Title: Habitat connectivity, more than species' biology, influences genetic differentiation in a habitat specialist, the short-eared rock-wallaby (*Petrogale brachyotis*)

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Abstract

It is difficult to assess the relative influence of anthropogenic processes (e.g. habitat fragmentation) versus species' biology on the level of genetic differentiation among populations when species are restricted in their distribution to fragmented habitats. This issue is particularly problematic for Australian rock-wallabies (*Petrogale* sp.), where most previous studies have examined threatened species in anthropogenically fragmented habitats. The short-eared rock-wallaby (*Petrogale brachyotis*) provides an opportunity to assess natural population structure and gene flow in relatively continuous habitat across north-western Australia. This region has reported widespread declines in small-to-medium sized mammals, making data regarding the influence of habitat connectivity on genetic diversity important for broad-scale management. Using non-invasive and standard methods, 12 microsatellite loci and mitochondrial DNA were compared to examine patterns of population structure and dispersal among populations of *P. brachyotis* in the Kimberley, Western Australia. Low genetic differentiation was detected between populations separated by up to 67 km. The inferred genetic connectivity of these populations suggests that in suitable habitat *P. brachyotis* can potentially disperse far greater distances than previously reported for rock-wallabies in more fragmented habitat. Like other *Petrogale* species male-biased dispersal was detected. These findings suggest that a complete understanding of population biology may not be achieved solely by the study of fragmented populations in disturbed environments and that management strategies may need to draw on studies of populations (or related species) in undisturbed areas of contiguous habitat.

Keywords: *Petrogale brachyotis*; rock-wallaby; mitochondrial DNA; microsatellites; habitat connectivity; genetic diversity.

Introduction

Habitat fragmentation is a major threat to global biodiversity through its negative impacts, both direct and indirect, on population dynamics (Fahrig 2003; Frankham et al. 2010; Gilpin and Hanski 1991; Harris 1984). Fragmentation (loss of habitat and connectivity) prevents or reduces dispersal, restricts gene flow and results in increased genetic drift (fixation and loss of alleles) and thus genetic differentiation among populations (Frankham et al. 2010; Lacy 1987; Lancaster et al. 2011; Stow and Sunnucks 2004). As well as reduced diversity, fragmentation can also lead to increased inbreeding, due to reduced dispersal and limited mate choice, and therefore inbreeding depression (Banks et al. 2005; Frankham et al. 2010). This results in populations with reduced fitness and adaptability and so increases their risk of extinction (Frankham et al. 2004, 2010).

Landscape characteristics alone, however, do not determine population structure, since individual species' biology will also contribute to the extent of differentiation between populations (Brouat et al. 2003; Kraaijeveld-Smit et al. 2007; Meyer et al. 2009; Öckinger et al. 2010). Life-history traits including dispersal ability and sex-biased dispersal, mating system, habitat specialisation and generation time, all impact population dynamics and the distribution of genetic diversity (Ewers and Didham 2006; Hedrick 2000; Henle et al. 2004; Prugh et al. 2008; Slatkin 1994). Even in continuous habitat these factors alone can lead to genetic differentiation (Cabe et al. 2007; Hazlitt et al. 2006a; Kraaijeveld-Smit et al. 2007). It can, therefore, be difficult to separate the effects of life-history traits from those of habitat fragmentation when assessing population structure. In addition, investigating the factors that influence population differentiation in contiguous habitat provides insight into genetic structure prior to the impacts of fragmentation (Macqueen et al. 2008). Comparison of genetic connectivity between populations within continuous and fragmented landscapes often reveal large differences attributed to anthropogenic processes (Stow et al. 2001). Fragmented populations may not solely result from habitat fragmentation, since other factors such as resource availability, climatic conditions, presence of competitors and predators can also limit the spatial distribution of species (Fischer et al. 2005). Genetic studies can be valuable in identifying the impact of anthropogenic processes and so enable better informed conservation management decisions (Lindenmayer and Fischer 2006).

The impacts of fragmentation versus life-history processes have rarely been investigated in mammals, yet are fundamental to understanding the factors that influence population declines (Amos and Balmford 2001; Peacock and Smith 1997; Walker et al. 2008; Wolff et al. 1997). To date, population genetic studies of rock-wallabies (*Petrogale* spp.) have focused on threatened and highly fragmented populations in southern Australia, including the yellow-footed (*P. xanthopus*), brush-tailed (*P. penicillata*) and black-footed (*P. lateralis*) rock-wallabies (Browning et al. 2001; Eldridge et al. 1999, 2001; Hazlitt et al. 2006a; Piggott et al. 2006a; Pope et al. 1996). These rock-wallabies are now mostly present in small, isolated colonies with limited dispersal and gene flow between them (Hazlitt et al. 2004, 2006a; Jarman and Bayne 1997). Rock-wallabies appear to have lower genetic diversity within populations and higher genetic divergences between populations compared to other macropodoids (Eldridge et al. 2010). This has been linked to their habitat specificity (i.e. rocky outcrops and escarpments) and therefore naturally patchy distribution (Eldridge et al. 2010; Hazlitt et al. 2006a; Piggott et al. 2006a). However, this assumption may be flawed (Eldridge et al. 2010), with these studies being biased towards rock-wallaby species that have declined as a result of habitat clearance/fragmentation and the impact of introduced predators (Maxwell et al. 1996). Additional analyses of rock-wallabies in more continuous habitat would elucidate whether this apparent low diversity is a function of species' biology or a result of anthropogenic processes.

The widespread and abundant short-eared rock-wallaby (*P. brachyotis*) from northern Australia (Fig. 1) provides a good candidate to examine the effects of life-history strategies versus anthropogenic processes within rock-wallabies. A recent study of *P. brachyotis* in the Northern Territory, northern Australia, aimed to assess population structure in more continuous habitat, but nevertheless revealed highly structured populations (Telfer and Eldridge 2010). This study suggests that rock-wallabies are predisposed to being highly structured as a result of their strong association with rocky habitat. Even though the Northern Territory retains largely intact vegetation between populations of *P. brachyotis*, the sandstone gorges and outcrops, which represent core rock-wallaby habitat (sampled by Telfer and Eldridge 2010), were quite disjunct and this seems likely to have led to the highly structured genetic diversity found in this species. The Kimberley, in northern Western Australia differs from the areas

sampled by Telfer and Eldridge (2010) in that it contains not only more extensive dissected sandstone plateaus and ranges, but also greater connectivity of rocky habitat, with less separation of these broader sandstone habitats across the landscape (Fig. 1). Therefore, this landscape provides an opportunity to assess the population biology of a rock-wallaby within intact and more continuous rocky habitat. A recent broad-scale phylogeographic study of *P. brachyotis* found high levels of genetic differentiation between the Kimberley and Top End, as well as identifying three distinct lineages within the Kimberley (Potter et al. 2012a). Although the Kimberley is relatively unaffected by the threatening processes, described above, which have impacted eastern and southern parts of Australia, widespread declines of small-to-medium sized mammals have recently been reported (Fitzsimons et al. 2010). It is therefore important to assess the population biology of taxa in this region, to better understand the influences of landscape on genetic differentiation. *P. brachyotis* in this region could also provide a suitable reference for the genetic diversity and population structure of a pre-decline or 'healthy' population of rock-wallaby, which could be used to set appropriate goals for the management of declining and threatened populations of rock-wallabies throughout Australia (Eldridge et al. 2004a), as well as declining mammals in northern Australia.

Rock-wallabies occur in small colonies (~5-100 individuals) and show high site fidelity (Eldridge 2008). Studies of rock-wallaby mating system and life-history are limited and there are no data for *P. brachyotis*, however, available evidence suggests sex-biased dispersal and strong female philopatry results in inbreeding avoidance and maintains genetic diversity (Pope et al. 1996; Hazlitt et al. 2004, 2006b, 2010, Piggott et al. 2006a). Despite limited inter-colony gene flow, intra-colony genetic diversity appears to be maintained via kin avoidance and mating dispersal (Spencer et al. 1997; Hazlitt et al. 2006b).

The current study uses trapping and non-invasive methods to investigate the genetic diversity and fine-scale population structure of *P. brachyotis* across the Kimberley to determine the effects of continuous versus fragmented habitat on population biology. The aims were to use microsatellite genotypes and mtDNA sequences to: (i) assess genetic diversity and population differentiation of *P. brachyotis* in a relatively connected environment versus two fragmented island populations, and (ii) investigate life-history strategies within *P. brachyotis* (e.g., sex-biased dispersal) to assess the impact of fragmentation on these processes.

Materials and methods

Study site and sample collection

Samples were collected from six sites (March 2007 – August 2010) within the Kimberley region of northwest Australia (Fig. 1). Four sites were sampled from the relatively intact environment of the northwest Kimberley (West Kimberley) over a hierarchical spatial scale from ~1 km to 67 km apart (Table 1; Fig. 1). In addition, two isolated populations located on islands in Lake Argyle in the East Kimberley were sampled (~3.5 km apart). These islands were formed in 1972 when the Ord River was dammed creating Lake Argyle. The East and West Kimberley sites were ~290 km apart (Fig. 1) and span the three distinct lineages identified by Potter et al. (2012a) within the Kimberley (West Kimberley-WK; East Kimberley-1, EK1; and East Kimberley-2, EK2) (see Table 1).

A total of 84 *P. brachyotis* (51 tissue and 33 fecal) samples were analysed. One *P. penicillata* (brush-tailed rock-wallaby) was used as an outgroup for phylogeographic analyses (see Potter et al. 2012b). Tissue samples (ear biopsies) were obtained from live-trapped *P. brachyotis* from four sites (Couchman-CN, Cyprus Valley-CV, Monsmont Island-MI and Bullanyin Island-BI; Fig. 1). For the purposes of the population analyses all samples were pooled over years, because major changes in allele frequencies between years are unlikely due to the long life-span of rock-wallabies (e.g., up to 12 years; Bee and Close 1993). Rock-wallabies inhabit rugged terrain (Van Dyck and Strahan 2008) and in the Kimberley access is limited, therefore 33 fresh faecal samples were collected to supplement tissue samples. Fresh faeces were collected from three sites (CN, King Edward River-KER, Pump Hill-PH; Fig. 1), individually stored in brown paper envelopes and kept frozen at -20°C from the day of collection until processing. The sample sizes for each of the six populations do not necessarily correlate to population density, but were based on sample availability and PCR success.

DNA extraction, amplification, microsatellite genotyping and mtDNA sequencing

Genomic DNA was isolated from tissue samples using the Gentra DNA Isolation Kit (Gentra Systems) and from faecal samples using the QIAamp DNA Stool Kit (QIAGEN), according to the manufacturer's instructions and the method of Piggott and Taylor (2003) with modifications (see Supplementary Material).

Polymerase chain reaction (PCR) was used to amplify two mitochondrial gene segments from tissue samples; *CR* (control region), L15999M (5'-ACCATCAACACCCAAAGCTGA-3') and H16498M (5'-CCTGAAGTAGCAACCAGTAG) (Fumagalli et al. 1997); *ND2* (NADH dehydrogenase subunit 2), mmND2.1 (5'-GCACCATTCCACTTYTGAGT-3') and mrND2c (5'-GATTTGCGTTCGAATGTAGCAAG-3') (Osborne and Christidis 2001). PCR-amplifications were carried out in 25μL reactions with ~100ng genomic DNA, 1 x PCR buffer (Applied Biosystems), 0.20mM dNTPs, 2.5mM $MgCl_2$, 2 pmol primers and 0.1 U Ampli*Taq* Gold^{TM} polymerase (Applied Biosystems). Thermocycling conditions were: initial denaturation at 94°C for 9 min; 34-40 cycles of 45 sec at 94°C (denaturation), 45 sec at 48°C (*ND2*)/55°C (*CR*) (annealing), and 1 min at 72°C (extension); and a final extension for 6 min at 72°C. PCR products were purified using Millipore MultiScreen PCR_{384} Filter Plates (Millipore) and directly sequenced on an AB 3730*xl* DNA Analyzer (Applied Biosystems) at the Australian Genome Research Facility (AGRF).

All individuals were screened with a total of 12 polymorphic microsatellite loci: Pa297, Pa593, Pa597 from the allied rock-wallaby (*P. assimilis*; Spencer et al. 1995); Me2, Me14, Me15, Me16, Me17 from the tammar wallaby (*Macropus eugenii*; Taylor and Cooper 1998); Y76, Y148, Y170 from *P. xanthopus* (Pope et al. 1996); G26-4 from the eastern-grey kangaroo (*M. giganteus*; Zenger and Cooper 2001). In addition, an SRY male-specific marker from *P. penicillata* (O'Neill et al. 1997) was used to sex the faecal samples.

Primer mixes (PM) consisted of 1μL of each fluorescently labelled primer (100μM) in 25μL volumes in the following combinations: PM1 – Y170, Pa297, Pa593; PM2 – Y148; PM3 – Y76, Me15; PM4 – SRY; PM5 – Me17, Me2; and PM6 – Pa597, Me14, Me16, G26-4.

PCR-amplifications of tissue samples were carried out in 10µL reaction volumes following the methods of Miller et al. (2010) using a Multiplex PCR kit (Qiagen); with approximately 30ng genomic DNA, 5µL Multiplex MasterMix, 0.1mM BSA, 0.2µM of each primer,. Thermocycling conditions were: initial denaturation at 95°C for 15 min; 34-50 cycles of 30 s at 94°C (denaturation), 90 s at 58°C (Primer Mix 1, PM 2, PM4), 56°C (PM3) and touchdown 60-50°C (60°C, 57°C, 55°C, 52°C and 50°C for 10 cycles each; PM 5) (annealing), and 90 s at 72°C (extension); and a final extension for 10 min at 72°C. PCRs were pooled: Pool 1 included 4µL PM 1, 3µL PM 2, 4µL PM 3, and 4µL PM 4; and Pool2 included 4µL PM5, 4µL PM6. Pooled PCR products were cleaned using Millipore MultiScreen PCR_{384} Filter Plates (Millipore) following the manufacturer's protocols. Serial dilutions of 1:40 for Pool 1 and 1:20 for Pool 2 were then genotyped at the Australian Genome Research Facility (AGRF) with an automated ABI 3730xl capillary sequencer (Applied Biosystems).

Multiplex PCR-amplifications of faecal samples were carried out in 25µL reactions using the Qiagen Multiplex kit (Qiagen); with 15µL Multiplex MasterMix, 2.5µL Q solution, 0.2µM of each primer and 2µL of DNA extract. The following conditions were used for amplification: initial denaturation 94°C for 5 min; 50 cycles of 30 s at 94°C (denaturation), 30 s at 56°C (PM 1 and 3), 54°C (PM2) and touchdown 60-54°C (60°C, 58°C, 56°C for 10 cycles each, 54°C for 20 cycles ; PM 4-6) (annealing), and 30 s at 72°C (extension); and a final extension for 30 min at 72°C. Primer mixes PM1 and PM3 were pooled together, as were primer mixes PM4, PM5 and PM6 into two separate MultiMix PCR reactions unlike the individual PCRs used for the tissue samples. PCR samples were pooled as above and then 1µL of pooled product was added to 0.3µL Liz500 and 8.7µL Hi-Di for genotyping and analysed on a 3730 DNA Analyser (Applied Biosystems).

DNA from non-invasive techniques can produce yields with lower quality and quantity. We, therefore, replicated all PCRs three times, according to Piggott et al. (2006b), to control for allelic dropout (stochastic non-amplification of one allele), the occurrence of false alleles and ensure reduction in type 1 errors (novel genotypes which are incorrect). The microsatellite DNA fragments were scored using GENEMAPPER v4.0 (Applied Biosystems) for allele size and quantification. To ensure duplicate samples

of faecal DNA or samples from trapped animals were not included in the analysis, only unique multilocus genotypes were used for population analyses. Following Piggott et al. (2006b) we accepted a heterozygous genotype if each allele was present in two out of three replicates and only accepted a homozygous allele if only one allele was identified in all three replicates. A *post hoc* method was implemented, whereby samples that matched at all but four alleles were assigned the same identity (one individual removed from further analyses). This approach aims at eliminating overestimation biases of population abundance because the probability of individuals differing at two or more loci was high (Ruibal et al. 2009). Allelic dropout and false alleles were estimated for each microsatellite locus and sample using GIMLET (v3.2; Valière 2002), with total number of positive amplifications and genotyping errors per locus and sample computed. Following the methods of Ruibal et al. (2009), the frequencies of allelic dropout were then used to estimate a locus-specific rate using the equations ADO_1 from Broquet and Petit (2004).

Population genetic and phylogenetic analyses

Mitochondrial DNA sequences were edited using SeqEd (version 1.0.3; Applied Biosystems), then aligned using CLUSTAL X (version 1.83; Thompson et al. 1997) and manually refined using SeAl (version 2.0a11; Rambaut 1996). DNASP (v5.10; Librado and Rozas 2009) was implemented to estimate the number of haplotypes (*H*), polymorphic sites, nucleotide diversity (π) and haplotype diversity (*h*) (Rozas et al. 2003). Intra-specific sequence divergence among mtDNA haplotypes was estimated for the combined mtDNA dataset with PAUP* (v4.0b10; Swofford 2002) using the Kimura 3-parameter model (Kimura 1981) selected by MODELTEST 3.06 (Posada and Crandall 1998), based on the Akaike Information Criterion (AIC).

Conformance to Hardy-Weinberg equilibrium and linkage disequilibrium for each microsatellite locus within each population was assessed using GENEPOP (v3.2; Raymond and Rousset 1995) employing the Markov chain method with 1000 iterations and *P*-values adjusted using the sequential Bonferroni procedure (Rice 1989). The presence of null alleles was estimated using MICRO-CHECKER (v2.2.3; Van Oosterhout *et al.* 2004). Allelic diversity (*AD*; average number of alleles per locus), observed and

expected heterozygosity (H_O, H_E respectively) were calculated for each population in ARLEQUIN (v3.11; Excoffier et al. 2005), and allelic richness (*AR*; allelic diversity corrected for sample size) was estimated using FSTAT (v2.9.3; Goudet 1995). Differences in H_E, *AD* and *AR* between the populations were tested using a Wilcoxon signed rank test with loci as the pairing factor using PASW Statistics, Release Version 17.0.2 (SPSS, Inc.). The number of rare alleles (frequency ≤ 5%) and the number of unique alleles (A_U) were calculated in GenAlEx 6 (Peakall and Smouse 2006).

Multilocus F_{IS} was also calculated at each locus for each population using FSTAT (version 2.9.3; Goudet 1995), and then tested by 1000 permutations using Weir and Cockerham's estimator (Weir and Cockerham 1984) an analogue of Wright's F statistic (F_{IS}; Wright 1951). F_{IS} was also calculated for males and females individually for each population.

Genetic structure

To assess whether individuals sampled at specific localities represent discreet genetic populations, STRUCTURE 2.1 (a Bayesian clustering method; Pritchard et al. 2000) was used to infer population structure. This method assumes no *a priori* geographical information and uses genotype data to infer population groups that minimise Hardy-Weinberg equilibrium and linkage disequilibrium. Analyses were performed on the entire microsatellite dataset and then on separated East and West Kimberley samples to identify any subtle structure between the sampling areas which may have been overlooked in the broader analysis. We estimated the most likely number of genetic clusters/groups (*K*) from between one to six for the entire dataset (total number of sampled sites), one to four for the West Kimberley samples and one to three for the East Kimberley samples (minimum three populations required for Δ*K* assessment below). The parameters used were the admixture ancestry model, correlated allele frequencies model, a burnin length of 10 000 iterations and a run length of 100,000 Markov chain Monte Carlo repetitions, with 6 iterations of each *K*. To estimate *K*, Ln P(*X*|*K*) (log likelihood value of *K* given the genotypes of sampled individuals, *X*) and Δ*K* values (a measure of the second order rate of change in the likelihood of *K*; Evanno et al. 2005) were assessed, with the lowest log likelihood value and highest Δ*K* value representing the most likely *K* (Pritchard et al. 2000).

An analysis of molecular variance (AMOVA based on Wright's F statistic F_{ST}; Wright 1951) was carried out in ARLEQUIN (v3.11; Excoffier et al. 2005) to examine the extent of population structuring, assessing mtDNA differentiation (pairwise Φ_{ST}) amongst the four populations from which tissue samples were collected (based on 10 000 permutations) and microsatellite differentiation (pairwise $F_{ST;}$ Wright 1951) among all six populations (based on 10 000 permutations). To visualise genetic differentiation among the six populations we used the microsatellite data to generate an unrooted neighbour-joining (NJ) tree based on Nei's standard genetic distance (Ds; Nei 1972) and 1000 bootstrapping support over individuals using the program POPULATIONS 1.2.32 (Langella 1999).

Concordance of the two mitochondrial fragments was assessed by independently generating phylogenetic trees for both *CR* and *ND2* to ensure concatenation of the data was appropriate. Maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference analyses were performed on the concatenated mitochondrial alignment of *CR* and *ND2* using RAxML (v7.0.3; Stamatakis 2006; Stamatakis et al. 2008), PAUP* (v4.0b10; Swofford 2002) and MRBAYES (v3.1.2; Huelsenbeck and Ronquist 2005; Ronquist and Huelsenbeck 2003) respectively. The program MODELTEST 3.06 (Posada and Crandall 1998) in PAUP* (v4.0b10) was used to determine the best-fit models, based on the AIC. Results from all analyses gave similar overall topologies, therefore only ML results are included. As the exact model suggested from MODELTEST 3.06 was not available in RAxML, the more complex General time Reversible (GTR) model was applied, with rate variation among sites modelled with a discrete gamma distribution (G), helping to account for rate variation among the non-coding *CR* and codon positions of *ND2*. Analysis used a random starting tree and bootstrap analyses were carried out for 1000 replicates, with multiple (100) searches per replicate, using the rapid Bootstrap analysis.

We tested for correlation (r) between genetic (microsatellite data) distance (Nei's D_A; Nei et al. 1983) and geographical distance using Mantel tests (Mantel 1967) implemented in AIS (Alleles in Space; Miller 2005) for all locations, for males and females individually and for the West Kimberley individuals alone, based on 1000 permutations and 10 distance classes.

Estimation of dispersal patterns using assignment tests

Putative first generation migrants and their population of origin were identified using the program GENECLASS 2.0 (Piry et al. 2004). Using the Bayesian method of Rannala and Mountain (1997) the likelihood of each genotype in each population was estimated, following the Paetkau et al. (2004) Monte Carlo re-sampling method. We used the comparison of the likelihood of each individual in its 'home' (sampled) population to the most likely of the other sampled populations, but took into account that all possible populations were not sampled (L=L_home). Individuals that were significantly different from their sampled population were only assigned to another population as the 'home' if the significance was above 0.99. Immigrants that did not meet the *P* value of 0.99 were considered immigrants from unsampled colonies. Methods followed Piggott et al. (2006a) and despite the small sample sizes for some populations, using the Bayesian method, with more than 10 loci, and F_{ST} values close to 0.1 or greater between populations, the procedure should be quite accurate (Cornuet et al. 1999; Paetkau et al. 2004). Putative migrants were checked for concordance with Bayesian clustering results of STRUCTURE 2.1.

Relatedness within populations

Individuals in each population were examined for overall pairwise relatedness and sex-specific relatedness using the program GenAlEx 6 (Peakall and Smouse 2006). Pairwise relatedness (*r*) estimates were calculated using the method of Queller and Goodnight (1989). An average of pairwise relatedness within populations was estimated using GenAlEx 6 using the Pops Mean option with 9999 permutations and 9999 bootstraps to estimate the 95% confidence interval around *r*. This was used to test the assumption that the average pairwise relatedness values did not differ significantly from random assortments of relatedness values (unrelated individuals).

Results

Genetic diversity

A total of 1182 base pairs (bp) of mtDNA was analysed (596 bp *CR*; 586 bp *ND2*) from 52 individuals (30 females, 21 males; and one *P. penicillata*). GenBank accession numbers are given in Table 1. Within the mtDNA fragment 120 sites were variable, of which 116 sites were parsimony informative. Within the *CR*, seven different indels were detected within *P. brachyotis*. Thirteen mtDNA haplotypes were identified (Table 1), with haplotype and nucleotide diversity highest in CV (h=52.6%, π=0.77%; Table 2).

A total of 40 females and 44 males (23 identified from faecal samples using the SRY marker) were genotyped for the six populations (see Supplementary Table 1for genotypes). All microsatellite loci in each population conformed to Hardy-Weinberg equilibrium after sequential Bonferroni corrections, except for PH which revealed a significant heterozygote deficit at one locus (Pa597). There was significant linkage disequilibrium after sequential Bonferroni corrections only within KER between the following sets of loci: Me14/Y148, Me15/Y148, Me14/Me15, Me15/Pa593, Pa297/Y76, Pa593/Y148. All other populations examined revealed linkage equilibrium, so analyses were carried out on the full dataset. No false alleles were detected, but 23 allelic differences were identified of the 1188 faecal amplifications, giving a genotyping error rate of 1.9%. The amplification success rate was 84% for both loci and samples, with allelic dropout rates of 2.8% detected across samples and 3.5% across loci.

The average number of alleles detected per locus was 6.13 ± 2.24. East Kimberley populations (MI and BI) had significantly lower *AD* values compared to West Kimberley populations (CN, CV, PH; *AD* = 4.1-4.3 vs. 6.3-8.3, P<0.05; Table 2). When corrected for sample size (*AR*), MI was significantly lower compared to three of the West Kimberley populations (*AR* = 3.6 vs. 5.3-5.6, P<0.05; Table 2), whereas BI was only significantly lower when compared to PH (H_E and *AR*; H_E = 0.661 vs. 0.771, *AR* = 4.4 vs. 5.4; P<0.05; Table 2). The West Kimberley H_E, *AD* and *AR* values were not significantly different between populations (P>0.05) except for *AD* between CN and KER (Table 2). No significant inbreeding (F_{IS}) was detected for any population at any locus (P>0.05), or for males or females within populations (Table 2). No rare alleles (frequency ≤ 5%) were detected. Unique alleles were detected in all populations ranging

from 1% (KER, PH) to 7% (BI; Table 2). A total of 71 alleles (48%) were only found in the West Kimberley populations and 25 alleles (17%) were unique to the East Kimberley populations.

Genetic structure

Initial STRUCTURE results for all the microsatellite data indicated a geographically distinct East and West Kimberley grouping (K = 2), with all individuals assigned to each cluster at probabilities >0.9. However, when the East and West Kimberley genetic clusters were analysed separately, the ΔK value was highest for two in East Kimberley analysis (K = 2) and two in the West Kimberley analysis (K = 2), indicating more subtle structure within these smaller regions (Fig. 2). Within the West Kimberley analysis, not all individuals were assigned to a cluster (probabilities >0.8), with sixteen individuals having a mixed assignment (probabilities <0.8) and three individuals (CN17, KER09, CN29) clustering with a group different to those corresponding to their sampling region (see Fig. 2). Generally, one cluster consisted of individuals from CN and KER, and the second cluster of individuals from CV and PH (Fig. 2). The East Kimberley assignments corresponded to the sampled localities of the two islands in Lake Argyle, with only one individual not assigned to a cluster (MI10; Fig. 2).

Population differentiation (Φ_{ST}) in mtDNA was highly significant between the four analysed populations (CV. MI, BI, CN) with values ranging from 0.856 to 0.995 (Table 3). For the purposes of looking at fine-scale genetic structure within the West Kimberley, we employed all four sites from this region as separate populations for analysis. All microsatellite pairwise population comparisons (F_{ST}) were significant after sequential Bonferroni corrections, excluding CV vs. PH (0.022-0.059; Table 3). The range of F_{ST} values between the East and West Kimberley populations and between the two East Kimberley populations were orders of magnitude higher (F_{ST} =0.184-0.274; F_{ST} =0.166 respectively; Table 3).

The unrooted NJ tree based on Ds between the six populations reveals closer genetic distances between the West Kimberley populations than those between the East Kimberley populations or between the East and West Kimberley (see Fig. 3). Bootstrap values (87-91%) provide strong support for more closely related populations in the West Kimberley.

The *CR* and *ND2* produced the same topology in independent phylogenetic analyses therefore only concatenated results are discussed. Three distinct groupings of haplotypes were apparent from the phylogenetic analyses (Fig. 4). One lineage comprised the haplotypes from MI (H, I, J), with the second lineage consisting of haplotypes from BI (K, L, M). The remaining haplotypes (A–G) from the CN and CV (West Kimberley) formed the third monophyletic group, with 0.2-2.9% sequence divergence (SD) between haplotypes (0.2-0.3% SD amongst haplotypes from CN; 0.4-2.9% SD CV). Between the fragmented MI and BI there was 5.6-5.9% SD.

Mantel tests indicated significant correlations of both male and female genetic divergence and geographic distances ($P<0.05$) across the Kimberley, with females having a slightly higher correlation of genetic similarity to geographic distance ($r = 0.73$ females; $r = 0.68$ for males). Results for the West Kimberley alone revealed significant, yet far lower, correlations between genetic and geographic distances ($r = 0.30$, $P<0.05$), with slightly greater genetic similarity to geographic distance for females ($r = 0.36$, $P<0.05$) than for males ($r = 0.25$, $P<0.05$).

Estimation of dispersal patterns using assignment tests

Four putative immigrants were detected amongst the six sites using GENECLASS 2.0, including individuals from CN, MI, KER and PH (CN29, MI10, KER09 and PH11 respectively). No first generation female immigrants were detected, with all four of these potential dispersers being males. Two individuals (CN29 and KER09) had the highest likelihoods of coming from PH and PH11 had the highest likelihood of coming from KER, but these individuals were not assigned to any population with a probability of 0.99. MI10, however, was excluded from all populations with no other sampled population having a higher likelihood of being ‘home’ despite the individual not having a high enough probability (0.99) of belonging to its ‘home’ population. Since all four individuals could not be assigned to a population with high probability (0.99) it is likely they have originated from sites not sampled in this study.

Relatedness within populations

All pairwise intra-population means showed that males and females were significantly related ($P < 0.05$). KER and PH appeared to have less significant relatedness, with bootstrap analysis indicating some overlap with the permuted relatedness values of the null hypothesis (unrelated individuals; results not shown). Females were significantly related (significant *r* means for intra-population analyses; $P<0.05$) for all sites except PH, whereas males were significantly related except at CV ($P<0.05$; Fig. 5).

Discussion

While both habitat fragmentation and species' biology can influence population biology, our study has demonstrated that for *P. brachyotis* habitat connectivity plays a critical role in maintaining gene flow and reducing genetic differentiation across the landscape. Numerous *Petrogale* taxa have declined in Australia and their remnant populations are actively managed (DEH 2006; DECC 2008; DERM 2010; Read and Ward 2010). The effects of fragmentation versus life-history traits on population biology in these species are unknown, since they now form small, isolated colonies and were not studied prior to their decline. Similar situations occur across the world, where limited information exists for species prior to their decline. Management of such taxa can be difficult without knowledge of natural pre-decline population dynamics and since such processes can be disturbed by fragmentation (e.g. Smith and Hellmann 2002), management can draw on studies of abundant closely related taxa in unmodified landscapes. This study provides the first comparison of rock-wallaby populations in a landscape containing large areas of interconnected habitat. For *P. brachyotis* genetic differentiation appears not to be a function of species' biology alone, highlighting the importance habitat connectivity plays in maintaining gene flow and reducing differentiation. This highlights that a detailed understanding of population biology may not solely be achieved by the study of fragmented populations in disturbed environments and that management strategies may need to draw on studies of related species in undisturbed areas of contiguous habitat.

Effects of Habitat

The greater habitat connectivity across the West Kimberley was associated with lower genetic differentiation within *P. brachyotis* compared to individuals from the island populations in the East Kimberley. The relatively low levels of genetic differentiation (F_{ST} values; 0.027-0.059) found at distances of up to 67 km are in contrast to those reported for rock-wallaby populations in closer proximity using similar combinations of microsatellite loci, but within more heavily modified landscapes; including *P. penicillata* (F_{ST} = 0.072, <10km; Hazlitt et al. 2006a), *P. xanthopus* (F_{ST} = 0.107, 10-70 km; Pope et al. 1996) and *P. lateralis* (F_{ST} = 0.238, <10 km; Eldridge et al. 2001). They are also far lower than that found in *P. brachyotis* from the Northern Territory which had limited contemporary and long-term gene flow between populations 1.2 km apart (F_{ST}: 0.085; Telfer and Eldridge 2010). In comparison, CV and PH, ≈1.2 km apart did not show significant genetic differentiation (F_{ST} : 0.022). Three first generation migrants were detected in the West Kimberley, indicating gene flow is occurring across this landscape, however, these immigrants are likely to have come from unsampled populations and further assessment of population and home range sizes may identify at what level fine-scale population structure exists at this location. The phylogenetic results of mtDNA provide no evidence for long term isolation of females between sites in the West Kimberley. Haplotypes from CV and CN formed a monophyletic group with only 0.2-2.9% SD compared to the two island populations which had 5.6-5.9% SD and formed two independent monophyletic groups. Although the similarity between sites in the West Kimberley could be interpreted as retained ancestral polymorphisms, the F_{ST} data, close genetic distances between populations and STRUCTURE results (showing two genetic clusters connected at distances up to 18 km, numerous individuals with admixed ancestry (~24%) and three individuals grouping with the genetic cluster opposite to samples from the same site), greater connectivity in this region seems more likely.

In contrast, the isolated island populations in the East Kimberley indicated high levels of genetic differentiation. Evidence of gene flow within the East Kimberley was limited by the sampling regime, however, the F_{ST} values between the two geographically close East Kimberley populations (0.166) were comparable to those found between the East and West Kimberley (0.184-0.274), indicating longer term isolation of these sites (longer than from 1972 when the Ord River Irrigation Scheme developed Lake

Argyle as a major storage reservoir and turned these sites into isolated islands). Long term isolation was also evident from mtDNA data, not only with significant Φ_{ST} values between islands, but distinct MI and BI lineages with up to 5.9% SD. This SD is similar to levels of divergences found between subspecies and ESUs in other rock-wallabies (e.g., 5.3% *P. xanthopus*, Pope *et al.* 1996; 5% *P. lateralis*, Eldridge et al. 2001; 7.6% *P. penicillata*, Browning et al. 2001), but over much greater distances (400-1000 km) apart. These results are consistent with the findings of a broader phylogeographic study (Potter et al. 2012a) which found that MI and BI belonged to distinct lineages that have been separated by the Ord River (= Ord Arid Intrusion; Eldridge et al. 2012) since the Pleistocene (~ 0.13 MYA).

The microsatellite diversity was also variable between the continuous and fragmented habitat, with fragmented populations of *P. brachyotis* having significantly lower microsatellite diversity (*AD*, *AR* and H_E) compared to those from the continuous populations (Table 2). West Kimberley *P. brachyotis* also generally have higher diversity than in other rock-wallaby species (e.g., *P. penicillata*, *P. lateralis*, *P. xanthopus*; see Table 4) which potentially reflect a larger population size and greater gene flow associated with this more continuously connected habitat. Eldridge et al. (2010) reported high levels of microsatellite diversity associated with macropodoid populations from relatively abundant or widespread species, whilst low levels were typically associated with populations showing a restricted distribution including those found on islands. Low levels of diversity are expected due to a loss of variation as a result of finite population size and inherent problems associated with small populations (e.g., inbreeding, genetic drift; Frankham 1997; Frankham et al. 2004, 2010). Low genetic diversity associated with a restricted distribution could explain the reduced diversity of *P. brachyotis* from the East Kimberley, located on small (50-100 ha) islands within Lake Argyle. This pattern of reduced genetic diversity is supported by other populations of rock-wallabies on islands (e.g., *P. lateralis*; Eldridge et al. 1999).

Effects of life-history strategies

As well as habitat impacts on population biology, life-history strategies appear to be in place within *P. brachyotis* to preserve genetic integrity, with no evidence for inbreeding in MI and BI despite low genetic diversity and high levels of relatedness for both males and females (Queller and Goodnight 1989; mean

estimator) within these populations (r = 0.251-0.797 MI; r = 0.118-0.693 BI). The presence of significantly related individuals is common within rock-wallaby populations and has been associated with strong female philopatry and spatial patterns associated with their habitat specificity (Hazlitt et al. 2004, 2006b; Piggott et al. 2006a). Aside from high relatedness, the results from *P. brachyotis* support other rock-wallaby studies which highlight inbreeding avoidance mechanisms despite limited gene flow between populations (e.g. male-biased dispersal; Hazlitt et al. 2004, 2006b). Amongst the six populations sampled in this study, four first generation migrants were detected all of which were male (supported by both GENECLASS and STRUCTURE results). Although the possibility of female dispersal cannot be discounted (Eldridge et al. 2001 has shown it occurs), the strong female philopatry indicated by the mtDNA results and low differentiation for microsatellite markers suggests these rock-wallabies most likely display sex-biased dispersal. Limited female-mediated gene flow is consistent with other rock-wallabies (Hazlitt et al. 2004, 2010; Piggott et al. 2006a) and supports suggestions that dispersal for macropodoids is generally male-biased (Eldridge et al. 2010). The isolation by distance results also indicate greater female genetic similarity to geographic distance compared to males, in both the overall and the West Kimberley analyses (r = 0.73 vs. 0.68; r = 0.36 vs. 0.25, respectively). Significant yet lower correlation values of isolation by distance in the West Kimberley may reflect a limit to dispersal distance between populations and highlight the influence continuous habitat may play in potential stepping stone methods of dispersal across the landscape. Mating system preferences such as kin avoidance and sex-biased dispersal have been suggested to facilitate inbreeding avoidance (Hazlitt et al. 2006b) and help maintain genetic diversity within *Petrogale* populations (Spencer et al. 1997). As the two island populations have only recently been isolated, it may not yet be evident if mating system strategies are in place or if the effects of genetic drift and inbreeding are just not detected.

Conclusions and future implications

Despite the Kimberley having relatively few threats in the past, human impacts associated with increased fire frequency and intensity, introduction of invasive species (e.g., cane toads), mining, grazing and predation by feral animals, threaten to cause faunal declines and extinctions (Braithwaite and Muller 1997; Eliot et al. 1999; Fitzsimons et al. 2010; McKenzie and Burbidge 2002; Woinarski 1992;

Woinarski et al. 2001). Our results indicate that West and East Kimberley populations of *P. brachyotis* are genetically distinct and could reflect a common pattern for other sandstone endemics (Potter et al. 2012a). This has implications for future management plans if threats described above do cause species to become endangered.

This study suggests that habitat connectivity plays a critical role in enabling dispersal within *P. brachyotis*, which exhibits high philopatry and strong habitat specificity. Despite evidence of male-biased dispersal and female philopatry, these results suggest that species' biology does not necessarily lead to genetic differentiation of rock-wallabies, with low genetic differentiation observed across suitable habitat. The results indicate that fragmentation alone may not influence the genetic differentiation of *P. brachyotis*, however, evidence suggests that connectivity between populations can reduce the effects of life-history traits within *P. brachyotis* and maintain higher genetic diversity/integrity. Our results suggest that patterns of genetic differentiation found in fragmented populations of rock-wallabies may not represent natural population structure but be a consequence of post-decline isolation and reduced gene flow, and therefore should be managed accordingly.

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Table 1

Population	**Sample ID**	**Latitude/Longitude**	**Sample Size**	**Haplotype**	**mtDNA lineage**
Couchman (CN)	ABTC99376, ABTC99377, ABTC99379, ABTC99380, ABTC99381, ABTC99382, ABTC99383, ABTC99384, ABTC99385, ABTC99386, ABTC99387, ABTC99388, ABTC99389,ABTC99390, ABTC99391, ABTC99392, ABTC99393, ABTC99394, ABTC99395, ABTC103715, CN01, CN02, CN03, CN04, CN05, CN06, CN07, CN08, CN09	204755 8321547	29	A (17) B (1) C (2)	WK WK WK
Cypress Valley (CV)	ABTC99396, ABTC100692, ABTC100693, ABTC100694, ABTC100695, ABTC100696, ABTC100697, ABTC100698, ABTC100699, ABTC100700, ABTC103330, ABTC103331, ABTC103332	231724 8361617	13	D (1) E (1) F(9) G(2)	WK WK WK WK
Monsmont Island (MI)	ABTC103495, ABTC103496, ABTC103497, ABTC103498, ABTC103499, ABTC101500, ABTC103501, ABTC103502, ABTC103503, ABTC103504, ABTC103505	467949 8198163	11	H (1) I (9) J (1)	EK1 EK1 EK1
Bullanyin Island (BI)	ABTC103506, ABTC103507, ABTC103508, ABTC103509, ABTC103510, ABTC103511, ABTC103512	464389 8198066	7	K (1) L (5) M (1)	EK2 EK2 EK2
King Edward River	KER01, KER02, KER03, KER04,	194873 8305693	11		

(KER)	KER05, KER06, KER07, KER08, KER09, KER10, KER11		
Pump Hill (PH)	PH01, PH02, PH03, PH04, PH05, PH06, PH07, PH08, PH09, PH10, PH11, PH12, PH13	232154 8360873	13

No mtDNA sequence data was obtained from the KER and PH scat samples. *MtDNA lineages (WK = West Kimberley; EK1 = East Kimberley ; EK2 = East Kimberley 2) follow Potter et al. (2012a).

Table 2

Sample Location	h (± SD)	π	AD (± SD)	AR (± SD)	H_E (± SD)	A_U	FIS
Couchman	0.279 ± 0.123	0.00063	8.3 (± 3.1)	5.3 (± 1.5)	0.745 (± 0.16)	9	-0.003
Cyprus Valley	0.526 ± 0.153	0.00775	6.9 (± 2.4)	5.6 (± 1.7)	0.755 (± 0.20)	7	0.041
Monsmont Is.	0.182 ± 0.144	0.00032	4.1 (± 1.5)	3.6 (± 1.3)	0.573 (± 0.19)	6	-0.035
Bullanyin Is.	0.286 ± 0.196	0.00025	4.4 (± 1.5)	4.4 (± 1.5)	0.661 (± 0.17)	10	0.030
King Edward River			6.3 (± 2.8)	5.2 (± 2.1)	0.763 (± 0.15)	2	-0.096
Pump Hill			6.8 (± 2.1)	5.4 (± 1.5)	0.771 (± 0.15)	2	0.042

Table 3

	CN	CV	MI	BI	KER
CN		0.856*	0.993*	0.993*	
CV	0.043*		0.935*	0.929*	
MI	0.256*	0.274*		0.995*	
BI	0.202*	0.209*	0.166*		
KER	0.027*	0.058*	0.248*	0.184*	
PH	0.051*	0.022	0.265*	0.201*	0.059*

*significant ($P < 0.05$) after Bonferroni correction.

Table 4

Taxon	No. of individuals (and site)	No. of loci	*AD*	H_E	Reference
Petrogale assimilis	128 (Black Rock)	5	11.6	0.86	(Spencer and Marsh 1997)
	15 (Little Black Rock)	5	8.6	0.85	(Spencer et al. 1995)
Petrogale brachyotis	32 (East Alligator)	10	7.8	0.78	(Telfer and Eldridge 2010)
	14 (Florence Falls)	10	3.4	0.55	(Telfer and Eldridge 2010)
	30 (Buley)	10	4.6	0.62	(Telfer and Eldridge 2010)
Petrogale penicillata	28 (Ingles Rd)[T]	11	5.2	0.70	(Eldridge et al. 2004b)
	15 (Crocodile Rock)[T]	7	5.1	0.65	(Piggott et al. 2006a)
	17 (Rocky Creek)[T]	7	5.1	0.65	(Piggott et al. 2006a)
	20 (Bowmans Rd)[T]	11	5.1	0.69	(Eldridge et al. 2004b)
	44 (Wolgan, Main)[T]	7	4.9	0.65	(Piggott et al. 2006a)
	54 (Hurdle Creek)[T]	12	4.5	0.67	(Hazlitt et al. 2006a)
	17 (Broke)[T]	11	4.0	0.64	(Eldridge et al. 2004b)
	15 (Perseverance Dam)[T]	12	3.4	0.57	(Hazlitt et al. 2006a)
	19 (Kangaroo Valley)[T]	12	2.4	0.37	(Paplinska 2006)
	8 (Rocky Plains Creek)[T]	11	1.9	0.34	(Browning et al. 2001)
Petrogale lateralis lateralis	19 (Tutakin)[T]	11	4.0	0.62	(Eldridge et al. 2004a)
	31 (Sales Rock)[T]	11	3.5	0.55	(Eldridge et al. 2004a)
	15 (Exmouth)[T]	10	3.4	0.62	(Eldridge et al. 1999)
	32 (Mt Caroline East)[T]	11	3.3	0.51	(Eldridge et al. 2004a)
	19 (Mt Caroline West)[T]	11	3.1	0.49	(Eldridge et al. 2004a)
	19 (Nangeen Hill)[T]	11	2.5	0.41	(Eldridge et al. 2004a)
	28 (Barrow Island)[T]	10	1.2	0.05	(Eldridge et al. 1999)
Petrogale lateralis pearsoni	38 (North Pearson Island)	10	1.6	0.13	(Jones Lennon et al. 2011)
Petrogale xanthopus celeris	50 (Hill of Knowledge)	4	7.5	0.69	(Pope et al. 1996)
Petrogale x. xanthopus	69 (Flinders Ranges)*[T]	7	7.8	0.75	(Zenger et al. 2003)

*Samples pooled from multiple localities. [T] Threatened taxon (Maxwell et al. 1996).

Figure Legends

Fig. 1 The distribution of the short-eared rock-wallaby (*Petrogale brachyotis*) across northern Australia (top left), showing (below) the collection localities for the six populations sampled from the Kimberley, Western Australia, Australia. Four sites (Couchman, Cyprus Valley, King Edward River and Pump Hill) in the West Kimberley were sampled across continuous habitat, while two sites (Monsmont Island and Bullanyin Island) in the East Kimberley represent fragmented populations. Sample sizes (n) are outlined

for each sampling locality. + represent the Northern Territory sites sampled by Telfer and Eldridge (2010). Increasing elevation is indicated by darker shading (up to ~1000 m).

Fig. 2 (a) Proportional membership (*Q*) of each short-eared rock-wallaby (*Petrogale brachyotis*) individual to the two clusters identified by STRUCTURE, in (a) the East Kimberley associated with the fragmented island populations; and (b) the West Kimberley. Each individual is represented by a single vertical bar and sample IDs are outlined in the figure.

Fig. 3 Unrooted NJ tree of populations of short-eared rock-wallaby (*Petrogale brachyotis*) from the East (Monsmont and Bullanyin Islands) and West Kimberley (Couchman, Cyprus Valley, King Edward River, Pump Hill) based on a matrix of Ds (Nei 1972) from microsatellite genotypes.

Fig. 4 Maximum likelihood reconstruction (RAxML) of short-eared rock-wallaby (*Petrogale brachyotis*) mtDNA haplotypes from West and East Kimberley. Bootstrap values are signified as percentages and Bayesian posterior probabilities as decimal values. Haplotypes (A-M) are grouped into geographic regions (BI – Bullanyin Island, MI – Monsmont Island, CN – Couchman, CV – Cyprus Valley; EK – East Kimberley, WK – West Kimberley).

Fig. 5 Mean pairwise relatedness for individual short-eared rock-wallabies (*Petrogale brachyotis*) from six sites in the Kimberley, (a) males; (b) females. Error bars surround the mean pairwise relatedness value based on bootstrap analysis; U and L are upper and lower 95% confidence bounds of the null hypothesis (no difference across populations) permuted from 10 000 runs.

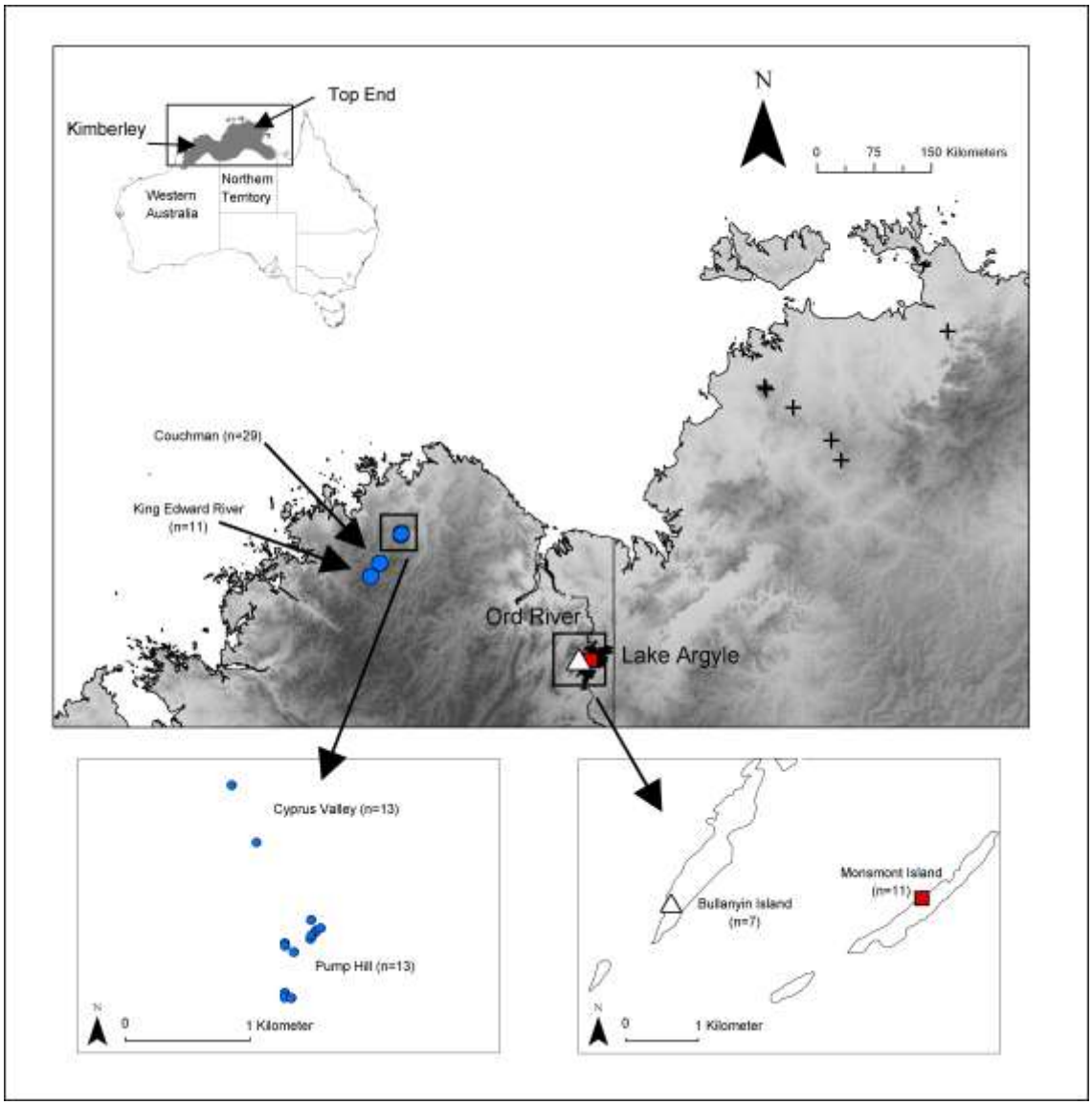
Table Captions

Table 1 Location of sampled short-eared rock-wallabies (*Petrogale brachyotis*), the number of identified mtDNA haplotypes and GenBank accession numbers.

Table 2 Summary of genetic diversity indices for six sampled short-eared rock-wallaby (*Petrogale brachyotis*) populations. *h* haplotype diversity, π nucleotide diversity, *AD* allelic diversity, *AR* allelic richness (allelic diversity corrected for sample size; n=7), H_E mean expected heterozygosity, A_U unique alleles, F_{IS} inbreeding levels. All F_{IS} values were not significantly different from zero.

Table 3 Genetic differentiation amongst sampled short-eared rock-wallaby (*Petrogale brachyotis*) populations (Couchman – CN; Cyprus Valley – CV; Monsmont Island – MI; Bullanyin Island – BI; King Edward River – KER; and Pump Hill – PH). Pairwise Φ_{ST} (mtDNA) above (excluding KER and PH) and F_{ST} (microsatellites) below the diagonal.

Table 4 Reported allelic diversity (*AD*) and average heterozygosity (H_E) at polymorphic microsatellite loci in 25 wild populations of *Petrogale* which were assessed using an assortment of the markers used in this study. (Taken from Eldridge et al. 2010, including more recent results).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Kimberley
Western Australia
Northern Territory
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Couchman (n=29)
King Edward River (n=11)
Ord River
Lake Argyle
Cyprus Valley (n=13)
Pump Hill (n=13)
N 0 1 Kilometer
Bullamyin Island (n=7)
Monsmont Island (n=11)
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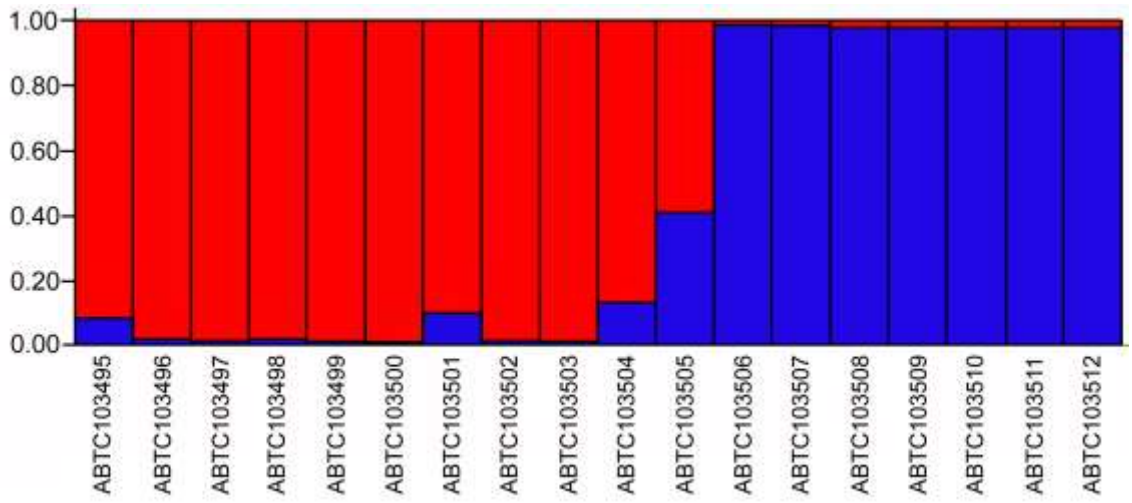
1


1.00
0.80
0.60
0.40
0.20
0.00
ABTC103495
ABTC103496
ABTC103497
ABTC103498
ABTC103499
ABTC103500
ABTC103501
ABTC103502
ABTC103503
ABTC103504
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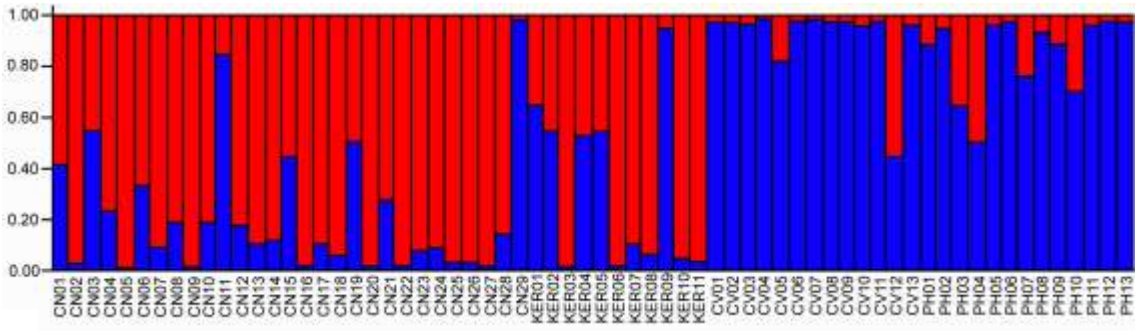
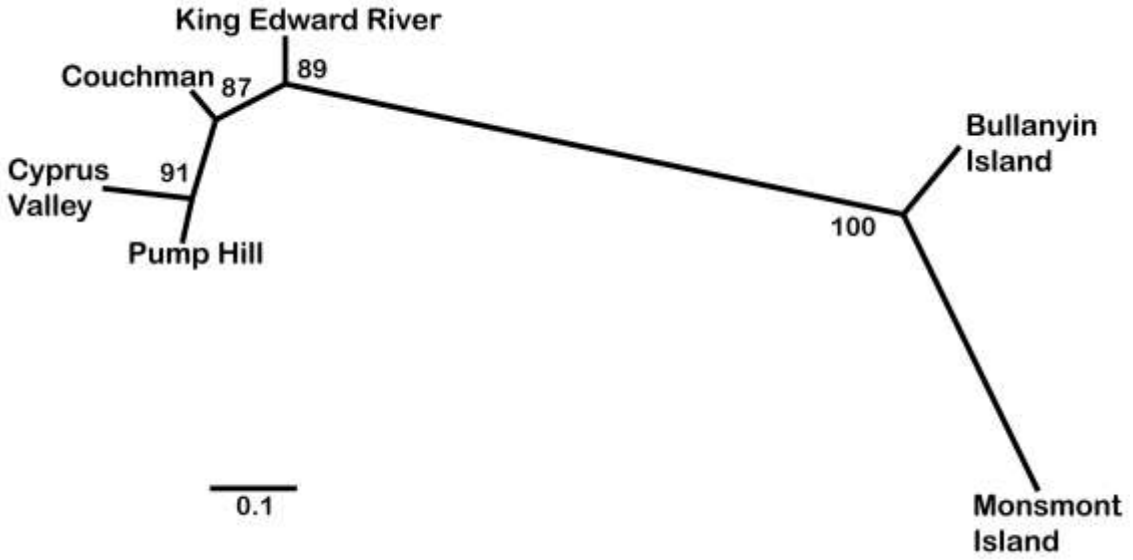
ABTC103512

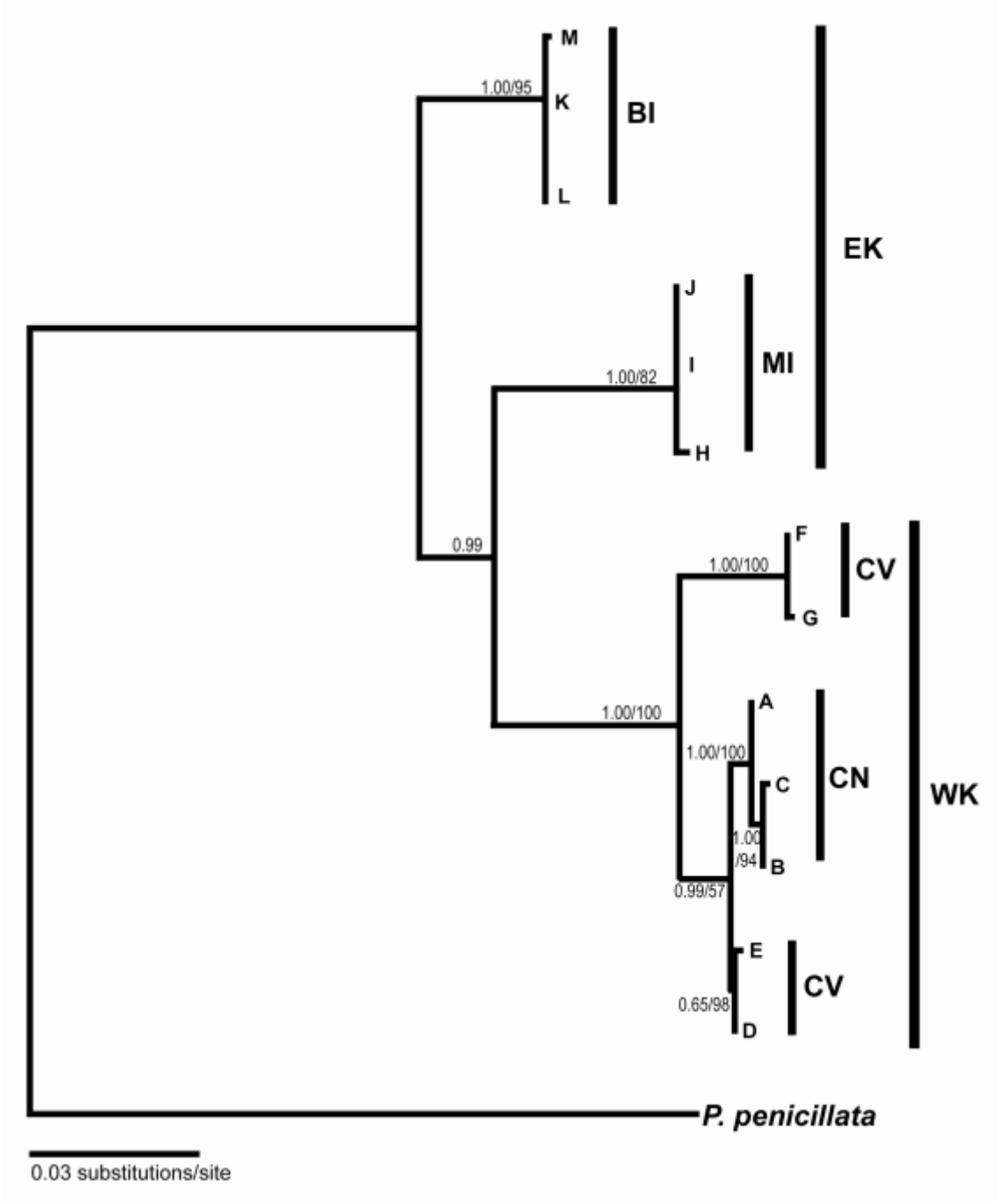
2

1


1.00
0.80
0.60
0.40
0.20
0.00
CN01 CN02 CN03 CN04 CN05 CN06 CN07 CN08 CN09 CN10 CN11 CN12 CN13 CN14 CN15 CN16 CN17 CN18 CN19 CN20 CN21 CN22 CN23 CN24 CN25 CN26 CN27 CN28 CN29 KER01 KER02 KER03 KER04 KER05 KER06 KER07 KER08 KER09 KER10 KER11 CV01 CV02 CV03 CV04 CV05 CV06 CV07 CV08 CV09 CV10 CV11 CV12 CV13 PH01 PH02 PH03 PH04 PH05 PH06 PH07 PH08 PH09 PH10 PH11 PH12 PH13

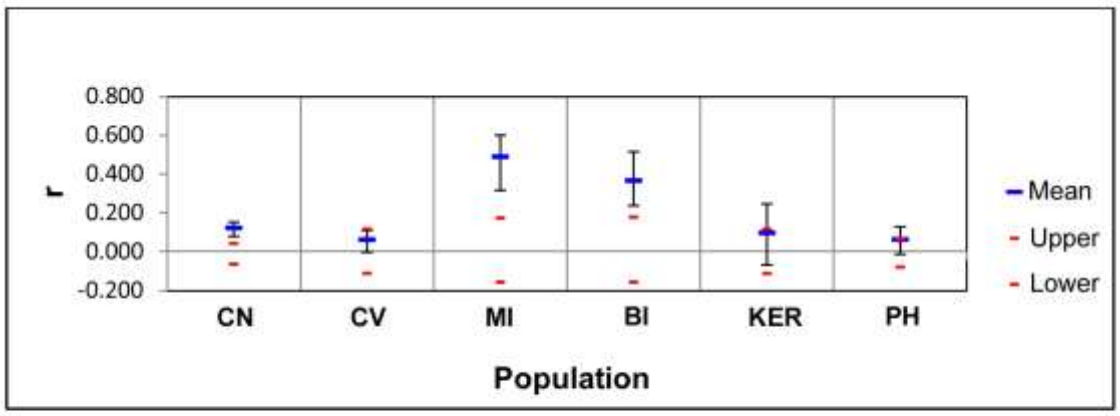
2


King Edward River
Couchman
87
89
Cyprus
Valley
91
Pump Hill
Bullanyin
Island
100
0.1
Monsmont
Island

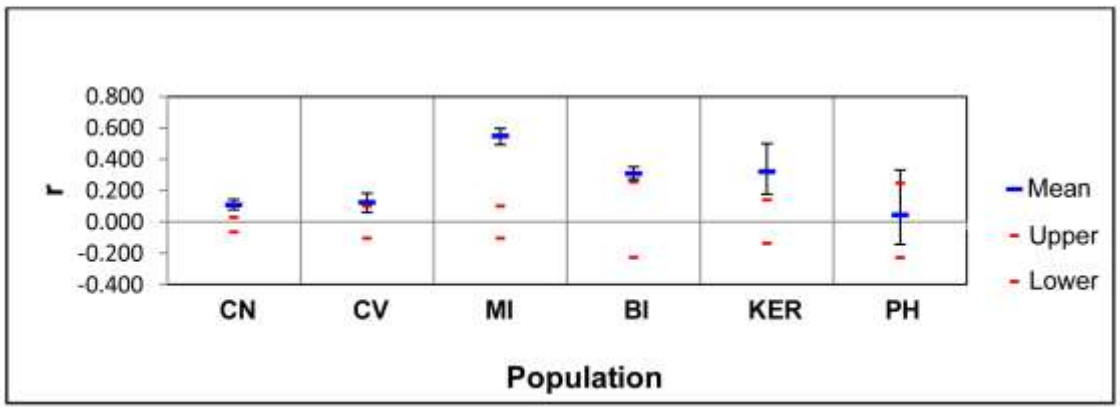

M
K
L
1.00/95
BI
EK
J
I
H
1.00/82
MI
0.99
F
G
1.00/100
CV
A
C
B
1.00/100
1.00
/94
0.99/57
CN
WK
E
D
0.65/98
CV
P. penicillata
0.03 substitutions/site

1

(a)


0.800
0.600
0.400
0.200
0.000
-0.200
r
CN
CV
MI
BI
KER
PH
Population
Mean
Upper
Lower

(b)


0.800
0.600
0.400
0.200
0.000
-0.200
-0.400
r
CN
CV
MI
BI
KER
PH
Population
Mean
Upper
Lower

1

2

Author/s:
Potter, S;Eldridge, MDB;Cooper, SJB;Paplinska, JZ;Taggart, DA

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