ACCEPTED VERSION

Lauren C. White, Katherine E. Moseby, Vicki A. Thomson, Stephen C. Donnellan, Jeremy J. Austin
Long-term genetic consequences of mammal reintroductions into an Australian conservation reserve
Biological Conservation, 2018; 219:1-11

© 2018 Elsevier Ltd. All rights reserved.

This manuscript version is made available under the CC-BY-NC-ND 4.0 license
http://creativecommons.org/licenses/by-nc-nd/4.0/

Final publication at http://dx.doi.org/10.1016/j.biocon.2017.12.038

PERMISSIONS

https://www.elsevier.com/about/our-business/policies/sharing

Accepted Manuscript

Authors can share their accepted manuscript:

[24 months embargo]

After the embargo period

- via non-commercial hosting platforms such as their institutional repository
- via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license – this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy
- not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article

26 March 2020

http://hdl.handle.net/2440/123845

Long-term genetic consequences of mammal reintroductions into an Australian conservation reserve

Lauren C. White[1, 2]*, Katherine E. Moseby[3, 4], Vicki A. Thomson[5], Steve Donnellan[6], and Jeremy J. Austin[1]

[1] Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia.

[2] Max Planck Institute for Evolutionary Anthropology, Department of Primatology, Deutscher Platz 6, Leipzig, 04103, Germany.

[3] Arid Recovery Ltd, Roxby Downs, South Australia 5725, Australia.

[4] University of New South Wales, Sydney, Australia

[5] School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia.

[6] South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia.

*corresponding author: lauren_white@eva.mpg.de

Keywords: Arid Recovery, bilby, bandicoot, stick-nest rat, bettong, genetic diversity, translocation, RAD-seq, admixture, inbreeding

Word count = 7,543

Figure count = 6

Table count = 4

Abstract

Reintroduction programs aim to restore self-sustaining populations of threatened species to their historic range. However, demographic restoration may not reflect genetic restoration, which is necessary for the long-term persistence of populations. Four threatened Australian mammals, the greater stick-nest rat (*Leporillus conditor*), greater bilby (*Macrotis lagotis*), burrowing bettong (*Bettongia lesueur*) and western barred bandicoot (*Perameles bougainville*), were reintroduced at Arid Recovery Reserve in northern South Australia over the last 18 years. These reintroductions have been deemed successful based on population growth and persistence, however the genetic consequences of the reintroductions are not known. We generated large single nucleotide polymorphism (SNP) datasets for each species currently at Arid Recovery and compared them to samples collected from founders. We found that average genetic diversity in all populations at the Arid Recovery Reserve are close to, or exceeding, the levels measured in the founders. Increased genetic diversity in two species was achieved by admixing slightly diverged and inbred source populations. Our results suggest that genetic diversity in translocated populations can be improved or maintained over relatively long time frames, even in small conservation reserves, and highlight the power of admixture as a tool for conservation management.

Introduction

Reintroduction programs aim to establish self-sustaining populations that do not require significant long-term management. Successful reintroductions generally increase a species' population size and geographic range, and restore ecological function to the area from which it was extirpated (Armstrong *et al*., 2015). Measuring an increase in population growth and size is most often how these reintroduction programs are judged to have succeeded (Ewen *et al*., 2012; Moseby *et al*. 2011). However, the ability of a population to persist in the long-term will also be strongly influenced by levels of genetic diversity (Cochran-Biederman *et al*., 2014, Weeks *et al*., 2015).

Reintroduced populations are susceptible to loss of genetic diversity due to founder effects, the isolated nature of reintroduction sites, and small population size (Frankham *et al*., 2010). These circumstances result in unavoidable inbreeding and genetic drift, leading to reduced fitness through the accumulation of deleterious alleles (genetic load), and the increased expression of recessive deleterious traits (inbreeding depression). Additionally, loss of genetic diversity will diminish the adaptive capacity of a population and limit its ability to cope with environmental change (Groombridge *et al.*, 2012).

Thus, most reintroduction programs adopt the preservation of genetic diversity as an explicit goal. Several guidelines can be followed to maximise genetic diversity in reintroduced populations, such as using large numbers of genetically diverse individuals as founders and encouraging rapid population growth after establishment (Jamieson and Lacy, 2012). However, it may not always be possible to follow these guidelines and many other interacting factors, such as the life-history traits and demographic history of a species, may affect genetic diversity in cryptic ways. It is therefore important that genetic monitoring is used in all reintroduction programs to evaluate success and guide management actions to maximise the retention of genetic diversity (Schwartz *et al.*, 2007).

Most studies assessing genetic diversity in reintroduction programs have sampled the source and reintroduced populations simultaneously a number of years after release—for example *Gongylomorphus bojerii*. (Michaelides *et al.*, 2015) and *Notionmystis cincta* (Brekke *et al*., 2011) — or by sampling just the reintroduced population at multiple time-points—such as *Vulpes velox* (Cullingham and Moehrenschlager, 2013) and *Mustela nigripes* (Cain *et al.*, 2011). In contrast, relatively few studies have explicitly tested changes in genetic diversity from founders to descendants over multiple generations (*e.g.* Maraes *et al.*, 2017). Such data is crucial for validating and establishing guidelines for maximising genetic diversity in reintroduced populations.

The Arid Recovery Reserve reintroduction program provides a model system in which to compare founder and descendant genetic diversity, as tissue samples were taken from founding individuals at time of release and stored explicitly for later genetic analysis. The reserve is a 123 km^2 fenced exclosure situated 20 km north of Roxby Downs in arid South Australia (Figure 1). A netting fence surrounds the reserve, and all European rabbits (*Oryctolagus cuniculus*), cats (*Felis catus*), and foxes (*Vulpes vulpes*) have been removed from a 60 km^2 sector at the southern end (Moseby and Read, 2006). Since 1998, this has allowed four species of locally extinct mammals to be reintroduced within the exclosure (Moseby *et al.* 2011), namely the greater stick-nest rat (GSNR, *Leporillus conditor*), greater bilby (*Macrotis lagotis*), burrowing bettong (*Bettongia lesueur*), and western barred bandicoot (WBB, *Perameles bougainville*). These species were all once widespread across the Australian arid zone, but their geographic ranges have been severely reduced due to competition with grazing stock and rabbits, and predation from introduced cats and foxes (Burbidge and McKenzie, 1989; Morton, 1990; Newsome, 1971; Richards 2005).

The reintroductions at Arid Recovery have been deemed successful based on the species' continued survival, population recovery after drought and increased abundance and

distribution within the reserve (Moseby *et al.*, 2011). However, the small number of founders (n=17 – n=122) and fluctuating population size in some species make loss of genetic diversity and inbreeding depression a concern, raising practical questions about the need for additional translocations (i.e. genetic rescue).

Here we compare genetic diversity, using large single nucleotide polymorphism (SNP) datasets, between founders and the descendant populations 18 years after the first reintroductions at Arid Recovery (seven years since the last animal was released). This allowed us to directly measure changes in genetic diversity and accumulation of inbreeding in the descendant populations. We make recommendations regarding the need for genetic rescue at Arid Recovery and, more broadly, comment on reintroduction strategies that can be used to maintain genetic diversity in small, reintroduced populations.

Materials and Methods.

Population History and Sample Collection:

The reintroduction histories of all four species at Arid Recovery are summarised in Table 1, and detailed descriptions are given in the Supplementary Information. Since release, the bettong population at Arid Recovery has increased rapidly with minimal population fluctuations. The WBB population has also increased without substantial bottlenecks, but at a slower rate than the bettongs (Moseby *et al*., 2011). Conversely the bilby and GSNR populations have often fluctuated significantly since release in response to seasonal conditions with populations doubling in size and then crashing to less than 100 individuals during droughts (Moseby and O'Donnell, 2003; Moseby and Bice 2004; Moseby *et al*., 2011).

Population sizes at Arid Recovery at the time of sampling were estimated from track count data for the GSNRs, bilbies and WBBs, and from mark-recapture data for the bettongs (Table 1). As of 2016 there were approximately 500 GSNRs, 500 bilbies, 6000 bettongs, and 1000 WBBs at Arid Recovery (Arid Recovery unpublished data; Moseby, *pers comm.*).

Founding individuals were DNA sampled as follows: a small (2mm) ear tissue sample were taken from bettongs and bandicoots, and a 2mm piece of the tail tip was taken from GSNRs. Samples were not taken from any of the bilby founders, the five WBB founders from Faure Island, and 32 of the GSNR founders originating from Reevesby Island (released in 1998 [n=6] and1999, [n=8]), and Monarto (released in 1998 [n=2] and 2003 [n=16]). Eight WBB ear-clip samples were collected on Faure Island in 2007, and these were used as a proxy for the Faure WBB founders. All samples were accessioned in the Australian Biological Tissue Collection (ABTC) at the South Australian Museum.

Post-release DNA samples were obtained during routine monitoring programs or targeted trapping and capture opportunities. WBBs and bettongs were sampled in 2014, while GSNRs and bilbies were sampled in 2016 (Table 1). Trapping at Arid Recovery was conducted under an ethics permit from the South Australian Wildlife Ethics Committee (58-2015). Ear tissue samples were taken using an ear punch or small sharp scissors and stored frozen in 70% ethanol. The numbers of samples collected for different populations and species are summarised in Table 1.

DNA Extraction and ddRAD-seq Library Preparation

DNA was extracted from tissue samples using a salting out method (Rivero *et al.* 2006) and the extracts quantified using the Quantus Fluorometer (Promega) as per manufacturer's instructions.

Double-digest Restriction Associated DNA sequencing (ddRAD-seq) libraries were made in batches of 96 including a library blank control following the protocol of Poland *et al*. (2012) with some modifications. Three hundred nanograms of DNA was digested at 37°C for 2 hours using 8 U of *PstI* (six-base recognition site, CTGCAG) and *HpaII* (four-base recognition site, CCGG) in 20 µL of 1x CutSmart Buffer (New England Biosciences [NEB]).

Uniquely barcoded adapters (see SI methods and SI Table 1) were then ligated to the DNA in 40 µL consisting of 20 µl of digested DNA, 200 U of T4 ligase, 0.1 ρmol of forward (rare) and 15 ρmol of reverse (common) adapters (SI Figure 1), and 1x T4 Buffer. The mixture was incubated at room temperature for 2 hours, and then heat killed at 65°C for 20 minutes. Ligation products were pooled into 12 pools of eight samples. Pooled libraries were purified using the QIAquick PCR purification kit (Qiagen) and eluted in 120 µL of EB buffer (Qiagen).

Polymerase chain reactions (PCR) to add the full-length Illumina adapters (Poland *et al*., 2012) were performed in eight replicates per library pool in 30 µL volumes containing 10 µL of purified library, 1x Hot Start Taq Master Mix (NEB), and 0.66 µM each of the forward and reverse primers (SI Figure 1). The PCR conditions were: 95° C for 30 seconds, 16 cycles of 95° C for 30 seconds, 65° C for 20 seconds, and 68° C for 30 seconds, followed by 68° C for 5 minutes, and 25° C for 1 minute. The eight replicates per library were re-pooled and purified as above, eluting in 30 µL of EB buffer (Qiagen). We used a two-step double-SPRI protocol (Lennon *et al.*, 2010) to select for fragments between 100 and 300 bp using a homemade SPRI bead mix (Rohland and Reich, 2012). Libraries were quantified using Tapestation 2200 (Agilent) and pooled at equi-molar concentrations. Pooled libraries were sequenced in 1x75 bp (single-end) high output reactions on the Illumina Next-seq at the Australian Genome Research Facility, Adelaide.

Sequence Processing

We used STACKS v1.35 pipeline (Catchen *et al*., 2013, 2011) to process the sequence data for each species separately, employing parameters recommended by Mastretta-Yanes *et al.* (2015) to minimise errors and maximise SNP recovery. Raw sequencing reads were de-multiplexed, truncated to 65 bp, and filtered for overall quality based on the presence of barcodes using the *process_radtags* module. Samples with fewer than 500,000 reads were excluded from further analysis. RAD loci were identified for each sample using the *ustacks* module, requiring a minimum stack read depth of three (m=3) and a maximum of two nucleotide mismatches (M=2) between stacks at a locus. Loci with more than three stacks (mls=3) and more reads than two standard deviations above the mean were filtered as they may map to multiple points on the genome. A 'deleveraging algorithm' was used to try to resolve over-merged loci. A catalogue of consensus loci among individuals for each species was constructed with the *cstacks* module using the *ustacks* output files. Loci were recognized as homologous across individuals if they mismatched at two or fewer bases (n=3). Alleles were identified in each individual against this catalogue using the module *sstacks*. The module *populations*, was used to remove potential homologs by filtering out loci with heterozygosity >0.7 and the resulting SNP datasets were output to a PLINK format file (i.e. ped and map files). Finally, the program PLINK (Purcell *et al*., 2007) was used to filter out loci with more than 25% missing data and minor allele frequencies of <0.05. Although removing loci with low minor allele frequencies prohibits tracing the loss of rare alleles, we believe this conservative step is necessary to avoid incorporating erroneously called SNPs.

Quality Control

Raw sequences from blank control samples were also run through the STACKS pipeline, matching the *ustacks* output to the consensus catalogue of all four species. Our aim

was to remove any potentially erroneous loci in our datasets that were also present in the library blank samples. However, upon inspection, none of the loci found in the blank controls were present in any of the final datasets, having been filtered at previous steps of the pipeline.

A subset of samples from each species was sequenced twice (four GSNRs, five bilbys, 12 bettongs and 10 WBBs) in separate libraries to allow the estimation of error rates. Replicate reads were subsampled to 1 million, 750,000, and 500,000 reads to control for sequencing depth. All subsampled replicates were run through the STACKS pipeline as above, matching the *ustacks* output to the previously constructed consensus catalogue for each species. Allelic error rate was then estimated by counting mismatching alleles at loci for which both replicates had been sequenced.

Genetic Diversity

For each species, samples were grouped by founder/descendant population so that comparisons could be made between each founding group and its descendant population. For each group we calculated observed and expected heterozygosity (H_O, H_E) using the program GENODIVE v2.0b27 (Meirmans and Van Tienderen, 2004), and allelic richness corrected for sample size (A_R) using the R package *hierfstat* (Goudet, 2005). Individual heterozygosity and inbreeding coefficients (*F*) were calculated in PLINK (Purcell *et al.*, 2007). We tested for significant differences in average individual heterozygosity and *F* between the reintroduced population and their founding groups (where available) using a Wilcoxon rank sum test, corrected for multiple testing.

Wang's pairwise relatedness coefficient (*PR*, Wang, 2002) was estimated for all pairs of individuals within each species using the R package *Related* (Pew *et al.*, 2015). *PR* measures the genetic relatedness of two individuals relative to the average genetic similarity

in the total sample (Hardy, 2003). Consequently, negative values may be obtained if two individuals are less related than the average in the reference.

Temporal Differentiation

PCA, pairwise F_{ST}, sNMF and Bayescan analyses were performed to test for differentiation between the founders and descendants. The bilby dataset did not include founder samples and so was excluded from these analyses

We visualised the variation in our datasets and differentiation between founders and descendants by performing a principal components analysis (PCA) in *adegenet v2.0.1* (Jombart, 2008). PCA is a statistical method for exploring datasets that have a large number of measurements; it reduces the variation in the dataset to a few principal components, which can then be projected onto a graph (Reich *et al*., 2008).

Genetic distance between founding groups (i.e. founders grouped by source population) and descendants was measured as pairwise F_{ST} in Arlequin v3.5. (Excoffier and Lischer, 2010) using the underlying pairwise distance matrix and 10,000 permutations. Significance values were corrected for multiple tests using the Bonferroni correction (Rice, 1989).

We then used the program sNMF v1.2 to estimate the proportional ancestry in each descendant dataset (Frichot *et al*., 2014). Similar to the widely-used program STRUCTURE (Pritchard *et al*., 2000), sNMF estimates the proportion of each individual's genome that originated from a specified number of gene pools (K). Unlike STRUCTURE, sNMF is capable of efficiently analysing large SNP datasets and is more robust to many of the demographic assumptions of Hardy-Weinberg and linkage equilibrium (Frichot *et al.*, 2014). We calculated ancestry proportions in our datasets by running ten replicates of K 1-20 with default parameters and chose the best-supported K as the one with the lowest cross-entropy criterion (CEC), as calculated in sNMF.

We tested for signatures of selection using the F_{ST}-outlier method implemented in Bayescan v2.01 using the default settings (Foll and Gaggiotti, 2008). Bayescan estimates the probability that each locus is subject to selection by teasing apart population-specific and locus-specific components of F-coefficients using a logistic regression. Using a reversible jump Markov chain Monte Carlo (MCMC) algorithm, the posterior probability of a locus being under selection is assessed by testing whether the locus-specific component is necessary to explain the observed pattern of diversity, which infers a departure from neutrality. A threshold value to detect selection was set using a conservative maximum false discovery rate (the expected proportion of false positives) of 0.05.

Results

Sequencing Results

We successfully sequenced 95 GSNR, 15 bilby, 71 bettong and 35 WBB samples, (summarised in Table 2 and SI Table 2), generating a large SNP dataset (1752-8703 SNPs) for each species. The WBB samples yielded fewer SNPs (n=1752) than the other species, despite similar sequencing success and locus discovery, suggesting lower average genetic diversity in this species. This is in agreement with previous studies showing very low genetic diversity in WBBs using microsatellite, mitochondrial (Smith and Hughs, 2008), and MHC (Smith *et al*., 2010) markers.

The average estimated allelic error rates, calculated between pairs of replicates subsampled to varying depths for each species was 1.2-6.6%, (SI Table 3-6). The error rate did not differ with sequencing depth for any species indicating that our cut-off of 500,000 reads per sample was appropriate.

Genetic Diversity and Inbreeding

Observed heterozygosity across all groups (i.e. founders from different sites and descendants) ranged from 0.14 to 0.31 and was lower than expected heterozygosity under Hardy-Weinberg equilibrium (HWE) for all populations except for the Faure Island WBBs (Table 3). Allelic richness ranged from 1.13 (Faure Island founder WBBs) to 1.34 (Arid Recovery descendant bettongs). The WBBs had the lowest genetic diversity of the four species, again consistent with previous studies (Smith and Hughes, 2008; Smith *et* al., 2010).

The bettongs and WBBs at Arid Recovery had higher diversity across all measures, than either of their founding groups. On the other hand, the Arid Recovery GSNR population had slightly lower diversity across all measures than their founders. Although we could not do similar comparisons with the bilby dataset, as founding samples were not available, we note that their diversity measures are similar to the other species at Arid Recovery.

We further explored genetic diversity by calculating individual heterozygosity (Figure 2). Average individual heterozygosity was significantly higher in the Arid Recovery bettongs compared to its two founding populations ($p < 0.05$), while all other comparisons between populations or groups were non-significant ($p > 0.05$). The distribution of individual heterozygosity within groups of all species demonstrates how genetic diversity is relatively even across individuals within each population, except for within the Arid Recovery WBBs. In this group, five individuals are much more heterozygous than all other samples. Most individuals in the WBB population have lower heterozygosity than the founding group, but the average has been driven up by the five outliers.

The Arid Recovery bettong and WBB populations' average inbreeding were lower than either of their founding groups (Figure 3). However, only the bettong population had significantly different average inbreeding compared to their founders ($p < 0.05$). The WBB inbreeding was highly variable, with most sampled individuals having higher coefficients

than the founders. The five Arid Recovery WBB individuals with high heterozygosity, and therefore, much lower inbreeding coefficients than the rest of the WBB group again drove this pattern. The Arid Recovery GSNR population had slightly higher (although non-significantly, $p > 0.05$) average inbreeding than either of their founding groups, and the Arid Recovery bilby population had comparable average inbreeding to the Arid Recovery GSNR and bettong populations.

Average pairwise relatedness (*PR)* between individuals was higher within the Arid Recovery GSNR population than in either of its founding groups (Figure 4). Conversely, average *PR* was lower in the bettong and WBB Arid Recovery populations compared to their founding groups (Figure 4). However, the *PR* in the WBBs was again quite varied, and lowest between the same five individuals that also had lower inbreeding and higher heterozygosity. The *PR* measured in the bettong and WBB populations also show that the two founding groups for each species (Bernier Island and Heirisson Prong in bettongs, and Bernier Island and Faure Island for the WBBs), were highly unrelated to each other and that the WBB Arid Recovery population was more related to its Bernier Island founding group than the Faure Island founding group, excepting the five outlier individuals, which were equally related to both founding groups. *PR* within the Arid Recovery bilby population was varied, but generally low.

Arid Recovery Differentiation from Founding Groups

The results of principle component analysis for the GSNR, bettong and WBB datasets are shown in Figure 5. The GSNR Arid Recovery population is identifiable as a cluster separate from both founding groups of Monarto and Reevesby Island individuals, although the total amount of variation explained by the first two principle components is low (2.24-3.4%). The Arid Recovery bettong population clusters as a group intermediate between its

two founding groups, Bernier Island and Heirisson Prong. Finally, the Arid Recovery WBB samples cluster with its Bernier Island founding group separate to the Faure Island proxy founders. The five WBB individuals with lower inbreeding and higher heterozygosity are the most intermediate between the rest of the Arid Recovery/Bernier Island group and the Faure Island cluster.

Pairwise F_{ST} values for each species are shown in Table 4 and are in general agreement to the PCA results. F_{ST} values between GSNR groups were significantly different from zero between Arid Recovery and the founding groups, but not between the Monarto and Reevesby Island animals. All pairwise F_{ST} values were significantly different from zero between all groups of bettongs, being highest between the two founding groups (Heirisson Prong and Bernier Island). Within the WBB dataset, pairwise F_{ST} was significantly different from zero between Arid Recovery and the Faure Island group, and between the two founding groups (Faure Island and Bernier Island), but not between Bernier Island and Arid Recovery.

The sNMF analysis inferred that the most likely number of ancestral gene pools was two for the GSNR and bettong datasets, and three for the WBB dataset (SI Figure 2). Results of the ancestry estimates are shown in Figure 6. The GSNR plot shows most individuals in this dataset are a mixture of two genepools, with Reevesby Island dominated by one (average of 80% 'blue' in the plot) and Arid Recovery dominated by the other (average of 85% 'red' in the plot). The bettong sNMF plot shows that the Arid Recovery population is a mixture of the Bernier Island (mainly all blue) and Heirisson Prong (mainly all red) founders with an average of 71% Heirisson Prong and 29% Bernier Island ancestry.

The WBB sNMF plot shows that most Arid Recovery individuals share their entire ancestry with the Bernier Island founders. However, seven individuals are estimated (under K=3) to have ancestry from a third source (shown in orange on the plot). When we plot the ancestry estimates for the WBB dataset under K=2 (as the known number of sources, Figure

6) we can see that those seven individuals are those with admixture from the Faure Island population. We also note that the five individuals with the most Faure Island ancestry correspond to the individuals that were found to be the least inbred and most heterozygous.

Bayescan analysis identified six loci under putative selection in the GSNR dataset, but none in the bettong or WBB datasets (SI Figure 3). These six loci represents 0.07% of the total GSNR dataset and had FST values of >0.19 compared to an average of 0.05 across all loci.

Discussion

Despite relatively small founding populations, but perhaps consistent with modest-to-large population growth in all four species over an ~18-year period, our results show that average genetic diversity in all populations of reintroduced mammals at Arid Recovery reserve are close to, or exceeding, the levels measured in their founding groups. We detect only a small reduction in genetic diversity and small increase in inbreeding since release in the GSNR population, while the bettong and WBB populations are, on average, more diverse and less inbred than their founding groups. These results are driven by the mixing of two diverged and individually inbred source populations, which has had a large positive impact on the genetic diversity of the descendant Arid Recovery populations. Our study suggests that additional translocations to Arid Recovery may not be necessary at this time, and highlights the power of admixture, even from small isolated populations, as a tool for conservation management to maximise genetic diversity in threatened taxa via genetic rescue.

GSNRs at Arid Recovery have retained 94-98% of genetic diversity (depending on the measure used) and show no significant increase in inbreeding compared to their founding groups. These results indicate that most of the genetic diversity captured in the founding

366 individuals from Monarto and Reevesby Island has been retained in the Arid Recovery
367 populations, possibly because of the larger-than-average number of founders released (n =
368 122).

369 However, we do detect a small amount of differentiation between the GSNR Arid
370 Recovery population and their founding groups, indicated by the small, but significant,
371 pairwise F_{ST} values, and both the sNMF analysis and PCA plot. This differentiation could be
372 due to selection. For example, unlike the other populations of reintroduced species, the Arid
373 Recovery GSNR population experiences high mortality due to heat stress during summer,
374 which may be acting as a selective pressure in this population (Moseby, *pers comm*). This
375 hypothesis is partially supported by our Bayescan analysis, which detected six loci under
376 putative selection in the GSNR dataset. However, F_{ST} outliers can also result from
377 demographic effects, such as wave-edge surfing in recently bottlenecked populations (Hofer
378 *et al.*, 2009; Klopfstein *et al.*, 2006). Given the probable small effective population size in the
379 Arid Recovery population that would limit natural selection (Frankham *et al.*, 2010), genetic
380 drift is a more likely explanation for the differentiation seen in the GSNRs here. Further field
381 experiments comparing fitness of locally sourced and translocated animals in the Arid
382 Recovery environment could be used to test the hypothesis of local adaptation in the Arid
383 Recovery population. Such research is crucial to understanding how drift and selection can be
384 differentiated and ultimately how either case should be treated in translocated populations,
385 particularly when animals are moved between climatic zones.

386 The bettong and WBB populations have increased average genetic diversity compared
387 to their founding groups. Allelic richness has increased in both populations by more than 7%
388 and measures of heterozygosity have increased between 40% and 80%. We found that in both
389 species these results were entirely driven by admixture between two diverged sources.

Within the Arid Recovery bettong population, ancestry proportions were relatively similar across individuals, likely reflecting the fact that the two groups of founding individuals (from Bernier Island and Heirisson Prong) were released within a year of each other and have had 16 years to interbreed. It is interesting that, on average, the majority of ancestry (as shown in the sNMF analysis) in Arid Recovery bettongs was from the Heirisson Prong founders, despite only 10 individuals being released from this source compared to 20 from Bernier Island. This may be due to the additional year that the Heirisson Prong founders had to acclimatize to the new habitat before the Bernier Island founders were released, potentially giving the first group an advantage over the second. Although, this pattern could also be driven by stochastic drift.

Within the WBB Arid Recovery population, the admixture is less evenly distributed than in the bettong population, likely because of the smaller number of individuals translocated from the second source, and the shorter time since the second release. Only five individuals were translocated from Faure Island in 2009 (eight years after the first release from Bernier Island), but their genetic impact on the population is clear. Individuals without Faure Island admixture were slightly more inbred and less genetically diverse than the founding groups, whilst the individuals with admixture had much lower inbreeding and much higher heterozygosity than any other sampled individual. The five outlier individuals had roughly half of their ancestry, as estimated by sNMF analysis, originating from Faure Island which indicates they may be F1 hybrids. The Faure Island WBBs released into Arid Recovery were first contained within a pen and allowed to breed with each other before being released into the wider reserve. Given that WBBs live for three to five years, sampling of F1 hybrids is possible. We expect this admixture in the WBBs to spread throughout the population in subsequent generations. However, to ensure the introgressed genetic diversity is

not lost through stochastic processes, the genetic composition of the WBB population should be retested in a biologically relevant time-frame (for example 5-10 generations).

The pattern of admixture in the WBBs compared to that observed in the bettongs suggests that, where possible, translocation programs should aim to mix a similar number of individuals from different genetic stock simultaneously and early on in the establishment of reintroduced populations to maximise the benefits of admixture on genetic diversity.

The bilby population at Arid Recovery had similar levels of inbreeding and genetic diversity to the GSNR and bettong populations within the reserve. We were, however, unable to assess how much inbreeding had accumulated or how much genetic diversity has been retained since release as samples from the bilby founders were not available. We emphasize the importance of collecting samples from founders during reintroduction programs for use in later genetic assessments, even when individuals are sourced from captive breeding facilities with studbooks. Genotyping samples from other extant populations of bilbies across Australia would improve our inference about how resilient this population is to genetic deterioration. Mortiz *et al*. (1997) examined genetic diversity across the wild bilby range using mitochondrial DNA and microsatellites. Repeating this analysis using SNP data would permit direct comparison with our dataset and allow recommendations on the need for additional translocations to be made.

Given that our results show Arid Recovery Reserve has been successful in maintaining or even increasing the genetic diversity in the species reintroduced there, we suggest additional reintroductions may not be necessary at this time. However, we note that our datasets did not allow us to detect the true impact of founder effects on the Arid Recovery populations. A founder effect is the reduction of genetic diversity in a new population compared to its source resulting from non-representative founding individuals (i.e. when not all genetic diversity present in a source population is ‘captured’ in the founding individuals;

Frankham *et al*., 2010). We would expect this effect to be exacerbated when serial founder events occur (i.e. when the founding source is itself a reintroduced or captive population), as is the case for some of the Arid Recovery species. Further sampling at source, the original source populations (in the case of serial founding events), and other remnant populations of each species should be prioritised to determine whether genetic diversity can be further increased in the Arid Recovery populations.

A further area of research that we were unable to address here, but that is critical to improving species reintroductions, is the impact of mating strategies, sex ratio and sex-specific reproductive skew on the maintenance of genetic diversity. Our current understanding of the species at Arid Recovery suggests that their mating strategies are similar (eg.polygamous and probably polygynous; the females have tight home ranges and the males' home ranges overlap with several females [Moseby, *pers comm.*]), precluding comparisons, and, unfortunately, we do not know the level of reproductive-skew (sex biased or otherwise) across founding animals. Future work could address the later by tracking haplotypes of sex-specific loci (Y-chromosome or mitochondrial DNA) from the founders to the descendant population. Alternatively, the impact of these processes, including mating strategy, on genetic diversity in reintroduced populations could be studied using in-silico simulations (eg. Fiumera *et al.* 2004)

Admixture as a Conservation Tool

Our results highlight the positive impact that admixture has had on genetic diversity in two of the reintroduced mammal populations at Arid Recovery. The impact of admixture and gene flow on genetic diversity is well established. Wright (1931) and Franklin (1980) estimated that just one migrant per generation would be enough to prevent population

464 differentiation, drift and loss of adaptive potential (although more recent work suggests 1-10
465 migrants per generation may be necessary to stop loss of diversity in wild populations; Mills
466 and Allendorf, 1996). Admixture of diverged populations was found to substantially increase
467 the genetic diversity in reintroduced populations of the peregrine falcon (*Falco peregrinus*;
468 Jacobsen *et al*., 2008) and Alpine ibex (*Capra ibex*; Biebach and Keller, 2012), even when
469 divergence between the source populations was low. Furthermore, genetic rescue (i.e.
470 deliberate introduction of individuals from other populations to restore genetic diversity and
471 fitness) is an effective strategy to increase the reproductive health of small, inbred
472 populations (Heber *et al*., 2013; Hedrick and Fredrickson, 2010; Madsen *et al*., 2004; Weeks
473 *et al*., 2015).

474 Despite the evident advantages, admixture has been underutilized as a conservation tool
475 due to concerns about outbreeding depression and the need to conserve locally adapted
476 variation within subpopulations (Frankham, 2015; Weeks *et al*., 2016, 2011). However,
477 outbreeding depression is unlikely when mixing animals from populations that share similar
478 environments, have the same karyotype, have previously exchange genes and/or have long
479 generation times (Frankham *et al*., 2010). Furthermore, Weeks *et al*. (2016) argue that many
480 populations previously perceived as genetically 'unique' and potentially locally adapted
481 using neutral genetic markers, are often more likely to have differentiated through random
482 genetic drift and are therefore the populations most likely to be in need of genetic restoration.

483 The source populations of the WBBs and bettongs at Arid Recovery are from similar
484 environments, all originating from islands in Shark Bay, Western Australia, and are therefore
485 unlikely to have different local adaptations. Additionally, a previous study found only minor
486 mitochondrial haplotype divergence between the two WBB remnant populations (Smith and
487 Hughes, 2008). Hence, the admixture at Arid Recovery is unlikely to have resulted in
488 outbreeding depression. Rather, the bettong population at Arid Recovery, which was admixed

from the outset of the reintroduction program, has seen the most significant population growth of all the reintroduced species at the reserve, suggesting a possible fitness advantage in the admixed animals. Further experiments examining the fitness levels of inbred compared to outbred/admixed bettongs is needed to test this hypothesis. Regardless of whether this admixture confers any fitness advantages in the Arid Recovery populations, mixing of the diverged source populations will contribute to the preservation of adaptive potential in these species.

Conclusion

Our high-resolution datasets have revealed the success of the Arid Recovery reintroduction programs in maintaining and maximising genetic diversity of the threatened mammal species released there. Our results suggest that additional translocations to Arid Recovery may be unnecessary at this time, and highlight the clear benefit to reintroduction programs of admixing slightly diverged populations to maximise genetic diversity and adaptive potential in threatened taxa. Comparison of the two admixture strategies employed in the bettong and WBB populations at Arid Recovery show future translocation programs that plan to mix different genetic stocks should aim to release equal numbers of animals from both sources simultaneously, early in the reintroduction program. This will promote balanced admixture of both sources in the descendant population.

Ultimately, we have demonstrated the benefits of genetic monitoring in reintroduction programs and advocate for it’s continued use at Arid Recovery and in other reintroduction programs in the future.

Data Availability: All de-multiplexed raw sequencing data are available from NCBI's short read archive (Accession number: PRJNA389954).

Acknowledgments: Arid Recovery is a conservation initiative supported by BHP Billiton, The University of Adelaide, The South Australian Department for Environment and Natural Resources and the local community. The Nature Foundation of South Australia generously provided funding for this project. We thank the Australia Wildlife Conservancy for providing western barred bandicoot samples from Faure Island. We are indebted to the many Arid Recovery staff and volunteers who assisted with this project including Katherine Tuft, Rebecca West, Reece Pedler, and Kimberly Solly.

Role of the Funding Source: This work was funded by the Nature Foundation of South Australia, which did not contribute to the design, interpretation or the writing of this study.

References

Armstrong, D., Hayward, M., Moro, D. & Seddon, P. (2015). *Advances in Reintroduction Biology of Australian and New Zealand Fauna*. CSIRO Publishing, Melbourne.

Biebach, I. & Keller, L.F. (2012). Genetic variation depends more on admixture than number of founders in reintroduced Alpine ibex populations. *Biological Conservation* **147**, 197–203.

Brekke, P., Bennett, P.M., Santure, A.W. & Ewen, J.G. (2011). High genetic diversity in the remnant island population of hihi and the genetic consequences of re-introduction. *Molecular Ecology* **20**, 29-45

Burbidge, A.A. & McKenzie, N.L. (1989). Patterns in the modern decline of western Australia's vertebrate fauna: Causes and conservation implications. *Biological Conservation, Australian Developments in Conservation Evaluation* **50**, 143–198.

Cain, C.M., Livieri, T.M. & Swansom, B.J. (2011). Genetic evaluation of a reintroduced population of black-footed ferrets (*Mustela nigripes*).*Journal of Mammalogy* **92**, 751-759

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A. & Cresko, W.A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology* **22**, 3124–3140.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H., 2011. Stacks: Building and genotyping loci de novo from short-read sequences. *G3 Genes Genomes Genetics* **1**, 171–182.

Cochran-Biederman, J., Wyman, K.E., French, W.E. & Loppnow, G.L. (2015). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology* **29**, 175-186

Cullingham, C.I. & Moehrenschlager, A. (2013). Temporal analysis of genetic structure to assess population dyamics of reintroduced swift foxes. *Conservation Biology* **27**, 1389-1398

Ewen, J.G., Armstrong, D.., Parker, K.A. & Seddon, P.J. (2012). *Reintroduction Biology: Integrating Science and Management*. Wiley-Blackwell, Oxford.

Excoffier, L. & Lischer, H. (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.

562 Fiumera, A. C., Porter, B. A., Looney, G., Asmussen, M. A. and Avise, J. C. (2004),
563 Maximizing offspring production while maintaining genetic diversity in supplemental
564 breeding programs of highly fecund managed species. *Conservation Biology* **18,** 94–
565 101.
566 Foll, M. & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate
567 for both dominant and codominant markers: A bayesian perspective. *Genetics* **180**,
568 977–993.
569 Frankham, R. (2015). Genetic rescue of small inbred populations: Meta-analysis reveals large
570 and consistent benefits of gene flow. *Molecular Ecology* **24**, 2610–2618.
571 Frankham, R., Ballou, J.D. & Briscoe, D.A. (2010). *Introduction to Conservation Genetics*,
572 *2nd ed*. Cambridge University Press, New York.
573 Franklin, I.R. (1980). Evolutionary change in small populations, in: C*onservation Biology:*
574 *An Evolutionary-Ecological Perspective*. Sinauer Associates.
575 Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G. & François, O. (2014). Fast and efficient
576 estimation of individual ancestry coefficients. *Genetics* **196**, 973–983.
577 Goudet, J. (2005). hierfstat, a package for r to compute and test hierarchical F-statistics.
578 *Molecular Ecology Notes* **5**, 184–186.
579 Groombridge, J.., Raisin, C., Bristol, R. & Richardson, D.S. (2012). Genetic Consequences of
580 Reintroductions and Insights from Population History, in: *Reintroduction Biology:*
581 *Integrating Science and Management*. Wiley-Blackwell, Oxford.
582 Hardy, O.J. (2003). Estimation of pairwise relatedness between individuals and
583 characterization of isolation-by-distance processes using dominant genetic markers.
584 Molecular Ecology **12**, 1577–1588.
585 Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B. & Briskie, J. (2013). The genetic
586 rescue of two bottlenecked South Island robin populations using translocations of

587 inbred donors. P*roceedings of the Royal Society of London B: Biological Sciences*
588 **280**, 20122228.
589 Hedrick, P.W. & Fredrickson, R. (2010). Genetic rescue guidelines with examples from
590 Mexican wolves and Florida panthers. *Conservation Genetics* **11**, 615–626.
591 Hofer, T., Ray, N., Wegmann, D. & Excoffier, L. (2009). Large allele frequency differences
592 between human continental groups are more likely to have occurred by drift during
593 range expansions than by selection. *Annals of Human Genetics* **73**, 95–108.
594 IUCN (2016). *Global re-introduction perspectives: 2016*. International Union for
595 Conservation of Nature, SCC Re-introduction Specialist Group.
596 Jacobsen, F., Nesje, M., Bachmann, L. & Lifjeld, J.T. (2008). Significant genetic admixture
597 after reintroduction of peregrine falcon (*Falco peregrinus*) in Southern Scandinavia.
598 *Conservation Genetics* **9**, 581–591.
599 Jamieson, I.G. & Lacy, R.C. (2012). Managing Genetic Issues in Reintroduciton Biology, in:
600 *Reintroduction Biology: Integrating Science and Management*. Wiley-Blackwell,
601 Oxford.
602 Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers.
603 *Bioinformatics* **24**, 1403–1405.
604 Klopfstein, S., Currat, M. & Excoffier, L. (2006). The fate of mutations surfing on the wave
605 of a range expansion. *Molecular Biology and Evolution* **23**, 482–490.
606 Lennon, N.J., Lintner, R.E., Anderson, S., Alvarez, P., Barry, A., Brockman, W., Daza, R.,
607 Erlich, R.L., Giannoukos, G., Green, L., Hollinger, A., Hoover, C.A., Jaffe, D.B.,
608 Juhn, F., McCarthy, D., Perrin, D., Ponchner, K., Powers, T.L., Rizzolo, K., Robbins,
609 D., Ryan, E., Russ, C., Sparrow, T., Stalker, J., Steelman, S., Weiand, M., Zimmer,
610 A., Henn, M.R., Nusbaum, C. & Nicol, R. (2010). A scalable, fully automated process

611 for construction of sequence-ready barcoded libraries for 454. *Genome Biology* **11**,
612 R15.
613 Madsen, T., Ujvari, B. & Olsson, M. (2004). Novel genes continue to enhance population
614 growth in adders (*Vipera berus*). *Biological Conservation* **120**, 145–147.
615 Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Piñero, D. & Emerson, B.C.
616 (2015). Restriction site-associated DNA sequencing, genotyping error estimation and
617 de novo assembly optimization for population genetic inference. *Molecular Ecology*
618 *Resources*, **15,** 28-41.
619 Miermans, P.G., & Van Tienderen, P.H. (2004). "GENOTYPE and GENODIVE: two
620 programs for the analysis of genetic diversity of asexual organisms. *Molecular*
621 *Ecology Notes* **4**, 792-794
622 Michaelides, S., Cole, N. & Funk, S.M. (2015). Translocation retains genetic diversity of a
623 threatened endemic reptile in Mauritius. *Conservation Genetics* **16**, 661-672
624 Mills, L.S. & Allendorf, F.W. (1996). The one-migrant-per-generation rule in conservation
625 and management. *Conservation Biology* **10**, 1509–1518.
626 Moraes, A.M., Ruiz-Miranda, C.R., Ribeiro, M.C., Grativol, A.D., Carvalho, C.S., Dietz,
627 J.M., Kierulff, M.C.M., Freitas, L.A. & Galetti, P.M. (2017). Temporal genetic
628 dynamics of reintroduced and translocated populations of the endangered golden lion
629 tamarin (*Leontopithecus rosalia*). *Conservation Genetics* 1-15
630 Moritz, C., Heideman, A., Geffen, E. & McRae, P. (1997). Genetic population structure of
631 the Greater Bilby *Macrotis lagotis*, a marsupial in decline. *Molecular Ecology* **6**, 925-
632 936
633 Morton, S.R. (1990). The impact of European settlement on the vertebrate animals of arid
634 Australia: a conceptual model. *Proceedings of the Ecological Society of Australia* **16**,
635 201–213.

636 Moseby, K.E. & Bice, J.K. (2004). A trial re-introduction of the greater stick-nest rat
637 (*Leporillus conditor*) in arid South Australia. *Ecological Management and. Restoration*
638 **5,** 118–124.

639 Moseby, K.E. & O'Donnell, E. (2003). Reintroduction of the greater bilby, *Macrotis lagotis*
640 (Reid) (Marsupialia : Thylacomyidae), to northern South Australia: survival, ecology
641 and notes on reintroduction protocols. *Wildlife Research* **30**, 15–27

642 Moseby, K.E. & Read, J.L. (2006). The efficacy of feral cat, fox and rabbit exclusion fence
643 designs for threatened species protection. *Biological Conservation* **127**, 429–437.
644 Moseby, K.E., Read, J.L., Paton, D.C., Copley, P., Hill, B.M. & Crisp, H.A. (2011).
645 Predation determines the outcome of 10 reintroduction attempts in arid South
646 Australia. *Biological Conservation* **144**, 2863–2872.
647 Newsome, A.E. (1971). Competition between wildlife and domestic livestock. *Australian*
648 *Veterinary Journal* **47**, 577–586.
649 Pew, J., Muir, P.H., Wang, J. & Frasier, T.R. (2015). related: an R package for analysing
650 pairwise relatedness from codominant molecular markers. *Molecular Ecology*
651 *Resources* **15**, 557–561.
652 Poland, J.A., Brown, P.J., Sorrells, M.E. & Jannink, J.-L. (2012). Development of high-
653 density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-
654 sequencing approach. *PLoS ONE* **7**, e32253.
655 Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using
656 multilocus genotype data. *Genetics* **155**, 945–959.
657 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J.,
658 Sklar, P., de Bakker, P.I.W., Daly, M.J. & Sham, P.C. (2007). PLINK: A tool set for
659 whole-genome association and population-based linkage analyses. *American Journal*
660 *of Human Genetics* **81**, 559–575.

661 Reich, D., Price, A.L. & Patterson, N. (2008). Principal component analysis of genetic data.
662 *Nature Genetics* **40**, 491–492.
663 Richards, J. (2005). *Western Barred Bandicoot, Burrowing Bettong and Banded Hare-*
664 *Wallaby Recovery Plan 2005 -2010*. Department of Conservation and Land
665 Management, Wanneroo, Western Australia
666 Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
667 Rivero, E. R., Neves, A. C., Silva-Valenzuela, M. G., Sousa, S. O. & Nunes, F. D. (2006).
668 Simple salting-out method for DNA extraction from formalin-fixed, paraffin-
669 embedded tissues. *Pathology-Research and Practice,* **202**, 523-529.
670 Rohland, N. & Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries
671 for multiplexed target capture. *Genome Research* **gr.128124.111**.
672 Schwartz, M.K., Luikart, G. & Waples, R.S. (2007). Genetic monitoring as a promising tool
673 for conservation and management. *Trends in Ecology and Evolution* **22**, 25–33.
674 Smith, S., Belov, K. & Hughes J. (2010). MHC screening for marsupial conservation:
675 extremely low levels of class II diversity indicate population vulnerability for an
676 endangered Australian marsupial. *Conservation Genetics* **11**, 269-278
677 Smith, S. & Hughes, J. (2008). Microsatellite and mitochondrial DNA variation defines
678 island genetic reservoirs for reintroductions of an endangered Australian marsupial,
679 *Perameles bougainville*. *Conservation Genetics* **9**, 547-557.
680 Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*
681 **160**, 1203–1215.
682 Weeks, A.R., Moro, D., Thavornkanlapachai, R., Taylor, H.R., White, N.E., Weiser, E.L. &
683 Heinze, D. (2015). Conserving and enhancing genetic diversity in translocation
684 programs, in: *Advances in Reintroduction Biology of Australian and New Zealand*
685 *Fauna*. CSIRO Publishing, Melbourne.

686 Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne,
687 M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A. &
688 Hoffmann, A.A. (2011). Assessing the benefits and risks of translocations in changing
689 environments: a genetic perspective. *Evolutionary Applications* **4**, 709–725.
690 Weeks, A.R., Stoklosa, J. & Hoffmann, A.A. (2016). Conservation of genetic uniqueness of
691 populations may increase extinction likelihood of endangered species: The case of
692 Australian mammals. *Frontiers in Zoology* **13**, 31.
693 Wright, S. (1931). Evolution in Mendelian populations. *Genetics* **16**, 97–159.
694

Figures and Tables

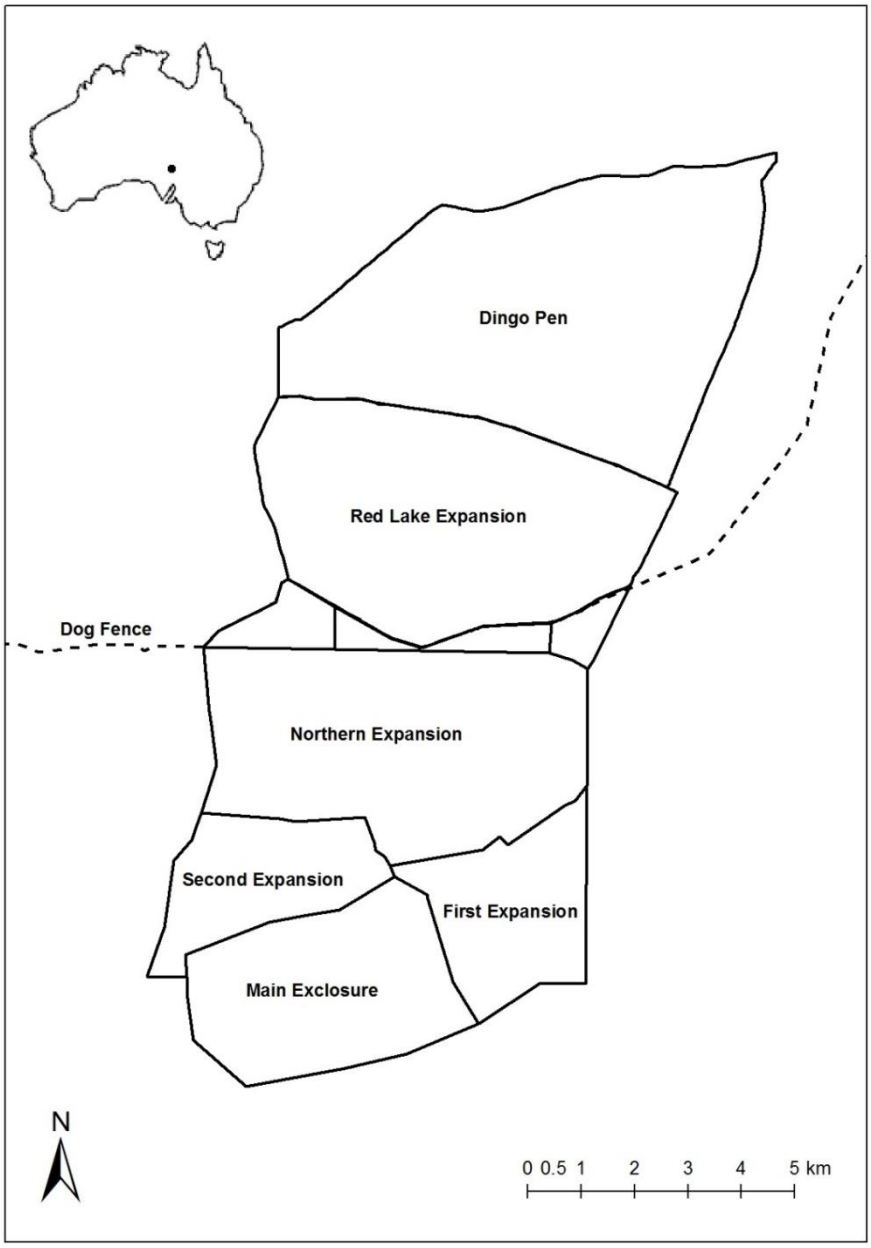

Figure 1. Location and lay-out of Arid Recovery reserve. Rabbits, cats and foxes have been removed from the four southern paddocks of the Reserve

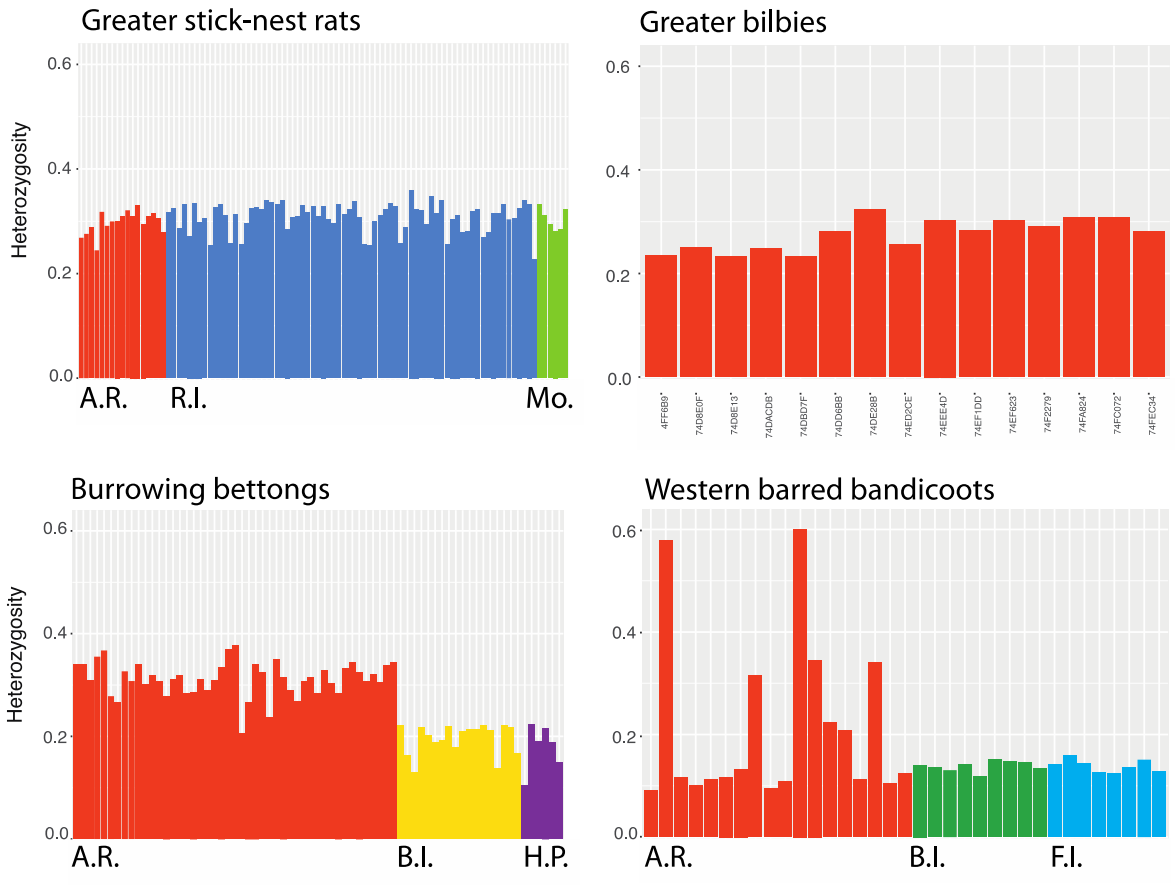

Figure 2. Individual observed heterozygosity calculated for each sampled individual of greater stick-nest rat (GSNR), greater bilby, burrowing bettong and western barred bandicoot (WBB). Each vertical bar represents an individual, and is coloured by population. Population names have been shortened: A.R —Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island.

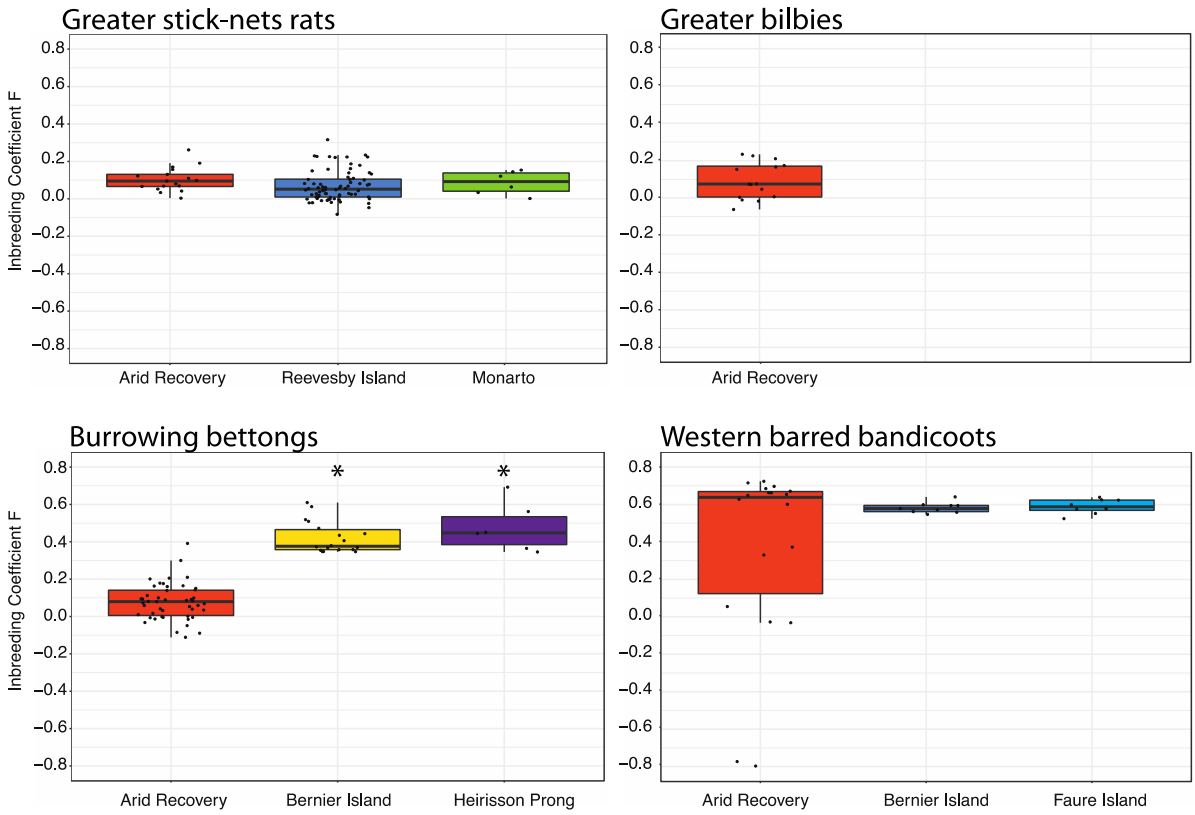

Figure 3. Individual inbreeding coefficients per population for founding groups (where available) and current Arid Recovery populations of greater stick-nest rats (GSNR), greater bilbies, burrowing bettongs and western barred bandicoots (WBB). Dots represent individual values. Middle horizontal lines represent the median, the boxes are bound by the 25th and 75th quartiles and vertical lines show the minimum and maximum range of values excluding outliers. Founding groups that had significantly different average inbreeding coefficients from their descendant Arid Recovery populations are denoted with an asterisk.

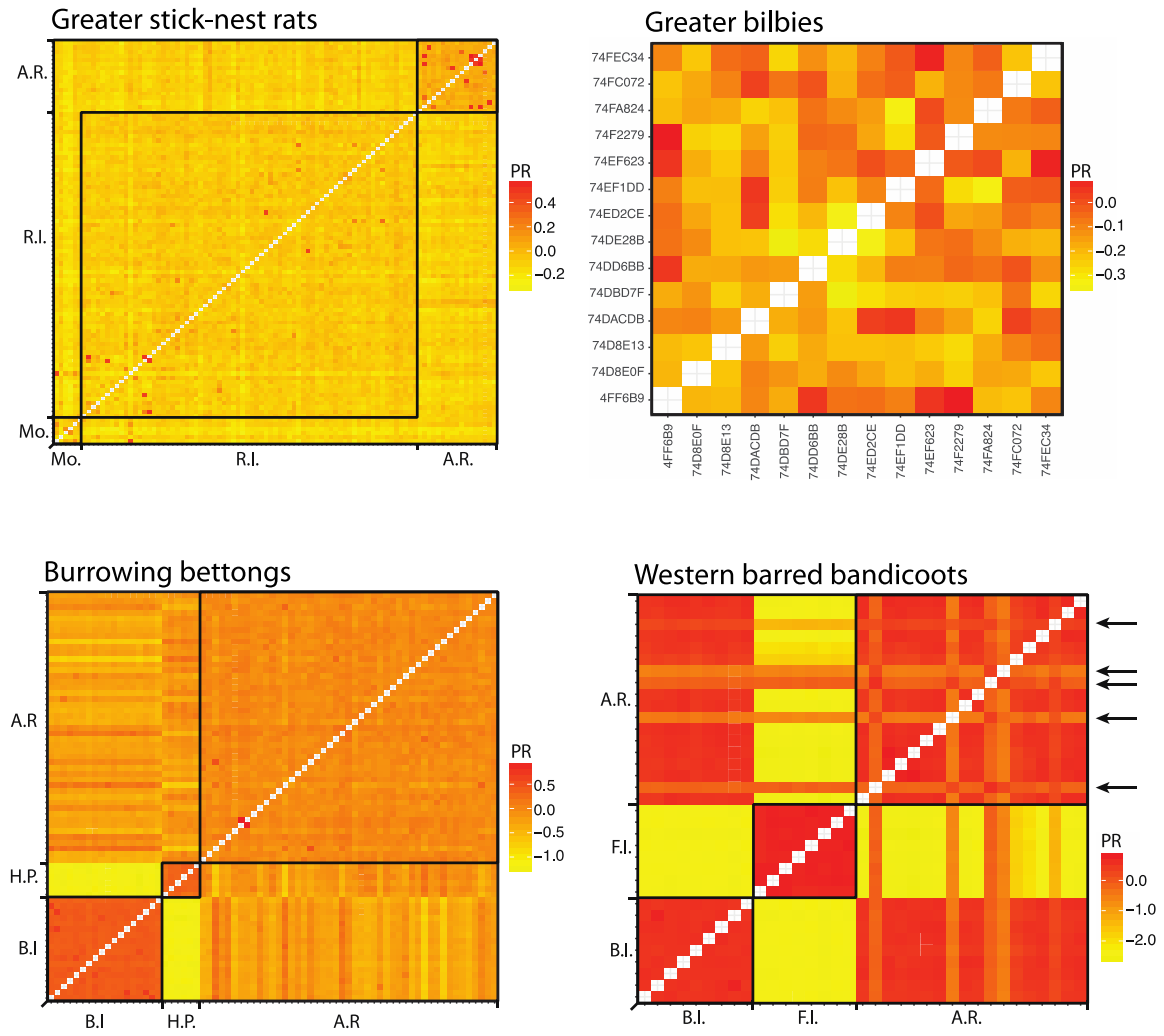

Figure 4. Heat map of pairwise relatedness (*PR*) calculated between each sampled individual within each species. Within population comparisons are bounded by black squares. Arrows on the WBB heat map highlight the five individuals with lower levels of inbreeding and average pairwise relatedness than the rest of the WBB Arid Recovery samples. Population names are shortened due to space requirements: A.R — Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island. Bilby PR is labelled by sample as founding individuals were not sampled.

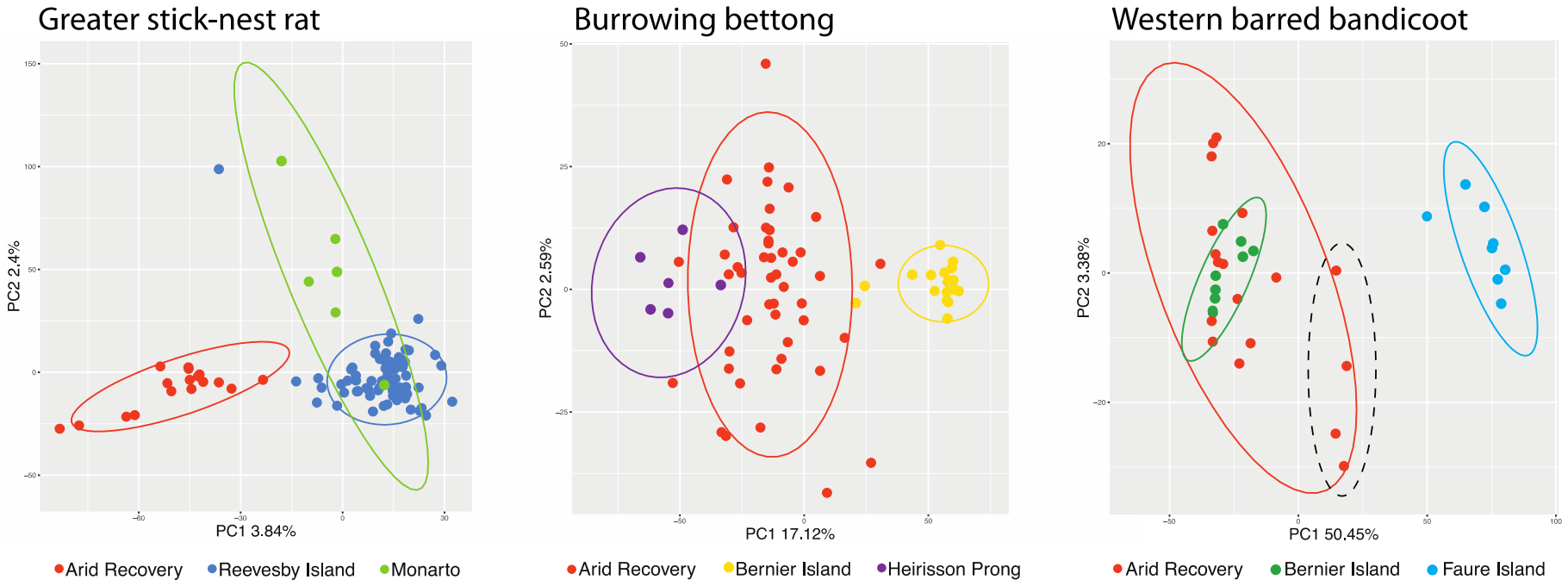

Figure 5. Relationships among founding groups and the descendant Arid Recovery populations of greater stick-nest rats (GSNR), burrowing bettongs and western barred bandicoots (WBB) based on principle coordinate analysis for principle components 1 and 2. Each dot represents an individual coloured by population. Solid ellipses represent the centre and 95% confidence interval of the points in each population. The dotted ellipse encompasses the five outlier WBB samples.

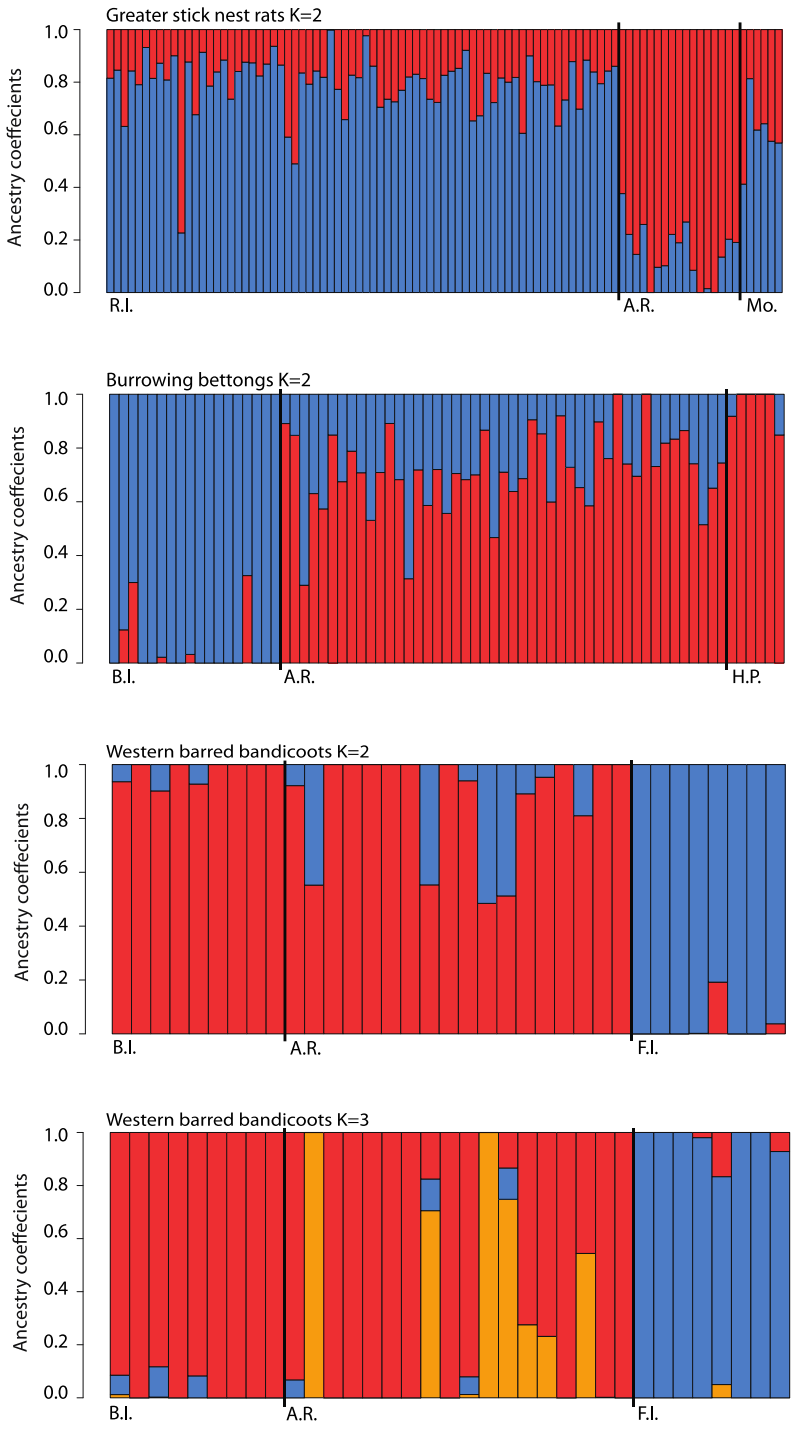

Figure 6. Genetic ancestry in individuals from Arid Recovery and their founding groups estimated using sNMF. Each vertical bar represents an individual. Population names are shortened due to space requirements: A.R — Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island.

Table 1. Reintroduction and genetic sampling history of the four species translocated to Arid Recovery Reserve (AR). Samples were not available from any of the bilby founding individuals, 32 GSNR founding individuals (12 from Reevesby Island and 18 from Monarto) and the Faure Island founding WBB individuals. We sourced eight WBB samples taken from Faure Island in 2007 as proxies for the AR founders, denoted here with an asterisk. Population size at AR was estimated at the time of sampling from track count data (GSNR, bilbies and WBB) or mark recapture data (bettongs).

Species	**Year of Translocation to AR**	**Number of founders (Male:Female)**	**Source Population(s)**	**Founders sampled at time of release**	**Year of Sampling at AR**	**Samples Collected at AR**	**Population Size at AR (at time of sampling)**
Greater Stick-nest Rat (GSNR)	1998,1999 & 2003	122 (65:57)	Reevesby Island (98), Monarto (24)	Reevesby:84, Monarto:6	2016	20	500
Greater Bilby	2000, 2001 & 2005	37 (21:16)	Monarto (9) Thistle Island (28)	-	2016	16	500
Burrowing Bettong	1999 & 2000	30 (11:19)	Heirisson Prong (10) Bernier (20)	All	2014	60	6000
Western Barred Bandicoot (WBB)	2001 & 2009	17 (4:13)	Bernier Island (12), Faure Island (5)	Bernier:10, Faure:8*	2014	18	1000

Table 2. Summary of sequencing statistics for each founder group and descendant population within each species.

Species	Population	Number of Samples successfully sequenced	Average Number of Reads	Average Number of Loci	Average Depth of Coverage	Number of SNPs in final dataset	Average Missing Data
Greater stick-nest rat	Reevesby Island	72	4148368.65	142615.26	20.66	8703	11.90%
Greater stick-nest rat	Monarto	6	2545679.00	110442.33	16.36	8703	15.80%
Greater stick-nest rat	Arid Recovery	17	4428737.94	158270.65	20.55	8703	6.80%
Greater bilby	Arid Recovery	15	5597898.73	97196.40	38.54	6880	13.23%
Burrowing bettong	Bernier Island	18	2427230.28	55023.33	32.76	3775	10.50%
Burrowing bettong	Heirisson Prong	6	748519.83	27520.17	20.45	3775	28.30%
Burrowing bettong	Arid Recovery	47	2633766.28	52221.19	35.50	3775	9.40%
Western barred bandicoot	Bernier Island	9	4775200.44	71154.11	44.71	1752	13.80%
Western barred bandicoot	Faure Island	8	2480600.38	69239.75	26.11	1752	11.70%
Western barred bandicoot	Arid Recovery	18	3821004.94	66350.67	41.68	1752	8.60%

Table 3. Average measures of genetic diversity in founding and descendant populations of mammals released at Arid Recovery, with standard deviation in parentheses. Allelic richness corrected for sample size (A_R), and expected and observed heterozygosity (H_E, H_O).

Species	**Population**	**H_E**	**H_O**	**A_R**
Greater stick-nest rat	Reevesby Island	0.33 (0.001)	0.31 (0.001)	1.33 (0.14)
Greater stick-nest rat	Monarto	0.33 (0.002)	0.30 (0.003)	1.32 (0.21)
Greater stick-nest rat	Arid Recovery	0.30 (0.002)	0.29 (0.002)	1.30 (0.17)
Greater bilby	Arid Recovery	0.31 (0.002)	0.26 (0.002)	1.28 (0.16)
Burrowing bettong	Bernier Island	0.21 (0.003)	0.20 (0.004)	1.21 (0.21)
Burrowing bettong	Heirisson Prong	0.23 (0.004)	0.18 (0.004)	1.22 (0.26)
Burrowing bettong	Arid Recovery	0.34 (0.002)	0.31 (0.002)	1.34 (0.13)
Western barred bandicoot	Bernier Island	0.15 (0.002)	0.14 (0.002)	1.15 (0.19)
Western barred bandicoot	Faure Island	0.13 (0.005)	0.15 (0.006)	1.13 (0.21)
Western barred bandicoot	Arid Recovery	0.24 (0.003)	0.21 (0.003)	1.24 (1.33)

Table 4. Pairwise F_{ST} values calculated between the founding groups and descendant Arid Recovery populations for the greater stick-nest rats, burrowing bettongs and western barred bandicoots. Significant values (after Bonferroni correction) are highlighted in bold.

Greater stick-nest rats (GSNR)

	Arid Recovery	**Reevesby Island**	**Monarto**
Arid Recovery			
Reevesby Island	**0.04352**		
Monarto	**0.05930**	0.02845	

Burrowing bettongs

	Arid Recovery	**Bernier Island**	**Heirisson Prong**
Arid Recovery			
Bernier Island	**0.19133**		
Heirisson Prong	**0.11992**	**0.53907**	

Western barred bandicoots (WBB)

	Arid Recovery	**Bernier Island**	**Faure Island**
Arid Recovery			
Bernier Island	0.03933		
Faure Island	**0.67165**	**0.8124**	