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Structure and fragmentation of growling grass frog metapopulations

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Abstract

Metapopulations occur in fragmented landscapes, and consist of demographically-independent populations connected by dispersal. Nevertheless, anthropogenic habitat fragmentation may be fatal to metapopulations, as it disrupts dispersal and gene flow, and undermines the balance between population extinction and colonization. Understanding the extent to which particular land-use practices disrupt dispersal and gene flow is therefore crucial for conserving metapopulations. We examined the structure and fragmentation of metapopulations of the endangered growling grass frog (*Litoria raniformis*) in an urbanizing landscape in southern Australia. Population clustering analyses revealed three distinct genetic units, corresponding to the three wetland clusters sampled. Isolation-by-distance was apparent between populations, and genetic distance was significantly correlated with the presence of urban barriers between populations. Our study provides evidence that urbanization fragments metapopulations of *L. raniformis*. Managers of *L. raniformis* in urbanizing landscapes should seek to mitigate effects of urbanization on dispersal and gene flow.

Introduction

Many species are organised as metapopulations. Examples range from herbs and forbs (Menges 1990; Ouborg 1993; Harrison and Ray 2002), through invertebrates and small vertebrates (Verboom et al. 1991; Hanski et al. 1994; Kindvall 1996; Sjögren-Gulve 1994; Moilanen et al. 1998), to larger vertebrates in both terrestrial and marine systems (Possingham et al. 1994; Lahaye et al. 1994; Harveson et al. 2006; Kritzer and Sale 2006). While the structure and dynamics of metapopulations varies considerably across these diverse guilds, a feature common to all is habitat patchiness arising from either natural or anthropogenic fragmentation. Metapopulations are defined as networks of spatially discrete populations connected by dispersal (Hanski and Simberloff 1997). Habitat fragmentation is therefore a necessary precursor to metapopulation formation, because it divides habitat into spatially patchy units, and curtails dispersal to the extent that mating between populations becomes non-random (Hanski 1998, 1999).

Despite this, metapopulations are sensitive to the level of habitat fragmentation they experience, particularly that arising from human land-use practices (Lindenmayer and Fischer 2006). While constraints on dispersal are required for metapopulations to form, sufficient dispersal between populations is also necessary to ensure that the rate of population extinction does not exceed the rate of patch colonization (Hanski 1998, 1999). Dispersal rate is clearly central to the rate of patch colonization; the more immigrants that a patch receives, the more likely establishment is (Ebenhard 1991). However, dispersal rate may also influence the rate of population extinction (Gaggiotti and Hanski 2004). From a demographic perspective, immigration from neighboring populations bolsters population size. Larger populations are less sensitive to demographic and environmental perturbations, and are therefore less prone to extinction (Brown and Kodric-Brown 1977; Hanski 1998, 1999). From a genetic perspective, immigration allows outcrossing, leading to the avoidance or alleviation of inbreeding depression in many species (Frankham et al. 2009). Immigration may also alleviate the deleterious effects of reduced genetic diversity in small, isolated populations (Reed and Frankham 2003).

There are two primary means by which anthropogenic habitat fragmentation can produce unsustainable reductions in the rate of dispersal within metapopulations. The first, and most intuitive, is its effect on the physical ability of migrants to move between patches. Increased inter-patch distances, the removal of dispersal corridors and the construction of particular types of human infrastructure may all reduce the physical capability of migrants to move across landscapes (Hanski 1999; Suter et al. 2007). The second is its effect on the size of the constituent populations. When habitat fragmentation reduces patch size or enhances edge effects, there will be, in most cases, a concomitant reduction in population size (Hanski 1998, 1999; Gaggiotti and Hanski 2004). Larger populations produce the most migrants, so changes in the size or quality of particular patches can lead to significant reductions in the overall rate of dispersal (Hanski 1999).

Of the various human land-use practices that fragment the habitat of plants and animals, urbanization is a key concern. Some 3.3 billion people currently inhabit urban centers worldwide, and this figure is expected to rise to 5 billion by 2030 (UNFPA 2007). The construction of housing and transport infrastructure to support these human population trends typically requires the destruction of habitat remnants, or parts thereof (McKinney 2002). Moreover, urban infrastructure represents a significant barrier to dispersal for many taxa, leading to considerable reductions in landscape permeability (Trombulak and Frissell 2000).

Urbanization-driven habitat fragmentation is of considerable concern for the conservation of amphibians (Cushman 2006; Hamer and McDonnell 2008). Amphibians are now one of the most threatened components of many ecosystems worldwide, with one third of all species currently at risk of extinction (Stuart et al. 2004; Beebee and Griffiths 2005). The effects of urbanization on amphibians can be multifarious; however, habitat fragmentation is a prominent risk, because many are reliant on metapopulation dynamics for persistence (Cushman 2006; Hamer and McDonnell 2008). However, habitat fragmentation is also problematic for the many amphibians that display patchy-population dynamics (*sensu* Harrison and Taylor 1997), in which individuals regularly migrate between wetlands in response to changing conditions, or between wetlands and surrounding terrestrial zones as part of seasonal changes in activity patterns (Marsh and Trenham 2001; Smith and Green 2005). Dispersal is obviously central to these dynamics, so processes that impede dispersal can also have significant detrimental outcomes for amphibians that display them (Semlitsch 2002, 2003; Cushman 2006; Baldwin et al. 2006).

This study extends recent ecological research on the metapopulation dynamics of the endangered growling grass frog (*Litoria raniformis*) in the urbanizing landscapes of Melbourne, Victoria, Australia (Heard et al. 2012). *Litoria raniformis* is a large, highly aquatic frog that was once widespread across south-eastern Australia (Pyke 2002), yet has suffered substantial population declines (Mahony 1999; Wassens 2008; Hamer et al. 2010). There is concern that urbanization fragments remnant populations of *L. raniformis* in the vicinity of Melbourne, and undermines their viability (Hamer and Organ 2008; Heard et al. 2010). However, empirical evidence of the effect of urban development on dispersal and gene flow has been lacking. We used microsatellite markers to investigate the pattern of population subdivision for *L. raniformis* across an urban gradient on the northern outskirts of Melbourne.

We predicted strong clustering of genetic similarity, consistent with the view that clusters of wetlands support discrete metapopulations of the frog (Heard et al. 2012). Between populations, we predicted isolation-by-distance effects consistent with a sharp decline in the rate of dispersal with distance, and resulting low rate of migration (Heard et al. 2012). Overlain on this pattern we predicted a signal of urbanization-driven habitat fragmentation, in the form of increased genetic distance between populations separated by major urban infrastructure.

Materials and Methods

Study area

This study was conducted in the Merri Creek catchment (MCC) on the northern fringe of Melbourne (Figure 1). The Merri Creek originates in the Great Dividing Range approximately 50 km north-east of Melbourne, and flows south to its confluence with the Yarra River approximately 5 km north-east of the central business district. The topography is undulating, with a largely volcanic geology. Land-use within the catchment displays a clear gradient from predominantly livestock-based agriculture in the north to urban development in the south (Figure 1). Current plans for Melbourne's urban growth propose development of much of the MCC over the next 20 years (DPCD 2009).

Tissue sampling

Litoria raniformis were sampled at 12 sites across the middle reaches of the MCC during the 2004/2005 and 2005/2006 breeding seasons (Figure 1). These sites were distributed in three distinct clusters (northern, central and southern), each separated by approximately 6 km. Site selection proceeded with dual aims of enabling detection of dispersal during a concomitant mark-recapture study (Heard et al. 2012), and to sample sites that varied in their distance to others in the network and in their degree of isolation by urban barriers. Sites included chains of pools along the Merri Creek and two of its ephemeral tributaries, and lentic wetlands including farm dams, natural and artificial ponds, swamps and quarries. Major barriers included housing and industrial estates, and two dual-carriage roads (Cooper Street and the Hume Freeway; Figure 1b). In the northern cluster, a single major barrier (the Hume Freeway) separated site N1 from all others (Figure 1c). Sites S1 and S2 in the southern cluster were separated by industrial estates (Figure 1e).

Each site was surveyed repeatedly between October and March, and capture by hand or net was attempted for all *L. raniformis* located. Tissue samples were obtained from all captured frogs by clipping the toe-pad on the left middle digit of the front left limb, following standard sterile techniques (Ferner 2007). Clipped toes were stored in 90% ethanol. To avoid double sampling (and for the purposes of the mark-recapture study) all frogs were marked by subcutaneous injection of either a Passive Integrated Transponder (PIT) tag (Trovan Ltd, East Yorkshire, UK), or a Visible Implant Alphanumeric (VIA) tag (Northwest Marine Technology, Shaw Island, WA, USA). Genetic contamination and disease transmission was avoided by sterilizing all equipment between each individual (by dipping equipment in ethanol and igniting). A sub-set of 198 individuals was selected for genetic analysis. Site locations and sampling intensities are summarized in Table 1.

Microsatellite analysis

Genomic DNA was extracted using a standard Proteinase K digestion and chloroform: isoamyl alcohol extraction protocol (Gemmell and Akiyama 1996). Eleven polymorphic microsatellite markers were used. Nine were developed specifically for this study system (Hale et al. 2011) and a further two taken from previous research on the closely-related species, *Litoria aurea* (Laurea2A, Laurea5M; Burns and Ferrari 2004). See Hale et al. (2011) for PCR information and conditions for the nine novel loci, as well as methods for fluorescently labelling fragments for all loci. PCR conditions for the two previously published loci were different than for the other loci: 95°C 5 min, 2 cycles of 95°C 30 s, 66°C 30 s, 72°C 60 s; 2 cycles of 95°C 30 s, 64°C 30 s, 72°C 60 s; 42 cycles of 95°C 30 s, 62°C 30 s, 72°C 60 s, followed by 72°C 5 min (adapted from Burns and Ferrari 2004). PCR products were diluted for fragment analysis which was carried out by the Australian Genome Research Facility (AGRF) on an Applied Biosystems ABI3730 DNA analyser using a LIZ-500 size standard. Scoring was completed using *Genemapper* version 3.1.2 and *Peak Scanner* version 1.0 (Applied Biosystems). All samples were manually screened for accuracy.

Statistical analyses

The frequency of null alleles was calculated using the program *FreeNA* (Chapuis and Estoup 2007). We tested for gametic disequilibrium between loci using Fisher's exact test, and deviations from Hardy-Weinberg Equilibrium (HWE) were tested using a U test. Both were implemented in *Genepop* version 4.0 (Rousset 2008). Parameters used for these analyses were a burn-in period (dememorisation) of 10,000, 100 batches and 1000 iterations. Two sites (C4 and C5) were excluded from these analyses due to low sample sizes. Observed and expected heterozygosity (H_O and $H_{E,}$ respectively) were estimated in *GenAlex* version 6.3 (Smouse and Peakall 2006). Sequential Bonferroni adjustments for multiple comparisons were made when required.

We estimated the number of genetic units in the full data set, as well as within each site cluster (northern, central and southern) using the Bayesian population clustering program *Structure* version 2.3.2 (Pritchard et al. 2000; Pritchard et al. 2010). We used a model that assumed uncorrelated allele frequencies (to reduce the likelihood of overestimating *K*) and admixture (the recommended starting point for the model). The LOCPRIOR model was used, which implicitly incorporates putative population information (in this case, geographic location). This model can help to detect genetic structure in the data, especially when the signal is relatively weak (Pritchard et al. 2010). We repeated each analysis with varying admixture states and excluding locational information. The results were concordant with the admixture/LOCPRIOR model. For all analyses, inferences were derived from 500,000 MCMC samples, after discarding the first 100,000 samples as a ‘burn-in’. The number of genetic units (*K*) was set *a priori* to range from 1-13 (to encompass 12, the number of sampling sites). Simulations were run 10 times for each proposed *K* between 1 and 13. To determine *K*, we plotted the mean estimated Ln probability of the data, Ln Pr ($X|K$), from the 10 runs to determine which value of *K* had the highest posterior probability or the smallest value of *K* among estimates at a plateau of highest values (Pritchard et al. 2010). Analysis of the three site clusters followed the same procedure, varying *K* from 1 to 8.

Pairwise F_{ST} between sites was estimated using *Arlequin* version 3.1 (Excoffier et al. 2005). As above, sites C4 and C5 were excluded from this analysis given sample size constraints. We used partial Mantel tests to assess the relationship between pairwise F_{ST} and both geographic distance and the presence of major urban barriers between sites. Partial Mantel tests allow the correlation between two distance matrices to be quantified whilst controlling for correlations with additional matrices (Smouse et al. 1986). Straight-line distances between wetland centres were used (see Tables 1 and 2) as *L. raniformis* is known to migrate overland through pasture, grassland and shrubland (see Heard et al. 2012), and was therefore capable of selecting the shortest route between any two of our sampling locations. Pairs of sites were defined as being separated by major urban barriers if the straight-line distance between them crossed housing or industrial estates, or dual-carriage roads. After Cushman et al. (2006), we ran two partial Mantel tests; one in which the correlation between pairwise F_{ST} and the presence of barriers was tested while partialling out distance, and the other in which the correlation between pairwise F_{ST} and distance was tested while partialling out the barrier effect. If distance and barriers both influence pairwise F_{ST} (as we anticipated), the correlation coefficients for both relationships should be independently significant. Partial Mantel tests were performed in *R* version 2.13.0 (R Development Core Team 2012) using the add-in package *ecodist* version 1.2.7 (Goslee and Urban 2012). Significance of the correlation coefficients was assessed using 10,000 random permutations of the first matrix, as described by Goslee and Urban (2007).

Results

A high frequency of null alleles (greater than 10%) was observed for four loci at three sampling sites. Locus Laurea5M showed a high frequency of null alleles at sampling site S1, Laurea2A and Lr8 at C1 and Lr3 at sampling site N3. Given the inconsistent pattern of null allele detection, all loci were retained for subsequent analyses. No significant gametic disequilibrium was observed for any locus pair across each population. Heterozygosity levels are presented in Table 1.

Bayesian population clustering tests including all samples (Figure 2a) revealed three distinct genetic units ($K = 3$) (average Ln Pr ($X|K$) = -6787.9). These units correspond with the three major geographic site clusters (northern, central and southern). Analysis of samples from within the northern cluster revealed two distinct genetic units (average Ln Pr ($X|K$) = -3140.2), separating samples from site N1 from all others in that cluster (Figure 2b). The analysis therefore suggests a genetic division between sites east of the freeway in this cluster from the remainder west of the freeway. However, the analysis suggests some gene flow between N1 and the other four sampling sites in the northern cluster, with admixture being particularly apparent in sites N2, N3 and N4 (Figure 2b). For the central cluster, in which no major urban barriers occurred between sites, $K = 1$ had the highest posterior probability (average Ln Pr ($X|K$) = -2236.5). There were clear genetic differences between the two sites in the southern cluster; $K = 2$ had the highest posterior probability (average Ln Pr ($X|K$) = -1232.44), and there was little admixture between the two sites (Figure 2c).

Pairwise F_{ST} values were always significant following sequential Bonferroni correction when sites were located in different clusters (Table 2). Within clusters, all pairwise F_{ST} values between sites separated by major urban development were significant. Of the eight within-cluster site pairings for which there was no major urban development between them, two displayed significant pairwise F_{ST} values. Partial Mantel tests revealed significant correlations between pairwise F_{ST} and both the presence of urban barriers ($r = 0.379$, two-sided $p = 0.019$) and geographic distance ($r = 0.569$, two-sided $p = 0.0003$). Figure 3 compares the relationship between pairwise F_{ST} and geographic distance for sites within the same cluster, with and without major urban barriers between them.

Discussion

Our study supports earlier ecological research on the spatial dynamics of *L. raniformis* in this region. On the basis of wetland occupancy and mark-recapture data, Heard et al. (2012) concluded that the dynamics of *L. raniformis* across northern Melbourne resemble classical metapopulation dynamics (*sensu* Hanski 1999), with clusters of wetlands spread over distances of 1-2 km supporting discrete metapopulations of the frog, and wetlands within clusters supporting demographically-independent populations connected by infrequent, distance-limited dispersal. Our Bayesian population clustering analyses revealed three distinct genetic units, corresponding with the three wetland clusters sampled (Figure 2a). They also suggested minimal admixture between the three clusters. These results clearly support the notion that clusters of wetlands support discrete metapopulations of *L. raniformis* across northern Melbourne. Bayesian population clustering analyses also revealed genetic sub-divisions within two of the three wetland clusters. These sub-divisions correlated with the occurrence of major barriers to dispersal in both cases (see further below); however, significant pairwise F_{ST} values between site N2 and both N3 and N5 in the northern cluster, which were not separated by urban barriers, are suggestive of additional population sub-division in this area. Given that sites N3 and N5 were the furthest from N2 (excluding N4, for which relatively few samples were obtained), this result is consistent with the strong isolation-by-distance effect suggested by the previous ecological study (Heard et al. 2012) and by our broader genetic data.

Nevertheless, the lack of genetic sub-divisions within the central cluster may be evidence of higher rates of dispersal by *L. raniformis* in some situations than those suggested by the previous demographic study (Heard et al. 2012). In this area, two of three sites for which adequate samples were obtained (C1 and C2) occurred along Merri Creek, and may have displayed relatively high rates of migration and genetic exchange for this reason. Higher rates of dispersal between adjacent sites, or between those connected by streams, could undermine their treatment as demographically-independent populations connected by infrequent dispersal, and instead point to a mixture of metapopulation and patchy-population processes within clusters (Harrison and Taylor 1997; Marsh and Trenham 2001; Smith and Green 2005; see also Hamer et al. 2008; Wassens et al. 2008). We acknowledge that treating some populations as being discrete from their neighbours may be problematic. However, it is also important to note that genetic panmixia does not necessarily imply demographic panmixia, because the rate of migration needed to maintain genetic cohesion may be far less than that required for demographic cohesion (Lowe and Allendorf 2010). Moreover, in our study area, the very sharp decay in the probability of dispersal with distance suggested by the previous mark-recapture study is an important determinant of wetland occupancy and colonisation by *L. raniformis*, including occupancy and colonisation of pools along streams (Heard et al. 2012). Given this, and the strong correlation between genetic distance and geographic distance detected in our study, we conclude that dispersal by *L. raniformis* is strongly distance-limited, and that metapopulation theory provides a useful overall model of the dynamics of the species in this region.

As well as inter-patch distance, this study provides evidence that major urban infrastructure affects the rate of dispersal and gene flow for *L. raniformis* in urbanizing landscapes. Bayesian population clustering tests identified genetic sub-division between all populations separated by major urban infrastructure (housing and industrial estates, and dual carriage roads), and pairwise F_{ST} values were correlated with the presence of urban barriers between populations. These results concur with those of numerous studies on the spatial dynamics of amphibians in urbanizing landscapes (Cushman 2006; Hamer and McDonnell 2008). In an early genetic study, Hitchings and Beebee (1997) found substantially increased genetic differentiation between populations of the Common Frog (*Rana temporaria*) in an urban setting relative to those within a rural landscape. The same result was observed for the Eastern Red-Backed Salamander (*Plethodon cinereus*) by Noël *et al.* (2007) in Canada. Reh and Sietz (1990), Vos *et al.* (2001) and Arens *et al.* (2006) explicitly tested the relationship between genetic distance and the extent of urbanization between populations of the Moor Frog (*Rana arvalis*) and Common Frog (*R. temporaria*) in Europe. Each of these studies indentified important correlations between genetic distance and urbanization.

These studies, and ours, provide evidence that urban infrastructure impedes dispersal and gene flow for amphibians. However, each has relied on correlative analyses to assess relationships between genetic distance and the extent of urbanization. Correlative analyses represent a relatively weak basis to infer cause and effect, because spurious relationships between the independent and supposed dependent variable may arise (Burnham and Anderson 2002; Williams et al. 2002; Cushman et al. 2006). Nevertheless, there are two reasons to believe that urbanization does indeed fragment populations of amphibians, including *L. raniformis*. Firstly, relationships between urbanization and both genetic and ecological measures of dispersal rate are consistently identified for amphibians (above), in which case the supposed cause and effect relationships between these variables becomes increasingly plausible (Burnham and Anderson 2002; Williams et al. 2002). Secondly, there are clear mechanisms underlying the supposed effects of urbanization on amphibian dispersal. Amphibians may actively avoid urban areas during dispersal, due to the physical barriers to movement these areas present, the lack of free-standing water or moist microhabitats, and the extensive light and noise pollution therein (see Eigenbrod et al. 2009). High mortality rates during attempts to cross urban infrastructure have also been documented for amphibians. For example, Hels and Buchwald (2001) studied the migration behaviour of six species of amphibian in Denmark, and calculated rates of mortality during road crossings. They estimated that the probability of surviving an attempt to cross a motorway could be as low as 0.02 for their study species.

In the preceding sections, we focused on the effect of urban barriers on the internal dynamics of metapopulations; however, urban barriers may also influence extinction and colonization dynamics of entire metapopulations. The dispersal kernels of amphibians often have long tails, resulting from occasional long-distance dispersal events (Smith and Green 2005). Such events may allow important genetic interactions, limiting metapopulation-wide reductions in genetic diversity through inbreeding and drift (Frankham et al. 2009). As such, the placement of urban infrastructure between metapopulations of *L. raniformis* may also threaten their persistence. Our study provides some evidence of this additive form of fragmentation for *L. raniformis*. Populations sampled in the southern wetland cluster appeared not only to be largely separated from each other, but also from the clusters further north (Figure 2a, c). Resulting reductions in genetic diversity are apparent for the populations in the southern cluster, with both observed heterozygosity and allelic richness being slightly lower for these populations than those in the central and northern clusters (Table 1; Hale 2010).

There are several ways to manage the effects of urbanization on dispersal and gene flow for *L. raniformis*. In the first instance, priority should be placed on preserving metapopulations that are, or will be, less susceptible to internal fragmentation. Metapopulations centered on in-stream habitat and adjacent off-stream wetlands are the obvious choice in this regard, because riparian corridors are usually reserved as open space in Melbourne's urbanizing landscapes (DPCD 2009), and because these flood prone areas have limited developable land. Within metapopulations, placement of major roads and other infrastructure may be manipulated to conserve dispersal pathways (Forman and Alexander 1998). Underpasses are known to facilitate dispersal for various animals, including amphibians (Ng et al. 2004; Mata et al. 2008; Glista et al. 2009), and may represent a means of mitigating barrier effects for

L. raniformis. However, while dedicated underpasses have been constructed for this species in recent years, their effectiveness remains unknown (Heard et al. 2010). Research on the efficacy and optimal design of these structures for *L. raniformis* would be of considerable value.

Maintaining dispersal and gene flow between metapopulations of *L. raniformis* may be achieved through sympathetic management of the stream network (where metapopulations are connected by streams) and/or the construction of dedicated connecting wetlands. In-stream habitat of *L. raniformis* is characterised by large, slow-flowing pools with dense aquatic vegetation (Heard et al. 2010). It is feasible to create or enhance these features through the installation of weirs or riffle beds, through physical deepening of pools, and through aquatic re-vegetation (Heard et al. 2010). Doing so strategically could establish new populations that connect otherwise disjunct metapopulations. Likewise, constructing or rejuvenating off-stream wetlands may represent a means of establishing new populations, and facilitating gene flow between wetland clusters. Such initiatives have proven successful for other frog species (Petranka et al. 2007; Brand and Snodgrass 2010).

Finally, genetic restoration through the translocation of individuals could be employed to offset the deleterious effects of inbreeding and drift for populations *L. raniformis* in urban landscapes (Tallmon et al. 2004; Hedrick 2005; Bouzat et al. 2009). However, translocation may also have detrimental effects. Outbreeding depression has been documented for amphibians (Sagvik et al. 2005; Sherman et al. 2008), and represents a legitimate concern. The spread of pathogens between populations is also a major risk, as highlighted by the extinction of numerous amphibian populations following exposure to the chytrid fungus, *Batrachochytrium dendrobatidis* (Skerratt et al. 2007). We caution that while genetic restoration could be employed to maintain or restore genetic diversity of *L. raniformis* in urbanizing landscapes, the potential demographic and genetic pitfalls associated with this technique require further study.

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Table 1. Sampling sites, number of individuals sampled (*N*), observed heterozygosity (H_O) and expected heterozygosity (H_E).

Site	Coordinates				
	Latitude	*Longitude*	*N*	H_O	H_E
S1	-37.6857	144.9602	20	0.614	0.623
S2	-37.6852	144.9789	20	0.616	0.617
C1	-37.6325	144.9579	20	0.714	0.681
C2	-37.6355	144.9639	19	0.679	0.688
C3	-37.6355	144.9684	20	0.723	0.677
C4	-37.6321	144.9669	3	--	--
C5	-37.6305	144.9607	4	--	--
N1	-37.5453	144.9439	20	0.650	0.625
N2	-37.5411	144.9494	20	0.755	0.672
N3	-37.5407	144.9605	20	0.695	0.674
N4	-37.5422	144.9599	12	0.742	0.694
N5	-37.5388	144.9713	20	0.739	0.691

Table 2. Pairwise F_{ST} values. The lower matrix displays the pairwise F_{ST} values (* = significant following sequential Bonferroni adjustment for multiple statistical tests), and the upper matrix shows both the straight-line distance between sites (in meters) and the presence or absence of major urban barriers between them (in parentheses, where 0 = no barrier and 1 = barrier present). Grey shading represents site clusters.

Site	S1	S2	C1	C2	C3	N1	N2	N3	N4	N5
S1	--	1616(1)	6071(1)	5557(1)	5482(1)	15664(1)	16053(1)	16073(1)	15923(1)	16264(1)
S2	0.059*	--	6332(1)	5712(1)	5509(1)	15896(1)	16233(1)	16154(1)	16006(1)	16252(1)
C1	0.082*	0.070*	--	695(0)	1111(0)	9611(1)	9986(1)	10008(1)	9857(1)	10232(1)
C2	0.054*	0.065*	0.011	--	463(0)	10192(1)	10547(1)	10526(1)	10367(1)	10707(1)
C3	0.058*	0.060*	0.021	0.011	--	10391(1)	10724(1)	10662(1)	10513(1)	10805(1)
N1	0.094*	0.134*	0.094*	0.073*	0.086*	--	634(1)	1542(1)	1482(1)	2477(1)
N2	0.087*	0.116*	0.060*	0.048*	0.047*	0.047*	--	998(0)	981(0)	1935(0)
N3	0.078*	0.093*	0.048*	0.042*	0.042*	0.048*	0.028*	--	152(0)	941(0)
N4	0.077*	0.099*	0.044*	0.028*	0.028*	0.047*	0.015	0.003	--	998(0)
N5	0.101*	0.113*	0.053*	0.053*	0.053*	0.068*	0.025*	0.018	0.017	--

Figure captions

Figure 1. Map of the study area. Panel *(a)* shows the location of the study area in Victoria, on the northern outskirts of the Melbourne metropolitan area (grey shading). Panel *(b)* displays the location of each site cluster (rectangles), with black dots in clusters representing sampling sites, and those in the surrounding landscape representing other locations known to be occupied by *Litoria raniformis* at the time of sampling. The grey shaded area depicts the distribution of major urban infrastructure (housing estates, industrial estates and dual-carriage roads). Panels *(c)*-*(e)* display the distribution of the sampling sites in each of the northern, central and southern clusters, respectively. Grey shading again depicts the distribution of major urban infrastructure. The black lines in panels *(b)*-*(e)* represent the Merri Creek and its tributaries.

Figure 2. Population structure of *Litoria raniformis* in the study area, as estimated by Bayesian population clustering analysis. Each column represents a Q value: the proportion which a given genotype belongs to a genetic unit of the given color. a) All sites from each of the three clusters; b) northern cluster; c) southern cluster. The central cluster did not display genetic subdivision, and is therefore not presented individually.

Figure 3. Plot of pairwise F_{ST} against distance (in meters) for sites within the same cluster. Site pairings separated by major urban barriers (dual-carriage roads, urban and industrial developments) are represented by filled circles; site pairings without urban barriers are represented by open circles.

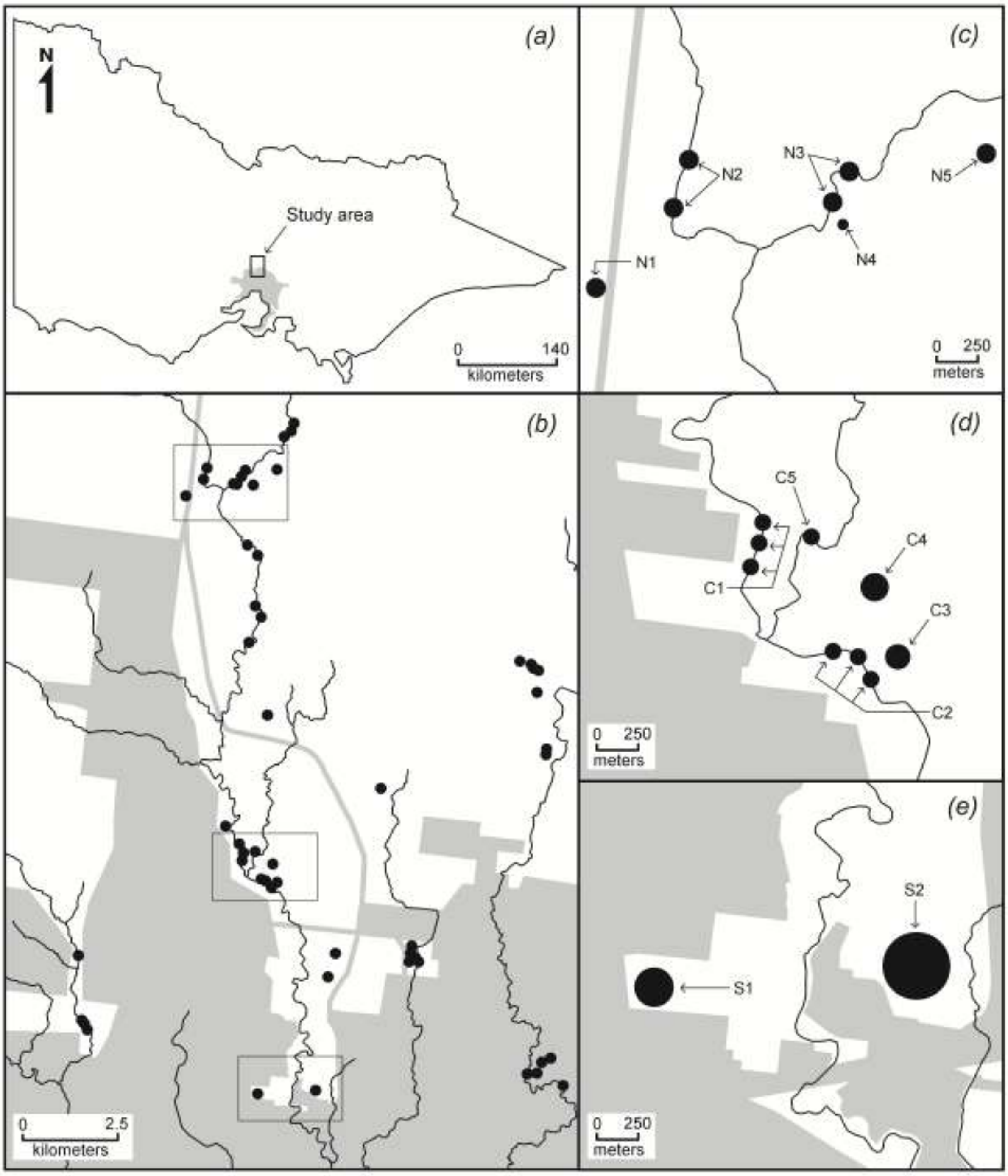
(a)
N
Study area
0 140
kilometers
(b)
0 2.5
kilometers
(c)
N1
N2
N3
N4
N5
0 250
meters
(d)
C1
C2
C3
C4
C5
0 250
meters
(e)
S1
S2
0 250
meters

Figure 1

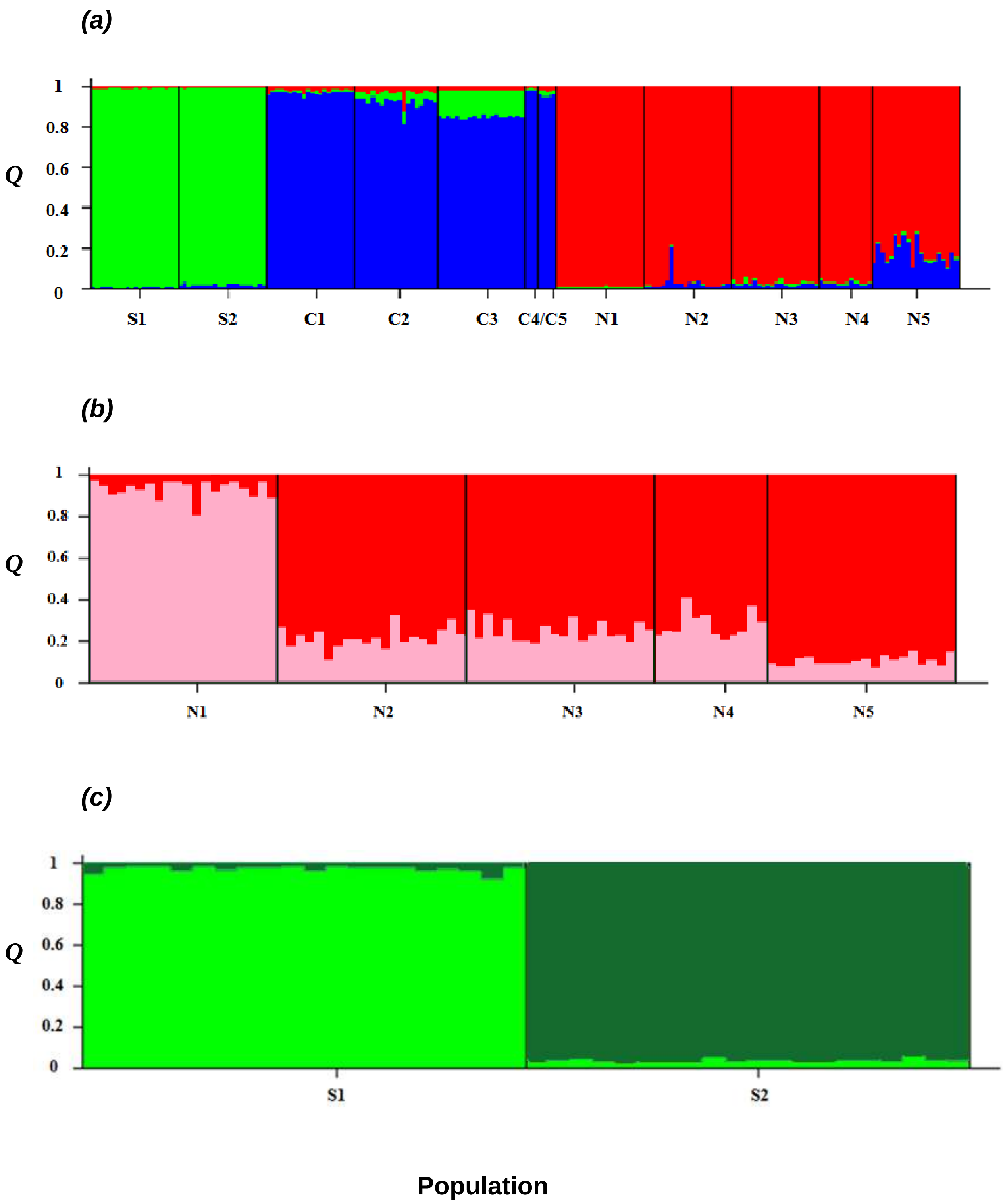
(a)
Q
1
0.8
0.6
0.4
0.2
0
S1
S2
C1
C2
C3
C4/C5
N1
N2
N3
N4
N5
(b)
Q
1
0.8
0.6
0.4
0.2
0
N1
N2
N3
N4
N5
(c)
Q
1
0.8
0.6
0.4
0.2
0
S1
S2
Population

Figure 2

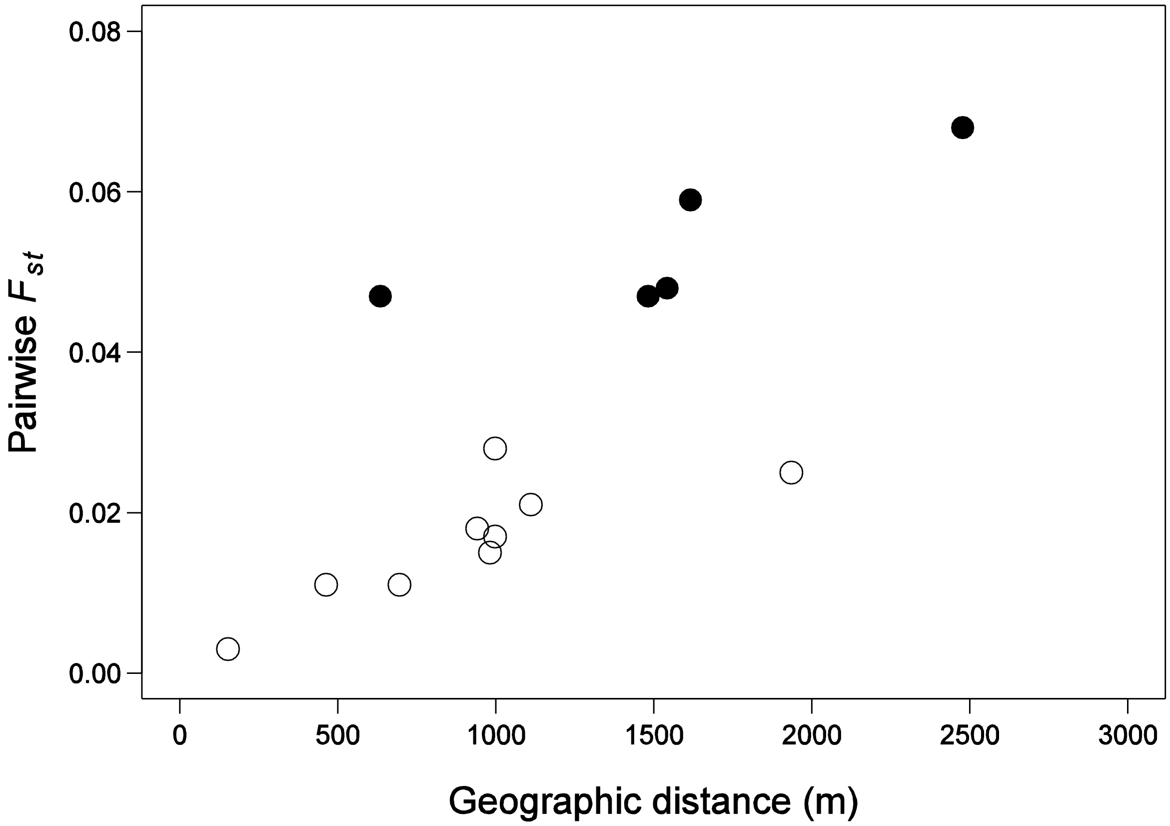
Pairwise F_{st}
0.08
0.06
0.04
0.02
0.00
0
500
1000
1500
2000
2500
3000
Geographic distance (m)

Figure 3