

Pleistocene climatic changes drive expansion and fragmentation in a widespread arid zone specialist, *Petrogale lateralis*

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Abstract

Organisms living in arid biomes are predicted to be at threat of extinction associated with ongoing climatic and anthropogenic change. Our understanding of species responses to Pleistocene climatic changes within these environments is still limited, particularly in Australia. Here we evaluate the demographic and evolutionary history of a widespread Australian marsupial, the black-footed rock-wallaby (*Petrogale lateralis*) whose contemporary distribution is highly fragmented across the arid biome and offshore islands. Combining genomic data from historical and modern samples we evaluate the divergence history of the five *P. lateralis* sub-species. The species has experienced a Pleistocene demographic expansion across the vast Australian arid biome, with subsequent fragmentation of populations and sub-species. Populations of the widespread sub-species *P. lateralis lateralis* are as divergent as sub-species within *P. lateralis* and there is negligible recent gene flow between most populations/sub-species. Individuals on islands have extremely low genetic diversity and high inbreeding coefficients, in contrast to the naturally fragmented mainland populations. Our results indicate historical connectivity of populations ~160-640 kya, and indications of bottlenecks for both island and some isolated mainland populations, providing important context for conservation management and potential genetic rescue. However, given the large ecological gradient and chromosomal variation within this widespread species, assessment of ecological differences will be important before decisions to mix across geographically distant populations and/or sub-species.

Keywords: phylogeography, *Petrogale lateralis*, arid biome, evolution, demographic history

Introduction

Arid biomes, including arid, hyper-arid and semi-arid regions (arid index <0.3 ; <100 - 300 mm annual precipitation), encompass $\sim 30\%$ of the global landmass and are under ongoing biodiversity loss and decline as a result of climate change and associated threats (Zhang et al., 2023). Unlike more mesic biomes (e.g., temperate and tropical regions) where we have a better understanding of organisms' responses to geologically recent climatic and environmental change, our understanding of arid adapted organisms is less well developed (Pepper & Keogh, 2020). Fossil and geological records in arid biomes are often limited and thus genetic information has been essential to understanding processes of speciation and diversification (e.g., Riddle & Hafner, 2006; Brito et al., 2014; Pepper & Keogh, 2020). Aridification began during the Miocene-Pliocene to form contemporary global arid biomes (Riddle & Hafner, 2006). Pleistocene climatic fluctuations caused range expansions from refugia in temperate areas of the Northern Hemisphere (Hewitt, 2000) yet led to localised persistence across multiple refugia during unfavourable times for low dispersal and/or habitat specialists, generating substantial phylogeographic structure in arid biomes (e.g., Brito et al., 2014; Burriel-Carranza et al., 2024; Riddle & Hafner, 2006). Genetic studies are now highlighting how these vast arid landscapes are associated with high levels of endemism and often "micro-hotspots" of biodiversity (Bruto et al., 2014; Burriel-Carranza et al., 2024).

Today, many arid zone species are threatened due to the amplified impact of climate change along with ecological disturbance in desert habitats (Loarie et al., 2009). Understanding responses of species to recent climatic change is pertinent to understand how to manage species for conservation (Hewitt, 2004a, 2004b; Byrne et al., 2008). Australian deserts are a case in point. Encompassing $\sim 70\%$ of the continent, the arid biome represents one of the largest desert ecosystems in the world, dominated by stony and sandy desert systems that

formed during the late Pliocene and mid-Pleistocene (Fujioka et al., 2005, 2009; Byrne et al., 2008; Pepper & Keogh, 2020). The landscape is heterogeneous, with rocky outcrops and range systems forming habitat islands amidst the deserts (Pepper & Keogh, 2020). The unstable and variable climatic conditions began oscillating from the Pliocene and intensified into the Pleistocene (see Byrne et al., 2008), with fluctuating moisture and climates during the glacial interglacial cycles, although with varied evidence for whether the glacial periods were hyper-arid or moisture rich (see Weij et al., 2024). Either way, climatic changes and instability during the glacial interglacial periods had different responses amongst organisms in the arid regions. Some widespread species (ecological generalists) had little or no divergence among populations, compared to others that had substantial phylogeographic structure with scattered rocky ranges as likely refugia (reviewed in Byrne et al., 2008; Pepper & Keogh, 2021). This period of climatic change also resulted in some groups undergoing rapid speciation and divergence (e.g., *Pseudomys* – Smissen & Rowe, 2018; lizards - Tejero-Cicuéndez et al., 2022; *Heteronotia* geckos - Fujita et al., 2010). Compared to reptiles and birds, there have been rather few studies of widespread arid zone mammals. The studies to date reveal low genetic structure and signatures of widespread expansion (e.g., delicate mice in the genus *Pseudomys*, Roycroft et al., 2024; dasyurid marsupials, Umbrello et al., 2020, red kangaroo *Osphranter rufus*, Clegg et al., 1998).

Many Australian taxa are threatened or have already become extinct since European colonisation in 1788 (see Burbidge et al., 2009; Woinarski et al., 2019). Arid Australian mammals have been the focus of intensive conservation initiatives where species are released within feral predator-free fenced exclosures and islands to manage population numbers and reintroduce species from sites where they have gone locally extinct (Garnett et al., 2018). Arid regions have been subject to the greatest number of Australian mammal losses, both

local and global extinction, and this biome has been suggested to be at risk due to changed land use in some areas, introduced herbivores, impacts of altered fire regimes, and introduced predator access in open environments (McKenzie et al., 2007; Johnson & Isaac, 2009, Woinarski et al., 2019). Recent comparative analysis of extinct and threatened Australian rodents identified elevated extinction in the arid biome (Roycroft et al., 2021). Further research to understand diversification of extant Australian mammals across the arid region and the processes shaping their genetic diversity is important to ongoing conservation initiatives of Australia's unique mammal fauna. The ecologies of mammals in desert ecosystems have been shown to vary despite shared climatic and environmental conditions, with some species exhibiting fluctuating population dynamics (i.e., boom-bust) associated with the resource availability (see Greenville et al., 2016). This indicates diverse responses to past climatic changes across the arid zone. A recent long-term empirical study of small mammals in the Simpson desert found gene flow during periodic population booms was important in maintaining genetic diversity in a species with fluctuating population dynamics (e.g., many arid species) (Stringer et al., 2024). Yet we still lack a broad understanding of how diverse organisms maintain their genetic diversity and how their genetic variation is partitioned across the Australian arid biome. We have little knowledge of how species persist in ongoing refugia during climatic and resource fluctuations, whether multiple areas of refugia exist and persist over climatic cycles, whether refugial areas are common amongst diverse organisms, and how they differ between ecological generalists and habitat specialists. Understanding how populations and species persist across the heterogenous arid landscape of Australia will be important to identifying how we can best protect species moving forward and how mammals in desert ecosystems persist under such variable environmental conditions.

Rock-wallabies (genus *Petrogale*) are a good case study to understand diversification processes across the arid zone, as they are habitat specialists, restricted to rocky habitats which in the arid zone of Australia are separated by large expanses of low-relief sandy or stony deserts (reviewed in Pepper & Keogh, 2020). Of the 17 *Petrogale* species, four occur in the arid biome. Dispersal does occur in rock-wallabies, but it is generally localised and limited (e.g., Pope et al., 1996; Hazlitt et al., 2006; Piggott et al., 2006). *Petrogale lateralis*, the black-footed rock-wallaby, has a large (~2.65 million km²) and naturally fragmented distribution across the central and western arid zone, within which five sub-species are recognised based on subtle differences in morphology and chromosome rearrangements (2n=20-22 karyotypes, including Robertsonian fusions and centric shifts; Briscoe et al., 1982; Sharman et al., 1990; Eldridge & Potter, 2020; Eldridge & Pearson, 2023; see Fig. 1). *Petrogale lateralis* offers a new biological perspective to explore how habitat specialists are genetically structured in the arid zone and their demographic response to past climatic changes. This will provide greater insight into where and how species have persisted across this heterogeneous landscape.

Petrogale lateralis has been the focus of numerous genetic studies that revealed low yet structured diversity among sub-species but with limited sampling across geographic isolates (Eldridge & Potter, 2020; Eldridge et al., 2021; Potter et al., 2012, 2017). Finer-scale genetic studies have been undertaken for *P. l. centralis* and *P. l. lateralis* and shown low diversity and limited contemporary gene flow between populations (Eldridge et al., 2004; Ruykys & Lancaster, 2015, West et al., 2019). A chromosomally heterogeneous population (2n=21-22) has been identified at Townsend Ridges in central eastern Western Australia (Eldridge & Pearson, 1997; Pearson, 2004), which mitochondrial data indicates is a hybrid population (Eldridge et al., 2021). Interestingly, experimental crosses between chromosomally divergent

P. l. centralis and *P. l. pearsoni* (fixed differences include a centric shift; see Fig. 1) produced fertile male and female hybrids and backcrosses (Close & Bell, 1997; R. L. Close, unpubl. data). However, these genetic studies have not yet had sufficiently comprehensive geographic and genomic coverage to fully understand the demographic history of the species and associated sub-species in response to past climatic changes.

Due to natural rocky habitat patchiness and widespread declines within the last 150 years the current distribution of *P. lateralis* is highly fragmented (Fig. 1). Two sub-species, *P. lateralis hacketti* and *P. l. pearsoni* are restricted to islands off the south coast of Australia.

Of the five sub-species, two are listed as Endangered (*P. l. lateralis* and *P. l. kimberleyensis*) and the other three are listed as Vulnerable under the Australian *Environment Protection and Biodiversity Conservation (EPBC) Act* 1999. *Petrogale l. centralis* and *P. l. lateralis* are actively managed for conservation with ongoing translocations, reintroductions, augmentations, and captive breeding used to bolster species numbers (e.g., Pearson et al., 2019; West et al., 2018; Nilsson et al., 2023). Some populations, particularly *P. l. lateralis* from Barrow Island and *P. l. pearsoni*, are highly inbred (Eldridge et al., 2001, 2004) and understanding the evolutionary history of *P. lateralis* across its' vast ecological and latitudinal gradient will assist in identifying future options for maximising genetic diversity through genetic rescue. In particular, how do island population dynamics compare to mainland populations, and do island populations need active management. In this study we incorporate both modern and historic museum samples to determine population structure and diversity across the range and evaluate their divergence history in response to past climatic changes. This study will not only shed light on how a widespread arid zone mammal has persisted and diversified across the Australian arid biome during the climatic changes of the

Pleistocene but also provide an important foundation for ongoing conservation management for the species.

Materials and Methods

Sampling and sequencing

Samples of *P. lateralis* were collected from the Australian Museum, Western Australian Department of Biodiversity, Conservation and Attractions, Western Australian Museum, and the Natural History Museum, London. In total, 97 samples were analysed from across the geographic range of *P. lateralis*, and included: *P. l. centralis* (n=10), *P. l. hacketti* (n=11), *P. l. kimberleyensis* (n=11), *P. l. lateralis* (n=41), and *P. l. pearsoni* (n=11), as well as six samples from Cotton Creek, Western Australia (karyotypes unknown) and seven samples from Townsend Ridges (putative hybrid population, Eldridge et al. 2020) (refer to Fig. 1 for distribution map of samples and Supp. Table 1 for sample information). Samples were collected from historical samples (1906-1959) and modern samples. The historical samples comprised biopsies from museum skins and some represented unique localities.

DNA was extracted from samples and sequenced as part of the Oz Mammal Genomics Framework initiative of BioPlatforms Australia. DNA from seven museum skin samples was extracted at the Australian National University's Ecogenomics and Bioinformatics Laboratory Trace DNA facility, using the DNeasy Blood and Tissue Kit (Qiagen) with modifications based on Joseph et al., (2016). Full details of this extraction method are available in Nistelberger et al., (2023). Extraction negative controls were included in each batch of extractions to monitor for contamination. DNA from two additional historical museum specimens (BMNH 1906.8.1.256 and BMNH 1939.2849) was extracted from approximately 10 mg of dried skin tissue, using the protocol described in Dabney et al., (2013) with

modifications as outlined in Brace et al., (2022). DNA extraction for these two samples was completed in the ancient DNA laboratory at the Natural History Museum, London. DNA from modern tissue samples was extracted either: 1) at the Australian Museum using a phenol chloroform method (Sambrook et al., 1989; 23 samples); 2) at the Australian Museum using a salting-out method (Sunnucks & Hales, 1996; 31 samples); or 3) by the Western Australian Department of Biodiversity, Conservation and Attractions using a salting-out method (34 samples). All DNA extracts were then prepared for a targeted exon capture approach where ~3000 exons were targeted for capture using a custom NimbleGen SeqCap EZ hybridisation kit (see Roycroft et al., 2022). Probes were designed from seven marsupial transcriptomes from across the order, as outlined in Bragg et al., (2016) and Duchene et al., (2018). Each sample was uniquely barcoded, and libraries prepared using the Meyer-Kircher library protocol (Meyer & Kircher, 2010), including some modifications from Bi et al., (2013). Samples were pooled in equimolar ratios (to a total of 1.2 ug) and hybridised with 5 ug of mouse cot-1 DNA following the manufacturers protocol for 72 hr. Hybridisation pools included libraries from 96 samples for DNA from modern specimens, and libraries from 64 samples for DNA from historical museum specimens (note that samples from other marsupial species were also included in each hybridisation pool to make up the total numbers). Post hybridisation, the sample was amplified in two separate enrichment PCRs (Phusion hot start kit, Thermo Scientific™; and IS5 and IS6 primers, Meyer and Kircher 2010) and cleaned up using the QIAquick PCR purification kit (QIAGEN). Quality control checks were made following the protocol of Potter et al. (2022). Samples were then sequenced across Illumina HiSeq 2500 and Illumina NovaSeq 6000 (100bp paired-end run) platforms at the ACRF Biomolecular Resource Facility at the Australian National University.

Bioinformatics and datasets

The raw sequencing data was processed, assembled and aligned using the Exon Capture Pipeline for Phylogenetics (ECPP, <https://github.com/Victaphanta/ECPP>) following the protocol of Roycroft et al., (2020) with some modifications. Briefly, we deduplicated raw paired reads using dedupe.sh (part of the BBTools package, Bushnell B. 2015, sourceforge.net/projects/bbmap) and quality trimmed using Trimmomatic (Bolger et al., 2014). We then *de novo* assembled the cleaned reads from each sample using Trinity v2.0.6 (Grabherr et al., 2011; Haas et al., 2014), and selected the highest-quality *de novo* assembly for each clade to be used as a reference for mapping. This approach has been shown to substantially improve in final data completeness and quality, especially from historical skins (Roycroft et al., 2022). We mapped the cleaned reads to the clade-specific reference using BBmap (version 35.82, Bushnell B. 2015, sourceforge.net/projects/bbmap) with a minimum depth of 10 to retain sites, and called variants using the mpileup2cns command in VarScan v2.3.7 (Koboldt et al., 2012). Resulting consensus sequences of all exons captured per sample were converted to fasta format and aligned using MAFFT (Katoh & Standley, 2013).

Loci with average per-locus heterozygosity >3% heterozygosity across samples (putative novel paralogs), and <90% sample completeness were removed from downstream analysis, leaving 1934 filtered exon alignments for final analysis. Final alignments were processed with BMGE (Criscuolo & Gribaldo, 2010) to remove poorly represented regions. From these final filtered alignments, single nucleotide polymorphism (SNP) datasets were extracted (not including the outgroups) using SNP-sites and scripts from https://github.com/tandermann/snp_extraction_from_alignments. Two SNP datasets were generated, one consisting of all the SNPs ('all SNP' dataset) and another consisting of a randomly selected SNP per exon ('single SNP' dataset).

Population structure and diversity

We estimated genetic clustering of individuals using the two SNP datasets. We used a hierarchical principal coordinate analysis (PCoA) using the package `dartR` (Gruber et al., 2018) in R (v. 3.6.3; R Core Team), where we sequentially removed the most divergent populations from the analysis and reanalysed the data to assess more fine-scale genetic clustering. This analysis was conducted on the ‘all SNP’ dataset. We also assessed population genetic structure using a spatial genetic clustering algorithm which estimates ancestry proportions from ancestral allele frequencies and geographic data in `tess3r` (Caye et al., 2018). We tested various numbers of K (number of genetic clusters) from 1-14 and used the cross-validation plot to assess the best value of K . As this approach can be arbitrary (François & Durand, 2010), we explored multiple values of K and also applied a hierarchical approach where divergent populations were removed to assess more fine-scale structure. This analysis was performed on both the ‘all SNP’ and ‘single SNP’ datasets.

We calculated average individual heterozygosity (H_o) of exon alignments for populations using `AMAS` (Borowiec, 2016), a tool for computing summary statistics. This summarises the data and enables estimation of heterozygous sites as a proportion of the entire dataset. We also calculated population inbreeding coefficients using `dartR` (Gruber et al., 2018) in R (v. 3.6.3; R Core Team) on the ‘all SNP’ dataset and the `gl.report.heterozygosity` function. We estimated effective inbreeding (equation $(1-(H_o/uH_e))$), where uH_e represents average uH_e of mainland populations (Frankham, 1997). We calculated pairwise sequence divergence between populations (D_{xy}) from the exon alignments, as well as within population variation (θ) in `PopGenome` R package (Pfeifer et al., 2014) using all sites (i.e., including missing data).

We tested for isolation by distance between mainland populations and for *P. l. centralis* using a Mantel test in dartR (gl.ibd function; Gruber et al., 2018). We used the individual approach using a Euclidean distance matrix compared to genetic distance. Analyses were run with 999 permutations on the ‘all SNP’ dataset.

Divergence history

Phylogenetic analysis of the concatenated dataset for all individuals was estimated using IQTree2 (Minh et al., 2020). The analysis was run using the MFP criterion and 1000 bootstrap replicates. We also assessed relationships using a phylogenetic network approach in SplitsTree4 using the NeighborNet analysis (Bryant & Moulton, 2002; Huson & Bryant, 2006). This approach allows visualisation of reticulation in a network as it clusters individuals and uses a matrix of uncorrected P distances to link individuals.

We then estimated divergence time of populations using a single individual per population using the concatenated nuclear dataset. We estimated the divergence time using MCMCTree (Rannala & Yang, 2007). We used soft bound calibrations based on the upper and lower confidence limits from a fossil calibrated divergence tree for *Petrogale* from Potter et al., (2012). Three calibrations were used: 0.53-1.79 mya for the node connecting all *P. lateralis* individuals, 0.13-0.59 mya for the node connecting the two *P. xanthopus* sub-species and 3.18-8.62 mya for the node connecting *P. xanthopus* to *P. lateralis*. The HKY nucleotide substitution model was applied with four discrete gamma categories and 0.5 alpha for gamma rates at sites. The analysis was run using the approximate likelihood approach (dos Reis & Yang, 2011), independent rates model and including all missing sites. The analysis was run for 10 million generations sampling every 1000 generations (total 10,000 samples) after a burnin of 1 million generations.

Population demographic history

We tested for evidence of population expansion of the mainland populations of *P. lateralis* as a species as well as for individual populations using the concatenated exon alignment data.

We assessed evidence of expansion by estimating *Tajima's D* (Tajima, 1989) in the PopGenome R package (Pfeifer et al., 2014) which assesses signals of non-neutral processes. We ran 1000 coalescent simulations for *Tajima's D* using Hudson's ms (Hudson, 2002) and significance was determined by comparing the observed *Tajima's D* to the top 0.05% of simulated data. We do not apply more elaborate tests for population demographic histories because of the low diversity overall, this would require full genome data.

As we were interested in exploring the degree of recent connectivity or isolation between currently fragmented mainland populations, we also estimated migration between populations and sub-species of *P. lateralis* using MIGRATE 4.0 (Beerli & Palczewski, 2010, Beerli et al., 2019). This Bayesian coalescent-based approach estimates mutation-scaled immigration rates (M) and population sizes (θ). We evaluated all pairwise migration rates between modern samples of mainland populations of *P. lateralis* for independent datasets of 100 loci due to computational limitations to ensure convergence on the same results. The analysis was run using a random starting seed, random starting values from the prior, the Jukes-Cantor sequence model with base frequencies 0.25, varying mutation rate estimated from the data and a uniform prior distribution for θ (0,0.01,0.001; minimum, maximum, Delta respectively) and M (0, 2000, 200). Two replicates were run for each analysis and included one long chain and four heated chains. A static heating scheme was used with temperatures set to 1, 1.5, 3 and 10^6 ordered from cold to hot, with sampling every 100 steps and run for 10 million steps after a burnin of one million. The mutation-scaled estimates were then

converted to real estimates from the following equations $\Theta_i = xN_e\mu$ and $M=m/\mu$, where μ is the mutation rate per generation and x is a multiplier that depends on the ploidy and inheritance of the data ($x=4$ for nuclear data). Here we used the mutation rate from Johnson et al., (2018). The product of $\Theta/4$ and M estimates the absolute rate of gene flow, here, the number of heterospecific genomes entering the population per generation.

Lastly, we evaluated the role of drift and migration in the evolutionary history of *P. lateralis*. We ran *TreeMix* (Pickrell & Pritchard, 2012) on the ‘single SNP’ dataset to estimate the population splitting history using allele frequency data. We used *P. l. centralis* as the outgroup based on the phylogenetic results above (IQTree2) and compared different admixture events ($m=0-30$). We ran the analysis with 1000 bootstraps, $k=1$ window size (since independent SNPs) with 10 replicates per iteration. We determined the best model of migration given the data using the package OptM in R (Fitak, 2021). OptM infers delta m based on the second-order rate of change in the log likelihood between different models. We ran the plotting funcs.R script associated with the *TreeMix* package in R and used the commands *plot_tree* and *plot_resid* to examine the *TreeMix* results and visualize the fit of the model to the data. The residual plots of covariance indicates populations that are not well-modelled, with positive residuals indicating underestimation of observed covariance between population pairs and negative values implying overestimation of the model. Good fit of the model to the data should be close to zero.

Results

Genomic datasets

We generated three different datasets to investigate the evolutionary and population history of *P. lateralis* using different approaches: (1) a diplotype sequence dataset of nuclear loci for 97

individuals (1,103,673 bp); (2) ‘all SNP’ dataset - a SNP dataset incorporating all SNPs across all exons (9168 SNPs); and (3) ‘single SNP’ dataset - a single SNP per exon dataset consisting of 1934 SNPs. Depending on the comparison for downstream analyses (pairwise analysis vs. all population), subsampled SNP datasets were generated from the full dataset to include only biallelic sites for that comparison. Most samples had low missing data (0.2-4%), including the skins (0.4-7%), with only one sample having more than 7% missing data (a modern *P. l. hacketti* sample with 27%) for the exon sequences (refer to Supp. Table 1 for missing data metrics).

Population structure and diversity

The hierarchical approach we applied identified multiple levels of population differentiation within *P. lateralis*. The island populations/sub-species were the most differentiated based on the PCoA analysis of ‘all SNPs’, with *P. l. pearsoni* diverging on PC1 (10.2%) and *P. l. hacketti* and *P. l. lateralis* from Salisbury Island and Barrow Island diverging on PC3 (6.6%). With individuals from islands removed, analysis of mainland individuals indicated differentiation of *P. l. centralis* (PC2 - 7.9%) and *P. l. kimberleyensis* individuals (PC3 – 5.5%), as well as individuals from the Kalbarri population of *P. l. lateralis* (PC3; Supp. Fig. 1). When mainland *P. l. lateralis* populations were analysed alone, individuals from Calvert Range separated on PC2 (10.4%), whilst individuals from Cape Range and Wheatbelt diverged on PC3 (8.5%; Supp. Fig. 1). The tess3r results, based on the ‘single SNP’ dataset, supported $K=4$ for all *P. lateralis* individuals. Genetic clustering resolved three island populations as discrete clusters, along with a cluster of mainland individuals, and some evidence of admixture (Supp. Fig. 2a). *Petrogale l. hacketti* and *P. l. lateralis* from Salisbury Island formed one genetic cluster, *P. l. lateralis* from Barrow Island a second, and *P. l. pearsoni* the third genetic cluster. Analysis of only the mainland populations and sub-species

indicated $K=8$ genetic clusters with some varied levels of admixture (Supp. Fig 2b). Most discrete geographic populations formed independent genetic clusters with small amounts of admixture; however, *P. l. kimberleyensis* split into two genetic clusters, as did *P. l. centralis*. Samples from Cotton Creek, Townsend Ridges and some of the *P. l. centralis* individuals were genetically admixed with ancestry from multiple mainland populations. When *P. l. kimberleyensis* and *P. l. centralis* were analysed separately, *P. l. kimberleyensis* supported two genetic clusters (Supp. Fig. 2c) whilst *P. l. centralis* did not show support for discrete genetic clustering but more a pattern of isolation-by-distance.

Genetic divergence between populations/sub-species (D_{xy}) revealed very low levels of divergence between populations of *P. l. lateralis*, populations of *P. l. kimberleyensis*, as well as between these populations and the other sub-species (0.0005-0.0007; Supp. Table 2). The highest divergence was between *P. l. centralis* and *P. l. pearsoni*, and between *P. l. centralis* and *P. l. lateralis* from Calvert Range (~ 0.0007). The lowest diversity was between *P. l. hacketti* and *P. l. lateralis* from Salisbury Island (0.0004). Despite low genetic divergence overall, significant isolation by distance was detected on the mainland and for *P. l. centralis* when analysed independently (Supp. Fig. 3).

Within each genetic cluster individual heterozygosity based on the diplotype sequence dataset was low, ~ 0.0002 for island populations and sub-species up to ~ 0.0007 for *P. l. centralis* (Fig. 3). Townsend Ridges had the second highest heterozygosity (~ 0.0005), midway between *P. l. centralis* and *P. l. kimberleyensis* populations. Mainland *P. l. lateralis* populations had similar heterozygosity levels (~ 0.0004) to other arid zone *Petrogale* taxa e.g., sub-species of *P. xanthopus*. Genetic diversity within these populations showed similar trends to heterozygosity, with the islands generally having almost an order of magnitude

lower theta values (~ 0.00003) compared to mainland populations (~ 0.0004), though *P. l. kimberleyensis* north also had lower theta values (0.0001) (Supp. Table 2). Again, *P. l. centralis* had the highest theta value (~ 0.0006), with as much diversity within this sub-species as comparisons between it and other populations/sub-species. The inbreeding coefficients showed similar trends to the heterozygosity results, with the islands and the mainland *P. l. kimberleyensis* north and *P. l. lateralis* Kalbarri populations having higher values (0.1-0.6) (Supp. Table 3). The remaining mainland populations/sub-species had lower inbreeding coefficients with *P. l. centralis* and Townsend Ridges having the lowest values.

Divergence history

Phylogenetic analysis of the nuclear exon sequence data supported monophyletic lineages for only two of the sub-species, *P. l. pearsoni* and *P. l. kimberleyensis* (Fig. 2). *P. l. centralis* formed a paraphyletic clade at the base of the phylogeny. *Petrogale l. hacketti* formed a lineage which included the proximate *P. l. lateralis* from Salisbury Island which have a different karyotype, as well as an individual from the now extinct Lucky Bay (ID: 364035) site on the adjacent mainland. *Petrogale l. lateralis* formed multiple well supported lineages (bootstrap support (bs) = 100) associated with identified genetic clusters (Supp. Figs. 1, 2). These lineages represented fragmented populations of *P. l. lateralis* clustered with *P. l. pearsoni* and *P. l. hacketti*, but with low support connecting these lineages (bs = 48-99). For *P. l. lateralis* there was strong support for Wheatbelt and Kalbarri populations to group together and weaker support for the placement of these populations with respect to populations at Barrow Island, Calvert Range and Cape Range. *P. l. kimberleyensis* supported two monophyletic populations (bs = 86). Individuals from Cotton Creek formed a monophyletic lineage (bs=100) separate from the other *P. l. lateralis* populations (bs=80).

The Townsend Ridges hybrid population formed a monophyletic lineage (bs=100) at the base of the tree, between *P. l. centralis* and individuals from Cotton Creek (bs=100).

The phylogenetic network largely clustered *P. lateralis* into sub-species, but again with the individual populations of *P. l. lateralis* showing distinct clustering (e.g., populations as divergent as sub-species; Fig. 2). There is evidence of reticulation amongst populations and sub-species, represented by webs towards the base of the network. *Petrogale l.*

kimberleyensis forms two separate clusters in the network. *Petrogale l. lateralis* from Salisbury Island clustered at the base of *P. l. hacketti*. Townsend Ridges was placed between *P. l. centralis* and *P. l. kimberleyensis* in the network. Unlike the phylogenetic tree, Cotton Creek clustered amongst the *P. l. lateralis* populations. There were long branches leading to island populations for *P. l. pearsoni* and *P. l. lateralis* from Barrow Island.

The divergence of populations of *P. lateralis* were estimated during the Pleistocene ~640 kya continuing to ~160 kya (Supp. Fig. 4). *Petrogale l. centralis* was the earliest sub-species to diverge (640 kya), followed by potentially admixed populations from Townsend Ridges and Cotton Creek (350-410 kya). *P. l. kimberleyensis* diverged ~320 kya and *P. l. pearsoni* diverged ~250 kya from the remaining *P. lateralis*. The proximate *P. l. hacketti* and *P. l. lateralis* from Salisbury Island diverged ~160 kya and the remaining *P. l. lateralis* populations diverged 190-260 kya. The two populations of *P. l. kimberleyensis* diverged ~260 kya. We note here that these estimates should be taken with caution due to potential overestimation below the species level (see Ho et al., 2005).

Population demographic history

Given low genetic differentiation overall, some evidence of admixture between genetic clusters, isolation by distance and recent branching in the phylogenetic analysis, we tested for spatial expansion using modern samples for all populations/sub-species, as well as a second analysis only including populations present on the mainland. Estimates of Tajima's D supported a signal of population expansion with significant negative values for both analyses ($p < 0.05$) (Supp. Table 2). Evaluation of Tajima's D for individual populations did not indicate any significant values.

Estimates of migration between genetic clusters indicated low levels of gene flow between populations/sub-species (0.11-0.34 immigrants per generation). The two independent analyses of 100 loci produced comparable results, with similar theta estimates between populations and sub-species but slightly higher for *P. l. centralis*, and some *P. l. lateralis* populations (Wheatbelt and Cape Range; Supp. Table 4). The highest levels of gene flow came from *P. l. centralis* and *P. l. lateralis* from Cape Range and the lowest were for migration into the Kalbarri population.

When evaluating the role of drift and migration in the evolutionary history of *P. lateralis* we see that genetic drift plays a major role in population divergence, most prominent for island populations/sub-species and very small remnant mainland populations (Fig. 3). Our results from *TreeMix* indicate one migration event best fits the data (Supp. Fig. 5). The migration event varied between different replicates but was more generally between *P. l. centralis* and the ancestor of the two *P. l. kimberleyensis* populations, however the direction of this migration varied but was more often from *P. l. centralis* into *P. l. kimberleyensis*. We note the best model was based on the delta m rather than the 99.8% threshold outlined by Pickrell & Pritchard, (2010) and caution at inferring too much from the migration edges, but rather

focus on the drift in the populations. The residual plot of covariance indicated a good fit of the model to the data for most pairwise comparisons, however some were slightly more positive implying an underestimation of the model to the data (Supp. Fig. 5).

Discussion

Arid environments represent heterogeneous landscapes impacted by irregular and fluctuating environmental changes that impact population dynamics in space and time. The organisms inhabiting these arid habitats show diverse and unique adaptations to persist in these often-harsh environments, yet in Australia we still lack a good understanding of the dynamics influencing species persistence. Incorporation of historical specimens into widespread genomic and geographic sampling of *Petrogale lateralis* has increased understanding of the demographic history of the species across the vast Australian arid biome. Our study exposes a highly fragmented distribution, with exceptionally low diversity and divergence between populations/sub-species caused by two disparate evolutionary processes in close succession. We see evidence of a demographic expansion of *P. lateralis* widely across the vast Australian arid biome during the intense climatic fluctuations of the Pleistocene. This was followed by more recent fragmentation of populations and sub-species, leading to small, isolated populations that diverged ~160-640 kya. Effects of this fragmentation were seen in the significant isolation-by-distance detected between mainland populations, and negligible gene flow between the now geographically isolated regions on the mainland. The lack of recent gene flow for such a widespread species likely drives low genetic diversity within populations and drift driving population divergence (Fig. 3).

The restriction of rock-wallabies to topographically complex rocky outcrops, has likely driven the contemporary divergence pattern we find in *P. lateralis*. This fragmentation has

been further exacerbated by local extinction of populations due to more recent anthropogenic impacts, including introduced predators and herbivores, as well as altered fire regimes (Woinarski et al., 2019; Lavery et al., 2021). Although restriction to isolated rocky range systems is characteristic of this genus, the low heterozygosity estimated for *P. lateralis* is far lower than for other conspecifics with similar habitat requirements (Fig. 3). Interestingly, we see here that other arid zone *Petrogale* taxa (*P. xanthopus celeris* and *P. x. xanthopus*) also show lower levels of heterozygosity than their more mesic relatives. This could be due to more recent divergence and differing ancestral population sizes, or due to fluctuating population dynamics (e.g., recent expansions) compared to more stable populations in mesic relatives. More broadly, mammals in the arid region, have either gone extinct locally or globally (e.g., Moritz et al., 1997; Hogg et al., 2024; Eldridge et al., 2018; White et al., 2018; Nistelberger et al., 2023), or have persisted widely (e.g., Roycroft et al., 2024; Neaves et al., 2012; Clegg et al., 1998; McLean et al., 2018; Umbrello et al., 2020). Most of the major declines and extinctions have involved Critical Weight Range mammals (Woinarski et al., 2015), with arid mammals having the greatest losses (McKenzie et al., 2007). These have been linked to both environment (mean annual rainfall and environmental change) but also species' specific differences (e.g., body weight, phylogenetic similarity, habitat preference) (McKenzie et al., 2007). Differing ecologies and habitat preferences are likely key drivers in diverse mammal responses to climatic changes across the arid zone, together with susceptibility to introduced predators.

Prior understanding of *Petrogale lateralis* evolution has largely come from shallow sampling at a sub-species level (e.g., Loupis & Eldridge, 2001; Potter et al., 2012, 2017; Eldridge & Potter, 2020), or broader geographic sampling with reduced genomic data to understand fine-scale evolutionary processes within a sub-species (e.g., Eldridge et al., 2001; West et al.,

2018; Nilsson et al., 2023). Here, the inclusion of historical museum specimens to increase geographic sampling has enabled robust species-wide analysis of broad evolutionary processes shaping current patterns of diversity. Our results reveal that some populations of *P. l. lateralis* are as divergent from each other as other *P. lateralis* sub-species are from one another, although this divergence amongst lineages is exceptionally low (0.0004-0.0007), particularly for rock-wallabies. These sub-species are currently differentiated primarily by karyotype and morphology (Eldridge & Potter, 2020), although our more extensive geographic and genomic sampling, and overall low genetic differentiation raises questions about their status. Related species from the east coast, which diverged across a similar timescale (Potter et al., 2012, 2017), show higher levels of divergence \sim 0.0006-0.001 (Potter et al., 2022), whilst the monsoonal *brachyotis* group display higher divergence again (0.008-0.002; Potter et al., 2024). This low population differentiation likely represents more recent processes of demographic expansion during the Pleistocene, sometime before 640 kya when populations started to diverge and become isolated.

The response of Australian arid biota from Pleistocene climatic changes appears to follow one of two patterns, either deep geographic structure particularly for low vagility species (e.g. Chapple et al., 2004; reviewed in Pepper & Keogh, 2020) where species have persisted across multiple localised refugia, or low diversity and demographic expansion in highly vagile widespread species (e.g., Kuch et al., 2005, Kearns et al., 2014; Roycroft et al., 2024). Like a majority of highly vagile species in this arid landscape, *P. lateralis* shows evidence of Pleistocene expansion across the Australian arid biome. However, *P. lateralis* differs from other studies to date, in that the recent expansion detected for this species has been followed in quick succession by fragmentation and isolation of populations, as well as reorganisation and fixation of chromosome variation at scale.

Habitat fragmentation and lack of contemporary connectivity in *P. lateralis* has resulted in small population sizes, especially for island populations that exhibit exceptionally low heterozygosity and increased inbreeding coefficients. These offshore islands were connected via land bridges to the mainland up until 8-12 kya when sea levels rose (Lewis et al., 2013). It is unclear from our results whether all of the island populations were isolated well before the rise of sea levels or whether this caused their fragmentation. We do find evidence of one individual from Lucky Bay on the mainland being closely connected to *P. l. hacketti* individuals on islands (Fig. 2) indicating these islands have not been isolated for a long period of time. We see an order of magnitude lower genetic diversity for island populations compared to mainland populations, consistent with previous genetic diversity results based on neutral loci (Eldridge et al., 2001; Lennon et al., 2011). Under increased risk of genetic drift and inbreeding, previous studies have found associations of small effective population sizes with fitness (Eldridge et al., 2001; Frankham et al., 2010). Our results (inbreeding, heterozygosity) indicate these islands have much lower diversity than their mainland counterparts despite becoming isolated at a similar time scale. These island populations need careful conservation planning, including consideration of reduced fitness and evolvability, genetic load, and loss of unique diversity if genetic rescue is contemplated.

Small population sizes have been suggested to play a role in fixation of chromosomal rearrangements in *Petrogale* (Eldridge & Close, 1993). Chromosome rearrangements have been associated with adaptation and can be a driver of divergence and speciation (Rieseberg, 2001; Navarro & Barton, 2003; Kirkpatrick & Barton, 2006; Guerrero & Kirkpatrick, 2014), yet they have also shown little effect on fertility and polymorphisms can persist in populations or be fixed through genetic drift (Walsh, 1982; Lande, 1985). *Petrogale lateralis*

represents an interesting species to explore the evolution of chromosome rearrangements because multiple novel karyotypes have formed in recent history. The polymorphic and hybrid karyotypes at Townsend Ridges provide an opportunity to examine these processes in action. The association of the Townsend Ridges with *P. l. centralis* and *P. l. kimberleyensis* supports previous hypotheses that it is a hybrid population of these two sub-species (Eldridge et al., 2021) based on genetic diversity and chromosome diversity. The low genetic differentiation between chromosomally divergent *P. l. hacketti* (found on three inner Recherche Archipelago islands) and proximate *P. l. lateralis* from Salisbury Island (an outer island in the Recherche Archipelago) is another interesting result that provides information on population dynamics. The recent connectivity of these island populations is further emphasised by their close relationship with a mainland individual at Lucky Bay. Our divergence dating suggests the Salisbury Island population diverged ~100 kya from *P. l. hacketti*. Fixation of the 6-10 fusion on the three inner islands of *P. l. hacketti* indicate this chromosome rearrangement was established in the area before sea levels rose (<15 kya). Further research is required to establish if chromosome variation is associated with localised adaptations or driven by genetic drift, as this will have important consequences for considerations of mixing populations for potential genetic rescue.

Petrogale lateralis covers a huge latitudinal and ecological gradient. Aside from the population structure detected for *P. lateralis lateralis*, our results indicate additional genetic structuring within *P. l. kimberleyensis*. Our results indicate isolation-by-distance (Supp. Fig. 3), consistent with a lack of phylogeographic structure from mtDNA from across most sites and historical connectivity across the vast distribution of *P. l. centralis* (West et al., 2018). *Petrogale l. centralis* centres on the vast rocky region in the centre of Australia, including the MacDonnell Ranges. Previous genetic studies using microsatellites found limited

contemporary dispersal and connectivity between populations within *P. l. centralis* in the Anangu Pitjantjatjara Yankunytjatjara (APY) Lands (West et al., 2018). The MacDonnell Ranges is a large ancient interconnected (~650 km long) mountain range composed of a variety of rock types, and has been identified as an area of refugia for arid zone specialists (Byrne et al., 2008; Pepper & Keogh, 2020). *Petrogale l. centralis* is the most divergent lineage in *P. lateralis* and additional sampling might detect more fine-scale population structure. As this sub-species inhabits the most arid areas of Australia it is likely that populations of *P. l. centralis* were better connected during wetter climates potentially during glacial maxima (see Weij et al., 2024). In contrast, *P. l. kimberleyensis* inhabits sandstone formations within the Fitzroy Trough at the arid-monsoon interface (Eldridge & Pearson, 2023). Our results indicate two genetic populations exist within this sub-species located north and south of the Fitzroy River. Large rivers are known to be barriers to gene flow within this genus (Bee & Close, 1993) and our results indicate these populations diverged ~260 kya.

The exceptionally low genetic diversity and lack of contemporary gene flow between populations of *P. lateralis*, emphasize the need for immediate and ongoing conservation management for this species. The islands harbour exceptionally low genetic diversity, illustrating potential genetic threats for isolated mainland populations if gene flow is not re-established. Mixing *P. lateralis* individuals between populations may be required to maintain genetic diversity. Successful recent mixing of *P. l. lateralis* from Kalbarri, Cape Range and Wheatbelt populations demonstrate the benefits of establishing gene flow between populations (i.e., higher genetic diversity), and showcases the need to think broadly about mixing in *P. lateralis* (Nilsson et al., 2023). Theory suggests that chromosome rearrangements could be associated with local adaptation and result in outbreeding depression (see Frankham et al., 2011). The presence of polymorphic chromosomal populations (i.e.,

Townsend Ridges) implies that change in structural variation may be frequent in this species and perhaps not such a significant factor in consideration of mixing. In addition, we know from experimental crosses that viable and fertile offspring are produced from crossing *P. l. pearsoni* with *P. l. centralis* (Close & Bell, 1997). However, given the large ecological and latitudinal gradient *P. lateralis* inhabits, we need to identify if local adaptation is present between populations/sub-species and if it is linked to chromosomal rearrangements. Small, isolated and inbred populations should be considered candidates for genetic rescue (Frankham, 2015). Where concerns exist about potential outbreeding depression or genetic swamping, mixing could be done experimentally and monitored. Lastly, chromosome information should be obtained for new populations (e.g., Cotton Creek; and Little Sandy Desert, Turpin et al., 2018) to inform their management.

This study builds on recent research showcasing how museum skins can enhance our understanding of the processes shaping species diversification and persistence in environments where the climatic and evolutionary history of organisms is less clear (e.g., Card et al., 2021; Roycroft et al., 2022; Potter et al., 2024). The inclusion of skin samples here revealed unique insight into the recent island connectivity with the mainland for *P. l. hacketti*, as well as sampling that enabled identification of two divergent populations for *P. l. kimberleyensis*. *Petrogale* always been a fascinating genus in which to explore chromosomal variation and its links to diversification. However, our results indicate that the simple chromosomal rearrangements within *P. lateralis* appear unlikely to cause outbreeding depression in this species and maximising genetic diversity is the most important priority for island and small remnant mainland populations. Our study highlights the need to look at the evolutionary history of species at genomic scale to understand the drivers of contemporary patterns to best inform conservation management. Further research is required to understand

similarities or idiosyncrasies in response to past climatic changes across arid Australia and how best to manage species and regions within the arid biome for conservation.

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Conflict of interest

The authors declare no conflicts of interest.

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Figures

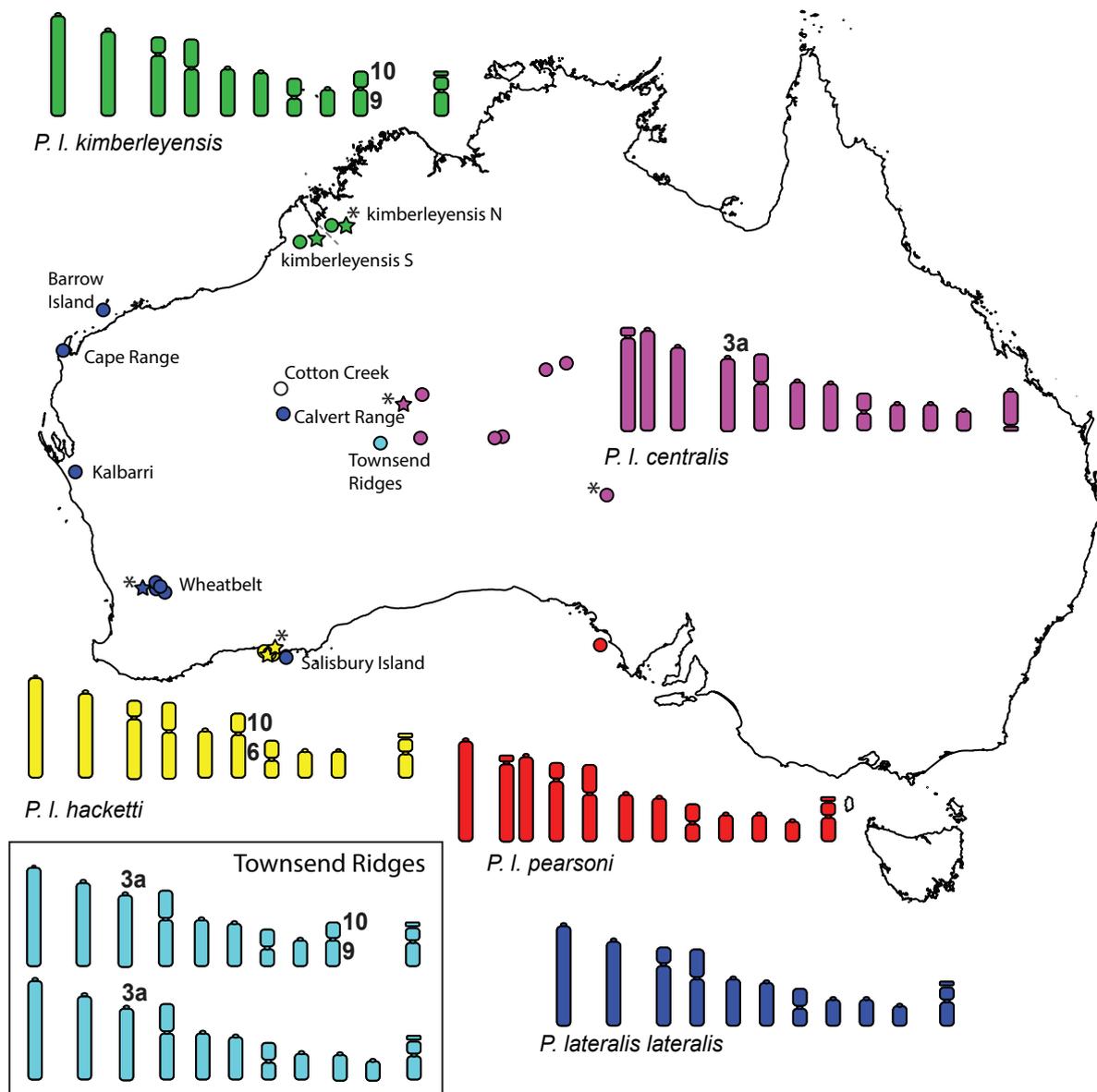


Figure 1 Sampling and karyotypes of *Petrogale lateralis* populations and sub-species across the arid biome of Australia and offshore islands. *Petrogale lateralis centralis* (purple), *P. l. hacketti* (yellow), *P. l. kimberleyensis* (green), *P. l. lateralis* (blue), and *P. l. pearsoni* (red). Sample locations are shown for modern (circle symbol), historical (star symbol), and extinct (asterisks) samples and coloured according to sub-species. Townsend Ridges are included in pale blue as this is a hybrid population of unknown sub-species and Cotton Creek is coloured

white also due to unknown sub-species allocation. There are various chromosomal rearrangements between sub-species. Here we highlight Robertsonian fusions by chromosome numbers in reference to the ancestral karyotype, (a) denotes acrocentric chromosome, and polymorphic karyotypes are shown for chromosome 1 for *P. l. centralis* and chromosome 2 for *P. l. pearsoni*. A single fusion is found in *P. l. kimberleyensis* and *P. l. hacketti*, a centric shift on chromosome 3 for *P. l. centralis* as well as a rearranged X chromosome, and Townsend Ridges has a polymorphic Robertsonian fusion and centric shift on chromosome 3.

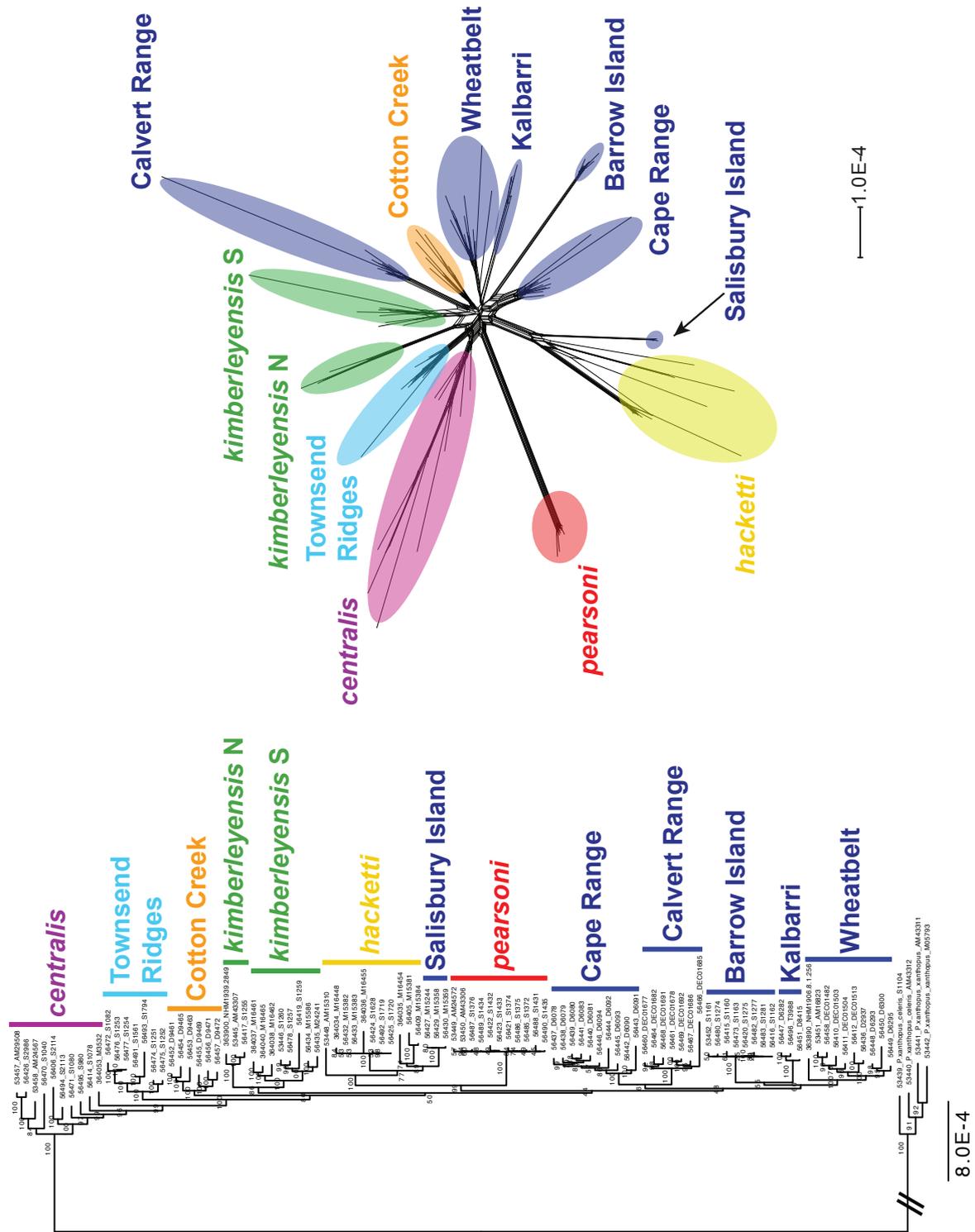


Figure 2 On left, a phylogeny of *Petrogale lateralis* populations and sub-species based on the concatenated nuclear alignment with bootstrap support values on nodes. *Petrogale xanthopus* sub-species are used to root the tree. On right, a phylogenetic network of the same dataset showing the star like shape of the populations/sub-species.

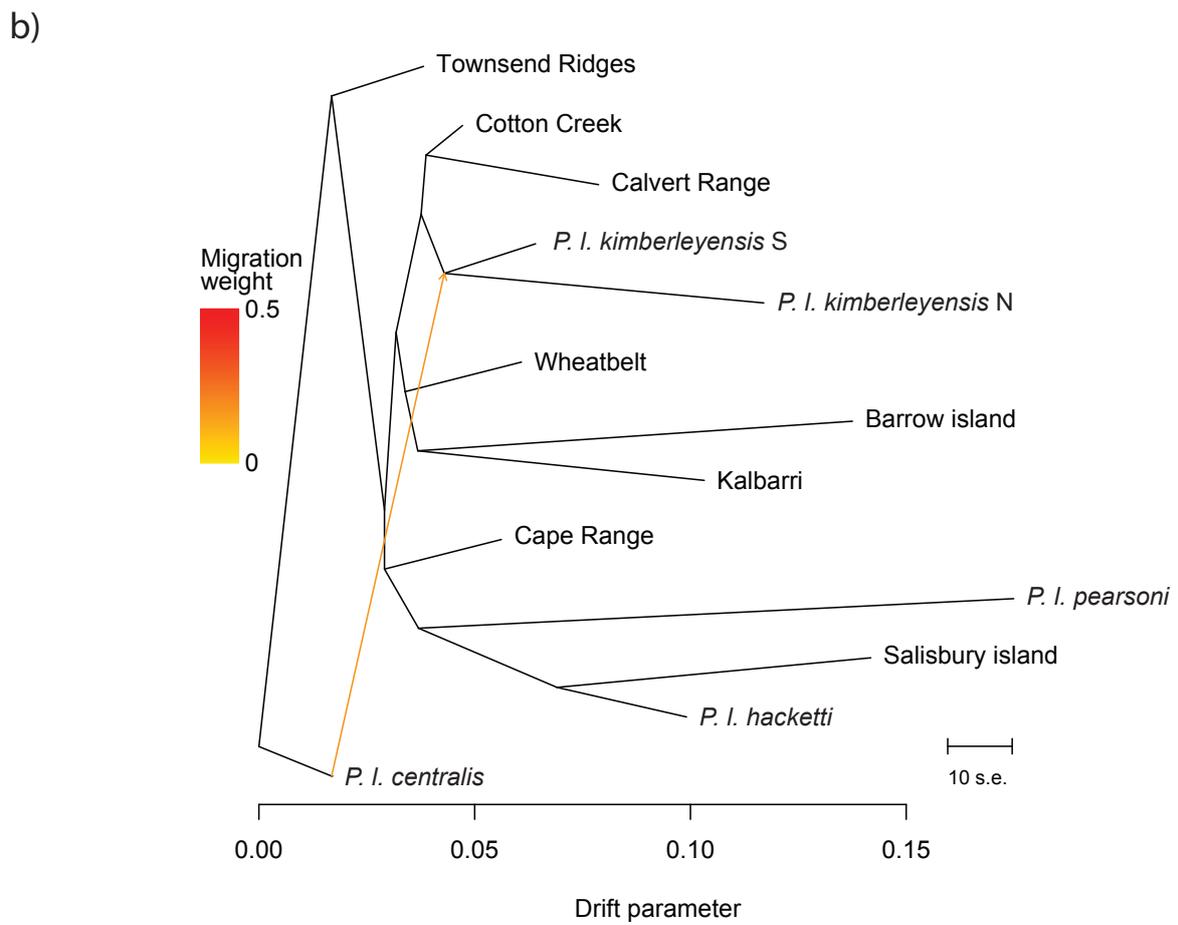
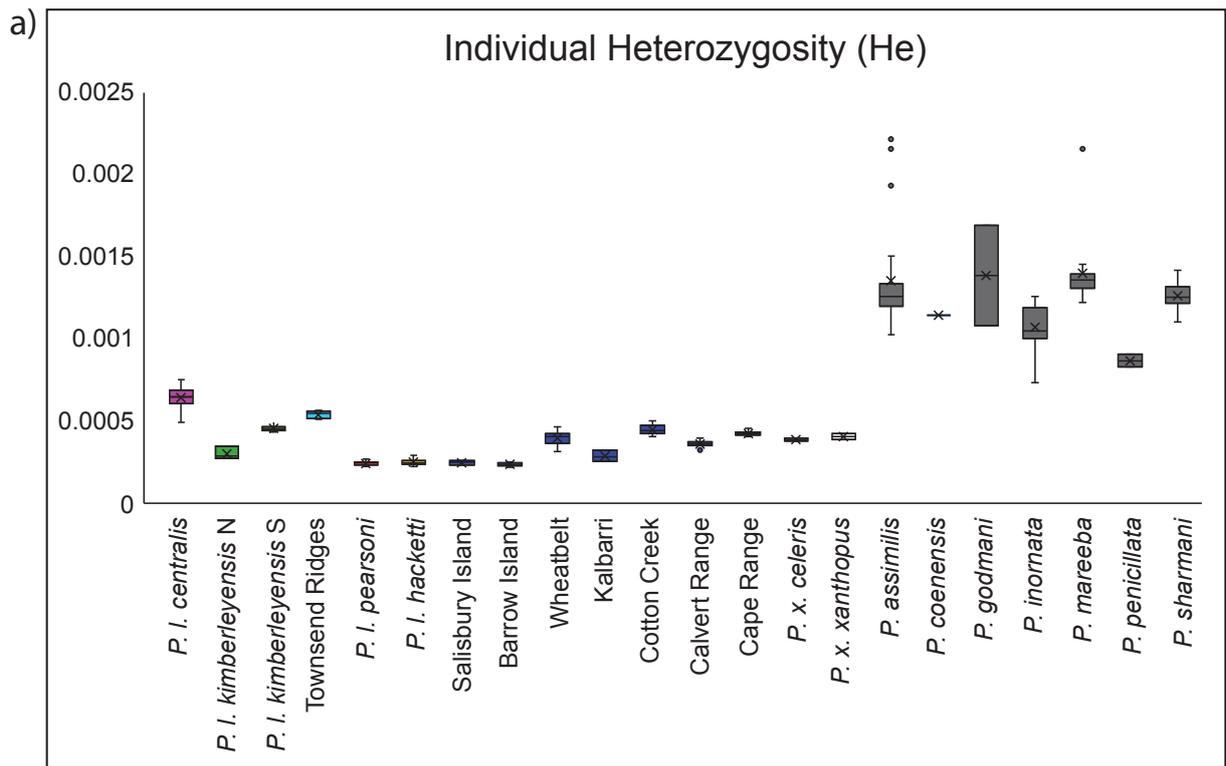


Figure 3 a) Graph of range and average individual heterozygosity values for populations/sub-species of *Petrogale lateralis* based on nuclear exon dataset, as well as estimates from *Petrogale xanthopus* (arid species), and mesic *Petrogale assimilis*, *P. coenensis*, *P. godmani*, *P. inornata*, *P. mareeba*, *P. penicillata* and *P. sharmani*. b) Population splitting results from *TreeMix* highlighting the role of drift in divergence, as well as potential migration between *P. l. centralis* and *P. l. kimberleyensis*.

Table S2 Estimates of theta and Tajima's D for populations/sub-species of *Petrogale lateralis* from PopGenome, and Dxy estimates between populations/sub-species.

Population	Wattersons theta (sites)	Theta	Segregating sites	Tajima D	Tajima D significance							
kimberleyensis_north	159.6667	0.000145	210	N/A								
kimberleyensis_south	397.3381	0.000360	957	0.4106	not significant							
pearsoni	74.53357	0.000068	194	-1.1838	not significant							
centralis	709.0259	0.000642	1747	0.6849	not significant							
Townsend	366.6664	0.000332	671	1.2914	not significant							
hacketti	444.4275	0.000403	1242	-0.8423	not significant							
Salisbury	13.33333	0.000012	16	N/A								
CapeRange	319.5914	0.000290	755	-0.0549	not significant							
Kalbarri	75.33333	0.000068	88	N/A								
Wheatbelt	367.3995	0.000333	831	0.4173	not significant							
Cotton Creek	375.2319	0.000340	681	1.2556	not significant							
CalvertRange	341.8787	0.000310	682	0.4028	not significant							
Barrow	52.94532	0.000048	123	-0.8226	not significant							
all modern samples				-1.824427	significant							
all modern mainland samples				-1.909045	significant							
*islands lowest diversity, centralis highest												
Dxy	kimberleyensis_north	kimberleyensis_north	pearsoni	centralis	Townsend	hacketti	Salisbury	CapeRange	Kalbarri	Wheatbelt	Cotton Creek	CalvertRange
kimberleyensis_south	0.000540859	0.000640861										
pearsoni	0.000656027	0.000688387	0.00073508									
centralis	0.00066783	0.000572726	0.000620671	0.00062034								
Townsend	0.000565015	0.00068321	0.000637416	0.00073543	0.000608175							
hacketti	0.00062129	0.00055348	0.000595339	0.0006632	0.000552401	0.00040848						
Salisbury	0.000545961	0.000512077	0.000599242	0.00064705	0.000513757	0.00055948	0.00049029					
CapeRange	0.000529665	0.000541006	0.000593729	0.00067565	0.000511655	0.00055958	0.000505939	0.00045754				
Kalbarri	0.000544451	0.000525154	0.000594908	0.0006605	0.000538608	0.00057271	0.000507632	0.00045518	0.00045579			
Wheatbelt	0.000541862	0.000509888	0.000564161	0.00063271	0.000499973	0.00056098	0.000509065	0.00046699	0.00046258	0.00046666		
Cotton Creek	0.000540843	0.000556101	0.000641185	0.00071706	0.000583121	0.00061844	0.000558768	0.00050852	0.000514	0.00053694	0.000493415	
CalvertRange	0.000585068	0.000553811	0.000614425	0.00068289	0.000567124	0.00059217	0.000520489	0.00046579	0.00048709	0.000475619	0.000559832	
Barrow	0.000571143											

1103559 valid sites in analysis

Table S3 Estimates of inbreeding coefficients where observed heterozygosity (H_o) was divided by average mainland unbiased expected heterozygosity (uHe) for all populations/sub-species of *Petrogale lateralis*.

Population/Sub-species	H_o	H_e	uHe	inbreeding coefficient
barrow_island	0.06494642	0.04863	0.051871	0.4874
calvert_range	0.13083003	0.111424	0.119995	-0.032596922
cape_range	0.12696261	0.109544	0.11531	-0.002072691
centralis	0.15604636	0.155634	0.163825	-0.231620837
cotton_creek	0.12854435	0.108663	0.118542	-0.014556827
hacketti	0.11063952	0.11448	0.119932	0.126759905
kalbarri	0.11181573	0.074641	0.089569	0.11747648
kimberleyensis_north	0.09978861	0.080946	0.097135	0.212402447
kimberleyensis_south	0.14230265	0.126384	0.134809	-0.123146409
pearsoni	0.07931932	0.060683	0.063573	0.37395959
salisbury_island	0.05251841	0.033488	0.040185	0.585490055
townsend_ridges	0.14366936	0.119845	0.129064	-0.133933386
wheatbelt	0.1428056	0.128249	0.134999	-0.127116022

average mainland uHe
0.126709875

Table S4 Estimates of theta and migration rates for 100 nuclear loci for populations/sub-species of mainland *P. lateralis*.

Population number	Population ID
1	kimberleyensis
2	centralis
3	wheatbelt
4	cape range
5	kalbarri
6	cotton creek
7	calvert range
8	townsend ridges

* samples combined for kimberleyensis as not enough individuals to split to two populations

dataset 100b	Population	Theta	dataset 100	Population	Theta
1	kimberleyensis	0.00116	1	kimberleyensis	0.0016
2	centralis	0.00147	2	centralis	0.00175
3	wheatbelt	0.00142	3	wheatbelt	0.00177
4	cape range	0.00127	4	cape range	0.00223
5	kalbarri	0.00077	5	kalbarri	0.00103
6	cotton creek	0.00105	6	cotton creek	0.0015
7	calvert range	0.00105	7	calvert range	0.0015
8	townsend ridges	0.00118	8	townsend ridges	0.0016

Migration	dataset100b	m*theta	/4 (4Ne)	Migration	dataset 100	m*theta	/4 (4Ne)
M2>1	576.994	0.66931304	0.16732826	M2>1	588.292	0.9412672	0.2353168
M3>1	553.214	0.64172824	0.16043206	M3>1	590.574	0.9449184	0.2362296
M4>1	589.097	0.68335252	0.17083813	M4>1	570.884	0.9134144	0.2283536
M5>1	608.066	0.70535656	0.17633914	M5>1	601.04	0.961664	0.240416
M6>1	565.888	0.65643008	0.16410752	M6>1	582.036	0.9312576	0.2328144
M7>1	558.897	0.64832052	0.16208013	M7>1	608.737	0.9739792	0.2434948
M8>1	570.586	0.66187976	0.16546994	M8>1	578.164	0.9250624	0.2312656
M1>2	564.659	0.83004873	0.207512183	M1>2	547.998	0.9589965	0.23974913
M3>2	546.458	0.80329326	0.200823315	M3>2	552.547	0.96695725	0.24173931
M4>2	581.935	0.85544445	0.213861113	M4>2	531.069	0.92937075	0.23234269
M5>2	602.605	0.88582935	0.221457338	M5>2	582.448	1.019284	0.254821
M6>2	550.447	0.80915709	0.202289273	M6>2	564.12	0.98721	0.2468025
M7>2	564.589	0.82994583	0.207486458	M7>2	563.804	0.986657	0.24666425
M8>2	578.333	0.85014951	0.212537378	M8>2	559.139	0.97849325	0.24462331
M1>3	575.26	0.8168692	0.2042173	M1>3	551.289	0.97574613	0.24393653
M2>3	564.08	0.8009936	0.2002484	M2>3	554.717	0.98184909	0.24546227
M4>3	588.259	0.83532778	0.208831945	M4>3	557.872	0.98743344	0.24685836
M5>3	593.675	0.8430185	0.210754625	M5>3	588.229	1.00576533	0.25144133
M6>3	578.075	0.8208665	0.205216625	M6>3	552.131	0.97727187	0.24431797
M7>3	596.723	0.84734666	0.211836665	M7>3	580.375	1.02726375	0.25681594
M8>3	563.361	0.79997262	0.199993155	M8>3	563.576	0.99752952	0.24938238
M1>4	510.66	0.6485382	0.16213455	M1>4	608.246	1.35638858	0.33909715
M2>4	501.687	0.63714249	0.159285623	M2>4	575.904	1.28426592	0.32106648
M3>4	493.611	0.62688597	0.156721493	M3>4	600.497	1.33910831	0.33477708
M5>4	553.323	0.70272021	0.175680053	M5>4	598.58	1.3348334	0.33370835
M6>4	518.529	0.65853183	0.164632958	M6>4	585.213	1.30502499	0.32625625
M7>4	525.757	0.66771139	0.166927848	M7>4	598.804	1.33533292	0.33383323
M8>4	511.059	0.64904493	0.162261233	M8>4	586.496	1.30788608	0.32697152
M1>5	580.378	0.44689106	0.111722765	M1>5	596.306	0.61419518	0.1535488
M2>5	535.099	0.41202623	0.103006558	M2>5	606.623	0.62482169	0.15620542
M3>5	565.41	0.4353657	0.108841425	M3>5	599.195	0.61717085	0.15429271
M4>5	572.692	0.44097284	0.11024321	M4>5	590.14	0.6078442	0.15196105
M6>5	571.461	0.44002497	0.110006243	M6>5	612.336	0.63070608	0.15767652
M7>5	593.176	0.45674552	0.11418638	M7>5	615.159	0.63361377	0.15840344
M8>5	561.304	0.43220408	0.10805102	M8>5	601.965	0.62002395	0.15500599
M1>6	586.334	0.6156507	0.153912675	M1>6	589	0.8835	0.220875
M2>6	552.614	0.5802447	0.145061175	M2>6	574.226	0.861339	0.21533475
M3>6	573.488	0.6021624	0.1505406	M3>6	603.445	0.9051675	0.22629188
M4>6	604.15	0.6343575	0.158589375	M4>6	606.587	0.9098805	0.22747013
M5>6	605.807	0.63609735	0.159024338	M5>6	629.522	0.944283	0.23607075
M7>6	592.149	0.62175645	0.155439113	M7>6	617.516	0.926274	0.2315685
M8>6	565.607	0.59388735	0.148471838	M8>6	588.657	0.8829855	0.22074638
M1>7	547.731	0.57511755	0.143779388	M1>7	568.864	0.853296	0.213324
M2>7	519.444	0.5454162	0.13635405	M2>7	569.622	0.854433	0.21360825
M3>7	537.675	0.56455875	0.141139688	M3>7	567.189	0.8507835	0.21269588
M4>7	538.774	0.5657127	0.141428175	M4>7	550.701	0.8260515	0.20651288
M5>7	572.152	0.6007596	0.1501899	M5>7	581.196	0.871794	0.2179485
M6>7	544.101	0.57130605	0.142826513	M6>7	553.281	0.8299215	0.20748038
M8>7	542.192	0.5693016	0.1423254	M8>7	551.644	0.827466	0.2068665
M1>8	570.914	0.67367852	0.16841963	M1>8	611.209	0.9779344	0.2444836
M2>8	561.443	0.66250274	0.165625685	M2>8	583.183	0.9330928	0.2332732
M3>8	547.728	0.64631904	0.16157976	M3>8	591.693	0.9467088	0.2366772
M4>8	569.279	0.67174922	0.167937305	M4>8	560.975	0.89756	0.22439
M5>8	577.207	0.68110426	0.170276065	M5>8	595.166	0.9522656	0.2380664
M6>8	568.825	0.6712135	0.167803375	M6>8	590.685	0.945096	0.236274
M7>8	585.958	0.69143044	0.17285761	M7>8	597.431	0.9558896	0.2389724

Figure S1 PCoA results from hierarchical analysis of individuals of *Petrogale lateralis* based on ‘all SNP’ dataset, where divergent populations/sub-species were removed to find additional sub-structure.

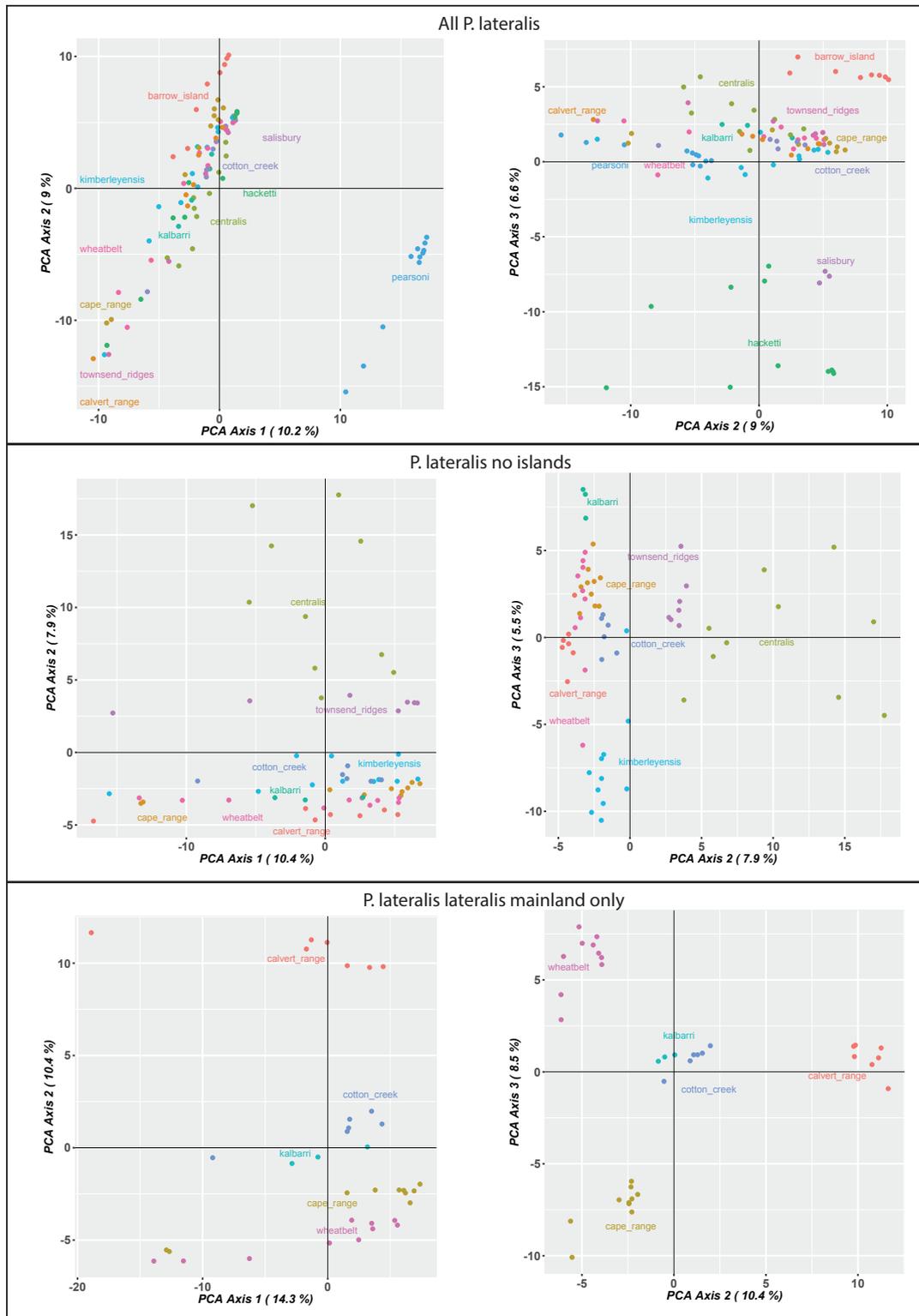
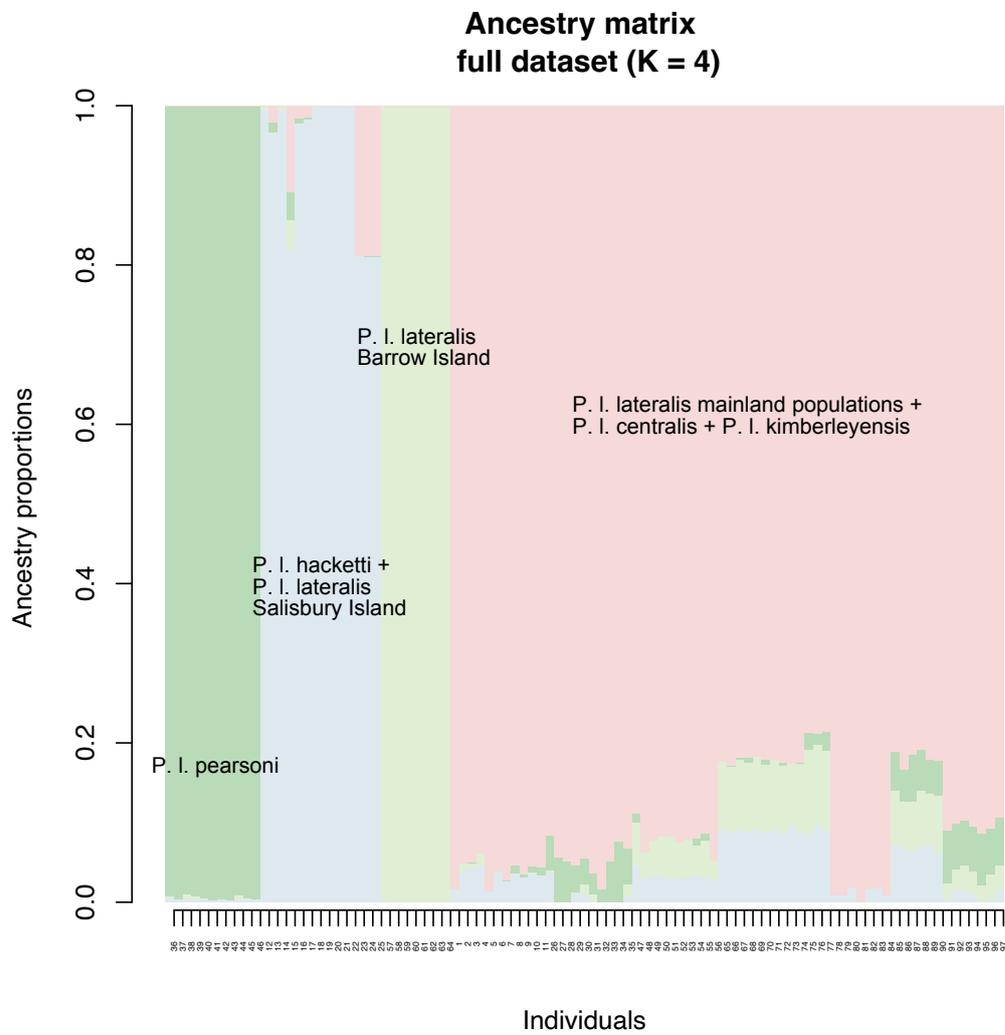
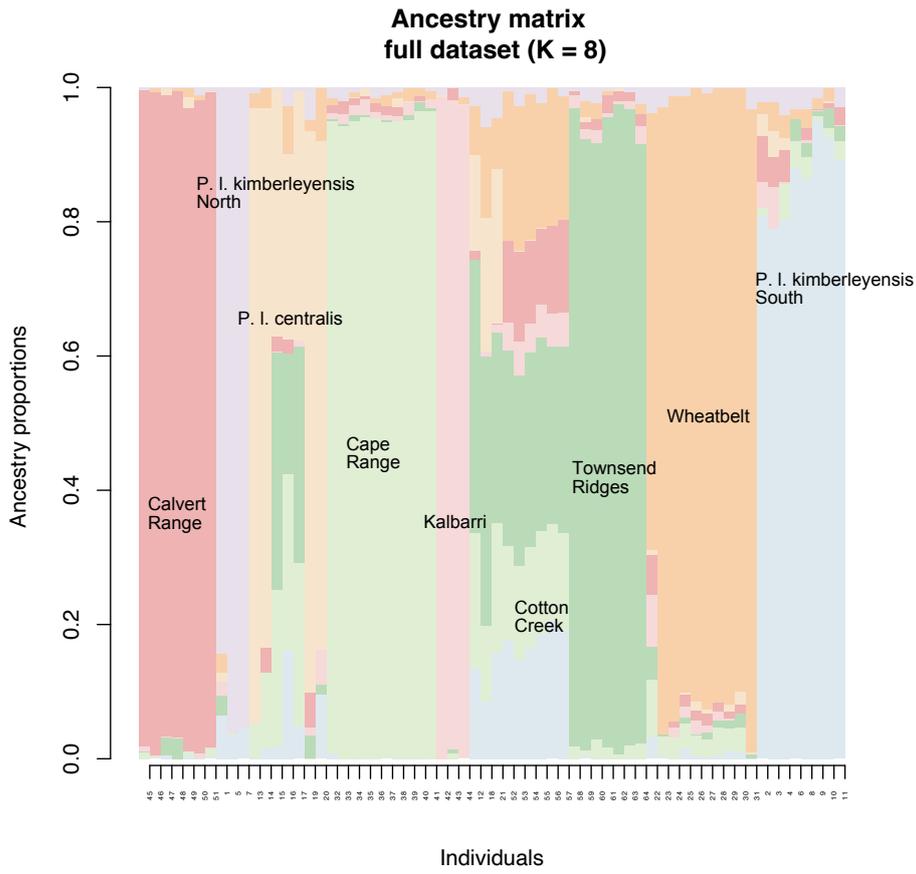


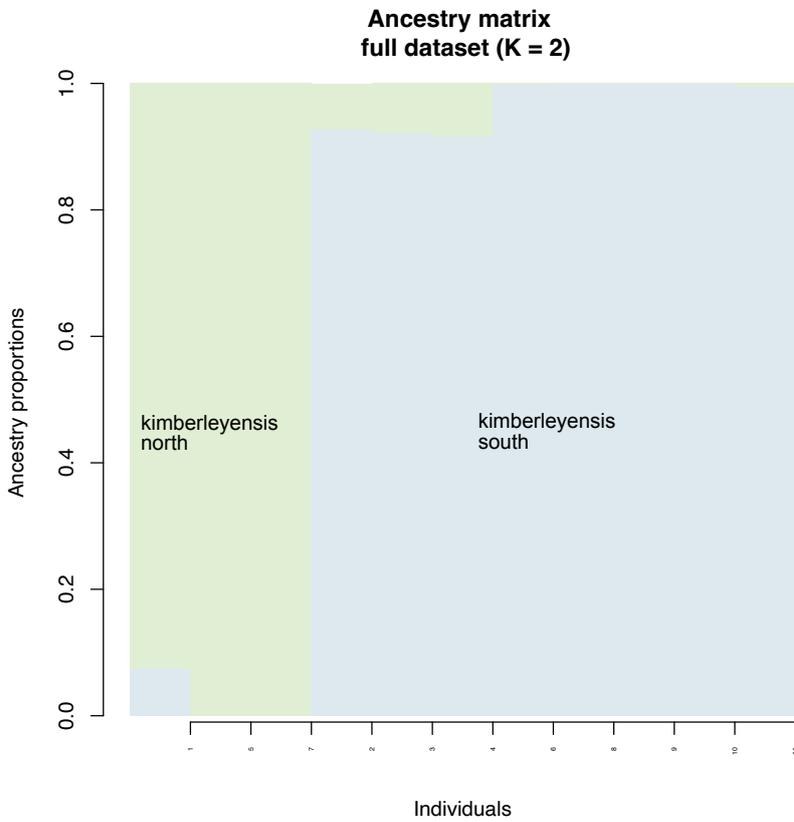
Figure S2 Tess3r results revealing genetic clustering of a) islands separate from mainland *Petrogale lateralis*, b) genetic clustering of mainland populations/sub-species, and c) genetic clustering of *P. l. kimberleyensis*.



a)



b)



c)

Figure S3 Isolation-by-distance of (a) all modern mainland individuals, and (b) modern *P. l. centralis* individuals. Both plots indicate significant isolation-by-distance ($p < 0.05$) based on 999 permutations.

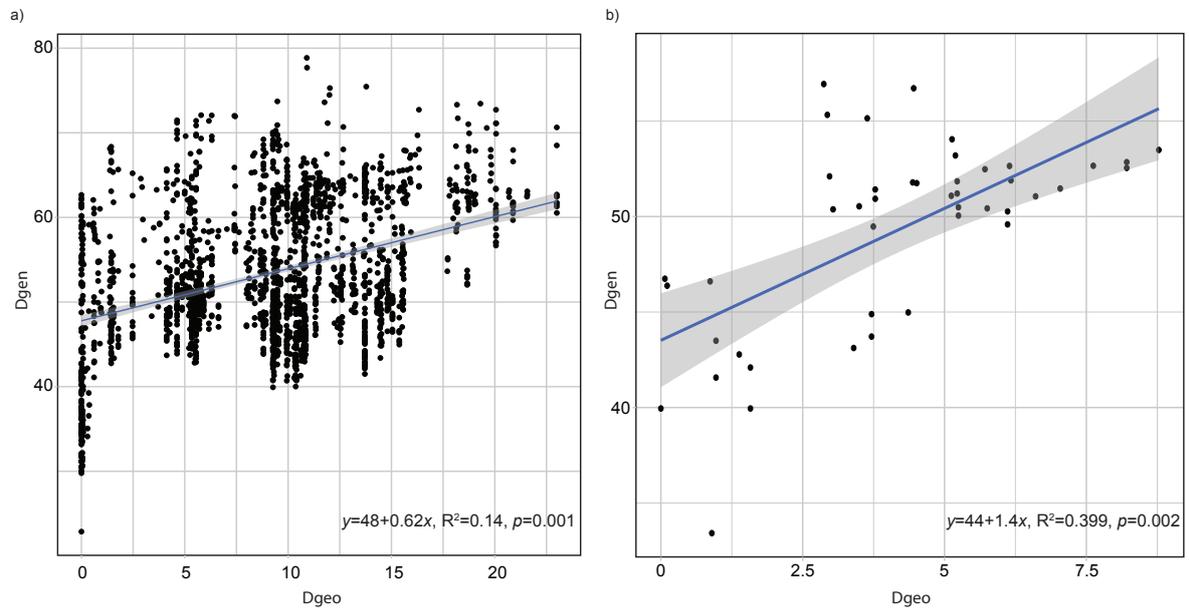


Figure S4 Phylogeny showing the divergence estimates based on MCMCTree analysis. The divergence times are highlighted at each node as well as the scale bar. The blue bars at each node represent the 95% HPD around the divergence estimates.

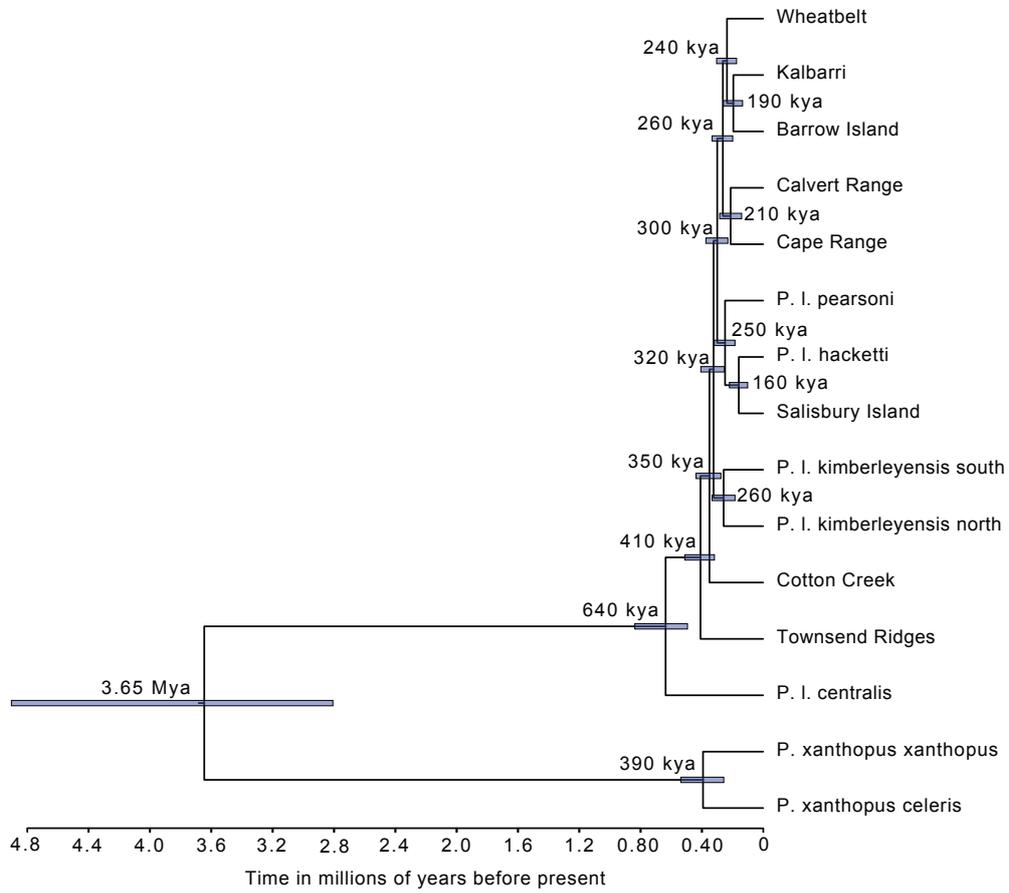


Figure S5 The OptM results showing (a) the mean log likelihood values of the varied migration edge estimates in *TreeMix* from the 10 replicate runs, (b) the delta m estimates highlighting $m=1$ as the best model fit for the data, and (c) the residual plot of covariance for one replicate of $m=1$, indicating some populations/sub-species are not well-modelled, with underestimation of observed covariance but in general most are good (close to zero).

