*: −0.34; $P = 0.0495$*).

H_s scores differed significantly between males and females in all five randomized data sets with individuals >40 mm, within four of five randomized data sets with individuals >40 mm, and within three of five randomized data sets with individuals >60 mm (mean for the five data sets; >40 mm – *F*: 0.72, *M*: 0.75; $P = 0.0225$*; >50 mm – *F*: 0.72, *M*: 0.75; $P = 0.0292$*; >60 mm – *F*: 0.72, *M*: 0.74; $P = 0.0569$ NS). Finally, vAIc did not differ significantly between the sexes in any of the randomized data sets (mean for the five data sets; >40 mm – *F*: 4.24, *M*: 4.84; $P > 0.05$ NS; >50 mm – *F*: 4.42, *M*: 4.38; $P > 0.05$ NS; >60 mm – *F*: 4.23, *M*: 3.68; $P > 0.05$ NS).

The first-generation migrant tests detected eight males and four females as migrants, consistent with the inference of male-biased dispersal from FSTAT (but not statistically significant in its own right). Mean SVLs for the first-generation migrants were 68.3 mm (range: 47–82 mm) for the eight males and 74.5 mm (range: 71–77 mm) for the four females. These patterns suggest that most dispersers were males, and at least some of these animals dispersed when they were juveniles. These results accord with a maximum difference in mAIc between sexes when small animals are included (i.e. all the individuals >40 and >50 mm). Hence, our data suggest (but do not conclusively prove) that dispersal in this system tends to be by males more often than by females.

Unidirectional gene flow among populations

In terms of the unidirectional migration rates (*M*) between pairs of populations, the Migrate analysis

Table 5 Results of sex-biased dispersal analyses, including the comparison between sexes of mAIc, vAIc and H_s in the three different categories of size classes (with number of males and females)

Individuals	mAIc	vAIc	H_s
>4 cm (88M/88F)	0.44, −0.44 (0.0092)**	4.24, 4.84 (0.05)	0.72, 0.75 (0.0225)*
>5 cm (88M/88F)	0.44, −0.44 (0.0125)*	4.42, 4.38 (0.05)	0.72, 0.75 (0.0292)*
>6 cm (78M/78F)	0.34, −0.34 (0.0495)*	4.23, 3.68 (0.05 NS)	0.72, 0.74 (0.0569 NS)

Females (*F*) and males (*M*) values are provided. All the values are means based on five randomized data sets with equal number of males and females within population and overall.
*P = 0.05; **P = 0.01.

suggested that migration occurs between most of the populations, with values ranging from 0.32 (WF1 into XFC1) to 2.94 (XFC1 into PNP1; Table S1). The population size parameter varied from 0.61 (WF5) to 1.09 (NP4).

Elevational gradients

AR, H_s' (from FSTAT analyses), and θ (from Migrate analyses) within populations increased in higher-elevation sites [linear regression, AR: $F_{1,12}$ = 15.79, R^2 = 0.59, P = 0.0022**; H_s' (arcsin square-root transformed {H_s}): $F_{1,12}$ = 12.87, R^2 = 0.54, P = 0.0043**; θ: $F_{1,12}$ = 7.09, R^2 = 0.39, P = 0.0221*; Fig. 2]. Because of the significant difference in mean allelic richness between the Blue Mountains and Newnes populations, a linear regression also was performed on the Blue Mountains populations only ($F_{1,9}$ = 5.9, R^2 = 0.42, P = 0.041*). No significant relationship between mean F_{ST} and site elevation was apparent ($F_{1,12}$ = 1.81, R^2 = 0.14, P = 0.20).

In addition, we estimated three measures of geographic isolation: the mean distance between a sampled site and all known populations, the shortest distance to the next known population, and the mean of the first five shortest distances to the closest known population. No significant relationships were found between any of these measures and AR, H_s' and θ (all $P > 0.05$, results not shown). Moreover, no significant relationship was found when we included elevation and one of these geographic-isolation measures in a multiple regression with AR, H_s' and θ as response variables (all $P > 0.05$), or if we included the elevation of sites and one of these distances as response variables (all $P > 0.05$, results not shown).

Structure of the populations and Bottleneck

The program STRUCTURE identified 10 clusters within our data set, using the method of Evanno *et al.* (2005). The clusters were geographically consistent: BH3–BH4, BH5, WF1–WF5–WF7, KT1, MH4, MRP1, WFL, NP4, PNP1 and XFC1 (Fig. 1). For a summary plot, see Fig. S1.

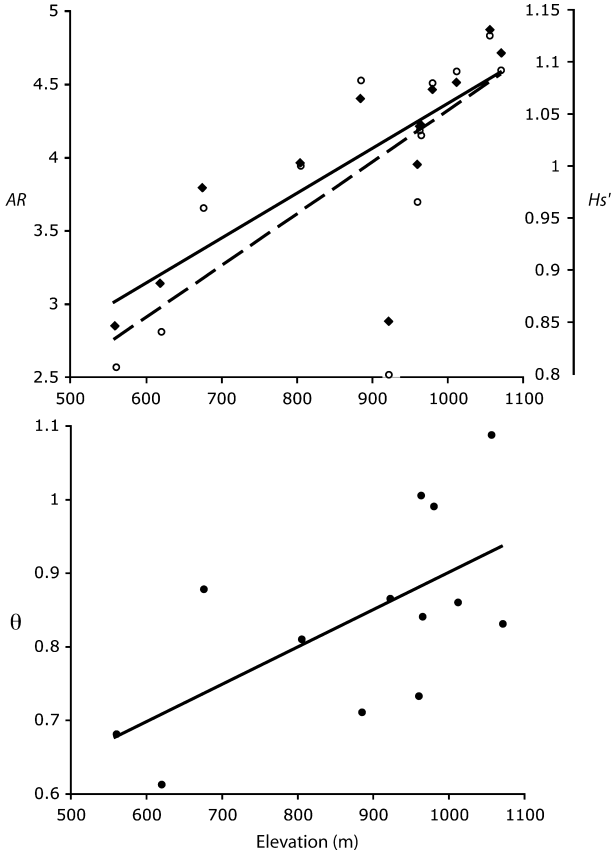

Fig. 2 Relationship between Allelic Richness (AR; black lozenge), transformed expected heterozygosity (H_s'; open circle) and population size parameter (θ; black circle) of skink populations compared with the elevation (*m*) of collecting site.

All the populations showed a significant excess of heterozygotes compared with their present allelic diversity under the GSM model, suggesting the signature of a recent bottleneck (see Materials and Methods section for more details). Additionally, the IAM (infinite allele model) and SMM models produced similar results (not shown). Detection of false positives is unlikely considering the low migration rate observed between our populations (see Pope *et al.* 2000).

Discussion

Population genetics of Eulamprus leuraensis

Levels of gene flow among our populations were low (Global F_{ST} = 0.128). First-generation migrants were detected, and unidirectional indices of migration were nonzero (*M*: 0.32–2.94): thus gene flow has occurred recently, but not often. This pattern is reinforced by the high number of clusters (*K* = 10) found by the structure analysis (most populations formed their own cluster), as well as by high pair-wise F_{ST} between populations (0.028–0.32). We found no overall pattern of genetic isolation by distance, but this relationship was significant if the analysis was restricted to the Blue Mountains populations only. This inconsistency may reflect the strong genetic isolation by elevation in the overall data set (including within the Blue Mountains). When our analyses included both the elevation and distance between sites, the trend for isolation by distance among the Blue Mountains sites was no longer significant.

Measures of F_{ST} found in other species of lizards are lower than the values observed in *Eulamprus leuraensis* (e.g. in *Anolis roquet*, F_{ST}: 0.0024–0.0213 – Johansson *et al.* 2008; *Phrynochephalus prewlzaski*, F_{ST}: 0.0062–0.0266 – Urquart *et al.* 2009; *Uma inornata*, F_{ST}: 0–0.13 – Hedtke *et al.* 2007; *Oedura reticulata* and *Gehyra variegata* in continuous forest population, F_{ST}: 0.003 vs. 0.004 respectively and in fragmented populations F_{ST}: 0.102 vs. 0.044 – Hoehn *et al.* 2007). The only published F_{ST} values in other species of lizards as high as those of *E. leuraensis* are cases of strongly fragmented habitats, such as between populations of an endangered skink species in New Zealand (*Cyclodina whitakeri* – between island and mainland populations – F_{ST}: 0.19–0.27, Miller *et al.* 2009; *Oligosoma grande* mean F_{ST}: 0.097, Berry *et al.* 2005) or between populations separated by more than 200 km in a lacertid species (*Lacerta viridis* – F_{ST} = 0.14–0.35; Böhme *et al.* 2007).

Because most populations of *E. leuraensis* are located within national parks and other protected areas, the impacts of urbanization and human activities should be limited. However, *E. leuraensis* is a specialized semi-aquatic species living in the wettest parts of montane swamps (LeBreton 1996), and thus the dry sandstone plateau areas adjacent to the swamps could be potential barriers to dispersal. Moreover, species in the genus *Eulamprus* have lower tolerance to water loss than do many other reptile species (Heatwole & Veron 1977), restricting their distribution to sites with water bodies. Although there are no large rivers in this region, small creeks could be used as dispersal corridors.

Dispersal appeared to involve males more often than females, with significant differences between the sexes apparent in some but not all comparisons that we conducted. We detected a significant signal of sex-biased dispersal among our populations in all three body-size categories that we tested (i.e. SVLs >40, >50 and >60 mm) for differences in mAIc and in two to three categories for the differences in expected heterozygosity (H_s; i.e. SVLs >40 and >50 mm). However, the sex bias in numbers of first-generation migrants detected (eight males, four females) was not statistically significant in its own right, neither did we detect significant sex differences in variance of AIc.

In general, male-biased dispersal and female philopatry appear to be the norm in mammals, whereas birds exhibit female-biased dispersal and male philopatry (Greenwood 1980). In reptiles, recent studies on crocodiles (Tucker *et al.* 1998), turtles (FitzSimmons *et al.* 1997; Casale *et al.* 2002; Chaloupka & Limpus 2002) and snakes (Rivera *et al.* 2006; Keogh *et al.* 2007; Dubey *et al.* 2008) all report male-biased dispersal. However, in lizards, the results are less consistent, with male-biased (*Lacerta vivipara* – Clobert *et al.* 1994; *Uta stansburiana* – Doughty & Sinervo 1994; *Lacerta agilis* – Olsson *et al.* 1996; – *Egernia stockesii* – Gardner *et al.* 2001; *Sceloporus occidentalis* – Massot *et al.* 2003; *Egernia frerei* – Fuller *et al.* 2005; *Anolis roquet* – Johansson *et al.* 2008; *Chlamydosaurus kingii* – Ujvari *et al.* 2008; *Phrynocephalus przewalskii* – Urquart *et al.* 2009) and female-biased dispersal (*Niveoscincus microlepidotus* – Olsson & Shine 2003; *Oligosoma grande* – Berry *et al.* 2005) documented in various species.

The proximate mechanisms underlying sex-biased dispersal in reptiles may differ among species, and may involve phenomena such as (i) local mate competition, with displacement of subadult males by larger rivals (Tucker *et al.* 1998; Johansson *et al.* 2008), such that males have to disperse further to find territory that is not already occupied by other males (Johansson *et al.* 2008) and (ii) extensive mate-searching movements by adult males (Rivera *et al.* 2006). Limited dispersal in females can be explained by advantages of an intimate knowledge of local refuges, risk factors and prey availability, and in some cases by the advantages of living in groups of related individuals that may provide enhanced vigilance and indirect protection of juveniles against predators (as in some Australian viviparous scincid species, genus *Egernia*; see e.g. Fuller *et al.* 2005). Finally, in oviparous (egg-laying) species of reptiles, female-biased dispersal could be linked to the constraint of finding suitable sites for oviposition, favouring dispersal (Bonnet *et al.*1999; Olsson & Shine 2003).

In *E. leuraensis*, tests based on mAIc and H_s values (but not on vAIc) suggested significantly male-biased dispersal, whereas analyses based on the first-generation

migrants revealed no significant difference between sexes. However, a tendency to male-biased dispersal (67% being males) was apparent, involving both subadult and adult males, consistent with the previous results. In addition, this species is viviparous, and pregnant female *Eulamprus* are vulnerable to predators because of reduced mobility (Schwarzkopf & Shine 1992). Thus, remaining in a well-known area, close to safe refuges, may enhance female survival. Detailed observational data are needed to clarify the plausibility of such mechanisms and test the apparent tendency to male-biased dispersal.

As well as revealing strong population structure and hinting at sex-biased dispersal, our analyses suggest potential recent bottleneck events in all the identified populations. Frequent bushfires in this region (LeBreton 1996), and dry years linked to climate change, could cause such bottleneck events as suggested for the Australian frillneck lizard (*C. kingii*; Ujvari *et al.* 2008). In addition, we found lower AR, H_s' and θ (population size parameters = $4Ne\mu$, where Ne is the effective population size and μ the mutation rate) at lower-elevation sites than at higher-elevation sites, meaning that a gradient of genetic diversity and number of breeding individuals per population is present at different elevations. This latter result could be explained by the combination of (i) less-demographic stability of the lowland populations or the occurrence of recent bottlenecks events, due to warmer and drier climatic conditions; (ii) a relatively recent colonization of the lowland (e.g. after the last glacial maximum); or by (iii) lower connectivity of the lowland populations, resulting in a loss of genetic diversity through time. Alternative (iii) is not supported by our results: for example, we found no relationship between site elevation and mean F_{ST} per population, and indeed the lowland populations WF1, WF5 and WF7 belonged to the same genetic cluster. Interestingly, an elevational gradient has also been recorded in shrews (*Crocidura russula*): the number of mitochondrial haplotypes decreases with altitude, due to smaller and sparser populations, undergoing frequent bottlenecks (Ehinger *et al.* 2002). But inconsistently, (i) haplotype and nucleotide diversities in the shrews showed no decrease with elevation, and (ii) genetic subdivision decreased in less-favourable habitats (i.e. at lower elevation), as between our three lowland populations of *E. leuraensis* (WF1, WF5 and WF7). Ehinger *et al.* (2002) suggested that (i) historical events may have shaped the present observed patterns or (ii) ongoing selection might maintain the present haplotype distribution. Elevational gradients in genetic diversity have also been reported in salamanders (*Ambystoma macrodactylum*; Giordano *et al.* 2007) with lower diversity at high elevations. In plants, higher genetic diversity has been reported at mid-elevations rather than in low- and high-elevation populations (e.g. Jump *et al.* 2003; Saenz-Romero & Tapia-Olivares 2003; Byars *et al.* 2009). In our study taxon, genetic diversity was maximal at high elevations (approximating the highest elevations of the Blue Mountains and Newnes Plateau) and not at low or mid-altitudes; optimal habitat for this montane species may occur at high elevation. In addition, a closely related species (*Eulamprus quoyii*) occurs in swamps at low elevation, and may exclude *E. leuraensis* from such sites (LeBreton 1996). Consequently, the observed gradients also might be due to interspecific competition.

Implication for the conservation of E. leuraensis

The reduced gene flow and genetic distinctiveness of remnant populations of *E. leuraensis* have important implications for conservation of this endangered species. Most populations are distinctive genetically and warrant separate management. The loss of a population (e.g. through predation by non-native mammals or habitat degradation, or most importantly via future global warming) would result in a loss of genetic diversity within *E. leuraensis* overall. In addition, low rates of gene flow among populations (reflecting low dispersal capability) in this semi-aquatic species imply that recolonization of swamps would be very slow. Consequently, a high priority should be given to preservation of the unique montane swamps that house these lizards. The elevational gradients in genetic diversity and effective population size also suggest that the survival of lowland populations could be at risk under global warming. Models of climatic variation predict that the area inhabited by *E. leuraensis* will become both warmer (by up to 5°C) and drier (by up to 40%) within the next century (http://www.climatechangeinaustralia.gov.au). Such changes may have a dramatic impact on the montane swamps and consequently on the lizards that depend upon them, for example, via potential drying out of swamps and increase in bushfire frequency and intensity. As *E. leuraensis* already occupies the highest parts of the Blue Mountains and Newnes Plateau, future colonization of habitats situated at even higher elevations will not be possible.

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Supporting Information

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

Table S1 Unidirectional gene flow and migration-rate ($M = m/\mu$) estimates within pairs of Blue Mountains water skink populations and population size parameter (θ) from Migrate (Beerli 2004)

Fig. S1 Summary plot of the individual assignment results of the Structure analyses for $K = 10$.

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