Genetic outcomes from the translocations of the critically endangered woylie

Carlo PACIONI1*, Adrian F. WAYNE2, Peter B. S. SPENCER1

1 School of Veterinary and Life Sciences, Murdoch University, Murdoch, 6150, Western Australia
2 Department of Environment and Conservation, Science Division, Manjimup, 6258, Western Australia

Abstract Translocations are an important conservation strategy for many species. However simply observing demographic growth of a translocated population is not sufficient to infer species recovery. Adequate genetic representation of the source population(s) and their long-term viability should also be considered. The woylie Bettongia penicillata ogilbyi has been subject to more formal translocations for conservation than any other marsupial that, up until recently, has resulted in one of the most successful species recoveries in Australia. We used mitochondrial and nuclear DNA markers to assess the genetic outcomes of translocated woylie populations. These populations have lost genetic variability, differentiated from their source population and the supplementation program on two island populations appears to have failed. We discuss the conservation implications that our results have for managing threatened species, outline some general recommendations for the management of present and future translocations and discuss the appropriate sampling design for the establishment of new populations or captive breeding programs that may mitigate the genetic ‘erosion’ seen in our study species. This research provides some practical outcomes and a pragmatic understanding of translocation biology. The findings are directly applicable to other translocation programs [Current Zoology 59 (3): 294–310, 2013].

Keywords Bettongia penicillata, Macropod, translocation, Supplementation, Microsatellites, mtDNA

Translocations are an important management strategy for biodiversity conservation (Griffith et al., 1989; IUCN, 1998; Seddon et al., 2007; Wolf et al., 1996). However a reduction of genetic diversity can occur, even in successfully established translocated populations, and can compromise the effectiveness of such strategies (Goossens et al., 2002; Stockwell et al., 1996). Ultimately, a reduction in genetic variability can impact the evolutionary potential (Frankham, 1996; Frankham et al., 1999) and reduce the fitness of populations by decreasing fecundity and survival rates, and increase the influence of inbreeding depression (Eldridge et al., 1999; O’Grady et al., 2006; Ralls et al., 1988). Consequently, genetic studies have been recommended to assess the appropriateness of translocations as an effective conservation option (IUCN, 1998; Moritz, 1999; Stockwell et al., 1996). Genetics in combination with demographic monitoring should be used for the assessment of translocation success (Goossens et al., 2002). Investigations of the genetic profile of founders and genetic variability of the translocated populations are particularly valuable in this regard. For example, these can help to predict the long-term viability of the new populations and quantify the success of subsequent supplementations (the addition of individuals to an existing population: i.e. assisted dispersal) (Goossens et al., 2002 and within).

Translocations have been an important tool in the re-establishment of species across their previous range (Fischer et al., 2000; Griffith et al., 1989; Moritz, 1999). For example, in Australia, 88 translocations (25 species) were carried out involving three states (Western Australia, South Australia and New South Wales) in one particular program (Mawson, 2004). Success however, has been hindered by inadequate control of threats such as removal of competitors and feral predators (Fischer et al., 2000). Macropods are the most common taxa involved in translocation programs in Australia (e.g. 59% translocations carried out under the Western Shield program involved members of this superfamily, Mawson, 2004) and several studies reviewed macropod translocations in an attempt to identify the most common problems and establish best practice management (e.g. Finlayson et al., 2010; Mawson, 2004; Short et al., 1992). It was concluded that the most important limiting factor in translocation success was the inadequate or partial control of feral predators and lack of long-term
monitoring to enable adaptive management. Finlayson et al. (2010) also recommended the use of captive bred founders because of the ability to manipulate sex ratio and implement predator avoidance training.

The woylie (brush-tailed bettong Bettongia penicillata ogilbyi) has been subject to more formal translocations for conservation than any other marsupial: 61 sites across Australia involving >3,400 individuals between 1977 and 2006 (Groom, 2010; Orell, 2004). It has been suggested that woylies should be used as a model to study translocation methodology in macropods because they are easily trapped, have high reproductive rates, abundant source populations (which have sharply declined since 2001; Groom, 2010), were distributed across a variety of climatic regions and because of the considerable knowledge gained in the last few decades about the ecology and physiology of this species (e.g. Finlayson et al., 2010; Mawson, 2004).

The average home range of a woylie is up to 35 ha (Christensen, 1980; Sampson, 1971), with males being more transient than females. Radio tracking and trapping data suggest that single individuals are capable of moving relatively long distances (3–5 km) (Christensen, 1980; Martin et al., 2006; Pacioni, 2010). Genetic data inferred female philopatry (neighbour-size of less than 1 km) and male-biased dispersal (neighbour sizes of between 1 and 3 km; Pacioni, 2010).

Females reach sexual maturity at 6–8 months and males at 10–12 months (Christensen, 1980; Sampson, 1971). Normally, females give birth to only one young at a time after a gestation of around 21 days (Smith, 1992). Natural populations show continuous reproduction throughout the year (Christensen, 1980; Sampson, 1971; Ward et al., 2008) with a birth interval of approximately 100 days (Christensen, 1980; Sampson, 1971). With embryonic diapause (Tyndale-Biscoe, 1984), females can give birth up to three young per year. The woylie diet mainly comprises hypogeal fungi (native truffles) although they also eat tubers, bulbs, seeds and invertebrates (Van Dyck et al., 2008; Zosky, 2012).

Until the arrival of Europeans (~200 years ago), the woylie was distributed across much of Australia: including the arid and semi-arid climatic regions, desert spinifex grassland, the Mediterranean jarrah forest and subtropical regions of eastern Australia.

Translocations were a key component in the recovery strategy of this species. This was in response to the species being reduced in range from more than four million km² to three small areas in southwestern Australia by the 1970s. Feral fox Vulpes vulpes predation and habitat fragmentation due to agriculture were identified as the main cause of this historical decline, although in some areas feral cats Felis catus played a major role (Burbidge et al., 1989; Christensen, 1980; Start et al., 1998; Van Dyck et al., 2008). The woylie was removed from the Endangered species list in 1996 having made a spectacular recovery as a result of feral predator control operations and the establishment of seven translocated populations within its previous range (Start et al., 1998). Despite this, the woylie is now listed as Critically Endangered, having declined by 90% between 1999 and 2006 (Groom, 2010; Wayne et al., in press) for unknown reasons.

Recent work has identified four genetically distinct and naturally occurring woylie populations. Two occur in the wheatbelt region (Dryandra Forest and Tutanning Nature Reserve) and another two in the jarrah forest of the Upper Warren region (Kingston and Perup; Pacioni et al., 2011; Fig. 1). A preliminary genetic study of the translocated populations in South Australia indicated a low level of genetic diversity – 80% band sharing at VNTR loci (Variable Number Tandem Repeat; Start et al., 1998; Start et al., 1994). No other genetic studies have been published for any other translocated populations, despite recommendations in the woylie recovery plan (Start et al., 1995).

We investigated the genetic profile of five key translocated woylie populations with the intent to (i) assess levels of genetic diversity and differentiation with the source populations, (ii) evaluate the success of the translocations and (iii), for the populations with a poorly documented translocation history, attempt to identify original source populations. We predicted that demographic attributes of the translocated populations would be reflected in their genetic characteristics. In particular, evidence of a bottleneck at Batalling would be expected given the slow growth in the first ten years (before more effective fox control was implemented). Despite the

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rapid growth of the population at Karakamia, due to a lack of introduced predators and few competitors, evidence of a bottleneck and lower genetic diversity might be expected due to the founder effect and the small population size. We would expect that the three South Australian populations would have the least genetic diversity as a result of their establishment through a breeding program that was not specifically designed to enhance the genetic diversity of the founders. Moreover, we evaluated the effectiveness of common statistics in detecting bottlenecks. This was possible because the demography of some translocated woylie populations was well documented and our data enabled an empirical assessment of the power of these statistics. Finally, we discuss the conservation implications that our results have for managing threatened species, outlining general recommendations for the management of present and future translocations.

1 Materials and Methods

1.1 Sample collection and population history

Genetic data was available from Pacioni et al. (2011) for the four distinct naturally occurring woylie populations (Dryandra Forest and Tutanning Nature Reserve in the Wheatbelt region and Kingston and Perup in the Upper Warren region. Table 1; Supplementary Material Table SOM1). The translocated populations at Batalling State Forest and Karakamia Wildlife Sanctuary in Western Australia (Fig. 1) were trapped using standard techniques in 2004 and tissue samples (collected using an ear punch) were used as a source for DNA profiling. Founders of these populations were sourced from Perup and Dryandra, respectively. The population at Batalling remained at very low density for the first ~10 years, but substantially increased (to ~3,000 individuals) following the commencement of aerial fox baiting in 1994 (Orell, 2004). In contrast, the population at Karakamia grew rapidly within the first 10 years (to ~500 individuals; Groom, 2010; Ward et al., 2008).

In 2006, samples were obtained from three additional

Table 1 Summary of the samples collected during the study of natural (bold) and translocated (italics) populations (sampling locations) in Western Australia (WA) and South Australia (SA) and measures of genetic variability

<table>
<thead>
<tr>
<th>Sampling locations</th>
<th>State</th>
<th>n</th>
<th>(N_e) (SE)</th>
<th>(N_A) (SE)</th>
<th>(N_{AR}) (SD)</th>
<th>(H_e) (SE) %</th>
<th>(H_L) (SE) %</th>
<th>(P4) (SE)</th>
<th>(P_{AR}) (SD)</th>
<th>(F_r)</th>
<th>(h)</th>
<th>(\pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryandra(^a)</td>
<td>WA</td>
<td>28</td>
<td>8.9 (± 0.9)</td>
<td>5.8 (± 0.7)</td>
<td>7.8 (± 2.3)</td>
<td>79.6 (± 3)</td>
<td>73.1 (± 5)</td>
<td>0.3 (± 0.2)</td>
<td>0.6 (± 0.7)</td>
<td>NA</td>
<td>0.82 (± 0.1)</td>
<td>0.022 (± 0.013)</td>
</tr>
<tr>
<td>Karakamia</td>
<td>WA</td>
<td>29</td>
<td>7.5 (± 0.8)</td>
<td>4.9 (± 0.7)</td>
<td>6.7 (± 2.3)</td>
<td>74.5 (± 4)</td>
<td>66.1 (± 7)</td>
<td>0.2 (± 0.1)</td>
<td>0.3 (± 0.5)</td>
<td>0.06</td>
<td>0.53 (± 0.06)</td>
<td>0.005 (± 0.003)</td>
</tr>
<tr>
<td>Tutanning(^a)</td>
<td>WA</td>
<td>32</td>
<td>5.5 (± 0.6)</td>
<td>3.2 (± 0.3)</td>
<td>4.8 (± 1.5)</td>
<td>64 (± 5)</td>
<td>64.5 (± 8)</td>
<td>0.6 (± 0.3)</td>
<td>0.7 (± 1.0)</td>
<td>NA</td>
<td>0.69 (± 0.04)</td>
<td>0.014 (± 0.007)</td>
</tr>
<tr>
<td>Kingston(^a)</td>
<td>WA</td>
<td>69</td>
<td>12.1 (± 1.4)</td>
<td>5.9 (± 0.6)</td>
<td>8.2 (± 2.5)</td>
<td>78.8 (± 4)</td>
<td>70.6 (± 6)</td>
<td>1.1 (± 0.4)</td>
<td>1.2 (± 1.1)</td>
<td>NA</td>
<td>0.59 (± 0.05)</td>
<td>0.018 (± 0.009)</td>
</tr>
<tr>
<td>Perup(^a)</td>
<td>WA</td>
<td>102</td>
<td>15 (± 1.8)</td>
<td>7.6 (± 0.9)</td>
<td>9.7 (± 2.7)</td>
<td>83.6 (± 3)</td>
<td>74.6 (± 4)</td>
<td>1.7 (± 0.7)</td>
<td>1.0 (± 1.1)</td>
<td>NA</td>
<td>0.61 (± 0.16)</td>
<td>0.016 (± 0.01)</td>
</tr>
<tr>
<td>Batalling State</td>
<td>WA</td>
<td>35</td>
<td>7.3 (± 0.6)</td>
<td>4.1 (± 0.4)</td>
<td>6.4 (± 1.6)</td>
<td>72.1 (± 4)</td>
<td>71.7 (± 5)</td>
<td>0.2 (± 0.1)</td>
<td>0.3 (± 0.3)</td>
<td>0.14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>St Peter Is.</td>
<td>SA</td>
<td>30</td>
<td>4.9 (± 0.5)</td>
<td>3.0 (± 0.3)</td>
<td>4.4 (± 1.2)</td>
<td>63.1 (± 4)</td>
<td>62 (± 5)</td>
<td>0.1 (± 0.1)</td>
<td>0.1 (± 0.2)</td>
<td>0.21</td>
<td>0.29 (± 0.12)</td>
<td>0.004 (± 0.002)</td>
</tr>
<tr>
<td>Wedge Is.</td>
<td>SA</td>
<td>32</td>
<td>4.2 (± 0.3)</td>
<td>2.8 (± 0.2)</td>
<td>3.9 (± 1.0)</td>
<td>60.2 (± 6)</td>
<td>55 (± 6)</td>
<td>0 (± 0)</td>
<td>0 (± 0.1)</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Venus Bay Is.</td>
<td>SA</td>
<td>14</td>
<td>2.7 (± 0.3)</td>
<td>2.1 (± 0.3)</td>
<td>2.7 (± 0.9)</td>
<td>42.3 (± 7)</td>
<td>45.5 (± 8)</td>
<td>0 (± 0)</td>
<td>0 (± 0.1)</td>
<td>0.47</td>
<td>0.26 (± 0.14)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(n=\)number of individuals genotyped at microsatellite loci. \(N_e=\)average number of alleles. \(N_A=\)average effective number of alleles. \(N_{AR}=\)average allelic richness. \(H_e=\)expected heterozygosity. \(H_L=\)observed heterozygosity. \(P4=\)average private alleles. \(P_{AR}=\)average private allelic richness. \(SE=\)standard error. \(SD=\)standard deviation. \(F_r=\)effective inbreeding coefficient (Frankham, 1998). \(h=\)haplotype diversity (mtDNA). \(\pi=\)nucleotide diversity (mtDNA). \(^a\)From Pacioni et al., 2011.
translocated populations established on three islands in South Australia (SA): St Peter Island, Wedge Island and Venus Bay Island. Woylies translocated to the South Australian islands were sourced from a breeding program that was established with only three individuals (two females and one male from Perth Zoo) of undocumented Western Australian origin (Delroy et al., 1986). Two attempts were made to increase the genetic variability of the population on Wedge Is., (a release in 1994 of 10 males and another in 1995 of 15 males) but both failed with most animals found dead or not re-trapped in subsequent monitoring (Start et al., 1994, Van Weenen, 1996). In 1996, 15 woylies (10 males, five females) from Dryandra were released on St Peter Is, but only six (four males and two females) remained after two months (Van Weenen, 1996). Consequently the extent of their genetic contribution remains unknown. Additional information was also collected; including year of establishment, total number of animals released and estimated population size (Table 2).

### 1.2 DNA extraction and amplification

A modified high-salt method (Miller et al., 1988) was used for the DNA extractions and a partial (~600 bp) section of the tRNA Proline end of the control region (or D-loop) was amplified using the primers H15999M and L16498M (Funagalli et al., 1997) using reaction conditions described in Pacioni et al. (2011). Amplification of 12 microsatellite loci (Supplementary Material Table SMO1) followed protocols given in Pacioni and Spencer (2010).

### 1.3 Sequence data analysis

Sequences were aligned in Geneious 5.6.2 (www.geneious.com) and then imported in TCS (Clement et al., 2000) to build the Maximum Parsimony Network. The alignment was also imported in DNAsp v5 (Librado et al., 2009) to set up the genetic structure and exported to Arequin (Excoffier et al., 2010) where we calculated the haplotype (h) and nucleotide diversity (π) (Nei, 1987), and performed the exact test for population differentiation (Raymond et al., 1995) with 100,000 Markov chain steps with 10,000 dememorisation steps.

### 1.4 Microsatellites analysis

Descriptive measures of population genetic diversity were all calculated using GENALEX 6.2 (Peakall et al., 2006) and included estimates of genetic diversity within populations: observed (H_o) and expected heterozygosity (H_E, Hartl et al., 1997); average of observed (N_A) and expected number of alleles (N_E, Brown et al., 1983); and average number of private alleles (P_A). In order to further enable the comparison of the genetic variability

### Table 2 Woylie population history including the type of population (being either ‘natural’, N or translocated, T, and the source of the translocated one in brackets), year of establishment, total number of released (TNR) animals and population census size (Nc), and genetic contribution (given as a proportion) of each population to the six genetic clusters identified with STRUCTURE (Pritchard et al., 2000) under the admixture with correlated allele frequencies model and geographic information included in the prior

<table>
<thead>
<tr>
<th>Sampled locality</th>
<th>State</th>
<th>Area (ha)</th>
<th>Type</th>
<th>Year</th>
<th>TNR</th>
<th>Nc 2001a</th>
<th>Nc 2006a</th>
<th>Genetic contribution to the genetic clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>1. Dryandra</td>
<td>WA</td>
<td>12,192</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td>6,000</td>
<td>400-500</td>
<td>1.00 0 0 0 0 0</td>
</tr>
<tr>
<td>Karakamia</td>
<td>WA</td>
<td>275</td>
<td>T (Dryandra)</td>
<td>1994</td>
<td>31b</td>
<td>500 500</td>
<td>0 0 1.00 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2. Tutanning</td>
<td>WA</td>
<td>2,369</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td>300 300</td>
<td>0 0 0.99 0.01 0 0</td>
<td></td>
</tr>
<tr>
<td>3. Kingston</td>
<td>WA</td>
<td>25,000c</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td>20,000d</td>
<td>1,000d</td>
<td>0 0 0.03 0.97 0 0</td>
</tr>
<tr>
<td>4. Perup</td>
<td>WA</td>
<td>60,000e</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td>20,000d</td>
<td>1,000d</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>5. Batalling</td>
<td>WA</td>
<td>3,617</td>
<td>T (Perup)</td>
<td>1983</td>
<td>52c</td>
<td>3,000 400-500</td>
<td>0 0 0 1.00 0 0</td>
<td></td>
</tr>
<tr>
<td>6. South Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St Peter Is.</td>
<td>SA</td>
<td>3,493</td>
<td>T (Unknown)</td>
<td>1989</td>
<td>113g</td>
<td>2,000-3,500 2,000-3,500</td>
<td>0.01 0 0.01 0.01 0.01 0.97</td>
<td></td>
</tr>
<tr>
<td>Wedge Is.</td>
<td>SA</td>
<td>947</td>
<td>T (Unknown)</td>
<td>1983</td>
<td>11a</td>
<td>1,500-3,000 1,500-3,000</td>
<td>0 0 0 0 0 0.99</td>
<td></td>
</tr>
<tr>
<td>Venus Bay Is.</td>
<td>SA</td>
<td>15</td>
<td>T (Unknown)</td>
<td>1980</td>
<td>7a</td>
<td>30 30</td>
<td>0 0 0 0 0 0.99</td>
<td></td>
</tr>
</tbody>
</table>

Sample localities are grouped by genetic cluster. WA refers to animals from Western Australia and SA from South Australia. *(Groom, 2010), bI. Williams personal communication, cApproximate extent of occurrence, forest habitat is contiguous across a much larger area (i.e. not isolated and as discretely defined as wheatbelt and island populations., dKingston and Perup combined, e(Start et al., 1995), f(Nelson et al., 1992), gAdditional releases (Dryandra stock) were carried out in 1996 (Van Weenen, 1996), h(Delroy et al., 1986)
among populations, we calculated the average allelic richness (\(N_{AR}\)) and average private allelic richness (\(P_{AR}\)) based on 14 diploid individuals using the rarefaction method implemented in HP-RARE (Kalinowski, 2005), which compensated for differences in sample size producing unbiased estimates of allelic richness. We also calculated \(F_{IS}\) (Hartl et al., 1997) for the wild populations using GENALEX 6.2 (Peakall et al., 2006).

We carried out assignment testing using STRUCTURE 2.2 (Pritchard et al., 2000). STRUCTURE uses a Bayesian assignment approach to determine the most likely number of inferred populations \((K)\) and the extent of the contribution from each inferred population to each animal’s genotype. Analysis of the data was repeated with both, admixture and no admixture models (Pritchard et al., 2000). In addition, the hypothesis that the allele frequencies were or were not correlated, have been tested under the admixture model. We also tested whether adding information on the geographic location into the prior probability would alter the results. To determine the most likely number of populations, we analyzed the posterior probability of the data given \(K\) (\(\log Pr(X/K)\)) (Pritchard et al., 2000) and the second rate of change of the likelihood distribution, \(\Delta K\) (Evanno et al., 2005). Each set of STRUCTURE results were based on 20 independent runs from one to ten inferred populations \((K = 1-10)\), using a ‘burn-in’ period of 100,000 iterations followed by \(10^6\) iterations of a Markov Chain Monte Carlo. Population differentiation was also estimated by calculating estimators of \(F_{ST}\) in GENALEX 6.2 (Peakall et al., 2006) under the AMOVA (analysis of molecular variance) framework (Peakall et al., 1995) using 1,000 permutations to test significant differences from zero.

By extension from Frankham (1998) the effective inbreeding coefficient, \(F_e\), between translocated and source populations was calculated as \(F_e = 1-H_f/H_s\), where \(H_f\) and \(H_s\) are translocated and source populations, respectively.

Genetic evidence of population bottlenecks was investigated by testing for an excess in heterozygosity (Cornuet et al., 1996) and mode-shift (Luikart et al., 1998b), using the program BOTTLENECK (Piry et al., 1999). Due to the relatively small number of loci analyzed \((n = 12)\), a Wilcoxon sign-rank test was estimated, as recommended by Piry et al. (1999). A mixed model of microsatellite mutation was assumed, with single step mutations accounting for 95% of all mutation events, and a variance among multiple steps of 12, as suggested by Piry et al. (1999). Additionally, we also used the M-ratio method (Garza et al., 2001), which evaluates the ratio of the total number of alleles to their size range. M-ratio drastically decreases in the event of a bottleneck and it recovers slowly afterwards. This test is anticipated to produce positive results for at least 100 generations post-reduction (Garza et al., 2001). We calculated the M-ratios \((M)\) with \(M\_P\_VAL\) (Garza et al., 2001). Recommended mutation parameters were used to simulate the M-ratio at equilibrium (Garza et al., 2001): 0.12 for the proportion of mutations larger than one-step, 2.8 as the average size of non one-step mutations \((\Delta E)\) and a mutation rate \((\mu)\) of \(5 \times 10^{-4}/\text{locus/generation}\). As the (historical) effective population size is unknown, we used a range of theta values (i.e. 1.2, 2, 4 and 40) for each population.

### 1.5 Statistical analysis

A number of demographic parameters, including (but not limited to) minimum trap success rates and evidence of recruitment, have been evaluated to establish the success of woylie translocated populations (Finlayson et al., 2010; Groom, 2010; Mawson, 2004). Based on these assessments, all the translocated populations investigated were considered (demographically) successful. As mentioned earlier, we aimed to include a genetic component in the evaluation of the success of the translocation program. To this end, we considered a translocation ‘successful’ if it resulted in a population with similar genetic diversity levels (i.e. non-significant difference in \(N_{AR}\) and \(H_E\) for microsatellites, and haplotype and nucleotide diversities for mitochondrial DNA) compared with its source population. In case the source population was unknown, we compared the translocated population parameters with the mean calculated across wild populations excluding Tutanning. Tutanning population was excluded because there is evidence that this population is genetically depauperate (Pacioni, 2010; Pacioni et al., 2011) and has not significantly recovered from the historical bottleneck (Sampson, 1971; Start et al., 1995).

We compared using SPSS (v.19), \(N_{AR}\) and \(H_E\) with the non-parametric Wilcoxon signed-rank test, which paired the data by locus. Haplotype and nucleotide diversities were compared using t-tests adjusted for unequal sample size in Excel 2007 (Microsoft Corporation 1985–2007).

### 2 Results

#### 2.1 Sequence data analysis

In addition to the 15 haplotypes that had been already identified in the naturally occurring populations
(Pacioni et al., 2011), three new haplotypes were identified: two in Karakamia and one in all three South Australian islands (Fig. 2. GenBank accession number: HQ141336-HQ141337; JX9133530). Karakamia shared two haplotypes with Dryandra, which is the known source population, and the two larger South Australian islands shared one of their two haplotypes with Dryandra (Fig. 2). As expected, Batalling shared its two haplotypes with Perup, being its source population.

Haplotype and nucleotide diversity of natural populations ranged between 0 (only one haplotype in Tutanning after removal of sequences with ambiguities) and 0.69, and 0 and 0.018, respectively (Table 1). Among the translocated populations, Karakamia had surprisingly high haplotype diversity and it was significantly higher than Dryandra ($P<0.001$). On the contrary, Batalling the South Australian island populations had significantly lower haplotype diversities compared to its source population (Perup) and the average of wild populations, respectively ($P<0.001$ and $P<0.01$).

**Fig. 2  Maximum parsimony network of haplotypes found in woylie populations**

Dots correspond to one point mutation and letters indicate haplotypes already identified in wild populations (Pacioni et al., 2011). Pie charts represent the proportional distribution of each haplotype between populations and size of pie charts is proportional to the overall frequency of each haplotype.
translocated populations, except Karakamia, had significantly lower nucleotide diversity \((P<0.001)\) than the nucleotide diversity of the source or average across wild populations. The exact tests for population differentiation were significant \((P=0.026\) for the pair Karakamia-Dryandra and \(p<0.001\) for all others) except for the pairs Dryandra-St Peter Is \((P=0.053)\) and Wedge Is-Venus Bay Is \((P=0.5)\).

### 2.2 Microsatellites analysis

Inspecting the posterior probability (Pritchard et al., 2000) produced by STRUCTURE, the most probable number of inferred populations \((K)\) was six except for the no admixture model without geographic information, in which case it was five (Supplementary Material Table SMO2).

The second rate of change of the likelihood distribution, \(\Delta K\) (Evanno et al., 2005), of the analyses that did not include the geographic information in the prior showed the highest peak at \(K=2\) (where one of the clusters included all Western Australian populations and the other all South Australian populations) when using the non-admixture or admixture with non correlated allele frequencies models, or \(K=6\) with the admixture and correlated allele frequencies. The analyses that did include the geographic information generated the highest \(\Delta K\) with \(K=6\) (Supplementary Material Table SMO2). Therefore, we concluded that \(K=6\) was the most probable number of inferred populations. From now on, we refer to these groups as "genetic clusters", because "inferred populations" would not correspond to a biologically meaningful definition since some of these clusters grouped together populations that are geographically distinct (i.e. island populations or fenced area). Because there were only marginal differences in the proportion of membership of the sampling locations in each of the six genetic clusters among the different STRUCTURE runs, we report only the results for the run with the highest log-likelihood (Table 2). The assignment test correctly detected the known source population of Karakamia, which clustered together with Dryandra. Batalling and the three South Australian islands clustered in two distinct groups (Table 2). Nevertheless, the Bayesian assignment identified the common origin of the South Australian populations, which clustered together (despite significant \(F_{ST}\) values) and, in the analyses conducted with no geographic information in the prior, STRUCTURE correctly identified the link between Batalling and Perup. In fact, around 9% (range 8.1–10.6) of Perup genetic profiles were assigned to Batalling.

The AMOVA among populations was significantly different from zero (Table 3) with values ranging from 0.037 to 0.271. The \(F_{ST}\) value of the pair Dryandra-Karakamia was relatively small (0.046) and Perup-Batalling was moderate (0.065). Pairwise \(F_{ST}\) values between the South Australian islands and the other wild populations were very high and ranged from 0.153 to 0.328. Venus Bay Island was also clearly different from the other two islands \((F_{ST} = 0.136\) Wedge Is and \(F_{ST} = 0.158\) St Peter Is), while these latter two were quite similar to each other \((F_{ST} = 0.037)\).

All loci were polymorphic in all populations except locus Y112, which was fixed in the Venus Bay Island population. \(N_{AR}\) was significantly lower \((P<0.05)\) in all translocated populations (compared with their respective source populations if known or with the average \(N_{AR}\) across all wild populations but Tutanning). Moreover, \(N_{AR}\) of St Peter Is. was significantly higher than \(N_{AR}\) in Wedge Is. \((P=0.023)\) and Venus Bay Is. \((p=0.008)\). Not surprisingly, all the other genetic diversity measures \((N_A, N_E, H_E, Pa\) and \(Pa_R)\) showed a similar trend (Table 1, Supplementary Material Table SMO3).

### Table 3 Descriptors of population differentiation: (microsatellite) pairwise \(F_{ST}\) values

<table>
<thead>
<tr>
<th></th>
<th>Dryandra</th>
<th>Tutannig</th>
<th>Kingston</th>
<th>Perup</th>
<th>Batalling</th>
<th>Karakamia</th>
<th>Saint Peter Is.</th>
<th>Venus Bay Is</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryandra</td>
<td>0.152</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tutannig</td>
<td>0.089</td>
<td>0.164</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kingston</td>
<td>0.061</td>
<td>0.137</td>
<td>0.056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perup</td>
<td>0.111</td>
<td>0.183</td>
<td>0.096</td>
<td>0.065</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batalling</td>
<td>0.046</td>
<td>0.175</td>
<td>0.109</td>
<td>0.096</td>
<td>0.130</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karakamia</td>
<td>0.164</td>
<td>0.255</td>
<td>0.171</td>
<td>0.153</td>
<td>0.205</td>
<td>0.206</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saint Peter Is.</td>
<td>0.231</td>
<td>0.328</td>
<td>0.243</td>
<td>0.222</td>
<td>0.271</td>
<td>0.264</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>Venus Bay Is.</td>
<td>0.183</td>
<td>0.285</td>
<td>0.198</td>
<td>0.174</td>
<td>0.227</td>
<td>0.217</td>
<td>0.037</td>
<td>0.136</td>
</tr>
<tr>
<td>Wedge Is.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All pairwise \(F_{ST}\) values were significant \((P=0.001)\).
and $H_E$ of all translocated populations except Karakamia ($P = 0.1$) was significantly lower ($P < 0.05$). The two larger South Australian islands had significantly higher $H_E$ than Venus Bay Is. but there was no difference between them. The effective inbreeding coefficients ($F_e$) were very high for the island populations (0.208–0.469; Table 1) while it was relatively small for Karakamia (0.065) and intermediate in the Batalling population (0.137). On the other hand, $F_{IS}$ of the wild populations was 0.078 ($SE = 0.033$).

All the M-ratio tests detected a bottleneck in the translocated populations. Conversely, none of the populations showed a significant heterozygosity excess and only Venus Bay Is. showed signs of a genetic bottleneck with a mode-shift.

3 Discussion

3.1 Genetic diversity of the translocated populations

The loss of genetic diversity is the greatest genetic concern associated with translocated populations genetic (Goossens et al., 2002; Stockwell et al., 1996) because, by definition, they go through a bottleneck and usually have a small founder size (Cornuet et al., 1996; Frankham, 1995; Frankham, 1996; Frankham, 2007; Frankham et al., 1999). None of the translocated woylie populations can be considered successful on the basis of having levels of genetic diversity comparable with the original source populations. More specifically, all translocated populations had at least one significant difference compared with the original source populations with respect to allelic richness ($N_{AR}$), expected heterozygosity ($H_E$), haplotype ($h$) and nucleotide ($\pi$) diversity, (or when the source of the population was unknown, compared with the mean of these parameters for the large indigenous populations of Dryandra, Perup and Kingston combined). Furthermore, the inbreeding coefficient, $F_e$ was relatively high in all translocated populations, indicating a considerable inbreeding effect. Somewhat surprising was the finding that only $N_{AR}$ was reduced in the Karakamia population and it actually had higher $h$ than that found in Dryandra. We argue that the combination of the intermediate number of woylies used to establish this population, the substantial demographic growth since establishment and the further release of rehabilitated woylies (from unknown, presumably different, sources) partly counteract the negative consequences of the founder effect and genetic drift in this population. The significant reduction of $N_{AR}$, however, indicates that some loss of genetic material occurred or is occurring as a result of genetic drift. Additionally, our data supports the theoretical expectation that the number of alleles is the most sensitive parameter in detecting loss of genetic material (Frankham et al., 2002; Nei et al., 1975).

Pope et al. (2000) described populations of macro-pods with heterozygosities of >70% as having ‘substantial’ genetic diversity. On this criteria alone, we would have judged the Batalling and Karakamia populations to still be retaining valuable genetic diversity ($H_E = 0.721–0.745$). These values are also comparable with other Betongia populations ($B. lesueur; H_E: 0.68–0.7$, Donaldson et al., 2008; $B. tropica; H_E: 0.65–0.75$, Pope et al., 2000) and slightly higher than in other potoroids ($Potoroo longipes; H_E: 0.556$, Luikart et al., 1997, but Aeppurynus rufescens; $H_E: 0.83$, Pope et al., 2005, $P. gilbertii; H_E: 0.457$, Sinclair et al., 2002). Importantly, the heterozygosity at Batalling was significantly less than the source population (Perup), and there was evidence of inbreeding at both WA translocated populations (Batalling $F_e=0.14$ and Karakamia $F_e=0.06$). These observations highlight the importance of obtaining genetic baseline data as a reference for ongoing monitoring necessary to facilitate effective conservation and management decisions. Moreover, it should be noted that our terms of comparison are conservative given that the sampling of wild populations was conducted either during or shortly after the modern decline and after a known historical substantial bottleneck. Other studies have compared historical (using museum specimens) and modern levels of genetic diversity demonstrating that the latter are often not an optimal indication of the levels of genetic diversity that should be targeted (Bourke et al., 2010; Groombridge et al., 2000). Preliminary results from a similar investigation in the woylie demonstrated that this applies to this species too (Hunt, 2010).

The Batalling and St Peter Island populations were established at almost the same time and both reached roughly similar population sizes (~3,000). The major difference in the establishment of these populations was the number of founders. The small number of founders ($n=3$) in the original zoo breeding program were clearly insufficient to maintain adequate levels of genetic diversity and this is reflected in the lower genetic diversity now seen on the South Australian islands. High values of $F_e$ (0.21–0.47) suggest that active management is required in order to maintain or improve the conservation value of these populations (discussed below).
How the loss of neutral genetic diversity observed in this study corresponds with possible changes, if any, in the diversity of functional genes remains unknown. Conclusions from a study on neutral genetic diversity cannot be directly transferred to functional genes. However, to the best of our knowledge, low levels of adaptive diversity were always associated with low levels of neutral genetic variability (e.g., Aguilar et al., 2004; Miller, 2004; Jones et al., 2004; Seddon et al., 1999; Siddle et al., 2007). As for other taxa, possible changes and differences in adaptive diversity is an aspect of woylie conservation that remains to be investigated further.

3.2 Identification of source populations

South Australian islands shared one haplotype with the population at Dryandra and the lowest pairwise $F_{ST}$ were also with this population. While these results may suggest that Dryandra was the original source of the individuals in the breeding program used to stock South Australian islands, we argue that while this may be likely, these results in themselves are not sufficient to identify the source population of these translocated populations with an acceptable level of certainty. In fact, these clustered separately in our STRUCTURE analysis, were significantly differentiated from any other wild populations ($F_{ST}$ and exact test of differentiation) and one haplotype found in these translocated populations was not sampled from any other wild populations. It is possible that this haplotype was not sampled due to insufficient sampling from wild populations or because it had been lost as a result of woylie bottlenecks. A further difficulty in identifying the source population for the South Australian breeding program is caused by the historical connectivity between the wild populations in Western Australia and consequent mixing of mtDNA haplotypes (Pacioni et al., 2011). Because of this it is not possible to speculate on the possible origin of the unique haplotype using genetic distances between haplotypes.

Reports that the South Australian captive colony was founded from only two females (Delroy et al., 1986) are supported by this study, which identified no more than two haplotypes in the South Australian translocated populations. Furthermore, the analysis provides evidence that females released during the supplementation exercises in 1996 did not contribute any additional mtDNA genetic diversity to these populations. While it is possible that their haplotypes were not different from those already present or that low frequency haplotypes were not sampled, it is clear that no effective genetic augmentation was achieved.

3.3 Bottleneck tests

The inconsistent results from different bottleneck tests should be concerning for researchers and wildlife managers. This is particularly so given that, most of the time, managers lack the ‘a priori’ knowledge that was available for the woylie (e.g., detailed records of the time and extent of population declines). Several studies investigated, within a theoretical framework or using empirical validations, the power of the three methods used here (e.g., Busch et al., 2007; Cornuet et al., 1996; Le Page et al., 2000; Luikart et al., 1998a; Mock et al., 2004; Williamson-Natesan, 2005). While the M-ratio would appear to be the most promising method (McEachern et al., 2011, Williamson-Natesan, 2005), there are instances where this method did not perform as expected (Busch et al., 2007; Le Page et al., 2000). Similarly, the heterozygosity excess method failed to detect known genetic bottlenecks (e.g., McEachern et al., 2011; Mock et al., 2004) even though it was successful in other cases (e.g., Sinclair et al., 2002). Furthermore, a number of studies reported that its results were dependent on parameter settings (Busch et al., 2007; Le Page et al., 2000). Clearly, careful considerations need to be given to the factors that may influence the power of these analyses to appropriately interpret the results on a case by case basis.

In our situation, the demography of the populations probably influenced our results. The M-ratio power is maximised when the pre-reduction population size is large (Williamson-Natesan, 2005). The historical population sizes of wild woylie populations were very large and the bottleneck was substantial, probably making the M-ratio an optimal method (Garza et al., 2001) in this study. We found it difficult, though, to discriminate whether the results we obtained were due to the species historical decline (consequent to European settlement in Australia), the founder effect of translocated populations, or a combination of both. Interestingly, the M-ratio test, which should detect a bottleneck for at least 100 generations post-reduction (Garza et al., 2001), also resulted in the detection of bottlenecks in Dryandra, Tutanning and depending on the theta values used, in Kingston (results presented in Pacioni, 2010).

Two main aspects are probably responsible for the limited power of the heterozygosity excess test. Given the time since establishment, it is possible that our sampling occurred outside the temporal window where the statistical power of this test is relatively high (Cornuet et al., 1996), and the rapid, post-establishment growth of
the new populations (Nelson et al., 1992; Orell, 2004) most likely caused a heterozygosity deficit rather than heterozygosity excess (Busch et al., 2007; Cornuet et al., 1996; Pacioni, 2010; Smith et al., 2008).

Genetic drift of the small Venus Bay Is. population is likely to be responsible for the loss of rare alleles and ultimately responsible for the detection of a mode shift. The mode shift approach had limited statistical power when applied to other populations because the pre-reduction population sizes were large (Williamson-Natesan, 2005). Therefore, we argue that the demographic history of the studied populations limited the suitability of both the heterozygosity excess and mode-shift approaches to investigating bottlenecks.

3.4 Implication for species management and conservation

Our data suggests that it is likely that newly established populations will have reduced genetic diversity when compared to the source population, even when there is a relatively large number of founders ($n \sim 50$) and the population expands to $\sim 3,000$ individuals. Drift (and localised selection) will also subsequently alter the genetic trajectory from the original source population. The vulnerability of translocated populations to founder effects and genetic drift is further attested to by the degree of differentiation at Batalling (after only eight generations) and the three South Australian islands (7–10 generations) from their source populations. These populations not only had significant pair-wise $F_{ST}$ values, but also clustered independently in the Bayesian clustering analysis and were significantly differentiated when considering mtDNA data. The implications of these results for insurance populations that aim to replicate source populations is that this can only be achieved in the short-term unless there is ongoing intervention.

Karakamia was the only woylie population that did not cluster separately from its source population. However, we argue that the genetic profile of the Karakamia population will differentiate considerably from that of Dryandra unless active management is undertaken. Similar differentiation was found to some extent in other Australian marsupials such as the bridled nail-tail wallaby Onychogalea fraenata and the western barred bandicoot Perameles bougainville (Sigg, 2006; Smith et al., 2008).

St Peter Island and Wedge Island are important opportunities for the conservation of the species. They support large populations and their geographic isolation provides protection from key threats that are more difficult to control on the mainland (e.g. feral predators and diseases). While all of the large mainland populations have recently declined, the two island populations have remained stable (Groom, 2010). However, our data clearly show that they have about half of the alleles of mainland counterparts and $\sim 20\%$ less heterozygosity. Should the mainland populations not recover from the current decline, a substantial proportion of the species' genetic diversity would be lost because it is currently so poorly represented on the two island populations. We advocate, therefore, that the genetic rescue of the island populations is warranted.

Despite the apparent initial failures of the supplementation attempts in 1996, we believe that this management option should be revisited. The constant and effective addition of new “migrants” (i.e. genetic material) can augment the genetic variability and reduce the differentiation of a population from its source (e.g. Sigg, 2006; Smith et al., 2008; Spielman et al., 1992). Careful consideration of factors that may affect the success of integrating recruits into these populations should be considered (e.g. ‘soft’ transitional release versus ‘hard’ immediate release; wild versus captive; age and gender). For example, female woylie survival after dispersal is higher than males (Christensen, 1980), consequently, we suggest that releasing small numbers of wild-adapted young adult females may result in more successful supplementations. Population modelling should also be considered as it could provide valuable information to instruct how to optimise genetic augmentation (Allendorf et al., 2007; Seddon et al., 2007).

The number of founders and carrying capacity are evidently important factors that influence the final outcome and the genetic viability of the translocated populations in this and other species (e.g. Fischer et al., 2000; Griffith et al., 1989; Houlden et al., 1999; Larson et al., 2002; Maudet et al., 2002; Sigg, 2006; Smith et al., 2008; Wolf et al., 1996). Limited population size appears to have disadvantaged the Karakamia and Venus Bay Island populations by increasing their susceptibility to genetic drift, while small founder size appears to have been the main problem on the larger South Australian islands. Unfortunately, it was not possible to quantify the relative contribution of each of these components (e.g. using a general linear model) due to the restricted number of replicates (i.e. only five translocated populations). Nevertheless, we argue that the effect of genetic drift will become substantial at Karakamia in the long term because it is a ‘closed’ population of limited size. Genetic monitoring and active management of this population is therefore warranted.
All genetic diversity parameters of the population at Batalling were reduced. Growth of this population is thought to have initially been substantially limited by introduced predators (Orell, 2004). Our results provide pragmatic evidence of the detrimental effects of inadequately controlling key limiting factors, not only for the demography of a population, but also for its genetic diversity (see also Bouzat et al., 2009).

The use of wild caught animals has been recommended to reduce the differentiation of translocated populations (Smith et al., 2008) and it was demonstrated that the release of captive bred animals can have disadvantageous consequences even in supplementation efforts because common alleles could dilute rare alleles in the target population (Sigg, 2006). This is mainly related to the fact that wild caught animals usually offer the chance to add (in the recipient population) genetic material that was not captured in the captive colony. Adaptation to captivity is an additional potential issue that arises from captive breeding programs (Frankham, 2007). While captive breeding programs are a valuable option in specific circumstances, in light of the above considerations and the substantial genetic reduction we found in woylie populations, we disagree with the recommendation of Finlayson et al (2010) preferring captive to wild caught animals, at least as a general approach. Additionally, a number of population genetic tools can be used to improve the selection of wild caught founders (or supplementation animals). For example, by generating the genetic profiles of candidates, their diversity can be maximised and breeding success of introduced individuals assessed (Goossens et al., 2002; Sigg, 2006). The design of sampling protocols can be optimised by preliminary molecular investigations of the spatial organization of source populations so that the relatedness of selected individuals is minimised.

In conclusion, this study highlights the importance of a careful evaluation of critical factors that might affect each translocation phase: planning, execution and ongoing management. Based on our results, we have summarised below key considerations for the translocation of woylies; however, we argue that the same principles and considerations apply to other species.

Translocation planning and execution

- Select and prioritise translocation sites that have the capacity to support larger populations (e.g. >3,000 individuals).
- Consider the selection of sites that increase the potential connectedness between populations (i.e. restore former mixing prior to fragmentation). Alternatively, plan for regular supplementations (i.e. assisted migration).
- Adequately manage limiting factors (e.g. predators) especially to maximise potential initial growth but also to maximise population size.
- Use a large number of founders (e.g. >50).
- Founders should be selected to maximize genetic diversity or sourcing founders and supplementation stock from multiple natural populations where there is evidence of historical gene flow and the current isolation is a result of recent fragmentation (e.g. analysing their genetic profile, using preliminary spatial genetic analysis to establish sampling regimes to minimise relatedness).
- Collect and store tissue samples to generate genetic reference data.
- Possibly develop a population viability model to explore management options (PVA).

Ongoing management

- Regularly monitor the demographics and genetics of the translocated population in conjunction with key factors influencing population dynamics (e.g. predators, competitors, food resources, health and diseases, etc) within an effective adaptive management framework.
- Manage appropriately the key threatening processes specific to that population.
- Maintain and update resources and information developed in the planning phase (e.g. PVA modelling, genetic data)

Acknowledgements We would like to thank all the staff of the Department of Environment and Conservation (DEC), Australian Wildlife Conservancy and Department of Environment and Heritage (SA), including C. Ward, M. Maxwell, C. Vellios, N. Marlow, N. Thomas, P. Orell, C. Groom, F. Kirkpatrick, C. Gilbert, J. Kuiper and J. Van Weenen that contributed to sample collection. We are much obliged to P. Davies (DEC) for preparing Figure 1. We are grateful to M. Bunce, E. McLay, N. White, M. Allenoft, C. Rafferty, S. Stevenson and three anonymous reviewers for useful comments. Computer simulations were supported by iVEC through the use of advanced computing resources provided by the Informatics Facility located at Murdoch University (http://www.ivec.org). We are greatly grateful to D. Schibeci, for his assistance with the cluster computer and C. Blacow for IT support. This project was supported by the Australian Academy of Science, South Coast Natural Resource Management Inc, Woylie Conservation and Research Project (a DEC ‘Save Our Species’ project) and DEC Science Division (PhD Student Stipend to CP). This study was approved by the Murdoch and DEC Animal Ethics committees.
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Kalinowski ST, 2005. hp-rare 1.0: A computer program for
O’Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn DW et al., 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133: 42–51.


## Supplementary Material

Table SOM1 Details of the microsatellite loci amplified in the woylie *Bettongia penicillata ogilbyi* including the species where these were first developed

<table>
<thead>
<tr>
<th>Source species</th>
<th>Locus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bettongia tropica</em></td>
<td>Bt64</td>
<td>(PopeEstoup &amp; Moritz, 2000)</td>
</tr>
<tr>
<td><em>Bettongia tropica</em></td>
<td>Bt76</td>
<td>(PopeEstoup &amp; Moritz, 2000)</td>
</tr>
<tr>
<td><em>Bettongia tropica</em></td>
<td>Bt80</td>
<td>(PopeEstoup &amp; Moritz, 2000)</td>
</tr>
<tr>
<td><em>Petrogale assimilis</em></td>
<td>Pa593</td>
<td>(Spencer et al., 1995)</td>
</tr>
<tr>
<td><em>Petrogale xanthopus</em></td>
<td>Y105</td>
<td>(ZengerMcKenzie &amp; Cooper, 2002)</td>
</tr>
<tr>
<td><em>Petrogale xanthopus</em></td>
<td>Y112</td>
<td>(ZengerMcKenzie &amp; Cooper, 2002)</td>
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<tr>
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<td>(PopeSharp &amp; Moritz, 1996)</td>
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<tr>
<td><em>Petrogale xanthopus</em></td>
<td>Y170</td>
<td>(PopeSharp &amp; Moritz, 1996)</td>
</tr>
<tr>
<td><em>Petrogale xanthopus</em></td>
<td>Y175</td>
<td>(ZengerMcKenzie &amp; Cooper, 2002)</td>
</tr>
<tr>
<td><em>Potorous longipedis</em></td>
<td>Pl2</td>
<td>(Luikart et al., 1997)</td>
</tr>
<tr>
<td><em>Potorous longipedis</em></td>
<td>Pl26</td>
<td>(Luikart et al., 1997)</td>
</tr>
<tr>
<td><em>Macropus eugenii</em></td>
<td>T17-2</td>
<td>(Zenger &amp; Cooper, 2001)</td>
</tr>
</tbody>
</table>

Table SMO2 Simulation summary of STRUCTURE for different models and prior probability

<table>
<thead>
<tr>
<th>K</th>
<th>Without Geographic information</th>
<th>With Geographic information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admix Correlated Frequencies</td>
<td>Admix no Correlated Frequencies</td>
</tr>
<tr>
<td>1</td>
<td>-20075.3 (0.3)</td>
<td>-20074.4 (0.1)</td>
</tr>
<tr>
<td>2</td>
<td>-18200.7 (41.8)</td>
<td>-18288.3 (78)</td>
</tr>
<tr>
<td>3</td>
<td>-17247.3 (52.1)</td>
<td>-17278.9 (48.8)</td>
</tr>
<tr>
<td>4</td>
<td>-16475.7 (57.8)</td>
<td>-16519.5 (62.8)</td>
</tr>
<tr>
<td>5</td>
<td>-15805.2 (79.9)</td>
<td>-15938 (231.6)</td>
</tr>
<tr>
<td>6</td>
<td>-15444.7 (0.8)</td>
<td>-15523 (134.9)</td>
</tr>
<tr>
<td>7</td>
<td>-15331.7 (13.6)</td>
<td>-15337.8 (23.7)</td>
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<td>-15323.4 (83.6)</td>
<td>-15249.7 (49.5)</td>
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<td>9</td>
<td>-15929.7 (846.1)</td>
<td>-15162.6 (33.6)</td>
</tr>
<tr>
<td>10</td>
<td>-15461.9 (230.9)</td>
<td>-15114.9 (37.5)</td>
</tr>
</tbody>
</table>

Mean Ln (K) and standard deviation (between brackets) over 20 runs.
Table SOM3 Summary of measures of microsatellite variability given by locus and Hardy-Weinberg equilibrium test (carried out with HW QuickCheck, Kalinowski 2006) after Bonferroni correction

<table>
<thead>
<tr>
<th>Sampling locations</th>
<th>Locus</th>
<th>n</th>
<th>Ns</th>
<th>Ne</th>
<th>Hs %</th>
<th>He %</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryandra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T17</td>
<td>26</td>
<td>8</td>
<td>4.38</td>
<td>69.2%</td>
<td>77.1%</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Bt64</td>
<td>28</td>
<td>13</td>
<td>7.13</td>
<td>64.3%</td>
<td>86.0%</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Y175</td>
<td>28</td>
<td>11</td>
<td>7.26</td>
<td>89.3%</td>
<td>86.2%</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Pa593</td>
<td>28</td>
<td>8</td>
<td>6.32</td>
<td>78.6%</td>
<td>84.2%</td>
<td>ns</td>
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ns: non-significant; sig: significant; NA: not applicable (monomorphic)