Genetic connectivity of little penguin colonies in Australia

Sandra Vardeh[1], Jennifer Sinclair[2], Kylie Cairns[3], Belinda Cannell[4], Lee Rollins [3], and William Sherwin[3]

[1]UNSW-Sydney
[2]UNSW Sydney
[3]University of New South Wales
[4]The University of Western Australia

June 2, 2023

Abstract

The Australian range of little penguins, Eudyptula minor, extends around southern Australia, with range-edge sites near the large cities of Perth (west) and Sydney (east). Both range-edges are closer to the equator than the range-core, being likely to experience similar heating with climate change. As a result, movement to one range-edge is not an option for little penguins, unlike in many other species. Therefore, adaptation at the range edge might be very important for little penguins. Capacity for future adaptation depends upon the variability each site holds, and the amount of exchange between sites. In peripheral sites, incoming dispersal might either forestall demographic collapse and replenish genetic variation (good), or overcome local adaptation and increase disease transmission (bad). We aimed to establish the genetic variability in each site, and the exchange (dispersal) of individuals between sites. Genetic markers included biparentally-inherited microsatellites, and maternally-inherited mitochondrial DNA sequence. For microsatellites, no site appeared to have critically low variation, including the peripheral sites, however there was a significant but slight trend of increased variation from east to west. In contrast, mitochondrial DNA showed a pattern of significantly reduced variation at the two range-edges, possibly indicating differential dispersal patterns in males and females. There appear to be two main genetically distinct groups, in the west and the east, but analysis of lifetime dispersal patterns across the Australian range also suggests complex dispersal, sometimes with high dispersal or similarity between locations that are not adjacent. Our work suggests that despite some differentiation, little penguin sites are interdependent due to complex dispersal patterns, and all have valuable genetic variation. In particular, the peripheral sites are not depauperate of variation, and are moderately connected to the remainder of the distribution, so possibly may be able to adapt in response to climate warming.

Genetic connectivity of little penguin colonies in Australia

Vardeh S[1], Sinclair J[1], Cairns KM[1,2], Cannell BL[3,4], Rollins LA[1], Sherwin WB[1*]

1 Evolution and Ecology Research Centre, School of Biological Earth and Environmental Sciences UNSW-Sydney, Sydney NSW 2052 AUSTRALIA

2 Centre for Ecosystem Science, School of Biological Earth and Environmental Sciences UNSW-Sydney, Sydney NSW 2052 AUSTRALIA

3 Environmental and Conservation Sciences, Murdoch University, Murdoch,WA, 6150, AUSTRALIA

4 Oceans Institute/School of Biological Sciences, University of Western Australia, Stirling Hwy, Crawley 6009 AUSTRALIA

* W.Sherwin@unsw.edu.au

Abstract

The Australian range of little penguins, *Eudyptula minor* , extends around southern Australia, with range-edge sites near the large cities of Perth (west) and Sydney (east). Both range-edges are closer to the equator than the range-core, being likely to experience similar heating with climate change. As a result, movement to one range-edge is not an option for little penguins, unlike in many other species. Therefore, adaptation at the range edge might be very important for little penguins. Capacity for future adaptation depends upon the variability each site holds, and the amount of exchange between sites. In peripheral sites, incoming dispersal might either forestall demographic collapse and replenish genetic variation (good), or overcome local adaptation and increase disease transmission (bad).

We aimed to establish the genetic variability in each site, and the exchange (dispersal) of individuals between sites. Genetic markers included biparentally-inherited microsatellites, and maternally-inherited mitochondrial DNA sequence. For microsatellites, no site appeared to have critically low variation, including the peripheral sites, however there was a significant but slight trend of increased variation from east to west. In contrast, mitochondrial DNA showed a pattern of significantly reduced variation at the two range-edges, possibly indicating differential dispersal patterns in males and females. There appear to be two main genetically distinct groups, in the west and the east, but analysis of lifetime dispersal patterns across the Australian range also suggests complex dispersal, sometimes with high dispersal or similarity between locations that are not adjacent. Our work suggests that despite some differentiation, little penguin sites are interdependent due to complex dispersal patterns, and all have valuable genetic variation. In particular, the peripheral sites are not depauperate of variation, and are moderately connected to the remainder of the distribution, so possibly may be able to adapt in response to climate warming.

Keywords

Dispersal, migration, peripheral populations, *Eudyptula minor*

Introduction

The range of little penguins, *Eudyptula minor* , extends from Perth around southern Australia to central New South Wales and New Zealand/Aotearoa. Impacts of pollution and other disturbance are expected to be particularly high near the two extremes of the Australian range which have large cities including Perth and Sydney. Anthropogenic disturbance is known to impact the colony near Perth (Cannell, 2016). Furthermore, being closer to the equator than the range-core, both range-edges are exposed to impacts of climate change (Cannell et al., 2012). As a result, conservation managers need to know to what extent populations are being impacted in various ways including genetically, as well as knowing to what extent populations are exchanging individuals that may reduce some demographic and genetic impacts, but increase others, such as disease transmission (Allendorf et al., 2022).

In general, populations at the range edge might be self-sufficient demographically and genetically, or they may be 'sink' populations, persisting because of dispersal from the centre of the range, with some studies supporting each possibility (Sagarin and Gaines, 2002) (note that in this document we use 'dispersal' to identify movement from place of birth to place of breeding, and thus distinguish it from 'there and back' seasonal migration). A meta-analysis found that 64.2% of studies detected a decline in genetic diversity towards a species range edge (Eckert et al., 2008). However, it is interesting to note that a review found that 37% of species occurred exclusively in their historical periphery compared to 2% solely in the historical core (Channell and Lomolino, 2000).

In a changing climate, the options for a species are extinction, adaptation, or movement of range. In the case of the little penguin, range-edge populations in the west and the east occur in areas that are likely to become less suitable as the climate warms, so movement to either extreme is unlikely to be a useful option. Populations at range edges might show adaptive genetic specialisation, if the dispersal into the population is not so great that it overcomes any local selection; therefore, sometimes peripheral populations have been assigned conservation values that are high (Lawton, 1993, Lesica, 1995, Turpie et al., 2000, Peterson, 2001)

whereas other authors rate them as low priority, assuming they would be sinks (Griffith et al., 1989, Pearl, 1992, Curnutt et al., 1996, Wolf and al, 1996).

In summary, the maintenance of genetic variation and connectivity is of great importance for the future of any species, and may be especially important for peripheral populations where dispersal might either augment declining populations, replenish genetic variation needed for adaptation, or overcome local adaptation if the exchange is too high.

Our study species, the little penguin, is the only penguin nesting in mainland Australia and Tasmania (Stonehouse, 1975). The little penguin is now primarily restricted to coastal islands free from terrestrial predators, and is found along the southern Australian coast from Perth in Western Australia (WA) to Port Stephens in New South Wales (NSW, Figure 1). These penguins are threatened by urbanisation, introduction of feral pests and invasive weeds, and climate change. The latter may affect little penguin survival and reproductive success, which appear to be influenced by ENSO events ('El Niño Southern Oscillation') (Cannell et al., 2012). The little penguin range-edge populations in the west and the east occur in areas that are likely to become less suitable as the climate warms, so movement to one extreme of the species range is probably not a useful option, unlike in species with north-south distributions. Instead, adaptation at the range edge might be very important for little penguins. As noted above, such adaptation is not impossible, but depends upon the connectivity with other populations being adequate to maintain a standing pool of genetic variation upon which selection can act, but not so great that adaptation is overwhelmed by input of non-adaptive genotypes from other parts of the range.

Intriguingly, there is possibly some adaptive difference in one range-edge population. Historically, the largest known little penguin colony in Western Australia, Penguin Island, is near their northern limit 50 km south of Perth (Wienecke et al., 1995, Wienecke, 1995) and penguins from this island are heavier and larger than elsewhere (Klomp and Wooller, 1988). Previous genetic studies have provided some evidence that Penguin Island individuals are genetically differentiated from other Australian populations (Wienecke, 1993, Peucker et al., 2009) (Burridge et al., 2015).

Little penguins show a range of movement behaviours. Little penguin movement includes multi-day foraging trips during incubation, and short foraging trips of a day or more (Collins et al., 1999, Johannesen et al., 2002, Saraux et al., 2011, Cannell, 2016, Wienecke, 1993). Ninety-three percent of penguins breed within 500 metres of birth site (Dann, 1991, Dann et al., 1991, Dann et al., 1996, Norman et al., 1991), so there might be genetic structure across their distribution. However, there is also some evidence of a small amount of long-distance, long-term movement, (Dann, 1991, Dann et al., 1991, Dann et al., 1996, Norman et al., 1991). It is possible that long-distance movements relate to the two strong southbound currents on the west and east coasts of Australia. Off Western Australia, the Leeuwin current runs south along the West Australian coast and turns eastward at Cape Leeuwin to continue along the south coast of WA (Cresswell, 1990, Cresswell and Golding, 1980). Off eastern Australia, the East Australian Current also flows southward (Suthers et al., 2011).

In this study we assessed genetic variation and connectivity of little penguins in Western Australia (WA), South Australia (SA), and NSW, particularly focusing on the connectivity between the range's northern edges (WA, NSW) and the centre (SA), and considering the possible impact of the Leeuwin and East Australian currents. We were also interested to know whether we could confirm the genetic differentiation of the morphologically unusual population near Perth (Penguin Island).

Materials and Methods

Genetic Diversity Within Sites and Regions

Collections were made from 22 sites around Australia (Figure 1). At most sites, blood samples from the metatarsal vein in heparinised capillary tubes (50-100 µl) were stored in 1 ml of Longmire's buffer (Longmire and etal., 1988) at ambient temperature until DNA was extracted following (Crandall and etal., 1999). Blood samples from SA were collected on FTA® cards and extracted following a variation of method #4

for nucleated erythrocytes (Smith and Burgoyne, 2004), in which (1) a 4 mm^2 square of FTA® paper was taken (2) 30 min wash in 200 µL of 100 mM Tris free base, 0.1% SDS; (3) 10 min wash in 200 µL DNAzol®; (4) two 5 min washes in 200 µL molecular grade water; (5) 10 min wash in 200 µL 95% ethanol; (6) DNA was resuspended in 50 µL of 10 mM Tris, 0.1 mM EDTA (Vardeh, 2015). Individuals were genotyped for ten microsatellite loci (nuclear DNA, inherited from both parents), and one mitochondrial DNA sequence (mt DNA, inherited from mother only) (Vardeh, 2015). When carrying out genetic analyses, *E. minor* samples were grouped according to collection site (Figure 1). After checking for homogeneity among sites within regions, for many analyses we pooled sites into seven geographic regions shown in the legend of Figure 1: Perth (PER), Albany (ALB), Esperance (ESP), Kangaroo Island (SA), South Coast NSW (SC), Sydney (SYD) and North Coast NSW (NC). Data were curated to remove individuals which were known offspring of others in the sample, or which had poor quality genetic data.

Microsatellite genetic diversity in *E. minor* penguins was estimated by calculating measures which have a spectrum of sensitivity to rare versus common alleles (Sherwin et al., 2017, Sherwin et al., 2021). The q =0 measure allelic richness (A_R) is highly sensitive to rare alleles, some of which may be vital for future adaptation, and was calculated in in FSTAT v2.9.4 (Goudet, 1995). All other measures were calculated using GenAlEx v6.5 (Peakall and Smouse, 2012). The q =1 measure Shannon's information index (^1H, Sherwin et al 2017) weights alleles by their relative occurrence. The q =2 measures chiefly represent the diversity of very common alleles: observed heterozygosity (H_O); and Hardy-Weinberg expected heterozygosity (a measure of diversity available for production of the next generation, if random-mating, H_E). Finally, Wright's fixation index (F_{IS}) measures depression of H_O relative to H_E due to mating between close relatives, or other causes such as selection for or against heterozygotes. For microsatellites, association between diversity (q= 0,1,2) and distance around the Australian coast was investigated by regression analysis in EXCEL.

Mitochondrial sequence chromatograms were assessed in Geneious Prime v2022.2.1 and trimmed to 281 bp of the mitochondrial control region consistent with (Vardeh, 2015). GenAlEx v6.5 was used to collapse the mitochondrial sequences into haplotypes and calculate the number of haplotypes (N_h, a q =0 measure highly sensitive to rare variants) and Shannon's information index (^1H aq =1 measure, using log base=2). DNAsp v6.12.03 (Rozas et al., 2017) was used to calculate haplotype diversity (h) and nucleotide diversity (π) – both of these are q =2 measures. For mitochondrial DNA, association between diversity (q= 0,1,2) and distance around the Australian coast was investigated by quadratic curve fitting using mycurvefit.com. Tajima's D and Fu and Li'sD^* statistics - sensitive to selection and demographic changes - were also calculated in DNAsp v6.12.03 from mitochondrial sequences, for regions and for sites.

Differentiation Between Sites and Regions

For microsatellites, pairwise differentiation was assessed in two ways, by Shannon's mutual information (I using log base=2) andF_{ST} , which were calculated in GenAlEx v6.5.I gives more emphasis to rare alleles than F_{ST}. Statistical significance levels were calculated using 999 permuations and corrected for multiple comparisons using Bonferroni adjustments (Rice, 1989). Using R v4.2.1, we tested for isolation by distance using Mantel tests implemented in the package 'Adegenet' (Jombart, 2008). The R packages 'Poppr' v2.9.3 (Kamvar et al., 2014) and 'ade4' (Dray and Dufour, 2007) were used to carry out an Analysis of Molecular Variance (AMOVA) to detect population differentiation at different hierarchical levels. Principal component analyses (PCA) and discriminant analysis of principal components (DAPC) analyses were completed using the 'Adegenet' package in R. For the DAPC analysis, the most appropriate number of principal component variables to incorporate in the DAPC analysis was chosen using 'ascores' as implemented in 'Adegenet' (Jombart et al., 2010). Population structure was investigated using STRUCTURE v2.3.4 (Falush et al., 2003) with Admixture LocPrior models. STRUCTURE analysis was carried out with 1 million MCMC replicates, a burn in of 150,000 and ten independent runs. Models were run with hypothetical population cluster (K) values of 1-7. The number of population clusters (K) was identified by determining the model with the best fit to the data, using the Evanno method (Evanno et al., 2005) implemented in Structure Harvester (Earl and vonHoldt, 2012). The CLUMPAK server main pipeline (Kopelman et al., 2015) was used to average the cluster population membership of individuals across the 10 independent runs. The

average population membership of individuals across multiple modelled K values were visualised using the R package 'pophelper' (Francis, 2017).

Unlike microsatellite DNA, mtDNA is inherited only from the mother, so its dispersal is limited to female lineages and first-generation sons. For mtDNA, pairwise differentiation was again assessed in two ways, by Shannon's mutual information (I) and F_{ST} . We calculated pairwise Shannon's mutual information (I) from the mitochondrial haplotype data using log base=2 in GenAIEx v6.5. Statistical significance levels were calculated using 999 permutations. Pairwise F_{ST} values based on the mitochondrial sequence data were calculated in Arlequin v3.5.2.2 (Excoffier and Lischer, 2010) with 999 permutations. We corrected for multiple comparisons using Bonferroni adjustments to the p-values (Rice, 1989). AMOVA was carried out using the packages 'Poppr' v2.9.3 and 'ade4' in R, to detect mitochondrial population differentiation at different hierarchical levels. Using the mitochondrial data, principal component analyses (PCA) and discriminant analysis of principal components (DAPC) analyses were carried out as for the microsatellites above.

Dispersal Between Sites and Regions

From microsatellite data, dispersal was assessed in two ways, across historical and contemporary timescales. Historical dispersal between*E. minor* populations was estimated using BayesAss v3 (Wilson and Rannala, 2003) with a burn in of 5,000,000, sampling of 5,000,000 iterations and a random seed. The only exception was the WA region analysis which required a burn in of 10,000,000 and sampling of 10,000,000 iterations to reach convergence. Convergence of each run was examined in Tracer v1.7.1 (Rambaut et al., 2018). Where possible, the mixing parameters deltaA, deltaF and deltaM were adjusted for each dataset such that the acceptance rate of the mixing parameters was between 20-60%, based on the BayesAss v3 manual. Thus the parameters (deltaA deltaF deltaM) were assigned the following values respectively: for full dataset subdivided by state 0.1, 0.6, 0.3; for WA dataset subdivided by region 0.05, 1, 0.8; for NSW plus SA dataset subdivided by region 0.6, 0.4, 0.7. Three independent runs were carried out for each dataset and set of conditions, allowing us to calculate mean dispersal estimates (m , where m *100 is the percent of individuals dispersing each generation) for each location-pair in the analysis. Chord diagrams depicting percent dispersal per generation between locations were constructed in R with the packages 'circlize' (Gu et al., 2014) and 'tidyverse' (Wickham et al., 2019) using a script written by Tom Jenkins (Holland et al., 2017). The number of contemporary dispersing individuals detected was assessed in GeneClass2 (Piry et al., 2004) with 10,000 permutations and an alpha value of 0.01.

The possibility of sex-biased dispersal was investigated by a variation of assignment index analysis (AIc) with 10,000 permutations of microsatellite data, which was carried out in R using the package 'hierfstat' (Goudet, 2005) based on the WA microsatellite dataset, for which we had sex information.

Using the mitochondrial sequence data, dispersal between regions was estimated in MIGRATE-n v5.0.4 (Beerli, 2016) using 5 long chains, a burn in of 500,000, and 15,000,000 MCMC samples with 5000 recorded steps at increments of 500 and 6 replicates. Runs had a static heating scheme of 4 chains with temperatures 1.0, 1.50, 3.0 and 1000000.0. Initial priors are required for Theta ($\theta = 2N\mu$, where N is effective population size and μ is mutation rate per generation) and$M$ ($= m/u$, where m *100= percent dispersal per generation). These were set to unity with uniform distributions of$\vartheta_{\mu\nu}$ =0.0, $\vartheta_{\mu a\xi}$ =0.10,$\vartheta_{\delta\epsilon\lambda\tau a}$ =0.01 and M_{min} =0,M_{max} =1000, M_{delta} =100. We estimated dispersal using the full dataset subdivided by state, the WA dataset subdivided by region, and the NSW-plus-SA dataset subdivided by region. Chord diagrams depicting dispersal between populations were constructed as for the microsatellites above, except that unlike the microsatellites, the output from MIGRATE is not in percent dispersal per generation (m *100), but given as M=μ/μ . Of course, the mutation rate μ is unknown, but if we make the reasonable assumption that μ is constant for all sites, then the Mvalues show relative size of m *100. To plot M in the chord diagrams we divided each estimated M value by the modal estimated$M$ value, making M proportional between regions, within each analysis (ie within each figure part).

Results

Local (alpha) genetic diversity

After curation of the data to remove individuals which were known offspring of others in the sample, or which had poor quality genetic data, some sites had only small numbers (<10), so most analyses in this paper focus on larger regional assemblages. Both microsatellite diversity and mitochondrial DNA (mtDNA) diversity showed association with geographic distance via the sea around the linear Australian distribution of this species, but the two marker types had different patterns of association (Figure 2, Tables A1, A2). Microsatellites showed a linear pattern of higher diversity in the west than the east, for all measures: allelic richness, Shannon-based number of effective alleles, and heterozygosity-based number of effective alleles (in order of sensitivity to rare alleles, Figure 2a, Table A1a). In contrast, mtDNA showed a pattern of reduced variation at each end of the species distribution, best fitting a quadratic relationship (Figure 2 b,c,d, Table A2a). Mitochondrial π is not shown in Figure 2 because it is difficult to convert to the common scale of numbers of alleles used in the other three mitochondrial plots, but Table A2a demonstrates that π follows the same pattern of maximum diversity at the centre of the distribution, and minimum at the edges. The mtDNA data did not support long-term demographic change or departure from neutrality (Table A3).

When analysing diversity within sites across Australia, microsatellite data showed no evidence of recent mating of close relatives (F_{IS} ˜0 , Table A1b). Note that this does not imply that there is high (or low) variation, becauseF_{IS} is only a measure of how the available variation is paired up within individuals each generation. In Table A1, the other statistics discussed above (ie not F_{IS}) tell us how much variation there is within each site or region.

Differentiation

Microsatellite data showed evidence of isolation by distance across Australia (Mantel test: individuals R^2=0.458, p-value 0.001; or site R^2=0.579, p-value 0.001). There are significant AMOVA results for genetic differentiation at state, site and individual but not regional levels (Table 1a). The two differentiation measures with different sensitivities to rare and common alleles were mostly concordant: based on Shannon's mutual information (I) andF_{ST}, WA regional populations were differentiated from each other and from the SA and NSW populations (Tables 2a, A4, A5). The SA population was differentiated from the NSW populations. The NSW populations were not differentiated from each other, except that for$I$, which is more sensitive to rare alleles, the NC and SC NSW populations were differentiated from each other (Table A4).

DAPC analysis of microsatellite data provided evidence of population structure between western and eastern Australia with the WA regions differentiated from the NSW and SA regions (Figure 3a). The SA region was mildly differentiated from the NSW regions. There was little evidence of population structure within NSW. There appeared to be population structure within the WA region, with Perth and Albany being more distant from each other and Esperance falling between these two clusters. A PCA analysis showed similar results that were less clear, as is expected from PCA's mathematical basis (Jombart et al 2010).

Using microsatellite data in a search for homogeneous random-mating geographic groupings (STRUCTURE Admixture-locprior-model), the Evanno method indicated that the best fit to the data was a K value of 2 (ie, two homogeneous populations Figure A1). The Admixture Locprior model indicated population structure across Australia with WA *E. minor*individuals differentiated from the SA and NSW populations (Figure 4). For the favoured model of K=2, SA shows a mix of WA and NSW genotypes, and the NSW-SC site FI shows some WA/SA genotypes. The NSW sites showed little other evidence of genetic structuring (K=3 is also shown in Figure 4, for interest).

Using mitochondrial DNA, a Mantel test found evidence of isolation by distance across Australia, although this evidence was weaker than for microsatellites, being significant when analysed for individuals (R^2=0.217, p-value 0.001), but not by site (R^2=0.138, p-value 0.084). An AMOVA based on the mitochondrial dataset found significant variation between different regions, and between individuals within sites, but not between states or between sites within regions (Table 1b). Mitochondrial sequence data showed significant differentiation between penguins from different geographic regions across Australia (Tables 2b,A6,A7). The WA regions were differentiated from NSW regions, and WA regions were also differentiated from SA, with the

exception that Albany was only differentiated from SA with I , the measure that is more sensitive to rare alleles. Within WA the Perth region was differentiated from both Albany and Esperance, but Albany and Esperance were not differentiated from each other. The SA population was differentiated from NSW regions (except not from NC, when using I). There was no significant differentiation within NSW for mtDNA.

Using mitochondrial sequence data, DAPC analysis of the 338 individuals found less evidence of population structure than was observed at microsatellite markers (Figure 3b), thus agreeing with the AMOVA (Table 1b). However, in the DAPC the Perth population was distinct from NSW, SA and other WA populations. A PCA analysis showed similar but less clear results, as is expected from its mathematical basis.

Dispersal

For microsatellites, analysis of dispersal was performed in two ways: using BayesAss that detects average 'historic' dispersal over many generations, and using GeneClass that only detects first-generation dispersers. In a continent-wide analysis, BayesAss3.0 detected evidence of dispersal from WA to SA (19.4%) and from SA to NSW (31.5%) with only limited dispersal from SA to WA (1%) and none from NSW to SA or between WA and NSW (Figure 5a). First-generation disperser detection analysis performed in GeneClass2 identified only ten individuals that were likely to be dispersing between geographic regions (Table A8a). There were movements towards and away from the two range limits (northwest and northeast).

Using mtDNA, continent-wide historic dispersal analysis identified relatively low levels of dispersal from WA into SA and NSW and from SA to NSW (Figure 5b). However, we observed higher dispersal from SA to WA. Dispersal from NSW to SA was double the dispersal observed from SA to NSW, although proportionally less than dispersal from SA to WA. The reader is reminded that the relative widths of the bands in Figures 5a and 5b can be qualitatively compared, but not quantitatively. This is because Figure 5a is in units of percent of the population exchanged per generation ($100^{*}m$) , whereas the MIGRATE analysis in Figure 5b shows $M{=}\mu/\mu$, where μ is the (unknown) mutation rate. Nevertheless, one can make a qualitative comparison, such as observing that in Figure 5b there is more dispersal from SA to WA than from NSW to WA, and the microsatellite Figure 5a also shows more dispersal from SA to WA than from NSW to WA; however the absolute widths of the bands cannot be compared between Figures 5a and 5b.

We then further analysed dispersal using finer divisions of the dataset. Between the three WA regions, historic dispersal analysis performed using BayesAss3.0 and microsatellite data found evidence of moderate dispersal (Figure 5c). There was unequal dispersal between the Albany and Perth populations, with dispersal from Albany to Perth (2.5%) roughly one-sixth the dispersal from Perth to Albany (18.8%). There was similar dispersal from Albany to Esperance (14.3%) as observed from Esperance to Albany (19.2%). There was limited evidence of dispersal from Perth to Esperance (1.3%) but moderate dispersal from Esperance to Perth (7.9%). A GeneClass2 analysis to identify first generation dispersers between WA sites found only five dispersing individuals (Table A8b), four of which were moving in the direction from the centre of the range (approximately the centre of SA, Figure 1) towards the north-western periphery of the range. For analysis within SA-plus-NSW, GeneClass2 failed to converge, possibly because of the lower variation within and between locations there. A corrected assignment index (AIc) analysis using the ten microsatellite loci observed no evidence for sex-biased dispersal in the WA dataset (female mean AIc=-0.1001303, male mean AIc= 0.1018567, test statistic = -0.369 and p-value 0.705). Sex information was very incomplete for SA and NSW, so the AIc analysis could not be performed.

Using mitochondrial DNA within WA, historic dispersal analysis in MIGRATE-n indicated limited dispersal into the Perth region from Albany or Esperance, however there was notable southern dispersal from Perth to Albany and Esperance (Figure 5d). There also appeared to be higher levels of dispersal from Esperance to Albany, than from Albany to Esperance.

Between SA and NSW regions, historical dispersal analysis using microsatellite data in BayesAss3.0 identified evidence of geneflow from SA into the NSW regions (Figure 5e). For instance, there was evidence of moderate geneflow from SA to NC NSW (13.6%) and from SA to SC NSW (14.4%) but lower geneflow from SA to SYD (4.1%). There was no evidence of geneflow from the NSW populations into the SA population. Within

NSW, the highest exchanges were from SC to both SYD (18.7%) and NC (6.5%) populations, from SYD to SC (10.4%) and from the NC to SC (9.9%) and SYD (17.0%) populations.

Using mtDNA for SA-plus-NSW regions, historical dispersal analysis in MIGRATE-n indicated: extensive dispersal from NC to SYD (51.4) and SC (51.4); and little other exchange, including to and from SA (Figure 5f).

Discussion

In summary, we used an array of measures that have different sensitivity to rare and common alleles, and their results generally agreed. There are no locations that appeared to have critically low genetic variation, though some are lower than others. Differentiation and inferred dispersal between localities showed a broad pattern of east-to-west differentiation, but very patchy details, suggesting complex dispersal patterns. Complex dispersal is also suggested by contrasts between differentiation inferred from biparentally-inherited microsatellites and uniparental mitochondrial DNA. Certainly, it is not possible to support a simple prediction that strong southward dispersal is caused by the Leeuwin and East Australian currents.

That neither the east nor the west of the Australian distribution showed pronounced reduction of microsatellite variation at the peripheries is a good sign for possible adaptive potential at the margins, where the effects of global warming are expected to be most severe, if potentially adaptive loci show the same pattern as the microsatellites. The slight but significant reduction of microsatellite variation in the east (Figure 2a) was surprising, because the east is central to the species' range in Australia and New Zealand. This reduced variation is possibly because the microsatellites were derived using WA samples, giving an ascertainment bias, ie more variants detected in the locations where the microsatellites were first characterised. Such bias is sometimes seen (but also sometimes not seen) when microsatellites derived from one species are used in another (Hutter et al.)1998, but in the current study, all locations were the same species. Alternatively, the difference in genetic variation is real, though slight, and would suggest that WA locations are especially important reservoirs of genetic variation – this would be the best interpretation to use in precautionary conservation.

In contrast to microsatellites, the mitochondrial DNA showed a pattern consistent with many other species, of reduced variation at the northern peripheries of the range (Figures 2b,c,d, (Eckert et al., 2008)). This result possibly reflects patterns of female-specific movement, despite the lack of evidence for sex-bias from microsatellites, possibly compounded by the lower effective size for mtDNA compared to microsatellites. This will be discussed below together with other information about dispersal.

As well as being important because they contain higher diversity, certain sites or regions might be important for evolution and conservation because they are strongly differentiated from other sites. For microsatellites, AMOVA indicated that high variation occurs between states rather than between regions within states (Table 1). WA regional populations are differentiated from each other and from the SA and NSW populations, based on *I,* F_{ST} and DAPC (Table 2a, Figure3a). STRUCTURE agrees with this, except finds little differentiation within WA (Figure 4). In contrast, DAPC indicates that Perth and Albany are more distant from each other and Esperance falls between these two population clusters, which is surprising given that Albany is geographically intermediate to the other two regions – possibly the result of complex dispersal patterns or population histories (Figure 3a). The pattern of mtDNA differentiation is not identical to the pattern for microsatellites, with most variation being within regions within states rather than between states, again suggesting different dispersal patterns for males and females (Table 1b). As with the microsatellites, mitochondrial DNA showed that WA regions are differentiated from NSW (Table 2b, Figure3b).

The microsatellite differentiation just presented may be due to dispersal patterns, or may be due to very different histories. As well as assessing differentiation between sites, there are programs that attempt to identify either historic or contemporary dispersal, which are two of the factors affecting differentiation between sites. The measures of contemporary dispersal (GeneClass2) detected very few individuals dispersing in the current generation (Table A8), so this discussion will be limited to the average dispersal over historical generations, which is also more relevant for medium- to long-term ecology, evolution, and conservation

(Figure 5). It appears that the west is a significant contributor to southern populations, and via those to eastern populations in Australia. Microsatellite DNA gave evidence of dispersal from WA to SA (19.4%) and from SA to NSW (31.5%) with only limited dispersal from SA to WA (1%) and none from NSW to SA or between WA and NSW (Figure 5a). The AIc analysis for microsatellites showed no evidence for sex-biased dispersal, but this analysis is very weak because it only shows sex-bias if the bias has been very strong in the most recent generation, after which mating between incoming dispersers and residents would obliterate any pattern of sex-bias for biparental microsatellites. [Note that this limitation is only for detection of sex-bias in dispersal from microsatellites, and does not apply to the other microsatellite dispersal analyses above, which sum the effects of biparental dispersal over many generations.]

In contrast to microsatellites, mtDNA has little sensitivity to male dispersal, and sums female dispersal over many generations, so might be expected to give a different assessment of dispersal to microsatellites. Already above, we have hinted at the possibility of sex-biased dispersal due to different patterns of within-site variation for microsatellites and mitochondrial DNA. Indeed, the dispersal analysis bears out this interpretation. Historic dispersal analysis using mitochondrial DNA showed evidence of east to west dispersal from NSW to SA and SA to WA (Figure 5b), unlike the microsatellite pattern. Thus, as mentioned earlier, it is possible that the direction and rate of dispersal are different in males and females, at a continent-wide scale, creating the contrast between the microsatellite and mitochondrial differentiation patterns (Figure 5). Another possible explanation is that the different inferences of dispersal (Figure 5) and within-site variation (Figure 2) from mtDNA compared to the nuclear microsatellites, could be due to the relatively lower effective population size of mtDNA.

There is no clear evidence of overall movement from the peripheries (Perth, north coast NSW) towards the centre of the range (SA), which we postulated could be caused by the southward Leeuwin and East Australian currents respectively. The results in Figures 5 a and b show examples where movement is greater to the peripheries or from the peripheries, and the finer-scale analyses are also equivocal (Figures 5c,d,e,f).

Between the three WA regions, the microsatellite and mitochondrial results for historic dispersal found evidence of moderate dispersal, suggesting that the regions are interdependent, and should therefore all be considered as important parts of the WA little penguin range (Figures 5 c,d). However, these patterns do not appear to explain why Perth and Esperance are more similar to one another than they are to Albany, despite Albany's intermediate geographic position. This result indicates that we require further data to understand not only dispersal, but also any possible differences between these locations, such as different selective regimes.

Between regions within SA and NSW, microsatellites showed dispersal from SA to north coast NSW, and within NSW, whereas mitochondrial DNA showed little evidence for dispersal between SA and NSW, again suggesting different patterns of dispersal for males and females (Figures 5 e,f).

The little penguin range-edge sites in the northwest and the northeast occur in areas that are not only near the large cities of Perth and Sydney, but are also both near the northernmost extent of the range, and thus likely to become less suitable as the climate warms. Therefore movement to one extreme of the range is not an option for avoiding effects of climate change. As a result, adaptation at the range edge might be very important for little penguins. Thus, conservation managers need to know to what extent populations are exchanging individuals, which may reduce some demographic and genetic impacts, but increase others, such as disease transmission (Allendorf et al., 2022). In these peripheral populations, dispersal might either forestall demographic collapse (good), replenish genetic variation (good), or overcome local adaptation (bad). Microsatellite results suggest that no Australian site has critically low genetic variation, and that western regions are significantly, but slightly, more variable; this contrasts with mitochondrial DNA which indicates low variability at the northern peripheries of the Australian range. Our work also suggests that the Australian populations are interdependent due to complex dispersal patterns, possibly including sex-biased dispersal direction, plus possible dispersal between sites that are not adjacent, skipping intermediate sites.

Finally, is adaptation already occurring in the Perth region, as previous research suggests (Klomp and

Wooller, 1988)? The DAPCs based on mtDNA in Figure 3b suggest that Perth is unusual relative to other WA populations and the rest of the continent. However, Figure 3a and the differentiation values in Tables A4 and A5 do not support the idea that the Perth little penguins are genetically unusual relative to the rest of the Australian range. Of course, the current analysis only sampled a very small portion of the genome, so it is possible that some genes are strongly differentiated between Perth and the remaining regions.

Data Accessibility Statement

Data will be on Dryad ……..

Competing Interests Statement

The authors have no competing interests

Author Contributions

All authors contributed to the writing of this paper. Vardeh genotyped the NSW and SA samples as part of her PhD, and rescored all SA WA and NSW data to ensure consistency. Sinclair genotyped the WA samples and supervised Vardeh. Cairns reanalysed all data in the combined SA WA and NSW dataset. Cannell collected WA samples and contributed extensive background on little penguin biology. Rollins cosupervised Cairns. Sherwin supervised Vardeh, Sinclair and Cairns, and wrote the draft of the paper.

Acknowledgements Various collectors aided the authors, including: Susanne Callaghan, Gemma Carrol, Martin Coates, Peter Collins, Diane Colombelli-Negrel, Nic Dunlop, Nick Carlisle, Martin Fortescue , Chris Lloyd, Paul Lynch , Rowena Morris, Lisa O'Neill, Laurence Penman, David Priddel, Katie Surrey, Cassandra Taylor, Melissa Tighe, and David Vardeh. Funders included: Australian Research Council LP0669207 (to Bradley Wooller and Sherwin) ; WA Dept Biodiversity, Conservation & Attractions (to Sherwin); Dept of Conservation and Land Management WA, Marine Conservation Branch (to Cannell); and WV Scott Foundation (to Vardeh and Sinclair). Taronga Conservation Society allowed preliminary trials of preservation methods. Wildlife and ethics permits were: Murdoch University Animal Ethics Committee- W2002/06; WA Department of Environment and Conservation 9SF 5763 and SF 6212; UNSW-ACEC 127B; NSW NPWS SL100821; Taronga Conservation Soc – 4c/11/11; Australian Federal AU-COM2012-139. We acknowledge the indigenous custodians of all the lands and water occupied by the little penguin, and where this work took place.

\sout

FIGURES

Figure 1 Locations sampled, with colour-coding showing regional groupings of sites , and number of individuals analysed in brackets. Codes for individual sites are WA-PER (Garden Is. GDI; Penguin Is PGI); WA-ALB (Muttonbird Is. MBI; Mistaken Is. MKI; Cheyne Is. CHI); WA-ESP (Woody Is. WDI; Wickham Is. WKI); SA Kangaroo island (Antechamber Bay ACB; Emu Bay EMB; Kingscote KSC; Penneshaw PNS; Vivonne Bay VVB); NSW South Coast SC (Montague Is. MI; Tollgate Is. TI; Brush Is. BrI; Bowen Is. BI; Five Is. FI); NSW-Sydney SYD (Manly M; Lion Is. LI); NSW North Coast NC (Cabbage Tree Is. CI; Broughton Is. Bro). Note that for each laboratory analysis, the number of individuals reported throughout this manuscript is the number that had unambiguous genotypes, so is often lower than the numbers on this map.

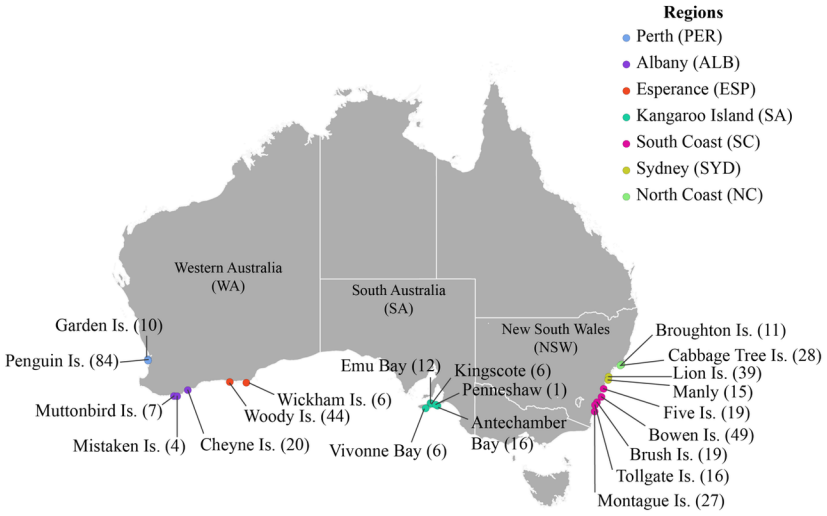


Figure 2. Alpha (within-location) genetic diversity versus distance from northernmost region in WA. Distances are taken from a point at the centre of each group of collection localities. Regions are, from west to east (left to right on the horizontal axes): WA-PER (Garden Is. GDI; Penguin Is PGI); WA-ALB (Muttonbird Is. MBI; Mistaken Is. MKI; Cheyne Is. CHI); WA-ESP (Woody Is. WDI; Wickham Is. WKI); SA Kangaroo island (Antechamber Bay ACB; Emu Bay EMB; Kingscote KSC; Penneshaw PNS; Vivonne Bay VVB); NSW South Coast SC (Montague Is. MI; Tollgate Is. TI; Brush Is. BrI; Bowen Is. BI; Five Is. FI); NSW-Sydney SYD (Manly M; Lion Is. LI); NSW North Coast NC (Cabbage Tree Is. CI; Broughton Is. Bro). Diversity measures are Ar Allelic richness, ${}^1D$ effective number of alleles (Shannon-log-2), and ${}^2D$ effective number of alleles (Heterozygosity-based) (Sherwin et al., 2017, Sherwin et al., 2021). Tables A1 and A2 contain data and equations for ${}^1D$ and ${}^2D$. All curves showed significant association between alpha genetic diversity and geographic distance, except for Nh .

Microsatellite Data; statistics for the linear regressions were: - Ar Genetic Diversity = 5.48-0.00029$*$(geographic distance) R^2=0.62 p=0.036 - ${}^1D$ Genetic Diversity = 2.28-0.00012$*$(geographic distance) R^2=0.85 p=0.0029 - ${}^2D$ Genetic Diversity = 2.33-0.00015$*$(geographic distance) R^2=0.85 p=0.0032

(b) MtDNA, Number of haplotypes Nh , statistics for the quadratic curve fit were: Genetic Diversity = 9.815 - 0.00882$*$(geographic distance)+0.00000207$*$(geographic distance)^2 R^2=0.541 p=0.21

(c) MtDNA ${}^1D$, statistics for the quadratic curve fit were: Genetic Diversity = 3.32+0.0031$*$(geographic distance) -7.44$*10^{-7}*$(geographic distance) ^{2} R^2=0.93 p=0.0054

(d) MtDNA ${}^2D$, statistics for the quadratic curve fit were: Genetic Diversity = 3.53+0.012$*$(geographic distance)- 0.0000029$*$(geographic distance) $^2R^2$=0.97 p=0.00071

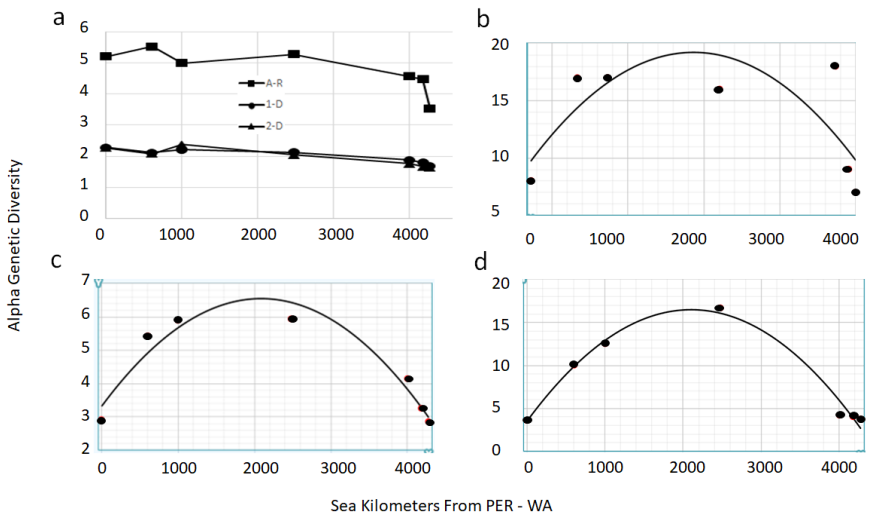
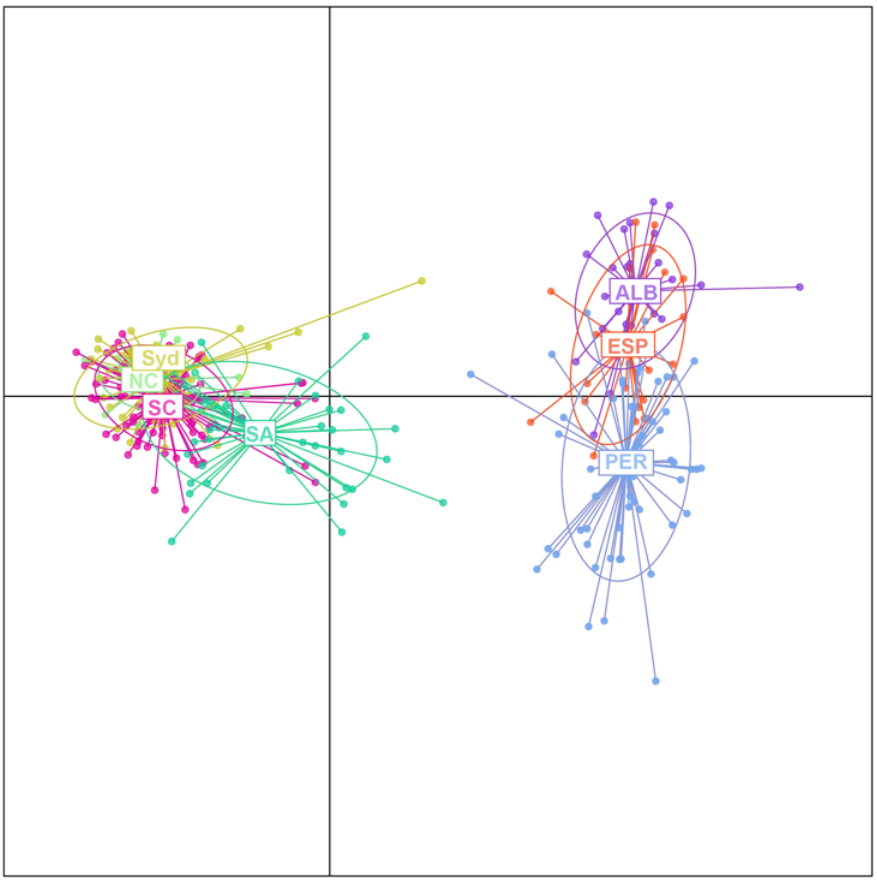


Figure 3. Population genetic structure using DAPC analysis of: (a) 348 individuals sampled across Australia based on 10 microsatellite loci; (b) 338 individuals based on 281 bp of mitochondrial sequence data. Individuals are coloured and grouped based on the geographic region from which they were sampled (Figure 1).

(a)



(b)

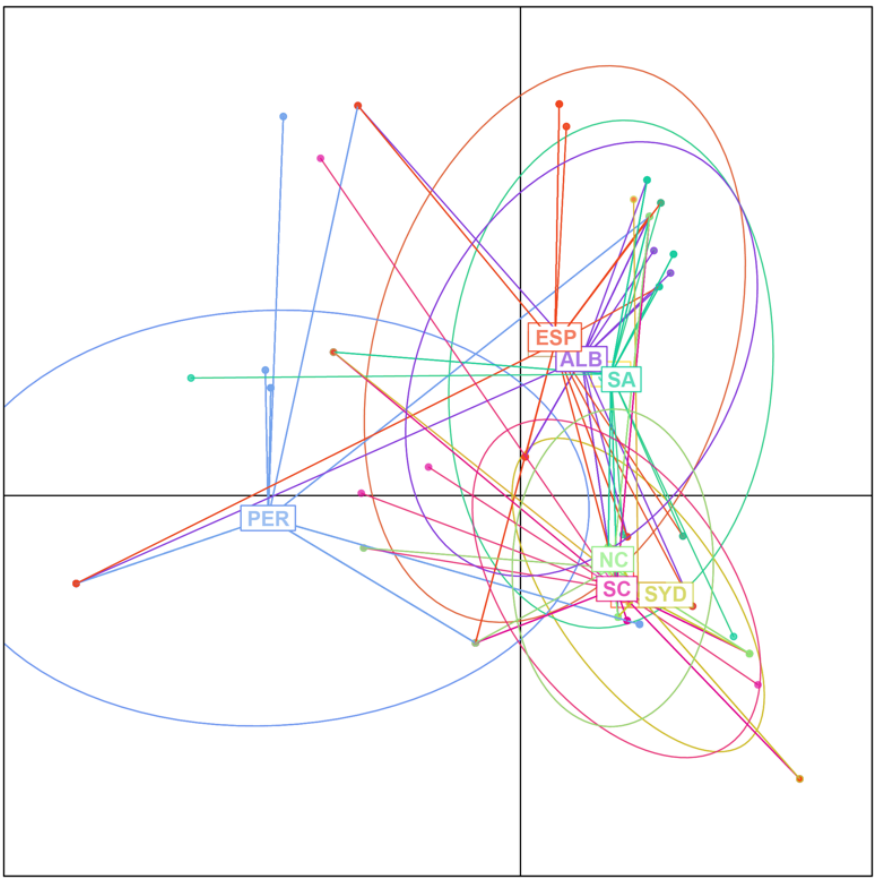
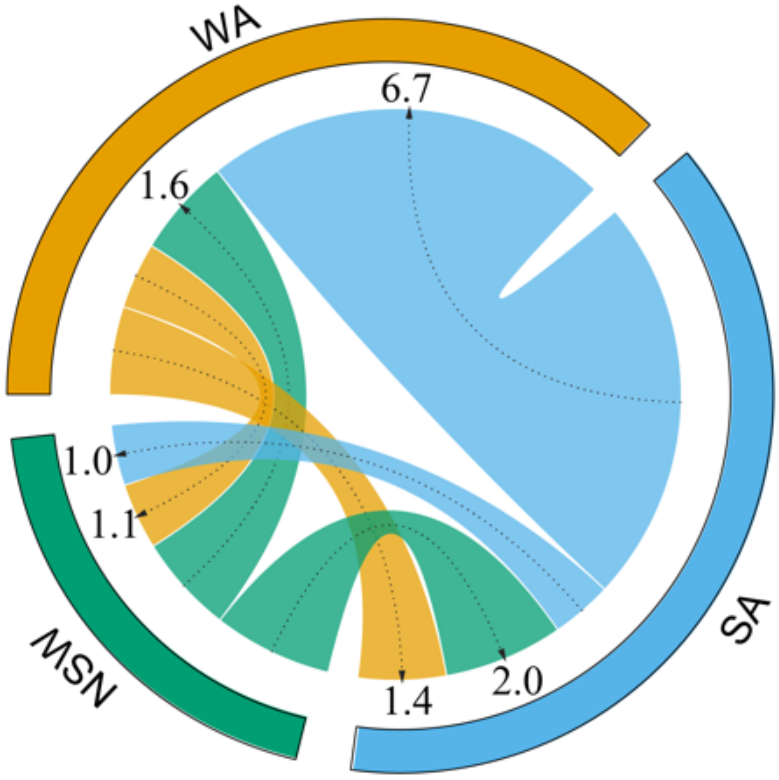


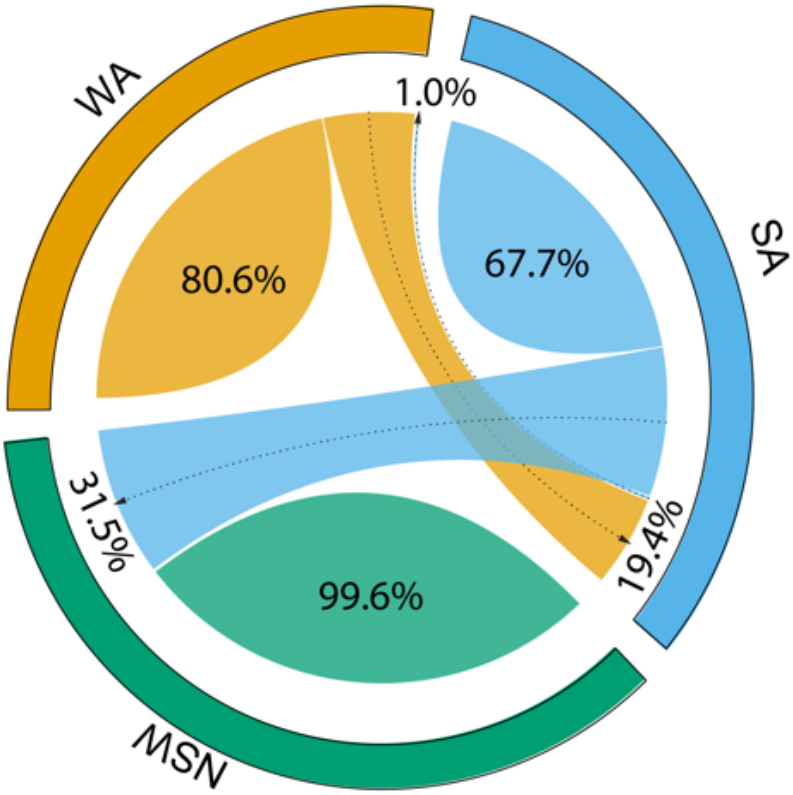
Figure 4. Population genetic structuring using STRUCTURE(Admixture LocPrior model) based on 10 microsatellite loci characterised in 348 samples from WA, SA and NSW (Figure 1). Figure created using pophelper in R and shows the favoured model of two populations K=2, as well as K=3.

Figure 5. Historical dispersal (a) Dispersal between WA, SA and NSW using genetic data from ten microsatellite loci and 348 individuals, showing percent of the population exchanged per generation (100*m) from BayesAss 3.0 simulations – this analysis program and presentation style applies to all diagrams in the left column. (b) Dispersal between WA, SA and NSW based on mitochondrial sequence data and 338 individuals as modelled in MIGRATE-n v5.0.4; the values reported are calculated by dividing the mutation-scaled dispersal rate (M) by the modal value observed between the three regions, making the values equivalent to proportional dispersal between the regions – this analysis program and presentation style applies to each diagram in the right column. (c) Dispersal between Perth, Albany and Esperance using genetic data from ten microsatellite loci and 117 individuals. (d) Dispersal between WA regions based on mitochondrial sequence data and 159 individuals. (e) Dispersal between South Australia (SA), North Coast NSW (NC), Sydney (SYD) and South Coast NSW (SC) using genetic data from ten microsatellite loci and 231 individuals. (f) Dispersal between SA and NSW regions based on mitochondrial sequence data and 179 individuals.

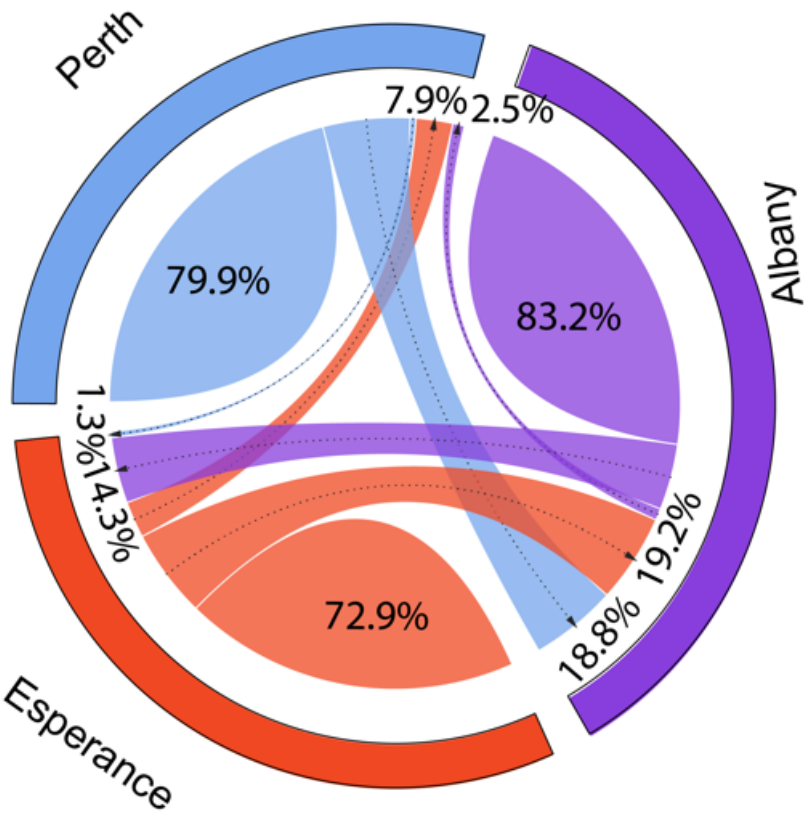
Microsatellite m*100 Mitochondrial DNA M/(modal$M$)

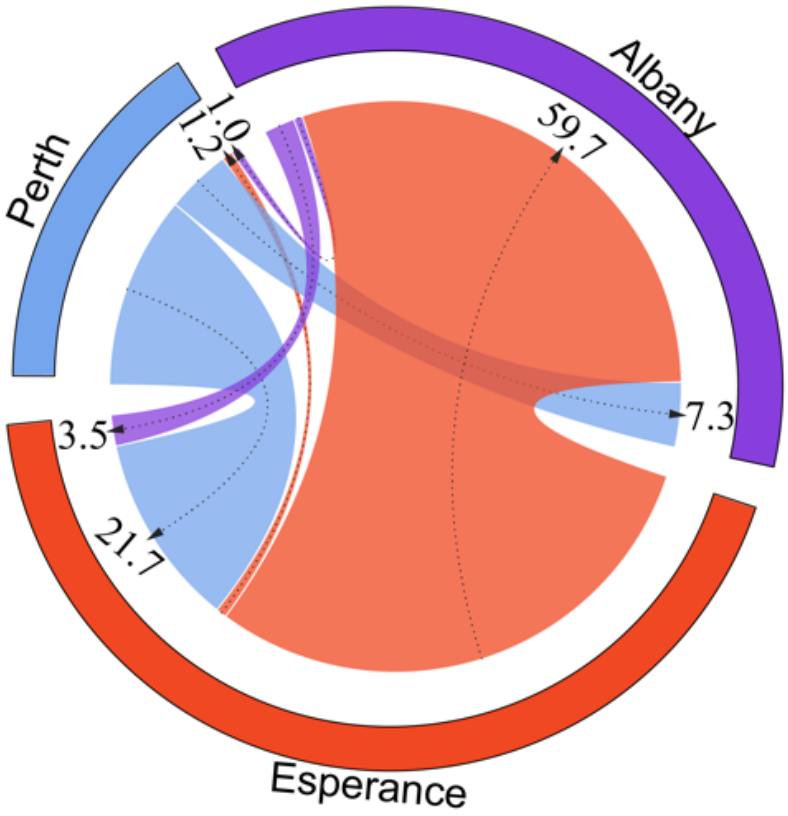
(a) (b)



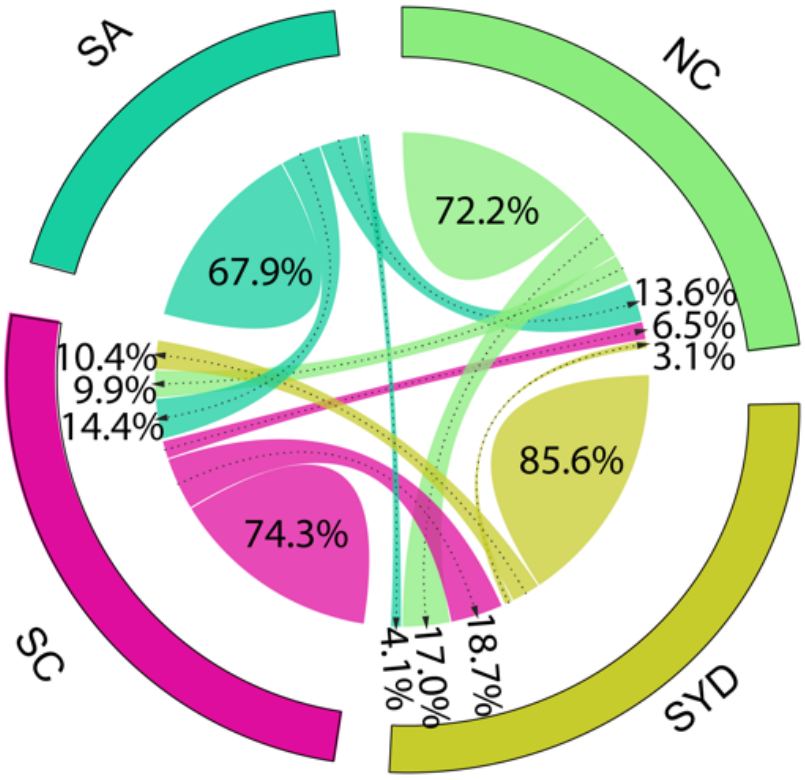


(c) (d)





(e) (f)



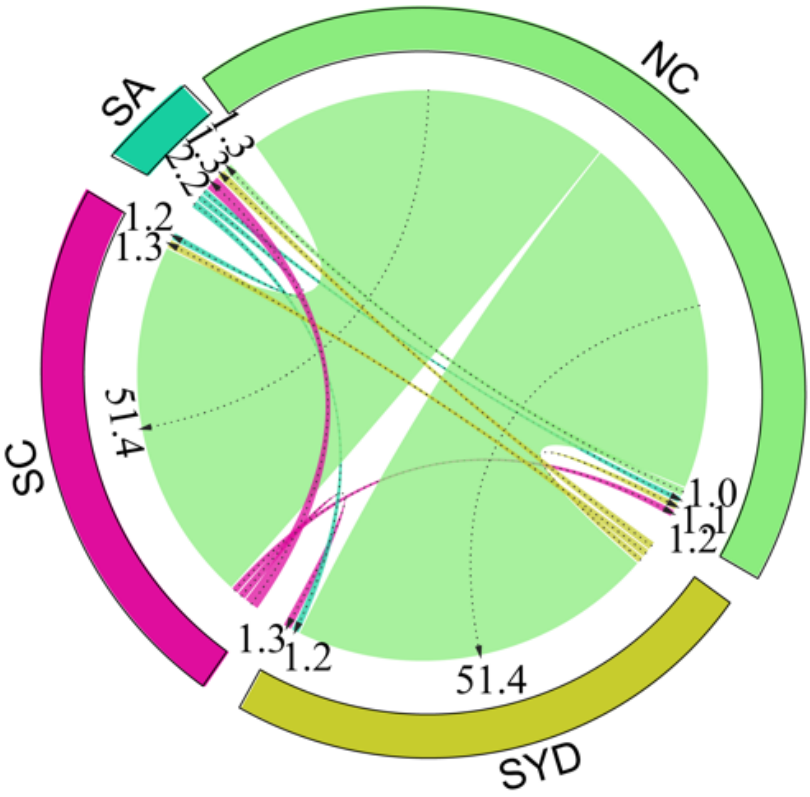


Table 1. Genetic differentiation characterised using analysis of molecular variance (AMOVA) conducted in 'Poppr' with 'Ade4' in R using 999 permutations. Significant values in red. (a) microsatellite data (b) mitochondrial DNA. **(a)**

Source	**df**	**Estimated Variation**	**% Estimated Variation**	**p-value**
Between states	2	0.541	17.363	0.02
Between regions within states	4	0.029	0.947	0.37
Between sites within regions	14	0.137	4.396	0.01
Between individuals within sites	327	2.406	77.294	0.01
Total	347	3.113	100.000	

(b)

Source	**df**	**Estimated Variation**	**% Estimated Variation**	**p-value**
Between states	2	0.180	4.890	0.30
Between regions within states	4	0.762	20.673	0.05
Between sites within regions	11	0.015	0.420	0.19
Between individuals within sites	320	2.730	74.018	0.01
Total	337	3.688	100.000	

Table 2. Genetic differentiation between seven geographic regions. Pairwise population matrix of F_{ST} and Shannon's mutual information (I using log base = 2). Pairs that are significantly differentiated for both measures (I and F_{ST}) are asterisked; (a) characterised using ten microsatellite loci; full data are in Tables A4 and A5; (b) using 281 bp of mtDNA sequence from 338 individuals; full data are in Tables A6 and A7. **(a)**

	Perth **PER (n=65)**	Albany **ALB (n=31)**	Esperance **ESP (n=23)**	Kangaroo Is. **SA (n=45)**	South NSW **SC (n=123)**	Sydney **SYD (n=48)**	North NSW **NC (n=34)**
PER							
ALB	*						
ESP	*	*					
SA	*	*	*				
SC	*	*	*	*			
SYD	*	*	*	*	-		
NC	*	*	*	*	-	-	

(b)

	Perth **PER (n=83)**	Albany **ALB (n=30)**	Esperance **ESP (n=46)**	Kangaroo Is. **SA (n=39)**	South NSW **SC (n=104)**	Sydney **SYD (n=30)**	North NSW **NC (n=27)**
PER							
ALB	*						
ESP	*	-					
SA	*	-	-				
SC	*	*	*	*			
SYD	*	*	*	*	-		
NC	*	*	*	-	-	-	

Location	Location Code	Code	n	A_R (s.e.)	A_R (s.e.)	^1H (s.e.)	^1H (s.e.)	H_O (s.e.)	H_O (s.e.)	H (s.e.)

Appendix Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven re-

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

^1H = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. ($^1D = e^{^1H}$)

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity $=1 - \sum_i \left[{p_i}^2\right]$. ($^2D = \frac{1}{(1-H_E)}$).

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

$s.e$. = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	$^1\boldsymbol{H}$	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)

Location	**Location**	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

&

Appendix

Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven regions; sites with less than 10 individuals are greyed.

(a)

Location	**Location**	**Location**	**Code**	**Code**	n	A_R **(s.e.)**	A_R **(s.e.)**	^1H **(s.e.)**	^1H **(s.e.)**	H_O **(s.e.)**	H_O **(s.e.)**	H (s.e.)
Perth WA	Perth WA	Perth WA	PER	PER	63	5.21 (0.80)	5.21 (0.80)	1.19 (0.21)	1.19 (0.21)	0.56 (0.10)	0.56 (0.10)	0 (
Albany WA	Albany WA	Albany WA	ALB	ALB	31	5.54 (1.11)	5.54 (1.11)	1.08 (0.20)	1.08 (0.20)	0.49 (0.08)	0.49 (0.08)	0 (
Esperance WA	Esperance WA	Esperance WA	ESP	ESP	23	5.00 (0.75)	5.00 (0.75)	1.14 (0.17)	1.14 (0.17)	0.59 (0.09)	0.59 (0.09)	0 (

Location	**Location**	**Location**	**Code**	**Code**	n	A_R **(s.e.)**	A_R **(s.e.)**	^1H **(s.e.)**	^1H **(s.e.)**	H_O **(s.e.)**	H_O **(s.e.)**	H (s
Kangaroo Is. SA	Kangaroo Is. SA	Kangaroo Is. SA	SA	SA	41	5.28 (1.10)	5.28 (1.10)	1.09 (0.24)	1.09 (0.24)	0.50 (0.11)	0.50 (0.11)	0 (
South Coast NSW	South Coast NSW	South Coast NSW	SC	SC	110	4.57 (0.10)	4.57 (0.10)	0.91 (0.22)	0.91 (0.22)	0.40 (0.10)	0.40 (0.10)	0 (
Sydney NSW	Sydney NSW	Sydney NSW	SYD	SYD	46	4.48 (1.02)	4.48 (1.02)	0.84 (0.22)	0.84 (0.22)	0.37 (0.10)	0.37 (0.10)	0 (
North Coast NSW	North Coast NSW	North Coast NSW	NC	NC	34	3.53 (0.75)	3.53 (0.75)	0.74 (0.18)	0.74 (0.18)	0.38 (0.10)	0.38 (0.10)	0 (

(b)

Location Code	**Location Code**	n	n	A_R **(s.e.)**	^1H **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

^1H = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. ($^1D = e^{^1H}$)

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity $=1 - \sum_i \left[{p_i}^2\right]$. ($^2D = \frac{1}{(1-H_E)}$).

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

$s.e$. = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

&

Appendix

Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven regions; sites with less than 10 individuals are greyed.

(a)

Location	Location	Location	Code	Code	n	A_R (s.e.)	A_R (s.e.)	^1H (s.e.)	^1H (s.e.)	H_O (s.e.)	H_O (s.e.)	H (s.e.)
Perth WA	Perth WA	Perth WA	PER	PER	63	5.21 (0.80)	5.21 (0.80)	1.19 (0.21)	1.19 (0.21)	0.56 (0.10)	0.56 (0.10)	0 (
Albany WA	Albany WA	Albany WA	ALB	ALB	31	5.54 (1.11)	5.54 (1.11)	1.08 (0.20)	1.08 (0.20)	0.49 (0.08)	0.49 (0.08)	0 (
Esperance WA	Esperance WA	Esperance WA	ESP	ESP	23	5.00 (0.75)	5.00 (0.75)	1.14 (0.17)	1.14 (0.17)	0.59 (0.09)	0.59 (0.09)	0 (
Kangaroo Is. SA	Kangaroo Is. SA	Kangaroo Is. SA	SA	SA	41	5.28 (1.10)	5.28 (1.10)	1.09 (0.24)	1.09 (0.24)	0.50 (0.11)	0.50 (0.11)	0 (
South Coast NSW	South Coast NSW	South Coast NSW	SC	SC	110	4.57 (0.10)	4.57 (0.10)	0.91 (0.22)	0.91 (0.22)	0.40 (0.10)	0.40 (0.10)	0 (
Sydney NSW	Sydney NSW	Sydney NSW	SYD	SYD	46	4.48 (1.02)	4.48 (1.02)	0.84 (0.22)	0.84 (0.22)	0.37 (0.10)	0.37 (0.10)	0 (
North Coast NSW	North Coast NSW	North Coast NSW	NC	NC	34	3.53 (0.75)	3.53 (0.75)	0.74 (0.18)	0.74 (0.18)	0.38 (0.10)	0.38 (0.10)	0 (

(b)

Location Code	Location Code	n	n	A_R (s.e.)	^1H (s.e.)	H_O (s.e.)	H_E (s.e.)	F_{IS} (s.e
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

^1H = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. ($^1D = e^{^1H}$)

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity $=1 - \sum_i \left[{p_i}^2\right]$. ($^2D = \frac{1}{(1-H_E)}$).

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

$s.e$. = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

&

Appendix

Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven regions; sites with less than 10 individuals are greyed.

(a)

Location	**Location**	**Location**	**Code**	**Code**	n	A_R **(s.e.)**	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_O **(s.e.)**	H (s.e.)
Perth WA	Perth WA	Perth WA	PER	PER	63	5.21 (0.80)	5.21 (0.80)	1.19 (0.21)	1.19 (0.21)	0.56 (0.10)	0.56 (0.10)	0 (
Albany WA	Albany WA	Albany WA	ALB	ALB	31	5.54 (1.11)	5.54 (1.11)	1.08 (0.20)	1.08 (0.20)	0.49 (0.08)	0.49 (0.08)	0 (
Esperance WA	Esperance WA	Esperance WA	ESP	ESP	23	5.00 (0.75)	5.00 (0.75)	1.14 (0.17)	1.14 (0.17)	0.59 (0.09)	0.59 (0.09)	0 (
Kangaroo Is. SA	Kangaroo Is. SA	Kangaroo Is. SA	SA	SA	41	5.28 (1.10)	5.28 (1.10)	1.09 (0.24)	1.09 (0.24)	0.50 (0.11)	0.50 (0.11)	0 (
South Coast NSW	South Coast NSW	South Coast NSW	SC	SC	110	4.57 (0.10)	4.57 (0.10)	0.91 (0.22)	0.91 (0.22)	0.40 (0.10)	0.40 (0.10)	0 (
Sydney NSW	Sydney NSW	Sydney NSW	SYD	SYD	46	4.48 (1.02)	4.48 (1.02)	0.84 (0.22)	0.84 (0.22)	0.37 (0.10)	0.37 (0.10)	0 (
North Coast NSW	North Coast NSW	North Coast NSW	NC	NC	34	3.53 (0.75)	3.53 (0.75)	0.74 (0.18)	0.74 (0.18)	0.38 (0.10)	0.38 (0.10)	0 (

(b)

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

^1H = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. ($^1D = e^{^1H}$)

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity $=1 - \sum_i \left[{p_i}^2\right]$. ($^2D = \frac{1}{(1-H_E)}$).

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

s.e . = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()

	Location	Code	n	N_h	^1H	h (s.d.)	π (s.e.)
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

&

Appendix

Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven regions; sites with less than 10 individuals are greyed.

(a)

Location	Location	Location	Code	Code	n	A_R (s.e.)	A_R (s.e.)	^1H (s.e.)	^1H (s.e.)	H_O (s.e.)	H_O (s.e.)	H (s.e.)
Perth WA	Perth WA	Perth WA	PER	PER	63	5.21 (0.80)	5.21 (0.80)	1.19 (0.21)	1.19 (0.21)	0.56 (0.10)	0.56 (0.10)	0 (
Albany WA	Albany WA	Albany WA	ALB	ALB	31	5.54 (1.11)	5.54 (1.11)	1.08 (0.20)	1.08 (0.20)	0.49 (0.08)	0.49 (0.08)	0 (
Esperance WA	Esperance WA	Esperance WA	ESP	ESP	23	5.00 (0.75)	5.00 (0.75)	1.14 (0.17)	1.14 (0.17)	0.59 (0.09)	0.59 (0.09)	0 (
Kangaroo Is. SA	Kangaroo Is. SA	Kangaroo Is. SA	SA	SA	41	5.28 (1.10)	5.28 (1.10)	1.09 (0.24)	1.09 (0.24)	0.50 (0.11)	0.50 (0.11)	0 (
South Coast NSW	South Coast NSW	South Coast NSW	SC	SC	110	4.57 (0.10)	4.57 (0.10)	0.91 (0.22)	0.91 (0.22)	0.40 (0.10)	0.40 (0.10)	0 (
Sydney NSW	Sydney NSW	Sydney NSW	SYD	SYD	46	4.48 (1.02)	4.48 (1.02)	0.84 (0.22)	0.84 (0.22)	0.37 (0.10)	0.37 (0.10)	0 (
North Coast NSW	North Coast NSW	North Coast NSW	NC	NC	34	3.53 (0.75)	3.53 (0.75)	0.74 (0.18)	0.74 (0.18)	0.38 (0.10)	0.38 (0.10)	0 (

(b)

Location Code	Location Code	n	n	A_R (s.e.)	^1H (s.e.)	H_O (s.e.)	H_E (s.e.)	F_{IS} (s.e
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

^1H = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. ($^1D = e^{^1H}$)

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity =$1 - \sum_i \left[{p_i}^2\right]$. ($^2D = \frac{1}{(1-H_E)}$).

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

s.e . = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()

	Location	**Code**	n	N_h	^1H	h (s.d.)	π (s.e.)
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

&

Appendix

Table A1. Within-location (alpha) genetic variation calculated from ten microsatellite loci. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions shown in Figure 1. (b) Individuals from 21 sites across seven regions; sites with less than 10 individuals are greyed.

(a)

Location	**Location**	**Location**	**Code**	**Code**	n	A_R **(s.e.)**	A_R **(s.e.)**	^1H **(s.e.)**	^1H **(s.e.)**	H_O **(s.e.)**	H_O **(s.e.)**	H (s.e.)
Perth WA	Perth WA	Perth WA	PER	PER	63	5.21 (0.80)	5.21 (0.80)	1.19 (0.21)	1.19 (0.21)	0.56 (0.10)	0.56 (0.10)	0 (
Albany WA	Albany WA	Albany WA	ALB	ALB	31	5.54 (1.11)	5.54 (1.11)	1.08 (0.20)	1.08 (0.20)	0.49 (0.08)	0.49 (0.08)	0 (
Esperance WA	Esperance WA	Esperance WA	ESP	ESP	23	5.00 (0.75)	5.00 (0.75)	1.14 (0.17)	1.14 (0.17)	0.59 (0.09)	0.59 (0.09)	0 (
Kangaroo Is. SA	Kangaroo Is. SA	Kangaroo Is. SA	SA	SA	41	5.28 (1.10)	5.28 (1.10)	1.09 (0.24)	1.09 (0.24)	0.50 (0.11)	0.50 (0.11)	0 (
South Coast NSW	South Coast NSW	South Coast NSW	SC	SC	110	4.57 (0.10)	4.57 (0.10)	0.91 (0.22)	0.91 (0.22)	0.40 (0.10)	0.40 (0.10)	0 (
Sydney NSW	Sydney NSW	Sydney NSW	SYD	SYD	46	4.48 (1.02)	4.48 (1.02)	0.84 (0.22)	0.84 (0.22)	0.37 (0.10)	0.37 (0.10)	0 (
North Coast NSW	North Coast NSW	North Coast NSW	NC	NC	34	3.53 (0.75)	3.53 (0.75)	0.74 (0.18)	0.74 (0.18)	0.38 (0.10)	0.38 (0.10)	0 (

(b)

Location Code	**Location Code**	n	n	A_R **(s.e.)**	^1H **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-PER Garden Is.	GDI	GDI	5	1.56 (0.11)	0.99 (0.20)	0.58 (0.12)	0.51 (0.10)	-0.14 (0
WA-PER Penguin Is.	PGI	58	58	1.58 (0.11)	1.18 (0.21)	0.56 (0.10)	0.56 (0.10)	-0.01 (0

Location Code	**Location Code**	n	n	A_R **(s.e.)**	$^1\boldsymbol{H}$ **(s.e.)**	H_O **(s.e.)**	H_E **(s.e.)**	F_{IS} **(s.e**
WA-ALB Muttonbird Is.	MBI	7	7	1.58 (0.11)	0.95 (0.19)	0.61 (0.12)	0.54 (0.10)	-0.15 (0
WA-ALB Mistaken Is.	MKI	4	4	1.49 (0.10)	0.76 (0.17)	0.43 (0.09)	0.43 (0.09)	-0.01 (0
WA-ALB Cheyne Is.	CHI	20	20	1.49 (0.09)	1.07 (0.20)	0.47 (0.08)	0.48 (0.08)	0.01 (0.
WA-ESP Woody Is.	WDI	20	20	1.59 (0.08)	1.14 (0.17)	0.59 (0.1)	0.58 (0.08)	0.05 (0.
WA-ESP Wickham Is.	WKI	3	3	1.61 (0.08)	0.88 (0.13)	0.57 (0.11)	0.51 (0.07)	-0.09 (0
SA Antechamber Bay	ACB	16	16	1.48 (0.11)	1.00 (0.23)	0.47 (0.11)	0.47 (0.10)	0.04 (0.
SA Emu Bay	EMB	12	12	1.54 (0.10)	1.05 (0.22)	0.51 (0.10)	0.52 (0.10)	0.02 (0.
SA Kingscote	KSC	6	6	1.52 (0.09)	0.86 (0.16)	0.5 (0.10)	0.48 (0.08)	-0.04 (0
SA Penneshaw	PNS	1	1	1.30 (0.15)	0.21 (0.11)	0.3 (0.15)	0.15 (0.08)	-1.00 (0
SA Vivonne Bay	VVB	6	6	1.53 (0.11)	0.94 (0.21)	0.53 (0.13)	0.48 (0.10)	-0.06 (0
NSW-SC Montague Is.	MI	24	24	1.43 (0.11)	0.81 (0.21)	0.39 (0.11)	0.40 (0.10)	0.08 (0.
NSW-SC Tollgate Is.	TI	14	14	1.39 (0.10)	0.70 (0.20)	0.38 (0.11)	0.37 (0.10)	0.001 (0
NSW-SC Brush Is.	BrI	16	16	1.43 (0.10)	0.74 (0.20)	0.40 (0.11)	0.42 (0.1)	0.03 (0.
NSW-SC Bowen Is.	BI	40	40	1.40 (0.10)	0.93 (0.23)	0.41 (0.10)	0.44 (0.11)	0.03 (0.
NSW-SC Five Is.	FI	16	16	1.47 (0.11)	0.77 (0.20)	0.46 (0.12)	0.45 (0.11)	-0.03 (0
NSW-SYD Manly	M	13	13	1.43 (0.12)	0.69 (0.21)	0.39 (0.11)	0.42 (0.11)	0.05 (0.
NSW-SYD Lion Is.	LI	33	33	1.38 (0.10)	0.84 (0.22)	0.37 (0.10)	0.41 (0.10)	0.06 (0.
NSW-NC Cabbage Tree Is.	CI	23	23	1.34 (0.09)	0.75 (0.19)	0.33 (0.10)	0.33 (0.09)	0.14 (0.
NSW-NC Broughton Is.	Bro	11	11	1.43 (0.10)	0.60 (0.17)	0.49 (0.12)	0.41 (0.10)	-0.19 (0

n = Number of individuals analysed

q=0 measures

A_R = allelic richness averaged across 10 loci for each site

q=1 measures

${}^1H$ = Shannon's diversity index $=-\sum_i [p_i * Log2\,(p_i)]$, where p_i is the proportion of the ith allele in the population. $({}^1D = e^{{}^1H})$

q=2 measures

H_O = Observed heterozygosity = No. of Hets /

n

H_E = Expected heterozygosity $=1 - \sum_i \left[{p_i}^2\right]$. $({}^2D = \frac{1}{(1-H_E)})$.

Other measures

F_{IS} = Fixation Index = (He - Ho) / He = 1 - (Ho / He); note that it is meaningless to calculate F_{IS} when sites are pooled

s.e . = standard error.

.

Table A2. Within-location (alpha) genetic variation calculated from mitochondrial DNA (MtDNA) control region sequence, 281 base pairs (nucleotides) from 338 individuals. (a) Individuals from multiple collection sites (Figure 1) pooled within seven geographic regions. (b) Individuals from 21 sites across seven regions; sites with less than ten individuals are greyed.

Location	**Location**	**Code**	n	N_h	***1H***	h (s.d.)	π (s.e.)
Perth WA	Perth WA	PER	83	8	1.54	0.72 (0.04)	0.011 (0.001)
Albany WA	Albany WA	ALB	30	17	2.44	0.90 (0.04)	0.012 (0.001)
Esperance WA	Esperance WA	ESP	46	17	2.56	0.92 (0.02)	0.012 (0.001)
Kangaroo Is. SA	Kangaroo Is. SA	SA	33	16	2.57	0.94 (0.02)	0.014 (0.001)
South Coast NSW	South Coast NSW	SC	90	18	2.05	0.76 (0.04)	0.008 (0.001)
Sydney NSW	Sydney NSW	SYD	29	9	1.70	0.75 (0.08)	0.008 (0.001)
North Coast NSW	North Coast NSW	NC	27	7	1.51	0.73 (0.07)	0.005 (0.001)

(b)

	Location	**Code**	n	N_h	***1H***	h (s.d.)	π (s.e.)
PER	Garden Is.	GDI	8	4	1.32	0.82 (0.10)	0.012 (0.001)
	Penguin Is.	PGI	75	8	1.51	0.72 (0.04))	0.011 (0.001)
ALB	Muttonbird Is.	MBI	7	6	1.75	0.95 (0.10)	0.017 (0.003)
	Mistaken Is.	MKI	3	2	0.64	0.67 (0.31)	0.005 (0.002)

	Location	Code	n	N_h	^1H	h (s.d.)	π (s.e.)
	Cheyne Is.	CHI	20	11	2.06	0.87 (0.06)	0.011 (0.002)
ESP	Woody Is.	WDI	40	15	2.47	0.92 (0.02)	0.012 (0.001)
	Wickham Is.	WKI	6	5	1.56	0.93 (0.12)	0.014 (0.003)
SA	Antechamber Bay	ACB	14	7	1.81	0.88 (0.06)	0.014 ()
	Emu Bay	EMB	9	7	1.83	0.92 (0.09)	0.013 ()
	Kingscote	KSC	5	5	1.61	1.00 (0.13)	0.011 (0.003)
	Penneshaw	PNS	1	1	0.00	0.00 (0.00)	0.000 (0.000)
	Vivonne Bay	VVB	4	3	1.04	0.83 (0.22)	0.012 ()
SC	Montague Is.	MI	18	6	1.61	0.81 (0.07)	0.009 ()
	Tollgate Is.	TI	13	8	1.84	0.85 (0.08)	0.008 (0.002)
	Brush Is.	BrI	19	5	1.04	0.54 (0.16)	0.005 (0.001)
	Bowen Is.	BI	24	8	1.44	0.66 (0.11)	0.007 ()
	Five Is.	FI	16	10	2.06	0.88 (0.06)	0.011 (0.002)
SYD	Manly	M	14	4	0.90	0.50 (0.15)	0.009 (0.003)
	Lion Is.	LI	15	7	1.75	0.86 (0.06)	0.008 ()
SC	Cabbage Tree Is.	CI	16	5	1.33	0.73 (0.08)	0.005 (0.001)
	Broughton Is.	Bro	11	6	1.42	0.73 (0.14)	0.006 (0.002)

Table A4. Pairwise population matrix of mean Shannon's mutual information (I) values over 10 microsatellite loci (using log base=2) across seven geographic regions.

	Perth
	01PER
01PER	
02ALB	0.101
03ESP	0.070
04SA	0.127
05SC	0.160
06SYD	0.195
07NC	0.174
Bottom triangle: $I = {}^1H$-pooled - average ^1H-region [calculated for each pair of regions at each locus] log base 2	Bottom
Top triangle: I Probability for significance, based on 999 permutations	Top tria
Bonferroni correction for all pairwise comparisons = 0.05/21 = 0.002. Significant values in red font.	Bonferro

TableA5. Pairwise population matrix of mean F_{ST} values across 10 microsatellite loci across seven geographic regions.

01PER
02ALB
03ESP
04SA
05SC
06SYD
07NC
Bottom triangle: pairwise population F_{ST} values. Top triangle: F_{ST} Probability for significance, based on 999 permutations

Table A6. Pairwise population matrix of Shannon's mutual information (I) values from 281 bp mtDNA sequences (Using Log Base = 2) across seven geographic regions.

	Perth **01PER**
01PER	
02ALB	0.694
03ESP	0.683
04SA	0.814
05SC	0.899
06SYD	0.825
07NC	0.728
Bottom triangle: $I = {}^1H$-pooled - average ${}^1H$-region [calculated for each pair of regions at each locus] log base 2	Bottom
Top triangle: I Probability for significance, based on 999 permutations	Top tria
Bonferroni correction for all pairwise comparisons = 0.05/21 = 0.002. Significant values in red font.	Bonferro

Table A7 Pairwise population matrix of F_{ST} values calculated from mitochondrial data across seven geographic regions.

	Perth **01PER (n=83)**
01PER	
02ALB	0.354
03ESP	0.340
04SA	0.372
05SC	0.278
06SYD	0.287
07NC	0.274
Bottom triangle: pairwise F_{ST} values	Bottom triangle: pair
Top triangle: F_{ST} Probability for significance, based on 999 permutations	Top triangle: F_{ST} Pr
Bonferroni correction for all pairwise comparisons = 0.05/21 = 0.002. Significant values in red font.	Bonferroni correction

Table A8 First-Generation Dispersal, from Geneclass, based on 10,000 simulations for ten microsatellite loci, showing movements between the centre of the distribution (SA) to or from the limits of the distribution in the northwest (NW) or northeast (NE). (a) First-generation dispersal between geographic regions over the entire continent. Seven of the ten dispersers were in the eastern and central part of the distribution, dispersing in either direction (NE to Centre or Centre to NE). (b) First-generation dispersal between WA sites. One of the five dispersals was in the direction from the northwest limit towards the centre of the continental distribution (NW to Centre) and four were from the centre towards the northwest limit (Centre to NW)

Individual	**Source Population**	**Recipient Population**	**-LOG (L_home / L_max)**	**p-value**	**Direction of di**
EM003	PER	ALB	2.429	0.004	NW to Centre
EM0048	ALB	PER	2.133	0.004	Centre to NW
EM0051	ALB	PER	1.797	0.009	Centre to NW
BI113	SA	SC	2.019	0.004	Centre to NE
FI116	SA	SC	1.763	0.009	Centre to NE
M75	SA	SYD	1.889	0.006	Centre to NE
SA54	SC	SA	2.212	0.007	NE to Centre

Individual	Source Population	Recipient Population	-LOG (L_home / L_max)	p-value	Direction of di
SA51	SYD	SA	2.115	0.008	NE to Centre
SA86	NC	SA	2.363	0.004	NE to Centre
LI194	NC	SYD	2.423	0.001	NE to Centre

Individual	Source Population	Recipient Population	-LOG (L_home / L_max)	p-value	Direction of di
EM132	GDI	WDI	3.101	0.0049	NW to Centre
EM051	MBI	PGI	4.168	0.0003	Centre to NW
EM048	MKI	PGI	4.645	0.0002	Centre to NW
EM006	MKI	MBI	5.879	0.0011	Centre to NW
EM108	WKI	WDI	3.575	0.0024	Centre to NW

Figure A1. Evanno Delta K results for STRUCTURE modelling using the Admixture LocPrior model, calculated using Structure Harvester. Evanno's method indicates that K=2 is the best fit.

Literature cited

ALLENDORF, F. W., FUNK, W. C., AITKEN, S. N., BYRNE, M. & LUIKART, G. 2022. *Conservation and the genomics of populations,* Oxford, Oxford University Press.

BEERLI, P. 2016. *MIGRATE* [Online]. Available: https://peterbeerli.com/migrate-html5/index.html accessed August 2016 [Accessed].

BURRIDGE, C. P., PEUCKER, A. J., VALAUTHAM, S. K., STYAN, C. A. & DANN, P. 2015. Nonequilibrium Conditions Explain Spatial Variability in Genetic Structuring of Little Penguin (Eudyptula minor). *Journal of Heredity,* 106**,** 228-237.

CANNELL, B. 2016. How resilient are the Little Penguins and the coastal marine habitats they use. Report Year 3. *Report for City of Rockingham and Fremantle Ports.* Perth, WA: Murdoch University.

CANNELL, B. L., CHAMBERS, L. E., WOOLLER , R. D. & BRADLEY, J. S. 2012. Poorer breeding by little penguins near Perth, Western Australia is correlated with above average sea surface temperatures and a stronger Leeuwin Current. *Marine and Freshwater Research,* 63**,**914-925.

CHANNELL, R. & LOMOLINO, M. V. 2000. Trajectories to Extinction: Spatial Dynamics of the Contraction of Geographical Ranges.*Journal of Biogeography,* 27**,** 169-179.

COLLINS, M., CULLEN, J. N. M. & DANN, P. 1999. Seasonal and annual foraging movements of little penguins from Phillip Island, Victoria. *Wildlife Research,* 26**,** 705-721.

CRANDALL, K. & ETAL. 1999. Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfishes (Decapoda: Parastacidae). *Australian Journal of Zoology,* 47**,**199–214.

CRESSWELL, G. 1990. The Leeuwin current. *Corella,* 14**,**113-118.

CRESSWELL, G. R. & GOLDING, T. J. 1980. Observations of a south-flowing current in the southeastern Indian Ocean. *Deep-Sea Res A, Oceanogr Res Pap,* 27**,** 449-466.

CURNUTT, J. L., PIMM, S. L. & MAURER, B. A. 1996. Population variability of sparrows in space and time. *Oikos,* 76**,**131-144.

DANN, P. 1991. Distribution, Population Trends and Factors Influencing the Population Size of Little Penguins Eudyptula minor on Phillip Island, Victoria. *Emu - Austral Ornithology,* 91**,**263-272.

DANN, P., CULLEN, J. M., THODAY, R. & JESSOP, R. 1991. Movements and Patterns of Mortality at Sea of Little Penguins Eudyptula minor from Phillip Island, Victoria. *Emu - Austral Ornithology,* 91**,**278-286.

DANN, P., CULLEN, J. M. & WEIR, I. 1996. *National review of the conservation status and management of Australian Little Penguin colonies,* Canberra, The Australian Nature Conservation Agency.

DRAY, S. & DUFOUR, A.-B. 2007. The ade4 Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software,*22**,** 1 - 20.

EARL, D. A. & VONHOLDT, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources,* 4**,** 359-361.

ECKERT, C. G., SAMIS, K. E. & LOUGHEED, S. C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology,* 17**,** 1170-1188.

EVANNO, G., REGNAUT, J. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology,* 14**,** 2611-2620.

EXCOFFIER, L. & LISCHER, H. E. L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources,* 10**,** 564-567.

FALUSH, D., STEPHENS, M. & PRITCHARD, J. K. 2003. Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics,* 164**,** 1567-1587.

FRANCIS, R. M. 2017. POPHELPER: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources,*17**,** 27-32.

GOUDET, J. 1995. FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *Journal of Heredity,* 86**,** 485-486.

GOUDET, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes,* 5**,**184-186.

GRIFFITH, B., SCOTT, J. M., CARPENTER, J. W. & REED, C. 1989. Translocation as a Species Conservation Tool - Status and Strategy.*Science,* 245**,** 477-480.

GU, Z., GU, L., EILS, R., SCHLESNER, M. & BRORS, B. 2014. circlize implements and enhances circular visualization in R.*Bioinformatics,* 30**,** 2811-2812.

HOLLAND, L. P., JENKINS, T. L. & STEVENS, J. R. 2017. Contrasting patterns of population structure and gene flow facilitate exploration of connectivity in two widely distributed temperate octocorals.*Heredity,* 119**,** 35-48.

HUTTER, C. M., SCHUG, M. D. & AQUADRO, C. F. 1998. Microsatellite Variation in Drosophila melanogaster and Drosophila simulans: A Reciprocal Test of the Ascertainment Bias Hypothesis. *Molecular Biology and Evolution,* 15**,** 1620–1636.

JOHANNESEN, E., PERRIMAN, L. & STEEN, H. 2002. The effect of breeding success on nest and colony fidelity in the Little Penguin (Eudyptula minor) in Otago, New Zealand. *Emu - Austral Ornithology,*102**,** 241-247.

JOMBART, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics,* 24**,** 1403-1405.

JOMBART, T., DEVILLARD, S. & BALLOUX, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics,* 11**,** 94.

KAMVAR, Z. N., TABIMA, J. F. & GRÜNWALD, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ,* 2**,** e281.

KLOMP, N. I. & WOOLLER, R. D. 1988. The size of Little Penguins Eudyptula minor, on Penguin Island, Western Australia. *Records of the Western Australian Museum,* 14**,** 211-215.

KOPELMAN, N. M., MAYZEL, J., JAKOBSSON, M., ROSENBERG, N. A. & MAYROSE, I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources,* 15**,** 1179-1191.

LAWTON, J. H. 1993. Range, population abundance and conservation. *Trends Ecol Evol,* 8**,** 409-413.

LESICA, P. A., FW. 1995. When are peripheral populations valuable for conservation. *Conservation Biology.,* 9**,** 753-760.

LONGMIRE, J. L. & ETAL. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics,* 2**,** 14–24.

NORMAN, F. I., CULLEN, J. M. & DANN, P. 1991. Little Penguins Eudyptula minor in Victoria: Past, Present and Future. *Emu - Austral Ornithology,* 91**,** 402-408.

PEAKALL, R. & SMOUSE, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics,* 28**,** 2537-2539.

PEARL, M. 1992. Conservation of Asian primates: aspects of genetics and behavioral ecology that predict vulnerability. *In:* FIELDER, P. L. & JAIN, S. K. (eds.) *Conservation Biology: The Theory and Practice of Nature Conservation Preservation and Management.* New York: Chapman & Hall.

PETERSON, A. T. 2001. Endangered species and peripheral populations: cause for reflection. *Endangered species UPDATE,* 18**,**30-31.

PEUCKER, A. J., DANN, P. & BURRIDGE, C. P. 2009. Range-wide phylogeography of the Little Penguin (Eudyptula minor): evidence of long-distance dispersal. *The Auk,* 126**,** 397-408.

PIRY, S., ALAPETITE, A., CORNUET, J.-M., PAETKAU, D., BAUDOUIN, L. & ESTOUP, A. 2004. GENECLASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. *Journal of Heredity,*95**,** 536-539.

RAMBAUT, A., DRUMMOND, A. J., XIE, D., BAELE, G. & SUCHARD, M. A. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7.*Systematic Biology,* 67**,** 901-904.

RICE, W. R. 1989. Analyzing tables of statistical tests.*Evolution,* 43**,** 223-225.

ROZAS, J., FERRER-MATA, A., SÁNCHEZ-DELBARRIO, J. C., GUIRAO-RICO, S., LIBRADO, P., RAMOS-ONSINS, S. E. & SÁNCHEZ-GRACIA, A. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution,* 34**,** 3299-3302.

SAGARIN, R. D. & GAINES, S. D. 2002. The 'abundant centre' distribution: to what extent is it a biogeographical rule? *Ecology Letters,* 5**,** 137-147.

SARAUX, C., ROBINSON-LAVERICK, S. M., LE MAHO, Y., ROPERT-COUDERT, Y. & CHIARADIA, A. 2011. Plasticity in foraging strategies of inshore birds: how little penguins maintain body reserves while feeding offspring.*Ecology,* 92**,** 1909-1916.

SHERWIN, W. B., CHAO, A., JOST, L. & SMOUSE, P. E. 2017. Information Theory Broadens the Spectrum of Molecular Ecology and Evolution. *Trends in Ecology and Evolution,* 32**,** 948 - 963.

SHERWIN, W. B., CHAO, A., JOST, L. & SMOUSE, P. E. 2021. Correction: Information theory broadens the spectrum of molecular ecology and evolution. *Trends in Ecology and Evolution,* 36**,** 955-6.

SMITH, L. M. & BURGOYNE, L. 2004. Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. *BMC ecology,* 4**,** 4.

STONEHOUSE, B. 1975. *The Biology of Penguins,* London, Macmillan.

SUTHERS, I. M., YOUNG, J. W., BAIRD, M. E., ROUGHAN, M., EVERETT, J. D., BRASSINGTON, G. B., BYRNE, M., CONDIE, S. A., HARTOG, J. R., HASSLER, C. S., HOBDAY, A. J., HOLBROOK, N. J., MALCOLM, H. A., OKE, P. R., THOMPSON, P. A. & RIDGWAY, K. 2011. Editorial: The strengthening East Australian Current, its eddies and biological effects — an introduction and overview. *Deep Sea Research Part II: Topical Studies in Oceanography,* 58**,** 538-546.

TURPIE, J. K., BECKLEY, L. E. & KATUA, S. M. 2000. Biogeography and the selection of priority areas for conservation of South African coastal fishes. *Biological Conservation,* 92**,** 59-72.

VARDEH, S. 2015. *Population Genetics, Demography and Population Variability of Little Penguins (Eudyptula minor) in Australia.* Ph.D., UNSW-Sydney.

WICKHAM, H., AVERICK, M., BRYAN, J., CHANG, W., MCGOWAN, L. D., FRANÇOIS, R., GROLEMUND, G., HAYES, A., HENRY, L., HESTER, J., KUHN, M., PEDERSEN, T. L., MILLER, E., BACHE, S. M., MÜLLER, K., OOMS, J., ROBINSON, D., SEIDEL, D. P., SPINU, V. & TAKAHASHI, K. 2019. Welcome to the Tidyverse. *Journal of Open Source Software* .

WIENECKE, B. C. 1993. *The size and breeding patterns of Little Penguins Eudytptula minor in Australia: a comparative study.* Ph.D., Murdoch University, Western Australia.

WIENECKE, B. C. 1995. Adoption of chicks by Little Penguins Eudyptula minor on Penguin Island, Western Australia. *Emu - Austral Ornithology,* 95**,** 119-122.

WIENECKE, B. C., WOOLLER, R. D. & KLOMP, N. I. 1995. The ecology and management of little penguins on Penguin Island, Western Australia.*In:* DANN, P., NORMAN, I. & REILLY, P. (eds.) *The penguins: ecology and management.* Sydney: Surrey Beatty.

WILSON, G. A. & RANNALA, B. 2003. Bayesian Inference of Recent Migration Rates Using Multilocus Genotypes. *Genetics,*163**,** 1177-1191.

WOLF, C. M. & AL, E. 1996. Avian and Mammalian Translocations: update and reanalysis of 1987 survey data. *Conservation Biology.,*10**,** 1142-1154.