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Genetic diversity and biogeographic history inform future conservation management strategies for the rare sunset frog (*Spicospina flammocaerulea*)

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Abstract. Outlining the distribution of genetic variation, patterns of gene flow and clarifying the biogeographic processes underlying population history are critical components of a comprehensive conservation strategy for endangered or vulnerable species. We provide this information for the vulnerable sunset frog (*Spicospina flammocaerulea*) using a comprehensive genetic dataset (*ND2*) with samples from 17 of 22 geographic localities where this species has been found. From genetic, biogeographic and coalescent-based analyses, we document the existing genetic variation, likely movement patterns and explore the biogeographic history of *S. flammocaerulea*. While catchment-based genetic variation is well documented in other high-rainfall taxa in south-western Australia, a much more complex scenario including dispersal across ridge lines between catchments better explains the distribution of genetic variation and observed patterns of gene flow in *S. flammocaerulea*. The population history of *S. flammocaerulea* is strongly indicative of recent population contraction and expansion, which may be related to late Pleistocene climate fluctuations. This suggests that this species can adapt or move in response to fluctuating climates provided suitable habitats or expansion areas are available. However, like many other endemic taxa with limited geographic ranges in south-western Australia, the potential to shift distributions is hampered by being land-locked within an agricultural landscape, limiting management options in the face of climate change.

Additional keywords: conservation, phylogeography, population genetics, south-western Australia.

Introduction

Understanding the biogeographic processes underlying population history, the distribution of genetic variation and patterns of gene flow are critical components of comprehensive and sustainable conservation management of threatened and endangered species (Moritz 1994, 2002; Moritz and Faith 1998). However, this information is rarely available for either flora or fauna in any ecosystem. South-western Australia is known for its extreme diversity of plants and highly threatened ecosystems, but there are comparatively few datasets analysing genetic differentiation within species that might lead to an understanding of processes generating that diversity (but for plants see Coates and Hamley 1999; Byrne and Macdonald 2000; Byrne *et al*. 2003*a*, 2003*b*; Coates *et al*. 2003; Broadhurst *et al*. 2004; Byrne and Hines 2004; Wheeler and Byrne 2006; and for animals see Driscoll 1998*a*, 1998*b*; Reid 2002; Munasinghe *et al*. 2004; Gouws *et al*. 2006; Edwards 2007*a*; 2007*b*; Edwards *et al*. 2007, 2008). Genetic data documenting variation within species can be used for conservation management in two ways: (1) to assess common patterns of variation that reflect historical events such as climate shifts, patterns of isolation or tectonic activity that might define the spatial array of genetic diversity and inform management actions (e.g. reserve selection or management of fragmented landscapes: Kahindo *et al*. 2007; McRae and Beier 2007; Vandergast *et al*. 2008) or (2) to document dispersal routes as part of recovery planning and ongoing management of natural populations of endangered species (De Boer *et al*. 2008; Neel 2008).

The frog fauna of south-western Australia shows a range of patterns of genetic subdivision with important conservation implications. The *Geocrinia rosea* species complex is a series of four allopatric, geographically restricted frog species with direct development and very limited dispersal (Driscoll 1998*a*, 1998*b*; Conroy and Brook 2003; Driscoll and Roberts 2008). These four species all show very high levels of subdivision within species, reflecting both historical patterns of drainage-based difference and the impact of genetic drift and limited local dispersal (Driscoll 1998*a*, 1998*b*; Driscoll and Roberts 2008). Other species with wider distributions and habitat preferences have either strong patterns of catchment-based genetic structure (Edwards *et al*. 2008) or limited genetic structuring and widespread dispersal (Davis and Roberts 2005; Morgan *et al*. 2007). The sunset frog (*Spicospina flammocaerulea*) breeds in late spring or early summer in landscapes with a strong winter rainfall maximum and

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extreme Mediterranean climate. It breeds in peat swamps high in the landscape (Roberts *et al*. 1997, 1999; Dziminski *et al*. 2004). Breeding site and season might favour differentiation as drying swamps in spring and summer might limit the chances for downstream dispersal by tadpoles. However, many breeding sites are at the top of drainage systems, possibly allowing adult or juvenile dispersal between drainages across the tops of catchments (Roberts *et al*. 1999; Burbidge and Roberts 2001). On the basis of the observed variation in phylogeographic pattern in frogs from south-western Australia and the known biology of the sunset frog, we cannot make reliable predictions about the presence or absence of genetic structuring in this species.

Consequently, we analysed population processes and genetic diversity in the sunset frog as background for ongoing management of this species. The sunset frog is classified as 'Vulnerable' under IUCN Red List criteria (Burbidge and Roberts 2001). The species, discovered in 1994, is the single survivor of a lineage within the family Myobatrachidae most closely related to the genus *Uperoleia* (Roberts *et al*. 1997; Read *et al*. 2001). It is an aquatic ovipositor with aquatic larval development that breeds in late spring or early summer when rainfall is lowest in south-western Australia (Burbidge and Roberts 2001), a breeding strategy unique in the south-western Australian frog fauna and likely to be an ancestral state retained from historical occupation of a subtropical region (Roberts *et al*. 1997). It has a small range (300 km^2 total distribution with an area of occupancy probably less than 20 km^2) in the high-rainfall zone of the south-west and has been found only in perched peat swamps on first-order streams high in the landscape in the Frankland, Bow and Kent River catchments (Roberts *et al*. 1997). We conducted a comprehensive genetic study using *mtDNA* across the entire known distribution of *S. flammocaerulea*. We aimed to develop a picture of the distribution of genetic diversity, movement patterns, and underlying population processes that have shaped the distribution of this species with implications for its ongoing conservation management. We posed three questions related to the biogeographic history of *S. flammocaerulea* and relevant for informing conservation management in the species:

(1) What is the effective dispersal distance for female *S. flammocaerulea*?
(2) What are the patterns of gene flow and genetic diversity within *S. flammocaerulea*: can they be explained by catchment?
(3) Has *S. flammocaerulea* undergone population expansions and contractions concordant with recent, Pleistocene, or older, climate cycles?

Materials and methods

Tissue samples

In total, 184 individuals were sampled (toe-clips) from 17 sites across the species' distribution. Four or five animals per site were sampled from seven smaller populations, and 12–18 animals per site were sampled from 10 larger populations. Most animals sampled were adults; only two juveniles were sampled, which constitutes the total number of records of juveniles observed for the species. Tadpoles are also rarely observed and little is known of the biology of either tadpoles or juveniles of this species. Fig. 1 shows the distribution of sampling sites and the sample numbers

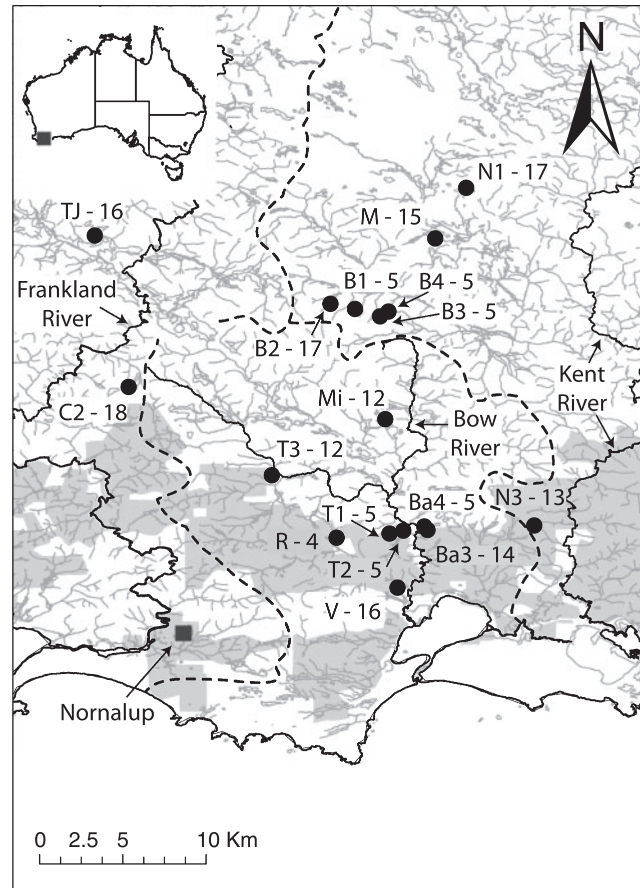

Fig. 1. Map of *Spicospina flammocaerulea* sampling sites [●] showing the major catchments (delineated by dotted lines) and rivers in the region, with a map of Australia (inset). Sampling sites represent all known and active (male calling activity) sites within the entire distribution of the species across two breeding seasons (2002 and 2003). Numbers following sites names represent the number of individuals sequenced from each site. Note the lack of a distinct boundary in the north-west of the Bow catchment bordering the Frankland catchment. Grey shaded areas represent agricultural and private land-use, unshaded areas are National Parks, unused Crown Land or Reserves.

from each site. Sampled populations represent every known site that was active (i.e. calling males could be heard) across two breeding seasons (2002–03). Both active searches and call surveys failed to locate individuals at a further five sites from which calling activity had previously been recorded, but several factors (e.g. time since fire) can affect calling activity (Bamford and Roberts 2003).

Molecular genetic methods

Genomic DNA extraction, polymerase chain reaction amplification and cycle sequencing procedures were carried out as outlined in Edwards (2007*a*). Primers used to amplify *ND2* were L4221 (5′-AAGGRCCTCCTTGATAGGGA-3′ – modified from Macey *et al.* 1998) and tRNA-trp (5′-CTCCTGCTTAGG GSTTTGAAGGC-3′ – modified from Read *et al*. 2001). Internal primers used for sequencing were L4437 (5′-AAGCTTTCGG

GGCCCATACC-3′: Macey *et al.* 1998), L5025spic (5′-CATG TGGGCTGAATGGTTT-3′), Myo-L4882 (5′-CMACVTGRCA AAAAYTHGCCCC-3′ – modified from Melville *et al.* 2004) and H4980 (5′-ATTTTTCGTAGTTGGGTTTGRTT-3′: Macey *et al.* 1998). Sequence data were edited (Sequencher 3.0, Gene Codes Corporation), aligned (ClustalX: Thompson *et al.* 1997) and checked by eye and a clear reading frame was observed in all sequences. Distinct haplotype sequences have been lodged in GENBANK (**accession numbers** – Appendix 1). Haplotype (H_D) and nucleotide (π) diversity were estimated using DnaSP ver. 4.50.3 (Rozas *et al.* 2003).

Phylogeographic patterns

Unrooted, statistical, parsimony haplotype networks were created using TCS 1.21.0 to display genetic relationships amongst *S. flammocaerulea* haplotypes presented as a network structure. Diversity of private alleles in each population was calculated using HP-RARE (Kalinowski 2005), with corrections for different sample sizes via rarefaction, as a measure of genetic distinctiveness or isolation (Kalinowski 2004). This is based on the premise that populations experiencing restricted gene flow will have a high number of private or unique alleles, and therefore high private allelic diversity. To map patterns of gene flow we calculated pairwise F_{ST}s between each population using Arelquin ver. 3.11 (Excoffier *et al.* 2005). Catchment-based patterns of genetic structure were tested using analyses of molecular variance (AMOVA), calculated in ARLEQUIN v3.11 (Excoffier *et al.* 2005). Genetic samples from each sampling site were grouped for hierarchical analyses according to the catchment in which they occurred (Kent, Bow or Frankland). Spatial autocorrelation analyses were used to estimate effective female dispersal and look for evidence of isolation by distance across the distribution-wide dataset by plotting patterns of spatial genetic structure using GenAlEx ver. 6 (Peakall and Smouse 2006). Spatial autocorrelation computes the statistic r, an index of genetic correlation (either positive or negative) amongst samples from specified geographic distance classes (Peakall *et al.* 2003). Because analyses can be sensitive to changes in size class (Peakall *et al.* 2003) we ran analyses using multiple size class bins at 2, 3, 4 and 5 km.

Demographic expansion analyses

Analysis of historical changes in population size, including the magnitude and the pattern and timing of expansion were analysed using a combination of summary statistics, mismatch distributions and coalescent-based simulations across the range of *S. flammocaerulea*. Tajima's D (D_T) (Tajima 1989), R_2 (Ramos-Onsins and Rozas 2002) and Fu's F_S summary statistics were all calculated using DnaSP ver. 4.50.3 (Rozas *et al.* 2003) to provide evidence of historical population expansion. The statistical significance of D_T, R_2 and Fu's F_S statistics were tested by generating random samples under the hypotheses of both selective neutrality and demographic equilibria using coalescent simulations with 10 000 permutations in DnaSP ver. 4.50.3 (Rozas and Rozas 1999; Rozas *et al.* 2003). In order to test whether estimated population expansion also corresponded to spatial expansion mismatch distributions were computed by comparing empirical distributions with those based on both a demographic expansion model (DEM) and a spatial expansion model (SEM) using the generalised least-squares method in ARLEQUIN ver. 3.11 (Excoffier *et al.* 2005). The statistical significance of parameters estimated by mismatch distribution tests were calculated by bootstrapping analyses 10 000 times.

To provide further evidence for population expansion, as well as evidence of contraction, in addition to estimating the magnitude of population expansion we estimated parameters θ and g for the distribution-wide data and for haplotype groups identified from the haplotype network. LAMARC ver. 2.1.2b (Kuhner 2006; Kuhner and Smith 2007) was used to obtain maximum-likelihood estimates of theta ($\theta = 2N_f\mu$, where N_f is the effective female population size and μ is the mutation rate) and an exponential population growth parameter (g). The θ parameter was estimated initially with g held at zero and with starting θ obtained using Watterson's (1975) estimate (θ_{NG}). The θ_G parameter was estimated jointly with g (initial value of $g = 0.1$). The search strategy employed for all runs consisted of 15 short chains (10 000 steps), five long chains (200 000 steps), sampled every 20th step, burn in = 1000, random starting trees, transition/transversion ratio = 2 and empirical base frequencies. Interpretations of the g parameter obtained from empirical data followed the methods outlined in Lessa *et al.* (2003) and Garrick *et al.* (2007). Estimates for parameters θ_{NG}, θ_G and g were repeated five times and the mean and standard deviation (s.d.) of these parameter estimates were calculated. The g parameter was considered to indicate population growth only when $g - (3 \times \text{s.d.}_{(g)}) > 0$, and, alternatively, population decline would be indicated when $g + (3 \times \text{s.d.}_{(g)}) > 0$ (Lessa *et al.* 2003; Garrick *et al.* 2007).

Where population expansion was detected, time since population expansion ($t = \tau/2\mu$: Rogers and Harpending 1992) was calculated using τ (estimated by the mismatch analysis) and the nucleotide substitution rate (μ) of the gene region used (Excoffier 2004). There are no appropriate external calibration points/fossils that might be used to calibrate a mutation rate for Australian frogs, therefore to provide approximate dates for expansions in *S. flammocaerulea* we adopted the mutation rate of 0.957% for every million years, calibrated for the entire *ND2* coding region in eleutherodactylid frogs (Crawford 2003). While rates can vary between branches, studies on vastly different groups of amphibians have shown similar rates of divergence to those originally proposed by Macey *et al.* (1998) (Crawford and Smith 2005). However, to incorporate the potential for variation in mutation rate we also calculated divergences using the lower and upper limits of rates reported for anurans in the literature for *ND2* (e.g. 0.69% for every million years for *Bufo* (Macey *et al.* 1998) and 2.4% for every million years for *Rana* (Plötner *et al.* 2001)).

Results

A 1140-bp sequence of *ND2* from 184 individuals across the whole known distribution of *S. flammocaerulea* yielded 41 haplotypes containing 42 variable sites. Haplotype diversity was high ($H_D = 0.862 \pm 0.018$), whereas nucleotide diversity was very low ($\pi = 0.00142 \pm 0.00008$). All 41 haplotypes were joined into a network spanning a maximum of 14 steps (Fig. 2*a*) at the 95% confidence level for parsimonious connections.

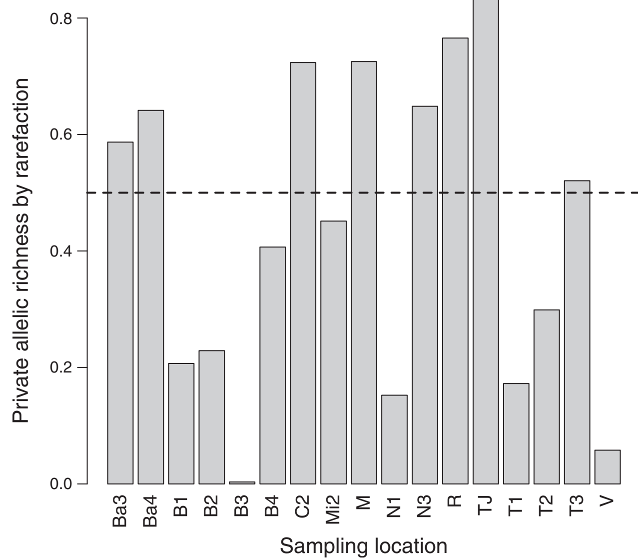

Fig. 2. Barplot of private allelic richness per sampling location corrected for differences in sampling intensity by rarefaction across the distribution of *Spicospina flammocaerulea*. Dashed line is at 0.5 private allelic richness; values below this line represent sampling locations with low to moderate numbers of private alleles and values above the line represent sampling locations with moderate to high numbers of private alleles.

Phylogeographic patterns

Patterns of private allele richness, when corrected for different sample sizes by rarefaction, showed several populations with moderate to high values (Fig. 2). Most of the populations with moderate to high private allele diversity were peripheral populations in the west (TJ, C2 and, to a lesser extent, T3), the south-east (N3), and the north (M). Additional populations in the south and central portion of the distribution also show high levels of private allelic richness (Ba3, Ba4 and R). Pairwise F_{ST}s (Table 1) show significant and high genetic structure ($F_{ST} > 0.3$) between R, M and N1 in comparison to most other populations. Other peripheral populations identified as potentially isolated with private allelic richness analyses (e.g. C2, TJ, T3, N3) also show significant, but more moderate F_{ST}s in comparison to other populations. Pairwise F_{ST}s were generally very low or non-significant amongst populations within the Bow River catchment. Analyses of molecular variance (AMOVA) show that catchments account for little of the genetic variation across *S. flammocaerulea* (12.21%: Table 2), with most genetic variation attributed to genetic variation within populations (67.92%: Table 2).

A much more complicated pattern is revealed when distribution-wide patterns of genetic structure are assessed without assuming a pattern of catchment-based genetic structure (Fig. 3). Two haplotypes (1 and 3) were observed in high frequency (Fig. 3*a*). Haplotype 3 occurs throughout the species' distribution and is detected at all sites excepting M and R, and is in very low frequency in another northern site (N1). Haplotype 1 is found only in the northern part of the species' distribution and is found in M, B1–4, C2, N1, Mi2 and T3. While individuals from TJ site appear to be very closely related to the southern haplotype group (indeed, five of the individuals sequenced share the common haplotype from this group) the remaining individuals had unique haplotypes found only within this site, a finding that is also supported by high private allelic richness when corrected for sampling effort (Fig. 2). Spatial autocorrelation analyses (Fig. 3*b*) show evidence of isolation by distance shown by the negative

Table 1. Pairwise F_{ST}s between individual sampling locations and estimates of nucleotide diversity (π) and haplotypic diversity (H_D) for each population

F_{ST} values in bold are significant at the $P \leq 0.05$ level. π, nucleotide diversity; H_D, haplotype diversity

	Sampling location																
	T3	M	R	B2	V	B1	B3	C2	T1	N3	T2	Ba4	Ba3	TJ	N1	Mi2	B4
π	0.0014	0.001	0.0009	0.001	0.0004	0.0012	0.0004	0.0018	0.0004	0.0014	0.0007	0.0019	0.0015	0.0013	0.0003	0.0004	0.0009
H_D	0.86	0.79	0.50	0.75	0.40	0.80	0.40	0.86	0.40	0.85	0.70	1.00	0.87	0.73	0.33	0.44	0.80
Sampling location																	
T3	–																
M	**0.09**	–															
R	**0.22**	**0.31**	–														
B2	0.02	**0.10**	**0.33**	–													
V	**0.33**	**0.41**	**0.47**	**0.30**	–												
B1	0.00	0.11	**0.34**	–0.08	**0.21**	–											
B3	**0.22**	**0.32**	**0.56**	0.15	0.00	0.00	–										
C2	**0.09**	**0.15**	**0.26**	**0.12**	**0.28**	0.06	**0.19**	–									
T1	**0.27**	**0.35**	**0.56**	**0.24**	0.00	0.12	–0.11	**0.21**	–								
N3	**0.13**	**0.18**	**0.28**	**0.17**	**0.31**	**0.12**	**0.23**	**0.13**	**0.22**	–							
T2	**0.16**	**0.24**	**0.39**	0.15	0.04	0.01	–0.06	**0.12**	–0.06	0.06	–						
Ba4	0.06	**0.12**	**0.23**	0.11	**0.27**	0.02	0.17	0.05	0.17	–0.06	–0.01	–					
Ba3	**0.11**	**0.17**	**0.26**	**0.12**	**0.14**	0.02	0.06	**0.08**	0.04	**0.08**	–0.01	–0.03	–				
TJ	**0.20**	**0.24**	**0.34**	**0.21**	**0.37**	**0.20**	**0.32**	**0.19**	**0.32**	**0.20**	**0.22**	0.14	**0.16**	–			
N1	**0.19**	**0.28**	**0.63**	**0.10**	**0.62**	0.22	**0.55**	**0.34**	**0.63**	**0.42**	**0.54**	**0.44**	**0.40**	**0.47**	–		
Mi2	**0.26**	**0.35**	**0.54**	**0.19**	0.04	0.06	–0.15	**0.23**	–0.05	**0.27**	–0.01	**0.22**	**0.10**	**0.34**	**0.54**	–	
B4	0.02	0.11	**0.34**	–0.01	**0.37**	–0.05	0.21	0.10	**0.29**	**0.15**	0.15	0.06	0.10	**0.22**	**0.23**	**0.26**	–

Table 2. The partitioning of genetic variance within *Spicospina flammocaerulea* across catchments as determined by an analysis of molecular variance (AMOVA)

d.f., degree's of freedom. ***, $P < 0.01$; *, $P < 0.05$

Source of variation (XY)	d.f.	Variance components (V_{XY})	Percentage of variation (%)	Fixation index (F_{XY})
Catchments as regions				
Among catchments	2	0.10482 (Va)	12.21	0.12208*
Among sites/catchment	14	0.17067 (Vb)	19.08	0.22640***
Within sites	167	0.58315 (Vc)	67.92	0.32084***
Total	183	0.85864		

slope. Values of r (x – intercept) obtained from multiple size class analyses suggest that effective female dispersal (i.e. resulting in a significantly positive genetic correlation) declines at around 8.1–10 km (Fig. 3*a* shows a representative analysis).

Demographic expansion analyses

Tests of neutrality and demographic analyses consistently show evidence of both demographic and spatial expansion (Table 3). Summary statistics Tajima's D_T and Fu's F_S, and the coalescent growth parameter g indicate demographic expansion and Ramos-Onsins's R_2 summary statistic (Ramos-Onsins and Rozas 2002) results largely mirror these findings. Mismatch analyses suggest significant spatial (SEM) and demographic (DEM) expansion. Using the values of τ obtained from the mismatch analyses, combined with a mutation rate of 0.957% for every million years (Crawford 2003), we estimated a single putative date for each of these expansions as estimates of τ did not differ between DEM and SEM models. These values show a date for the expansion of the whole species at 159 000 years ago (128 000–202 000 years ago). The mean time for expansion has a lower limit of 220 000 and an upper limit of 165 000 years ago using the lowest and highest published mutation rates for *ND2* (see Methods).

Discussion

Patterns of gene flow and genetic diversity in S. flammocaerulea

Biogeographic studies in the south-west high-rainfall zone of Australia have focussed on faunal assemblages that either require streams for survival, or have a life-history strategy that tie them to moist areas. These studies have indicated that catchments are important in capturing genetic diversity within this biodiversity hotspot, particularly in the high-rainfall province (Driscoll 1998*a*, 1998*b*; Munasinghe *et al*. 2004; Gouws *et al*. 2006; Edwards *et al*. 2008). However, this is not a universal trend, as other anuran species do not show signatures of catchment-based genetic structure (Davis and Roberts 2005; Edwards *et al*. 2007; Morgan *et al*. 2007). The current study of *S. flammocaerulea* presents a much more detailed and complex scenario.

Little genetic variance within *S. flammocaerulea* was explained by catchment, while high levels of gene flow within the Bow River catchment, and isolation of peripheral populations better explains the patterns of genetic structure and diversity in the species. Molecular analyses of peripheral versus central populations generally have shown high levels of isolation (high numbers of private alleles gained through genetic drift and evidence for restricted gene flow) and low genetic diversity (Eckert *et al*. 2008). Within *S. flammocaerulea*, populations at the periphery have high private allelic and haplotypic diversity, appear to be isolated and in some cases show equal or higher levels of nucleotide and haplotypic diversity when compared with central populations within the Bow River catchment. Under some evolutionary scenarios an increase in genetic diversity at the periphery of species ranges may be expected (i.e. under population expansion), which is a possibility for the current study (see below). However, our estimates of genetic diversity are low across the species and may be further limited given the *mtDNA* marker used in the current study (Eckert *et al*. 2008). Further testing to determine the exact causes of this pattern would require a more detailed understanding of the ecological requirements of *S. flammocaerulea* (Sexton *et al*. 2009).

Population expansion/contraction in S. flammocaerulea

Frog species previously studied across the south-west high-rainfall zone have either not been sampled at fine enough scales (Davis and Roberts 2005; Edwards 2007*b*; Edwards *et al*. 2007, 2008) or analysis techniques were not available at the time of the study (Driscoll 1998*a*, 1998*b*) to infer reliable estimates of the magnitude and timing of population expansion/contraction. Based on the occurrence of fixed novel genotypes in some sites and patterns of genotypic variance across river systems, Driscoll (1998*a*, 1998*b*) suggested that species in the *Geocrinia rosea* species complex, occurring to the west of *S. flammocaerulea*, showed evidence of range shifts and population expansion/contraction but no time scale was applied to these events. Other species show patterns of differentiation across some catchments associated with much older climate change (i.e. Miocene–Pliocene: Edwards *et al*. 2008), as well as evidence of population differentiation into catchment groups, probably associated with Pleistocene climate fluctuation across the region occupied by *S. flammocaerulea* (Edwards *et al*. 2007). However, detailed information on population contraction/expansion is lacking in these studies due to the nature of the data available for analysis.

Generalised additive distribution models (GAMs: Swan 2007) suggest that such range expansions and shifts in *S. flammocaerulea* can occur with very small changes in rainfall (e.g. comparing rainfall in the 1960s with current data). Although subject to relatively large error terms (Bromham and Penny 2003), estimates dating expansions of *ND2* haplotypes suggest that the demographic history of *S. flammocaerulea* is likely to have been strongly shaped by the dramatic changes in climate across south-western Australia during the late Pleistocene (e.g. Edwards *et al*. 2007; Byrne *et al*. 2008). These biogeographic patterns appear to be shaped by the same population processes (climate-driven population expansion/contraction) as seen in other frog species in the high-rainfall zone, but at much shallower time scales and with little evidence of restricted dispersal across catchment boundaries (Edwards *et al*. 2008; but cf. Driscoll and Roberts 2008).

A synthesis of our results and using a range of mutation rates nevertheless presents quite clear biogeographic scenarios relating the historical population processes in *S. flammocaerulea* to the

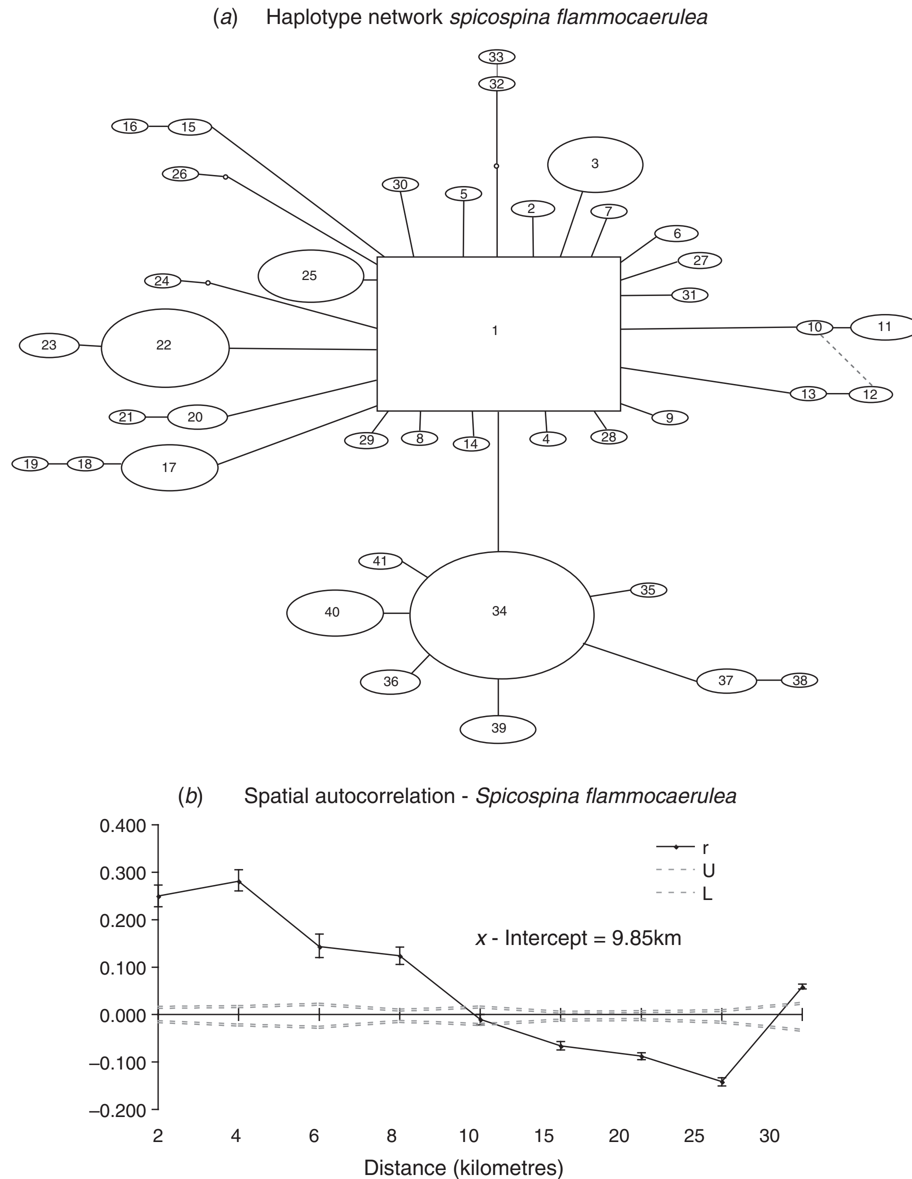

Fig. 3. Analysis of broad-scale phylogeographic structure within *Spicospina flammocaerulea* as shown in (*a*) a haplotype network, and (*b*) a spatial autocorrelation. The relative size of depicted haplotype elipses in the network increases with haplotype sampling frequency. Lines between haplotypes indicate a single mutational step and small circles indicate haplotypes that were either unsampled or extinct. The SA correlogram graphs the trend of positive genetic correlation (*r* +ve) between samples or negative genetic correlation (*r* –ve) between samples within defined distance classes. The *x*-intercept indicates the effective dispersal distance for females, a spike to +ve *r* values at greater distances suggests some long-distance dispersal, and values within the confidence limits indicate no significant spatial structure within a particular distance class. From multiple analyses with varying size classes this *x*-intercept varies between 8 and 10 km. The maximum parsimony network (*a*) spans all 41 *S. flammocaerulea ND2* haplotypes at the 95% confidence level of a parsimonious connection with a maximum of 14 steps. Information on the haplotypes and numbers present at each sampling site have been provided in Appendix 1. A representative SA analysis across the distribution of *S. flammocaerulea* (*b*) shows a trend of limited effective female dispersal above 9.85 km (*x*-intercept).

late Pleistocene. Being restricted by higher rainfall (Swan 2007), the species' distribution is likely to have contracted in relation to glacial cycles and expanded again during interglacial cycles. The species has some ability to cope with drying climates by occupying specific microhabitats, in the form of permanently wet peat swamps (Roberts *et al*. 1997), and explosively breeding

Table 3. Results of tests of neutrality/demography (Tajima's D_T, Fu's F_S, and R_2), mismatch analyses based both on models of demographic expansion (DEM) and spatial expansion (SEM), and coalescent analyses for the *Spicospina flammocaerulea ND*2 dataset

*, significant results at $\alpha = P < 0.05$, or non-overlapping standard deviations (*g* parameter only)

	Value	Range	*P*
No. of individuals	184		
No. of haplotypes	41		
Neutrality/demographic tests			
D_T	–2.2935*		$P < 0.01$
Fu's F_S	–48.8663*		$P < 0.01$
R_2	0.0186*		$P < 0.01$
Mismatch distributions			
DEM	$\tau = 1.73$*	1.4–2.2	$P < 0.05$
SEM	$\tau = 1.74$*	1.1–2.0	$P < 0.05$
Coalescent simulations			
θ_{NG}	0.0106036	0.008–0.014	
θ_G	0.0449122	0.02–0.07	
g	5612.038*	4770–6453	

following fire (Bamford and Roberts 2003). However, it still retains ancestral summer breeding regimes (breeding in November when rainfall is lowest in south-west Australia), which makes this species much more susceptible to past and modern climate change.

Conservation implications

Our current study analyses the patterns of genetic structure and gene flow in female *S. flammocaerulea*, providing valuable information on the distribution of genetic structure and population history for conservation management. We show here the preservation of catchment processes will not necessarily ensure the preservation of genetic diversity in high rainfall endemics and, despite the lack of restricted gene flow associated with riverine barriers, females generally do not disperse distances greater than 10 km, mirroring results seen in other species in the high-rainfall zone (Driscoll 1998*a*, 1998*b*). These results are based on *mtDNA* only and can therefore infer dispersal of females only: we need appropriate nuclear markers to infer dispersal patterns of males.

Our knowledge of population processes and habitat requirements show that *S. flammocaerulea* has undergone climate-induced distributional shifts and associated range contraction/expansion, both in the past (this study) and ongoing (Swan 2007). Range expansion/contraction processes are also thought to have contributed to contemporary genetic structure and diversity in other threatened high-rainfall endemics (Driscoll 1998*a*, 1998*b*). Distribution modelling of *S. flammocaerulea* suggested that the species is highly susceptible to changes in rainfall, which is likely to have caused shifts in the species' distribution both south and west towards the coast over the last 30 years (Swan 2007). *S. flammocaerulea* might be resilient to some level of predicted climate change providing that suitable habitats are available, allowing the species to shift its distribution. However, despite 11 of 17 sites sampled in this study occurring on currently protected land, all protected sites occur in the northern portion of the species' distribution. The remaining coastal areas in the south are used for agriculture (Fig. 1).

Habitat is unlikely to be available to allow further shifts in the distribution of *S. flammocaerulea* under predicted climate change (Hughes 2003), as *S. flammocaerulea*, like other high-rainfall endemics, appears to be land-locked by the adjacent agricultural matrix (Wardell-Johnson *et al*. 1995). This is particularly concerning given the level to which south-western Australia has already experienced reductions in rainfall, and is predicted to be one of the regions of Australia to see the most dramatic reductions in rainfall under models of future climate change (Solomon *et al*. 2009). Effort is needed to better understand patterns of genetic diversity and population isolation (using appropriate markers) in concert with information on habitat suitability and preferences in order to gain a clearer understanding of how distributional limits in *S. flammocaerulea* and other high-rainfall endemics. This is important for the determination of adaptive potential under future climate change (Eckert *et al*. 2008; Sexton *et al*. 2009; Behrman and Kirkpatrick 2011). This will also enable the prediction of distribution shifts under past and future climate change models (Kearney and Porter 2004; Kearney 2006) and will assist in concentrating habitat rehabilitation efforts for *S. flammocaerulea* and other high-rainfall endemics.

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Appendix 1. List of unique *S. flammocaerulea* haplotypes, the site where they were found, the frequency at which they were observed, and the haplotype GenBank numbers

Haplotype	Sampling site	Frequency	Accession #
1	T3	1	JN202731
	B2	4	JN202839-40, JN202848-9
	B3	4	JN202853-6
	B1	2	JN202834-5
	V	12	JN202784-90, JN202792-3, JN202796-8
	T1	4	JN202773-6
	C2	3	JN202740, JN202744, JN202749
	N3	2	JN202819, JN202826
	T2	3	JN202778, JN202780-1
	Ba3	5	JN202804-8
	TJ	2	JN202899, JN202903
	Ba4	1	JN202803
	Mi2	9	JN202758-60, JN202762-5, JN202767-8
	B4	1	JN202890
	N1	1	JN202888
2	T3	2	JN202727-8
3	R	3	JN202769-70, JN202772
	V	4	JN202783, JN202791, JN202794-5
	T3	1	JN202738
4	R	1	JN202771
5	N3	1	JN202820
6	T3	2	JN202732, JN2027327
7	T2	1	JN202782
8	B2	1	JN202847
9	Mi2	1	JN202766
10	N3	1	JN202822
11	Ba4	1	JN202799
	Ba3	1	JN202812
	N3	2	JN202828, JN202830
12	Ba3	2	JN202813-14
13	N3	1	JN202829
14	Ba3	1	JN202816
15	Ba3	1	JN202817
	Ba4	1	JN202800
16	Ba4	1	JN202801
17	C2	6	JN202739, JN202741, JN202750, JN202752-3, JN202755
18	C2	1	JN202748
19	C2	1	JN202756
20	T1	1	JN202777
	Ba3	1	JN202810
	N3	1	JN202827
21	Ba3	1	JN202811
22	B2	1	JN202838
	TJ	8	JN202897-8, JN202900, JN202902, JN202905-7, JN202910
23	TJ	3	JN202895-6, JN202909
24	B1	1	JN202833
25	N3	5	JN202818, JN202821, JN202823-5
	T2	1	JN202779
	Ba4	1	JN202802
26	C2	1	JN202745
27	T3	2	JN202733-4
28	B2	1	JN202846
29	Ba3	2	JN202809, JN202815
30	TJ	1	JN202908
31	B2	1	JN202852
32	TJ	1	JN2020904
33	TJ	1	JN202901
34	M	4	JN202858-9, JN202866-7
	T3	4	JN202729-30,JN202735-6
	B2	8	JN202836-7, JN202841–5, JN202851
	B1	2	JN202831-2
	B3	1	JN202857
	C2	2	JN202743, JN202746
	N1	14	JN202873–6, JN202878-84, JN202886-7, JN202889
	Mi2	2	JN202757, JN202761
	B4	2	JN202893-4
35	N1	1	JN202885
36	M	3	JN202865, JN202871-2
37	C2	3	JN202747, JN202751, JN202754
38	C2	1	JN202742
39	M	3	JN202860-1, JN202870
	N1	1	JN202877
37	M	5	JN202862-4, JN202868-9
	B2	1	JN202850
41	B4	2	JN202891-2