**Molecular detection of intra-population structure in a threatened potoroid,
Potorous tridactylus: conservation management and sampling implications**

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Abstract

Fine-scale genetic structure was investigated in three regional populations of the long-nosed potoroo (*Potorous tridactylus*) a threatened endemic marsupial. Two populations were from the Australian mainland and one from an island. Populations were sub-sampled at two sites, 6–8 km apart, connected by suitable habitat for dispersal. Factors influencing fine-scale structure were investigated by genotyping 157 individuals at 10 microsatellite loci and sequencing a ~621 bp region of the mtDNA control region. Results indicated that *P. tridactylus* populations exhibit significant intra-population structure, with significant F_{ST} and Φ_{ST} values recorded between subpopulations. This structure appeared mediated by small neighbourhood size, female philopatry and limited dispersal over 6–8 km, predominantly by males. Results highlighted several important features of *P. tridactylus* populations that have implications for conservation. Firstly, the small neighbourhood size suggests any investigations of intra-population structure should be conducted on a finer scale (e.g. 25–50 m) than many current monitoring programs. Secondly, the island populations were genetically depauperate, which may reflect processes occurring in many isolated ‘mainland island’ populations. Thirdly, the lower gene flow identified between populations separated by anthropogenically modified habitat suggests *P. tridactylus* is sensitive to changes in habitat configuration

Introduction

The extensive fragmentation of terrestrial habitats across the globe has resulted in the disruption of connectivity between populations of many species (Saunders et al. 1991; Haila 2002; Fischer and Lindenmayer 2007). The impacts of fragmentation vary between taxa and populations depending on factors including, the makeup of the intervening matrix (Ricketts 2001), as well as the species dispersal capabilities, demography, physiology and behaviour (Anthony and Blumstein 2000; Colson et al. 2013). Habitat specialists are often more sensitive to variation in local environments (Brouat et al. 2004) and therefore the extent of fragmentation can have pronounced effects on the persistence of such populations in disturbed areas (Frankham 1996; Keyghobadi et al. 2005). The loss of ecological connectivity between populations, hindering migration, also results in loss of genetic connectivity, leaving populations susceptible to a reduction in diversity and increased differentiation through genetic drift (Lacy 1987; Keyghobadi 2007; Lancaster et al. 2011). Genetic erosion and increased

inbreeding can also leave these populations at a higher risk of extinction (Spielman et al. 2004).

In species with habitat or diet specialisations, restricted dispersal capabilities or high site fidelity, fine-scale structure may also occur within populations confined to isolated habitat patches (Peakall et al. 2003; Keyghobadi et al. 2005; Coulon et al. 2006). This intra-population structure may vary across isolates, as it is likely to be highly influenced by factors such as behaviour, terrain and habitat (Ricketts 2001; Colson et al. 2013). Nevertheless, understanding intra-population structure, neighbourhood size and how demographic processes influence structure, will underpin successful management of these populations (Temple et al. 2006; Colson et al. 2013).

Fine-scale processes are notoriously difficult to determine using traditional field based approaches (Solberg et al. 2006). For species which are solitary, live in dense habitat or occur at low densities, it is often difficult to gather sufficient data for robust analyses using methods such as trapping or radio-telemetry (Piggott et al. 2006a; Ruibal et al. 2010). Recent molecular advances provide an insightful, cost effective approach as samples collected directly (e.g. tissue) or indirectly (e.g. scats or hair) provide alternative data to analyse population processes (Piggott et al. 2004; Ruibal et al. 2010). In addition, the increased information content of modern genetic markers (Sunnucks 2000) enable molecular signals to further elucidate fine-scale processes (Keyghobadi et al. 2005; Proctor et al. 2005; Piggott et al. 2006b).

Following European settlement of Australia in 1788 many native species underwent significant declines due to extensive land clearing for agriculture and urban settlement (Short and Smith 1994). This resulted in the distributions of many species retracting to small disjunct populations confined to native vegetation remnants (Saunders et al. 1991; Fischer and Lindenmayer 2007). Much of this habitat fragmentation occurred within the southeastern Australian mesic zone. Management of these isolated populations often occurs regionally or locally, and it is increasingly recognised that understanding population structuring, both within and across the fragmented landscapes, will promote more effective management of threatened species (Moritz 1994).

79 The long-nosed potoroo, *Potorous tridactylus*, is a small solitary macropodoid
80 marsupial distributed across the south-eastern Australian mesic zone, from southern
81 Queensland to eastern South Australia, and throughout Tasmania (Johnston 2008). Like
82 many Australian mammals, *P. tridactylus* experienced significant declines since
83 European settlement (Short 1998). Across their distribution adult potoroos range in
84 mass from 700 g to 1.5 kg (Norton et al. 2010a; Frankham et al. 2011a), and are
85 therefore vulnerable to predation by the introduced European red fox (*Vulpes vulpes*)
86 and domestic cat (*Felis catus*) (Seebeck 1978; Short 1998; Dexter and Murray 2009).
87 As for many other species, *P. tridactylus* population decline has also been accelerated
88 by widespread clearing and fragmentation of habitat for agriculture and urban sprawl
89 (Short 1998). Consequently *P. tridactylus* is now listed as ‘Vulnerable’ under the
90 *Environment Protection and Biodiversity Conservation Act* 1999. As a consequence of
91 its highly specialised diet of hypogaeal fungi (truffles), and its vulnerability to introduced
92 predators *P. tridactylus* requires environments with both dense habitat for shelter and
93 moist open areas to forage (Bennett 1993). In addition, the species rarely utilises
94 disturbed habitats (Bennett 1987; Frankham et al. 2011a). Thus *P. tridactylus* is now
95 confined to highly disjunct remnant patches of native vegetation across its range
96 (Seebeck 1981). Remaining areas of suitable habitat tend to occur in and around
97 protected areas (Seebeck 1981).

98 Due to the patchy distribution of *P. tridactylus*, regional management occurs on
99 a state by state basis within individually managed reserves (Martin and Temple-Smith
100 2010). The lack of information on intra-population structure and dispersal capabilities
101 of *P. tridactylus*, hinders effective management. Characteristics of potoroid life history
102 suggest that intra-population structuring is likely to occur: females appear philopatric
103 and show high levels of site fidelity (Johnson 1989; Johnson and Payne 2002; Pope et
104 al. 2005; Frankham et al. 2013), and dispersal is thought to be male biased, and occurs
105 over relatively small distances (Johnson 1989; Johnson and Payne 2002; Pope et al.
106 2005; Pacioni et al. 2011). For example, one of the largest movement events ever
107 recorded in a potoroid was 6.5 km, in a rufous bettong *Aepyprymnus rufescens* (Pope et
108 al. 2005), the largest of the extant potoroids (3.5 kg) and two to three times larger than
109 *P. tridactylus*.

Due to their dramatic population declines, potoroids are actively managed across Australia (Van Dyck and Strahan 2008). While the processes determining intra-population structure may differ between populations and taxa, knowledge of demographic processes will be useful in estimating the potential for intra-population structure within and across species. Such data would assist managers, who do not have the resources to conduct extensive genetic sampling, to estimate appropriate scales on which to focus management. It may also allow predictions of how management actions might affect population persistence across landscapes.

This study aims to use molecular data (nuclear microsatellite markers and mitochondrial DNA sequences) from three regional populations of *P. tridactylus* to explore population connectivity, neighbourhood size, dispersal and population dynamics at a local scale within continuous habitat.

Materials and Methods

Sample collection

Tissue samples were collected from two subpopulations within three regional populations of the *Potorous tridactylus* subspecies, *P. tridactylus trisulcatus* (Frankham et al. 2012): Southern Highlands (SH), in New South Wales, as well as East Gippsland (EG) and French Island (FI) both in Victoria (Fig.1). The SH subpopulations were managed as part of the Budderoo National Park and Barren Grounds Nature Reserve. The two SH sites were connected by contiguous forest, however a cleared area was located between the subpopulations (Fig. 1). The EG subpopulations were located within contiguous forest, in the Cape Conran Coastal Reserve (Fig. 1). The FI population was located on a 17,000 ha island in Western Port, which includes the 11,000 ha French Island National Park with the remaining area comprising mainly cleared agricultural land (Fig. 1). The SH subpopulation were Barren Grounds (BG) and Budderoo (BR), located ~ 6 km apart. For EG the two sampled sites were East Gippsland West (EG-W) and East Gippsland East (EG-E), located ~ 7 km apart: these are the sites referred to as East Coast Treatment and Non Treatment sites respectively, by Dexter and Murray (2009). The two French Island sites were, French Island East (FI-E) and French Island West (FI-W), located ~ 8 km apart, the straight line distance between the FI sites included stretches of cleared agricultural land. Although the degree

of fragmentation impacting each regional population differed, at all three sites the two sampled subpopulations were connected by habitat suitable for facilitating natural dispersal and gene flow.

Trapping was conducted at each site due to the presence of abundant *P. tridactylus* populations in largely contiguous native forest. Trapping of each regional populations was lead by different organisations and carried out either as part of state government management and/or monitoring programs, as well as ecological research directly targeting *P. tridactylus*. For individual site descriptions and trapping methods see Dexter and Murray (2009), Norton et al. (2010a) and Frankham et al. (2011a). Traps were set at varying intervals across the different sites: ~ 25 m apart on FI, ~ 100 m apart in the SH and ~ 300m apart on EG, with trap locations logged using GPS. A 2 mm diameter ear biopsy was taken from each individual *P. tridactylus* captured and stored in 70–95% ethanol. Samples were obtained from 49 individuals from SH (BG, 30; BR, 19), 61 from EG (EG-E, 32; EG-W, 29) and 47 from FI (FI-E, 36; FI-W, 11).

DNA extraction

Genomic DNA was extracted from 157 ethanol preserved tissue samples using Qiagen DNeasy Blood and Tissue kit (Qiagen GmbH, Hilden, Germany) following standard protocols.

Microsatellite DNA analysis

All individuals were genotyped at 10 microsatellite loci as previously described by Frankham et al. (2013). Genotyping was carried out at the Australian Genome Research Facility (AGRF), Melbourne. Scoring was carried out by two independent reviewers and completed using GENEMAPPER[®] version 4.1 (Applied Biosystems). To test for potential genotyping errors, nine samples genotyped as a pilot study, were re-genotyped with all remaining samples, and comparisons were carried out between 17 known mother-offspring pairs (Frankham et al. 2013).

Mitochondrial DNA analysis

A ~621 base pair (bp) fragment of Domain 1 of the mitochondrial DNA Control Region (CR) was also amplified from each individual genotyped using primers L15999M and H16498M (Fumagalli et al. 1997). PCRs were carried out in 25 µl

reactions with 1000 ng of genomic DNA; 10 x CoralLoad PCR Buffer (Qiagen); 25 mM MgCl_2 (Qiagen); 5 x Q-solution (Qiagen); Bovine serum albumin (New England Biolabs); 0.20 mM dNTPs; 2 pmol corresponding primers and Taq polymerase (0.5 unit Qiagen). Negative controls were included in each PCR. PCRs were performed on an Eppendorf Mastercycler EpS under the following conditions: initial denaturation (94 °C for 2 min); 36 cycles of denaturation (94 °C for 20 s); annealing (60 °C for 40 s) and extension (72 °C for 50 s) followed by a final extension (5 min at 72 °C). PCR products were cleaned using ExoSap-IT[®] (USB Corporation, Cleveland, Ohio, USA). Sequencing was carried out using the forward primer and resolved on an AB 3730xl Sequencer at AGRF Sydney, and verified with reference to chromatograms using SEQUENCHER v4.0.10 (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was conducted in MEGA version 5.0 (Tamura et al. 2011) using CLUSTAL W (Thompson et al. 1994). All gaps were coded as ‘-’ and treated as ‘indels’.

Microsatellite diversity and population bottlenecks

Deviations from Hardy Weinberg equilibrium (HWE) and linkage disequilibrium (LD) across all loci were tested for using GENEPOP v4.0.10 (Raymond and Rousset 1995) (Markov chain parameters; 5000 dememorizations, 500 batches, 5000 iterations). Standard diversity indices, including observed and expected heterozygosity (H_O and H_E), were calculated in GENALEX v6.0 (Peakall and Smouse 2006). Allelic diversity (AD), allelic richness (AR) corrected for minimum sample size ($n = 11$), and F_{ST} were estimated using FSTAT (Goudet 1995). Significant differences in diversity indices between and within regional populations were assessed via a Wilcoxon Rank Sign Test (Quinn and Keough 2002) using SPSS v17.0 (SPSS Inc 2008). Populations were tested for signatures of recent bottlenecks using the program BOTTLENECK (Piry et al. 1999). The Wilcoxon’s Heterozygosity Excess test was used under the Two Phase Model (TPM) with parameters recommended by Piry et al. (1999) (95% single step mutations, variance among multiple steps, 12).

Mitochondrial DNA diversity indices

Standard population diversity indices (h , haplotype diversity; π , nucleotide diversity) and pairwise differentiation (Φ_{ST}) amongst populations were estimated using ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010). A haplotype network for each regional population was constructed using TCS (Clement et al. 2000), which uses the

probability of parsimony calculated for pairwise comparisons (Templeton et al. 1992) to create the network.

Population structure (assignment tests)

Inter- and intra-population structure were investigated using the microsatellite data via Bayesian clustering methods implemented in STRUCTURE v2.3.1 (Pritchard et al. 2000) assuming no *a priori* population information. Analyses were conducted using the admixture model, alpha inferred from the data, independent allele frequencies and lambda set at 1.0. A burn-in of 300,000 runs was used with 500,000 iterations performed. The number of populations (K) was tested for: all populations together ($K = 1$ to $K = 10$); EG and SH mainland populations only ($K = 1$ to $K = 8$) and each regional population independently ($K = 1$ to $K = 5$). All simulations were carried out with 10 replicates of each K value. K was inferred from both posterior probability (Pritchard et al. 2000) and the delta log likelihood (Evanno et al. 2005) which was visualised using STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Gene flow and migration

Relative gene flow (migration) was estimated between subpopulations within each region via two methods. Firstly, using the method of Wright (1951) the number of migrants $N_{em} = [1/\theta_{ST} - 1]/4$ was calculated, using F_{ST} values estimated from the microsatellite data. Secondly, relative rates of female migration were compared, using Φ_{ST} calculated from mtDNA data, using the equation $N_{em} = [1/\Phi_{ST} - 1]/2$ (Slatkin 1993; Avise 2004). The Private Allele method (Barton and Slatkin 1986) was also employed in GENEPOP to investigate relative migration between populations. First generation migrants were also determined using GENECLASS 2 using the following methods: L_home/L_max likelihood ratio, Rannala and Mountain (1997) criterion, and Paetkau et al. (2004) algorithm, with 100,000 simulated individuals.

Neighbourhood size, spatial autocorrelation and relatedness

Spatial autocorrelation analyses of the microsatellite data were conducted using GENALEX v6.4 to estimate the neighbourhood size of males and females (separately) using 10 x 50 m distance classes (500 m). 1000 permutations of the data were used to create the 95% confidence interval around the null hypothesis ($r = 0$) and estimates of r were plotted at the end point of each distance class. 500 m was chosen as the maximum

distance class as preliminary analyses using distance classes up to 3000 m did not show any significant structure (data not shown).

Sex specific relatedness within each of the subpopulations was calculated in GENALEX v6.4. Male and female sample sizes for each sub-sample population were as follows; BG, 14 ♂, 16 ♀; BR, 12 ♂, 7 ♀; EG-W, 18 ♂, 11 ♀; EG-E, 20 ♂, 12 ♀; FI-E, 19 ♂, 13 ♀; FI-W, 7 ♂, 4 ♀. Pairwise relatedness (r) estimates were calculated using the Queller and Goodnight (1989) method and the Pop Mean option was used to determine an average of pairwise relatedness within the population using 1000 permutations and 1000 bootstraps to estimate the 95% confidence intervals around r .

Results

Microsatellite diversity and population bottlenecks

All individuals were genotyped successfully at 10 microsatellite loci. After correction for multiple sampling (Rice 1989) several loci were found to deviate significantly from HWE in some, but not all, populations [P112, Pa297, Y175 (EG), Pa297, Bt76 (SH) and G26.4 (FI)]. Results of preliminary analyses did not differ when data was run including and excluding these loci, therefore all were retained in the final analysis. All loci were in LD. Allelic diversity ranged from 2.90 to 9.70 (Table 1), and when corrected for the smallest sample size ($n = 11$) AR ranged from 2.16 to 5.66 (Table 1). Expected heterozygosity ranged from 0.47 to 0.80 (Table 1). Data per loci is presented in Table S1, supplementary material. No recent bottlenecks were detected in any of the populations. The results of the Wilcoxon Rank Sign tests indicated that there were no significant differences between any of the intra-population comparisons (AD , AR , H_O , H_E), however most inter-population comparisons were significant (Table 2).

Mitochondrial DNA diversity

~621 bp of the mtDNA control region was sequenced from 157 individuals, with 23 unique haplotypes identified (GenBank Accession numbers: KF979308 – KF979330). Twenty-one of the 23 haplotypes identified were recorded in multiple individuals, the two samples that represented singleton haplotypes were sequenced from two independent PCRs to confirm the sequence. The EG populations appeared to

265 harbour the most diversity and FI the lowest (Table 1). While some haplotypes were
266 shared between subpopulations, none were shared between the regions (Fig. 2). All FI
267 haplotypes were closely related with limited mutational steps separating each haplotype
268 on the island. Conversely, while SH and EG had some haplotypes that were closely
269 related (< 5 steps), these populations also included haplotypes that were over 5–10 steps
270 different from the others in the population, SH had the most missing haplotypes with
271 over 20 steps recorded between haplotypes (Fig. 2).

272

273

Population structure (assignment tests)

STRUCTURE analyses identified both inter-population and intra-population structuring across all sites in the microsatellite data (Fig. 3). Analysis of all regional populations suggested $K = 2$ was the best fit for the data, grouping the mainland SH and EG populations and separating the FI populations (Fig. 3a). Analysis of mainland populations only suggested $K = 2$ was the most likely fit for the data, separating SH and EG populations (Fig. 3b). Analysis of individual populations also revealed intra-population structuring with $K = 2$ proposed within the SH and EG subpopulations (Fig. 3c and d) and $K = 3$ across FI (Fig. 3e). The $K = 2$ subpopulations identified in SH and EG equated to the two sub-sampled populations, however the levels of admixture differed between subpopulations. Between the two SH subpopulations $Q > 0.9$, indicating low levels of gene flow. Conversely higher levels of admixture were present between the two EG subpopulations ($Q \leq 0.7$). Individuals from the two sub-sampled populations on FI were equally as likely to belong to any of the three populations suggested by STRUCTURE, with Q values suggesting high levels of admixture ($Q \leq 0.5$), although it is likely that the low genetic diversity on FI reduced the resolving power of data.

Gene flow and migration

F_{ST} and Φ_{ST} values were significant between all regional populations (F_{ST} range: 0.09–0.29, Φ_{ST} range: 0.34–0.91; see Table 3) and subpopulations (F_{ST} range: 0.03–0.08, Φ_{ST} range: 0.02–0.59; see Table 3). The only non-significant result was the Φ_{ST} value between the two FI subpopulations. Migration rates between regional populations were low. Analyses using the Wright (1951) and Slatkin (1993) methods suggested that there was less than one migrant per generation between FI and the other regional populations (Table 4). Some migration however was estimated to occur between EG and the SH. Migration rates were higher between the sub-sampled populations within SH, EG and FI, with the two EG subpopulations appearing to have the highest relative migration rates, with up to three times more migration between subpopulations using the Wright (1951) method (Table 4). The Private Allele method (Barton and Slatkin 1986) suggested more conservative estimates of migration between subpopulations. Although the relative migration rates between different populations were similar, values for EG

were the highest and those for FI the lowest. The migration rates estimated using the mtDNA data were consistently lower, suggesting that the estimate of overall migration was biased by male movements (Table 4). The higher rate of migration recorded in females on FI can be explained by the presence of only four haplotypes on FI that differed by only one base pair indel in each case.

GENECLASS 2 assignment testing suggested nearly all individuals (95%) were native to the subpopulation in which they were sampled. First generations migrants were rare, although two (out of 29) were identified in the EG-E site and three (out of 32) were identified in the EG-W site. Lower first generation migration was recorded across the SH subpopulations with only two (out of 19) identified in the BR subpopulation. No first generation migrations were identified between the FI populations. Overall, all but one first generation migrants were male.

Neighbourhood size, spatial autocorrelation and relatedness

The results of the spatial autocorrelation (Fig. 4) indicated that females sampled within 100 m of one another, across all populations, were significantly more closely related than by chance (SH: $r = 0.60$, $p = 0.003$; EG: $r = 0.38$, $p = 0.001$; FI: $r = 0.11$ $p = 0.05$). This was also the case for males within all populations, however relatedness tended to be lower (SH: $r = 0.28$ $p = 0.03$; EG: $r = 0.10$, $p = 0.02$; FI: $r = 0.11$ $p = 0.01$). Additionally, males were also recorded as being significantly more closely related at various other distance classes up to 300m. While data were available for individuals up to 16 km apart across two subsampled sites (EG), the results indicated that *P. tridactylus* movements and within population structure, occur on a very fine-scale, therefore only results up to 500m were shown (Fig. 4).

The results of the relatedness analysis were ambiguous. Only one subpopulation, FI-E showed the expected mammalian patterns of females being significantly more closely related than by chance ($p = 0.001$), and males showing a trend in the opposite direction ($p = 0.074$).

336 Discussion

337 Using molecular markers we have demonstrated the presence of significant fine-
338 scale structure within regional *Potorous tridactylus* populations. Spatial autocorrelation
339 analyses indicated that *P. tridactylus* have surprisingly small (100–300 m)
340 neighbourhood sizes, and dispersal occurred rarely between sites sampled 6–8 km apart,
341 even when these sites were connected by contiguous habitat. An insular population (FI)
342 was genetically depauperate, and *P. tridactylus* appeared sensitive to habitat
343 fragmentation modification as reduced migration was detected between sites impacted
344 by habitat modification.

345 Intra-population structure

346 Low but significant structure was detected (F_{ST} and Φ_{ST}) between all
347 subpopulations within each region (SH, EG, FI). The only non-significant result was the
348 Φ_{ST} between the two FI populations, although this may be an artefact of the low
349 haplotype diversity on the island. The level of structure and functional connectivity
350 between sub-sampled populations differed between regions. The highest levels of
351 connectivity (migration estimates and STRUCTURE analysis) were between the two EG
352 subpopulations (7 km apart), even though the SH subpopulations were geographically
353 closer (6 km). This might be explained by the EG subpopulations being connected by
354 uninterrupted contiguous habitat, while the SH subpopulations had a cleared area
355 between them which may represent a barrier to dispersal and gene flow (see Fig. 1).
356 This indicates that straight line distance between sites may not represent the shortest
357 distances for gene flow, which is often the case for forest dependent species in
358 fragmented landscapes (Coulon et al. 2004; McRae 2006). Gene flow between the two
359 FI subpopulations also appeared to be impacted by habitat fragmentation (Fig. 1), since
360 these pops are ~8 km apart (in a straight line across cleared land) but have relatively
361 lower gene flow compared to EG. These findings suggest that *P. tridactylus* is sensitive
362 to disturbance (see also Holland and Bennett 2009), whether it be complete
363 fragmentation or changes to habitat configuration (Keyghobadi 2007).

364 Investigation of intra-population structuring, using spatial autocorrelation
365 analyses, suggests different demographic processes for males versus females (Chesser
366 1991b). Previous field observations and genetic data for *P. tridactylus* have reported

several instances of female young settling in areas adjacent to their mothers (Johnson and Payne 2002; Frankham et al. 2013). This idea is supported by our spatial autocorrelation results from all three regional populations, which showed that females in close proximity were highly related (< 100 m) (Fig. 4). Given that genetic data from this study correlate with data from other potoroids (Pope et al. 2000; Johnson and Payne 2002; Pope et al. 2005; Pope et al. 2013), this is likely a universal behaviour across the family, driving both social and genetic population structure.

In species with strong female philopatry, dispersal is often male biased, thus reducing inbreeding and resource competition between relatives (Greenwood 1980). Dispersal behaviour can therefore have significant effects on genetic structure within populations (Chesser 1991a). The differences between migration estimates calculated from F_{ST} and Φ_{ST} (Table 4), while not an exact corollary of dispersal (Whitlock and McCauley 1999) do suggest that most dispersal in *P. tridactylus* populations was male-mediated. This is similar to the findings of Pope et al. (2005) who suggested that male *A. rufescens* dispersed over greater distances, and more frequently than females. As female young often settle close to their mothers, often in an adjacent home range, male potoroids may not actually need to disperse very far to mitigate any risk of inbreeding. Dispersal distance equating to two home ranges was estimated for male *B. tropica* (Pope et al. 2000; Pope et al. 2005), and our spatial autocorrelation results suggest a similar (extrapolated) estimate for *P. tridactylus*, since males sampled within 100–300 m were significantly related.

The sex-biases in philopatry and dispersal we found for *P. tridactylus* occurred at very small scale. This is not unprecedented for potoroids, as in several bettong species it has been suggested that dispersal occurs over small distances (Christensen 1980; Pope et al. 2000; Pope et al. 2005). Significant structure was observed between populations of the *B. tropica* only 12 km apart (Pope et al. 2000). One of the largest movement events recorded for a potoroid was from a study of paternity in *A. rufescens*, which identified a sire over 6.5 km from its offspring (Pope et al. 2005). The minimum distances between sub-sampled populations in our study were ~ 6 km (SH), ~ 7 km (EG), and ~ 8 km (FI), and all showed significant structure. Despite Dexter and Murray (2009) not recording any potoroos moving greater than 500 m across the EG site (using trapping records), we detected gene flow across the intervening distances between

subpopulations in both the SH and EG populations. These longer distance movements of individuals appear to be rare, and probably occur over significantly longer time frames than would occur during a trapping program. The low diversity on FI made it difficult to accurately estimate gene flow between those subpopulations.

The outcomes of the relatedness analyses also indicated that *P. tridactylus* tends to have small neighbourhood sizes. Only in the FI-E population, where samples were collected at 20–25 m intervals (Frankham et al. 2011a), and with all samples collected from within 650 m of each other, were we able to identify significant differences in male and female relatedness patterns in the data which correlated with the spatial autocorrelation results. Conversely the relatedness analyses of the SH and EG subpopulations did not yield any significant results. Samples from SH and EG populations were collected at 100–300 m trapping intervals, with sampled individuals from the same ‘population’ located up to 2000 m apart (SH) and 4500 m (EG). There are two possible explanations for these different outcomes. Firstly, samples from EG and SH were collected on a spatial scale too coarse to detect fine-scale sex differences in relatedness (Peakall et al. 2003). At this sampling scale several ‘neighbourhoods’ may have actually been sampled in SH and EG compared to the single ‘neighbourhood’ sampled on FI. Secondly, it is possible that the *P. tridactylus* population on French Island may behave differently to mainland populations due to the ‘island syndrome’ (Adler and Levins 1994; Goltsman et al. 2005). Different biological constraints on island populations often lead to reduced migration/dispersal in these insular populations. Additionally *P. tridactylus* on FI are larger than nearby mainland populations (Frankham et al. 2011a). This is another trait often characteristic of small mammal populations isolated on islands (Lomolino 1985; Adler and Levins 1994). However, contrary to what is expected with the ‘island syndrome’, *P. tridactylus* populations on FI did not occur at higher densities (Frankham et al. 2011a), nor show reduced fecundity compared to mainland populations (Goltsman et al. 2005). Thus, until mainland populations are sampled on the same scale as the FI population (20–25 m) it is difficult to tease apart these influences.

Inter-population structure

High levels of inter-population structure were detected between regional *P. tridactylus* populations. However patterns present in the mtDNA haplotype networks

(long branch lengths) suggest populations across the mainland may have been historically connected. Interestingly the high number of apparently missing haplotypes in the SH population (Fig. 2) compared to the EG population do suggest a decline has occurred in the SH populations at some stage, however the non-significant bottleneck result makes it hard to determine when this has occurred. Historical connectivity across the east coast mainland was also evident in the STRUCTURE results when all three regional populations were analysed together (Fig. 3a). This analysis separated FI while grouping the two mainland populations together. The FI population is believed to have been isolated from the mainland for around 8000–10,000 years (Marsden et al 1979), which is a considerable period for a species with a short generation time, such as *P. tridactylus* (~3–4 years) (IUCN 2012). The data showing a lack of any recent bottlenecks in the FI population suggests that the lower genetic diversity within the FI population is due to this long term isolation, leading to the gradual erosion of genetic diversity through genetic drift, a common phenomenon in island populations (Frankham 1997; Eldridge et al. 1999; Furlan et al. 2012). The dynamics of the FI population are of particular interest as many of the remaining regional populations of *P. tridactylus* now are isolated in patches of remnant habitat cut off from other populations by large tracts of hostile matrix (Seebeck 1981), such as grazed farmland. Such isolation would prevent migration (Holland and Bennett 2007) making these populations likely to function similarly to island populations (MacArthur and Wilson 1967). The analysis of intra-population data from FI therefore provides a blueprint for management of more recently isolated populations, which is of considerable importance given that the loss of diversity in mainland populations is likely to be accelerated by the presence of novel predators such as the fox, which is absent on FI (Andrew et al. 1984).

The isolation of mainland populations (SH and EG) is more recent than FI and is most likely associated with European settlement in Australia, similar to other Australian mammal species (Taylor et al. 2011). Our migration estimates did however suggest a small amount of gene flow between the SH and EG populations, which seems possible given the presence of unsampled populations in between. In light of long branch lengths present in the haplotype network, the migration estimates probably resulted from large historical populations existing across this corner of south-eastern Australia, leading to retention of ancestral alleles (Pacioni et al. 2011).

Conservation implications

In this study we determined that *P. tridactylus* has small neighbourhood sizes, male biased dispersal, limited gene flow (even between geographically close populations), and strong female philopatry. Any research aiming to further investigate these processes therefore needs to sample populations on an appropriately small scale (see also Peakall et al. 2003). The collection of DNA samples from the SH and EG regional populations were secondary to the main aims of the monitoring programs, thus data were collected at different spatial scales. Given the small scale (<100 m) upon which *P. tridactylus* populations appear to function these results highlight the need for future research to employ hierarchical sampling wherever possible, even for general monitoring, in order to obtain robust data on different scales (Peakall et al. 2003). This will maximise the value of any incidental/secondary data collected.

Data from this study indicating that the FI population was genetically depauperate compared to the two mainland populations also has significant implications for the conservation management of this species. This is of concern as many mainland *P. tridactylus* populations now occur in isolated patches of remnant habitat in a sea of cleared land (Seebeck 1981), i.e. they effectively occupy 'mainland islands'. Therefore a similar progressive loss of diversity and increased differentiation of these populations is likely to occur (Frankham 1998). Additionally island populations are often seen as 'security populations' for threatened species, free from many mainland population threats (Burbidge 1999). Since FI is the largest fox free area in Victoria, its *P. tridactylus* population is of considerable conservation value (Frankham et al. 2011a). Therefore the continued loss of genetic diversity in this populations is of concern (Frankham 1996) and the addition of novel genetic material may be required to maintain diversity in FI and to ward off the deleterious effects of inbreeding and drift (Weeks et al. 2011). This would also promote population longevity through providing adaptive potential to buffer against future climatic changes or stochastic events (Willi et al. 2006). Phylogenetic research carried out by Frankham et al. (2012) suggests all Victorian and southern New South Wales *P. tridactylus* populations (including SH) comprise the same subspecies. While there are many factors to consider in translocating individuals between populations, the risk of out-breeding depression does not appear to be a limiting factor if movement of individuals was carried out within this subspecies (Frankham et al. 2011b).

While data collected from these three regional populations has consistently suggested female philopatry and male biased dispersal, it is clear that many other factors may also influence genetic structure within populations or species. The intricate and complex relationships that exists within Australia's temperate forests between mycophagous mammals (such as potoroids), ectomycorrhizal truffle species and native trees/shrub (Claridge and May 1994; Maser et al. 2008) are also likely to play a significant role in shaping neighbourhood size and genetic structure in *P. tridactylus*. This is because fungi make up a significant part (70–80%) of a potoroos diet (Bennett and Baxter 1989; Bowie 2007), yet the diversity of truffle species can change over very fine spatial scales depending on season, soil fertility, topography, or host plant diversity (Johnson 1994; Danks et al. 2012). Truffle community structure will therefore also influence mycophagous mammal presence, population size and density (Danks et al. 2012). It is not surprising therefore that *P. tridactylus* also shows such high levels of fine-scale structuring, as this is often the case for habitat or diet specialists (Campbell et al. 2009). Future research should also aim to include these more complex questions regarding other ecological factors that may influence fine-scale intra-population structure within this species (McRae 2006; Spear et al. 2010).

While *P. tridactylus* is thought to require a complex mosaic of dense habitat for shelter and more open foraging areas (Bennett 1993), Norton et al. (2010b), noted that areas of apparently suitable habitat often lacked evidence of potoroo activity, which may result from either missing environmental components (Bennett 1993) or from behavioural barriers to dispersal (Scribner and Chesser 1993). This need for complex habitat and a specialised diet could be incorporated into future analysis via GIS data, resistance layers (McRae 2006; Spear et al. 2010) and least-cost path methods (Coulon et al. 2004), to reveal the roles of landscape features and food availability in driving fine-scale structure in *P. tridactylus*. These analyses should also be coupled with hierarchical sampling (fine-scale to regional) to identify the appropriate scale for data collection and to aid in identifying subtle barriers to gene flow that may not be immediately obvious.

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Fig 1 The current distribution of *Potorous tridactylus* (grey) in southeastern Australia showing the location of the three regional populations. Subpopulations sampled for this study are outlined in white in the enlarged sections with black shading representing forested areas, dark grey shading representing cleared or modified land and light grey representing sea. Population codes from Table 1.

Fig.2 Maximum Parsimony network of *Potorous tridactylus* mtDNA control regions haplotypes identified in each of the three regional populations. The size of the circle indicates the relative proportion of each haplotype, with the smallest circles representing singleton haplotypes. Black bars indicate unidentified haplotypes and dotted lines indicate alternative connections. a) Southern Highlands, b) East Gippsland and c) French Island.

Fig. 3 Assignment probabilities calculated by STRUCTURE version 2.3.3 for sampled *Potorous tridactylus* individuals. Q equals the proportional membership to

assigned clusters, with K the most likely number of clusters. a) samples from all three regional populations; b) mainland populations only; c) Southern Highlands populations; d) East Gippsland populations; e) French Island populations. Population codes from Table 1.

Fig. 4 Correlograms indicating the degree of spatial autocorrelation in sampled *Potorous tridactylus* populations using 50 metre distance classes; r is the genetic correlation co-efficient as a function of geographic distance, U and L represent the 95% confidence interval around the null hypothesis $r = 0$. a) Southern Highlands females; b)

Southern Highlands males; c) East Gippsland females; d) East Gippsland males; e) French Island females; f) French Island males.

Table 1 Summary of mitochondrial DNA and microsatellite diversity across three sampled

Population (<i>n</i> mtDNA/ <i>n</i> nuDNA)	mtDNA			nuDNA (microsatellite)			
	Haplotypes	<i>h</i> (s.e)	π (s.e)	<i>AD</i> (s.e)	<i>AR</i> (s.e)	<i>H_O</i> (s.e)	<i>H_E</i> (s.e)
Barren Grounds (BG) 28/30	3	0.54 (0.05)	0.95 (0.52)	5.50 (0.54)	3.87 (0.46)	0.67 (0.07)	0.70 (0.04)
Budderoo (BR) 20/19	4	0.57 (0.09)	1.79 (0.95)	6.80 (0.65)	4.28 (0.53)	0.68 (0.05)	0.73 (0.04)
East Gippsland –West (EG-W) 26/52	9	0.89 (0.03)	0.44 (0.27)	9.70 (1.19)	5.30 (0.70)	0.70 (0.04)	0.77 (0.03)
East Gippsland –East (EG-E) 56/32	9	0.85 (0.02)	0.34 (0.22)	9.10 (1.03)	5.66 (0.72)	0.73 (0.04)	0.80 (0.03)
French Island – East (FI-E) 38/40	3	0.33 (0.09)	0.03 (0.04)	3.50 (0.40)	2.44 (0.34)	0.51 (0.08)	0.51 (0.07)
French Island – West (FI-W) 11/11	2	0.18 (0.14)	0* (0.31)	2.90 (0.31)	2.16 (0.22)	0.54 (0.09)	0.47 (0.07)

Potorous tridactylus populations; *n*: sample size; *h* haplotypic diversity (standard error), π nucleotide diversity (standard error), *AD*: Allelic diversity (standard error); *AR*: Allelic richness (standard error) (corrected for *n*=11); *H_O*: observed heterozygosity (standard error); *H_E*: expected heterozygosity (standard error). * π not estimated due to a single base pair indel difference between haplotypes.

Table 2. Results of Wilcoxon Rank Sign Test of significance for pairwise comparisons amongst sampled *Potorous tridactylus* populations: +, significant *p* < 0.5; -, non significant (*p* > 0.5). The four diversity indices tested, in order from left to right in the table were: *AD*, Allelic diversity; *AR*, Allelic richness; *H_O*, observed heterozygosity and *H_E*, expected heterozygosity. Population codes taken from Table 1.

	BG	BR	EG-W	EG-E	FI-E	FI-W
BG						
BR	----					
EG-W	++-+	+- - -				
EG-E	++-+	-+-+	----			
FI-E	++-+	++-+	++++	++++		
FI-W	++-+	++-+	++-+	++-+	----	

Table 3. Levels of differentiation amongst sampled populations of *Potorous tridactylus*. F_{ST} derived from the 10 microsatellites below the diagonal and Φ_{ST} derived from mtDNA control region sequence above diagonal. Population codes from Table 1. Significant values are indicated *.

	BG	BR	EG-W	EG-E	FI-E	FI-W
BG	0	0.59*	0.36*	0.34*	0.76*	0.73*
BR	0.08*	0	0.74*	0.67*	0.75*	0.60*
EG-W	0.12*	0.08*	0	0.11*	0.91*	0.89*
EG-E	0.11*	0.09*	0.03*	0	0.92*	0.88*
FI-E	0.29*	0.26*	0.23*	0.25*	0	0.02
FI-W	0.27*	0.25*	0.13*	0.22*	0.07*	0

Table 4 Estimated number of migrants between *Potorous tridactylus* populations, based on F_{ST} derived from the 10 microsatellites below the diagonal, and Φ_{ST} derived from mtDNA control region, above the diagonal. Population codes from Table 1.

	FI E	FI W	EG-W	EG-E	BG	BR
FI E	—	23.31	0.05	0.04	0.16	0.17
FI W	3.15	—	0.06	0.07	0.18	0.33
EG-W	0.68	0.73	—	3.92	0.90	0.17
EG-E	0.73	0.85	5.24	—	0.97	0.24

BG	0.59	0.68	1.78	2.04	—	0.35
BR	0.66	0.72	2.40	2.42	2.65	—

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