Urban restoration of common species: population genetics of reintroduced native bush rats *Rattus fuscipes* in Sydney, Australia

A. L. Wright¹, J. R. Anson², V. Leo², B. R. Wright¹,³, T. M. Newsome¹ & C. E. Grueber¹

1 School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW, Australia
2 Australian Wildlife Conservancy, Perth, WA, Australia
3 Sydney School of Veterinary Sciences, The University of Sydney, Faculty of Science, The University of Sydney, Sydney, NSW, Australia

Keywords
- genomics; mammals; reintroductions; rewilding; translocations; urban restoration; *Rattus fuscipes*.

Abstract

Common species play a disproportionate role in shaping ecosystem structure and function, but are currently under-represented in conservation translocation initiatives. This represents a missed opportunity because common species are typically easier to source for restoration projects, and larger numbers of common species can feasibly be translocated without substantially impacting source populations. Reintroduction of common species is an important first step in the faunal restoration of severely impacted habitats, such as urban spaces. Common species typically retain higher genetic diversity than threatened species, but this also means that they may have more to lose via population bottlenecks that can occur from translocation. To inform efforts to translocate common species, we assessed genetic impacts of a reintroduction of the common native bush rat *Rattus fuscipes* to an urban reserve at North Head, Sydney (Australia). Using single-nucleotide polymorphism diversity, we found that differentiation between source populations was low. Nevertheless, admixture during reintroduction and follow-up translocations initially increased standardized observed heterozygosity of North Head-born bush rats and population NE, with a subtle corresponding decrease in within-population kinship. For 3 years following the last translocation, we detected a small decline in genetic diversity in the North Head population, although final statistics remained similar to the source populations. Our results indicate that no short-term interventions are necessary to further promote bush rat genetic diversity at North Head, but that continued genetic monitoring will be important to determine whether a trend in declining diversity continues as the population stabilizes. We conclude that translocation of a large number of individuals from multiple sources presents a suitable option for restoring an extirpated small mammal population whilst minimizing genetic effects typically associated with such management actions. Common species present viable candidates for translocations aiming to return biodiversity to disturbed or fragmented urban ecosystems.

Introduction

Common species have a disproportionate role in shaping ecosystem structure and function (Gaston & Fuller, 2008; Lindenmayer *et al*., 2011). Relatively small declines in the abundance of common species can therefore represent considerable losses of the ecosystem services they provide (Cameron *et al*., 2011; Winfree *et al*., 2015; Walsh, Carpenter & Zanden, 2016). Importantly, ‘commonness’ does not preclude a species’ susceptibility to the main threats driving global biodiversity declines (Gaston, 2010). In Australia, for example, a suite of formerly common species, especially small mammals, have experienced rapid declines in abundance and widespread range contractions (Lindenmayer *et al*., 2011; Woinarski, Burbidge & Harrison, 2015). Conservation practices have traditionally prioritized management actions based on assessments of extinction risk, resulting in a variety of restoration initiatives for rare or endangered species (Gaston & Fuller, 2008). For common species, early interventions may be the most effective way to preserve their important ecological functions and help ‘keep common species common’ (Watson & Watson, 2015).

Translocation of common species presents a viable means for returning functional diversity and important ecosystem
services to fragmented or disturbed habitats. Urban landscapes are among the most ecologically disrupted spaces and, if restored, they offer enormous potential to increase human engagement and appreciation of nature (Sweeney et al., 2019). Indeed, broader initiatives to restore or ‘rewild’ remote wilderness areas may be enhanced when similar practices are undertaken close to urban centres (Jepson, 2016). However, key to the persistence of common species in urban landscapes is an individual’s ability to move among habitat fragments (Holland & Bennett, 2010). Many key species, such as small mammals, have limited dispersal capabilities (Peakall, Ruibal & Lindenmayer, 2003), so translocations can play an important role in restoring the functional connectivity of remnant populations and/or recolonization of formerly occupied habitats. Translocations can help restore genetic connectivity among isolated populations, remediate localized extinctions or establish new populations in habitats outside native ranges (Seddon, 2010; IUCN/SSC, 2013). As a conservation tool, translocations can promote the resilience of common species and return functional diversity to fragmented habitats (Watson & Watson, 2015; Loth & Newton, 2018; van Heezik & Seddon, 2018).

While factors such as habitat suitability and the remediation of key threats are important for translocation success, there are also a number of genetic constraints (bottlenecks, genetic drift and inbreeding depression) which must be overcome to ensure long-term population viability (Jamieson, 2011; Ewen et al., 2012). If left unchecked, these demographic and genetic processes can reduce individual fitness and erode adaptive potential at the population level (Weeks et al., 2011; Frankham et al., 2017). With ongoing advancements in molecular technology, genetic monitoring strategies have become increasingly accessible to conservation managers (Narum et al., 2013). Methods such as reduced-representation sequencing (RRS) provide a cost-effective means for assaying genomewide diversity, even for species without previous genetic information or reference genomes (Galla et al., 2019; Wright et al., 2019). By genotyping thousands of single-nucleotide polymorphisms (SNPs) across numerous individuals, RRS techniques enable the detection of fine-scale population structure and genetic variation. The importance of genetic monitoring in species translocations is supported by the variety of conservation efforts involving endangered species (Weeks et al., 2011; Frankham et al., 2017; Furlan et al., 2020). However, the genetic effects of translocations on common species are less understood. Common species are typically found in large numbers or are widely distributed, and therefore often retain higher heterozygosity compared to threatened species. For example, heterozygosity of threatened taxa was lower in 77% of comparisons with taxonomically related non-threatened taxa (Spielman, Brook & Frankham, 2004). Retaining these high levels of heterozygosity is therefore an important consideration when translocating non-threatened or common taxa. While commonness may place species in a better position for translocation initiatives, they may also have more to lose via the population bottlenecks that may result from such management activities, highlighting the importance for genetic monitoring.

The translocation of common species has formed an important component of the ongoing ecosystem restoration at North Head, located approximately 10 km from the centre of Sydney, Australia (Fig. 1). In the 200 years since European colonization, this isolated patch of remnant bushland has experienced local extinctions of several native mammalian species (Banks, Cleary & Dickman, 2011; Saul, 2013). In 2014, a major species translocation involving the native bush rat Rattus fuscipes was undertaken by Australian Wildlife Conservancy (AWC) at North Head. The bush rat is one of the most common small mammals found along the southeast coastline of Australia (Parrott et al., 2007); however, due to persecution in the early 1900s, the species faced local extirpations throughout the Sydney Harbour foreshore region, including North Head (Banks et al., 2011). Since translocation, the North Head bush rat population has expanded, reaching an average density of 7 individuals per hectare across the site (Banks et al., 2011; O’Rourke et al., 2020). AWC implemented a number of measures to promote genetic diversity within the bush rat population at North Head. This included the mixture of different source populations for the initial reintroduction and during two subsequent translocations. The genetic admixture of individuals from different source populations is an increasingly common consideration in species translocations, aimed at enhancing the allelic diversity among founding individuals (Biebach & Keller, 2012; Thavornkanlapachai et al., 2019).

The current study investigates the population genetic changes that occurred during the establishment of bush rats at North Head. Using measures of individual and population diversity, as well as population differentiation, we examine temporal changes in the population genetic profile of North Head bush rats from 2014 to 2019 and relative to the source populations (represented by the animals caught for translocation). As an isolated site, we predict that the North Head bush rat population may lose genetic diversity over time. However, the rate of decline may be influenced by the level of genetic admixture achieved from mixing source populations and demographic processes (such as the population growth rate, which we do not assess directly here). From a population genetic perspective, if there was substantial genetic structure among the source populations, admixture may occur at North Head, elevating diversity of locally born individuals (‘admixture hypothesis’, Fig. 2). It is also plausible that a population bottleneck may have occurred following establishment, leading to a decline in diversity ('no admixture hypothesis', Fig. 2). The results will inform restoration planning for bush rat into the future, and for returning other common species to urban areas.

Materials and methods

Study site and translocation

North Head is a ~300 ha headland situated on the northern entrance of Sydney Harbour (33°48’S, 151°17’E). The headland forms part of the Sydney Harbour National Park and is actively managed by the Sydney Harbour Federation Trust.
Figure 1 Bush rat source locations used for the North Head translocation, showing the general location of the study area in Australia (a), the source locations relative to the North Head release site (b), and the sampling localities within the source site (c).

Figure 2 A conceptual diagram of the reintroduction of bush rats to North Head, and two alternate hypotheses for the effects of admixture on the descendant population. Bush rats were sourced from wilderness sites 29 km away from North Head (Figure 1), and initially reintroduced in 2014 with follow-up translocations in 2015 and 2016. If the source populations show genetic structure, the descendant population may have increased genetic diversity (admixture hypothesis). A population bottleneck during translocation may also result in reduced diversity of locally born offspring. In either case, diversity of the North Head population may decline in later years due to genetic drift if the descendant population remains isolated and fails to grow quickly (i.e. remains at small size for an extended period).
and NSW National Parks and Wildlife Services (Department of Planning Industry and the Environment) with active conservation management of the site and ecological monitoring carried out by AWC. North Head is isolated for dispersal-limited species, such as small mammals, since the site must be accessed via a complex urban matrix, whilst the remainder of the headland is bordered by ocean. Due to its mixed historical use, North Head hosts a mosaic of built infrastructure, open lawns and extensive remnant patches of native vegetation, including 35% Eastern Suburbs Banksia Scrub and 12% coastal sandstone heath (Hansen et al., 2020). As part of the ecosystem restoration, pest control is conducted for invasive populations of European rabbits Oryctolagus cuniculus and black rats Rattus rattus, and incursions by European red foxes Vulpes vulpes and cats Felis catus.

This study focuses on the translocation of bush rats to North Head initiated by AWC in 2014. A previous reintroduction attempt in 2011 was unsuccessful at establishing a bush rat population at North Head, likely due to the small number of translocated individuals (N = 25) (Callandar, 2018). The bush rat is a small (40–225 g), native Australian rodent that is typically abundant in forests and heathland regions of south and eastern Australia (Parrott et al., 2007). The species can reproduce year-round, with a peak breeding season of spring to early summer, and a typical litter size of 4–5 young each year (Warneke, 1971; Taylor & Horner, 1973). The average lifespan of the species is 12–15 months and individuals often do not survive to a second breeding cycle (Warneke, 1971). A total of 180 individuals were released at North Head during translocation events in 2014 (N = 73), 2015 (N = 54) and 2016 (N = 53). Pre-release monitoring was conducted across the headland to confirm the absence of bush rats at North Head prior to the translocations. The 2014 founders were jointly sourced from Muogamarra Nature Reserve (‘Muogamarra 1’ N = 53, 33°33’S, 151°10’E) and Ku-ring-gai Chase National Park (N = 20, 33°33’S, 151°13’E), New South Wales. These two sites are approximately 29 km from North Head, 3 km apart from one another, and separated by a multi-lane motorway, which presents a likely barrier to gene flow between them (Fig. 1). Individuals for the subsequent translocations were sourced from separate regions of Muogamarra (‘Muogamarra 2’ 2015, N = 54; ‘Muogamarra 3’ 2016, N = 28) and Ku-ring-gai Chase (2016, N = 25) (Fig. 1) to maximize the potential for translocating individuals from different population clusters. Source locations were selected based on high local densities of bush rats and comparable habitat composition to North Head: the predominant vegetation communities at both the source sites and North Head are heathland and eucalypt forest; although there is less eucalypt forest at North Head, the understory species associated with this community are similar to the source sites.

Bush rats were collected from the source locations using Elliott traps (Elliott Scientific, Upwey, Victoria) baited with oats, honey and peanut butter. Linear transects of Elliott traps were left out for up to four consecutive nights at each source location during each trapping event (2014, 2015 and 2016). Upon capture, the bush rats were aged, sexed, weighed, inspected for any injuries and assessed for translocation potential. Founder animals were immediately transported from the source locations to North Head in small cages in a climate-controlled vehicle and were released into breeder cages onsite. Ear-tissue samples were collected from all translocated individuals, and a microchip inserted, prior to release. Tissue samples were collected using a 2-mm ear punch, stored in 90% ethanol and immediately refrigerated. Tissue samples were additionally collected from any locally born individuals captured during post-translocation monitoring surveys conducted between 2014 and 2019, providing a catalogue of genetic material spanning the population’s establishment. The post-translocation monitoring was conducted at 20 sites across North Head. At each site, live-traps were deployed in a linear transect comprised of five Elliott traps and five cage traps (40 × 13 cm) spaced 10 m apart. Trapping was conducted biannually in May and December. All trapped individuals were scanned for microchips, and microchips were fitted at the initial point of capture if no microchip was present. In total, 613 bush rat samples were collected from locally born individuals during this period.

All animal work was carried out under Animal Research Authority - Biodiversity Conservation and Restoration, North Head TRIM 14/267 and 17/226 and Scientific Licences – Translocation of native bush rats into its former range, Sydney Harbour National Park SL100201 and Biodiversity Conservation and Restoration, North Head, SL 101333.

DNA extraction and sequencing

To assess genetic structure and temporal changes in North Head bush rat diversity, a subset of tissue samples were selected from founder groups and locally born animals (total N = 251). Samples were chosen to provide a representative subset of the source populations at Ku-ring-gai (2014 N = 14; 2016 N = 19) and Muogamarra (1: 2014 N = 36; 2: 2015 N = 31; 3: 2016 N = 21), and for North Head-born animals across the population’s establishment (2015 N = 25, 2016 N = 25, 2017 N = 25, 2018 N = 25, 2019 N = 30) (Fig. 1). Each group of samples selected for DNA extraction was chosen at random from each population/cohort, accounting for equal sex representation where possible.

DNA was extracted from tissue samples using the Isolate II Genomic DNA Kit (Bioline Pty Ltd), following manufacturer protocols with the exception that the digestion incubation was conducted overnight. DNA concentration was quantified using a Nanodrop 2000 Spectrophotometer (ThermoFisher Scientific), and extraction quality was assessed via 1% agarose gel electrophoresis (Astral Scientific), using 1 × TAE buffer and SYBR safe DNA gel stain (ThermoFisher Scientific) at 100 V for 30 min. Following the criteria of McLennan et al. (2019), each DNA sample was ranked based DNA extraction quality, with additional extractions performed to replace poor quality samples when necessary. In total, 282 samples were submitted for RRS via DArTseq™ (Diversity Arrays Technology Pty Ltd., Australia; DArT), with 31 individuals submitted twice as technical replicates to calculate genotyping concordance within and between plates. The DArTseq
reduced-representation sequencing approach targets non-repetitive regions of the genome with restriction enzymes *PstI* and *SphiI*, followed by sequencing of the resulting fragments on a HiSeq 2500 as 77-bp single-end reads (Cruz, Kilian & Dierig, 2013). DArT processed the sequencing reads using their in-house pipeline (Cruz et al., 2013), mapping reads to the Norway rat *Rattus norvegicus* reference genome Rnor6.0 (Gibbs et al., 2004, see also below), and returned a total of 97 345 SNP loci.

We used analysis of our technical replicates and found that genotype concordance, even prior to further filtering, was high (mean error rate = 1.87%; Supporting Information Table S1). We removed 10,668 loci that showed >1 replication errors. Subsequent filtering was conducted using the package dartR v 1.9.9.1 (Gruber et al., 2018) for R v 4.1.1 (R Core Team, 2021). We removed SNPs with <100% repeatability according to DArT internal controls, a call rate of <95% of individuals and individuals with a call rate of <95% of SNPs (one 2017 North Head individual removed at this step). Loci were filtered based on a minor allele frequency of 0.005: this filtering threshold (3/2N individuals) removes loci with minor alleles appearing in <2 individuals, to reduce the probability of retaining false alleles resulting from sequencing errors (Lott et al., 2020). SNPs occurring within the same sequence read have a higher likelihood of being linked (Gruber et al., 2018) and therefore only the one SNP was retained (at random) for sequence reads containing more than one SNP.

Our data were mapped to the Norway rat reference genome Rnor6.0 (Gibbs et al., 2004); so, we removed any SNPs that did not map unambiguously to autosomes (Supporting Information Table S2). We used ANNOVAR (Wang et al., 2010) to extract reference genome annotation information available from UCSC (Karolchik et al., 2004) to identify the functional classifications of the SNPs in our dataset. This enabled us to break down our SNP dataset according to whether SNPs fell within genes (summarized at Supporting Information Table S3), providing a ‘genomic’ dataset (all 9716 filtered and mapped SNPs), ‘genic’ dataset (3712 SNPs in and around genes, including introns, exons, UTRs, up/downstream and splice-site variants) and ‘exonic’ dataset (224 SNPs within exons of genes; full details of exonic SNPs provided at Supporting Information Table S4). To avoid linkage disequilibrium (LD) bias from clustered SNPs in our dimensionality reduction (e.g. PCA) and relatedness analyses (e.g. Zou et al., 2010), all three SNP datasets were pruned to a set of unlinked loci using the snpgdsLDpruning function in SNPRelate v 3.13 (Zheng et al., 2012), providing 532, 415 and 113 independent SNPs, respectively. This pruning did not have a substantive effect on our qualitative conclusions (see Results).

### Population structure and genetic change

A principal component analysis (PCA) based on the genetic covariance matrix from genotypes was generated using SNPRelate to obtain an overview of genetic structure across all sampled individuals. We additionally generated a PCA of our unpruned SNP dataset to determine whether LD pruning plausibly influenced our overall inferences. As the broad qualitative patterns were similar to those with the pruned dataset (see Results), subsequent analyses were based on the pruned data, to prevent any influence of SNP clustering bias (e.g. Zou et al., 2010). PCA was repeated for each SNP subset (genic, genic and exonic), and for all samples combined (N = 250), for the source individuals only (Ku-ring-gai and Muogamarra; N = 121) and for the North Head-born individuals only (N = 129 across 5 years).

We quantified differentiation among populations using SNPRelate to evaluate *F*_{ST} (Weir & Cockerham, 1984) and pairwise kinship via the maximum likelihood method (Milligan, 2003) (following Bragg et al., 2020). Comparisons using both methods were made between the two source populations, and between the two source populations and the North Head population, either in total (all North Head samples across five years analysed together, N = 129), or in the 2019 samples only (N = 30). We further generated a temporal comparison between 2015 and 2019 for North Head, to obtain a general insight into the change in population profile.

The R package inbreedR v 0.3.2 (Stoffel et al., 2016) was used to calculate standardized multilocus heterozygosity for each individual, generating individual observed heterozygosity metrics that are standardized across all loci and individuals. Standardizing across all individuals (N = 250) enabled us to observe relative changes in heterozygosity over time whilst incorporating population structure and accounting for any missing genotypes. We used linear regression in R to test temporal changes in standardized heterozygosity of North Head-born individuals, using the total number of days since the initial reintroduction as the predictor variable, and standardized heterozygosity as the response (Gaussian error); each individual was represented in the regression modelling dataset only once (N = 129).

To further quantify population genetic change in our study system, we evaluated the effective population size (N_{E}) using the linkage disequilibrium method, based on Pearson correlation approximation (‘ldNe’ method; Waples, 2006; Waples, Larson & Waples, 2016) using the R-package strataG v 2.4.905 (Archer, Adams & Schneider, 2017) with parametric 95% confidence intervals. This analysis was conducted using the full dataset of 9716 SNPs, to retain any LD signal. We calculated N_{E} for each population, and per year for the North Head population to monitor change over time.

### Results

Based on our LD-pruned dataset, qualitative assessment of population structure using PCA indicated some separation amongst the sample sets included in this analysis. Visible differentiation is observable between the North Head population and the two main source populations (Ku-ring-gai and Muogamarra), but the variance explained by the PC ordination was low (the first two PCs total only 3.14% of the variation) (Fig. 3a). Further, there was apparent differentiation between the two main source populations when these were analysed separately from the North Head samples, but again, total variance explained was low (PC1 + PC2 = 4.14%) (Fig. 3b). The three Muogamarra sites showed a high degree
of overlap (Fig. 3b). The low variation explained across our main PCAs suggests that although genetic diversity of these populations may be high (as suggested by the high number of SNPs returned from our sequencing), demographic drivers of population structure among individuals are weak on the scale that we conducted our study (e.g. <10 km total distance across the source sites; Fig. 1). LD pruning, which substantially reduced the number of loci available for analysis, unlikely had a material effect on our results, as similar conclusions can be drawn from a PCA using all SNPs (some differentiation is seen between North Head, Ku-ring-gai and Muogamarra, but low overall variance explained; Supporting Information Figure S1a). Our PCA-based qualitative observations of low differentiation are supported by quantitative estimates of population differentiation estimated using $F_{ST}$, where all pairwise comparisons were low ($<0.03$; Table 1). Between-population kinship values were also low, being close to or less than 2% for all inter-population comparisons (Table 1), indicating a low level of inter-individual relatedness among sites (and probably overall, see below), consistent with a large, panmictic population.

Incorporating the genomic reference annotations of our SNPs (all of which were mapped) resulted in smaller SNP datasets, with little change in population discrimination results: the use of genic and exonic SNPs saw marginal increases in our differentiation statistics, despite using fewer loci, but differentiation remained weak. Specifically, compared to PCA based on genomic SNPs (Fig. 3a), PCAs using genic or exonic SNPs saw marginal increases in the percentage variation explained by the first two PCs (Supporting Information Figure S1b and c, respectively), acknowledging that these values are still very small overall, and suggestive of little differentiation between populations. Patterns of slightly increased differentiation in the genic and exonic datasets (relative to the genomic dataset) were reflected in our $F_{ST}$ statistics, although again differentiation remained very low across the dataset (highest observed $F_{ST}$ overall = 0.0274; Table 1). Between-population kinship estimates varied little depending on the data subsets used (highest observed kinship = 0.0320; Table 1).

When using population structure to consider genetic change over time at the North Head site, PCA generated using only the North Head animals did not show clear discrimination across yearly cohorts (Fig. 3c), suggesting no
substantive change in the genetic profile of the population over time. Similarly, differentiation measured using $F_{ST}$ to compare temporal samples at North Head (comparing 2015 to 2019) showed values very close to zero ($F_{ST} < 0.005$ regardless of dataset used; Table 1).

Considering individual-level genetic change over time at North Head (population means of individual observed standardized heterozygosity), the first cohort of individuals born at the site (2015) showed a slight elevation in standardized heterozygosity when compared to the animals sourced from Ku-ring-gai and Muogamarra in 2014 (Fig. 4a), suggesting some minor genetic admixture from this initial reintroduction, consistent with low population differentiation between these two populations according to PCA and $F_{ST}$. The heterozygosity of North Head-born bush rats showed increases after the second translocation in 2015 (sourced from Muogamarra) and after the third translocation in 2016 (sourced from Ku-ring-gai and Muogamarra) (Fig. 4a). Examining the year-on-year differences in standardized heterozygosity between the source populations and the North Head population revealed that these increases were statistically significant in 2016 and 2017 (but not 2015) (Supporting Information Table S5). Linear regression of individual heterozygosity over time (North Head-born animals only) indicated a slight temporal decline in heterozygosity over the course of the study (Fig. 4b), which was statistically significant (estimate for days since first introduction $[-1.704 \times 10^{-5} \pm 0.0420]$, $R^2 = 0.0420$, $N = 129$ bush rats). However, the levels of individual heterozygosity observed amongst the bush rats produced at North Head by 2019 remained largely comparable to estimates for the source populations (Fig. 4a).

Although the population at North Head is likely still growing, the highest $N_e$ value at the site was seen in 2017, reflecting the first cohort born after the last bush rat supplementation in 2016, followed by a gradual decline in the two subsequent years (2018, 2019) (Table 2). This pattern is consistent with a trend in declining standardized observed heterozygosity (Fig. 4b), and a subtle trend towards increased within-population kinship over the last 3 years of the study (observable with genomic SNPs, but not with genic or exonic SNPs; Table 2). Together, these results show a pattern consistent with a slight increase in diversity through multiple supplementations whilst the population was being established, followed by a possible slight decline in genetic diversity over the 3 years after the final supplementation (2017–2019). We caution, however, that we only have 3 years of post-establishment monitoring, and that the pattern was not consistent across all data subsets (refer genic and exonic data in Table 2).

**Discussion**

The reintroduction of bush rats to North Head lays the foundation for the faunal restoration of an important urban site, by returning an extirpated common species to an isolated

---

**Table 1** Measures of population differentiation among bush rats populations in Sydney, based on SNPs obtained via reduced-representation sequencing (DArTseq).

| Population | Genomic SNPs | Genic SNPs | Exonic SNPs | Npairs |
|------------|--------------|------------|-------------|--------|
| Ku-ring-gai (33) | 0.0132 (0.0339) | 532 | 0.0158 (0.0361) | 414 |
| Muogamarra (88) | 0.0095 (0.019) | 531 | 0.0102 (0.0225) | 413 |

---

**Figures and Tables**

- **Figure 4** illustrates the genetic diversity changes in bush rats at North Head from 2014 to 2019.
- **Table 2** provides a summary of population genetic data across different datasets.

---

**References**

[1] A. L. Wright et al., *Measures of population differentiation among bush rat populations in Sydney, based on SNPs obtained via reduced-representation sequencing (DArTseq)* (Animal Conservation, 2022).

---

**Supplementary Information**

- Supporting Information Table S5 provides additional statistical analyses.
- Further details on the study design and methods can be found in the supplementary material.
patch of its former occupied habitat. The results of this study will inform future management of bush rats at the site, and assist in planning the restoration of other common species in urban areas. Using reduced-representation sequencing, we found that the newly established population of bush rats at North Head retained levels of heterozygosity comparable to the source populations. Although there was little population structure between the source sites used in this restoration project, combining populations, either at initial reintroduction or through the two follow-up translocations, resulted in a subtle increase in heterozygosity of locally born bush rats at North Head, and the effective population size at the site, supporting our admixture hypothesis (Fig. 2). However, we also detected a statistically significant temporal decline in individual heterozygosity amongst bush rats born at North Head, supported by declines in other diversity metrics. Together, these results show that, although the current levels of heterozygosity of the North Head bush rats are similar to those of the source populations, this diversity may continue to decline. We suggest that it would be valuable to continue monitoring the establishment of the bush rat population in coming years to determine whether the genetic diversity trends we observed continue as the population approaches its carrying capacity and enters demographic stabilization.

Species reintroductions can benefit from population admixture. Reintroductions of Alpine ibex Capra ibex (Biebach & Keller, 2012) and peregrine falcon Falco peregrinus ( Jacobsen et al., 2008) using multiple source populations of low genetic divergence promoted genetic diversity of the admixed population. However, the benefits of admixture are typically maximized when source populations are highly diverged. For example, by mixing independently inbred source groups, reintroduced western barred bandicoot Perameles bougainville and burrowing bettong Bettongia lesueur populations each showed substantial increases in heterozygosity, relative to their founder populations (White et al., 2018). Whilst similar admixture effects have been observed in translocations of a variety of threatened species (e.g. Rick et al., 2019; Lott et al., 2020, McLennan et al. (2020), corresponding evidence is less prevalent for common species. The results of our study show that admixture can also increase diversity when conducting translocations of common animal species, although the magnitude of the effect seen in the current study is small, likely because of low population structure between the two source populations.

Admixture has been underutilized in conservation translocations due to concerns regarding the loss of local adaptation (i.e. outbreeding depression) and non-random mating among founder individuals (Frankham et al., 2011). However, if populations share similar habitat (i.e. are subject to similar selection pressures), genetic divergence among isolated populations is more likely to result from random genetic drift, rather than local adaptation, a plausible situation for our North Head study population, which is largely isolated (Biebach & Keller, 2012). In our study, the source populations selected were of comparable habitat to North Head (see Methods), so selection pressures for this generalist species are also likely to be similar. Furthermore, the two source populations were very close to one another, and separated only by a major highway (Fig. 1), suggesting that any observed differentiation between those two sites is likely a result of genetic drift. Annotating our dataset against the Norway rat reference genome revealed little-to-no genetic differentiation at a selection of putatively functional genomic

Figure 4  (a) Box and whisker plot of individual standardized observed heterozygosity for bush rats sampled from source sites (Ku-ring-gai [blue; total N = 33] and Muogamarra [red; total N = 88]) as well as those born on North Head (grey; total N = 129) across the course of our study (2014–2019). The thick horizontal bars indicate the median, boxes indicate the interquartile range and the whiskers indicate the range of values <1.5 interquartile range; open circles indicate values greater than 1.5 x interquartile range. (b) Trend in individual heterozygosity according to date of first capture (North Head-born animals) at North Head. Fitted line is a linear regression (see Results).
Table 2 Diversity measures for bush rat populations in Sydney, based on SNPs obtained via reduced-representation sequencing (DArTseq)

| Population          | N_{ind} | N_{50} (95% CI)b | N_{kin} | Genomic SNPsd | Genic SNPsd | Exonic SNPsd |
|---------------------|---------|------------------|---------|---------------|-------------|--------------|
| Ku-ring-gai (all)   | 33      | 119.90 (118.99; 120.83) | 528     | 0.0224 (0.0265) | 0.0253 (0.0294) | 0.0355 (0.0442) |
| Ku-ring-gai 2014    | 14      | 74.11 (73.12; 75.11) | 91      | 0.0264 (0.0336) | 0.0260 (0.0349) | 0.0343 (0.0451) |
| Ku-ring-gai 2016    | 19      | 85.47 (84.56; 86.39) | 171     | 0.0218 (0.0287) | 0.0285 (0.0336) | 0.0382 (0.0474) |
| Muogamarra (all)    | 88      | 244.80 (243.27; 246.35) | 3828    | 0.0137 (0.0201) | 0.0154 (0.0213) | 0.0268 (0.0358) |
| Muogamarra 1, 2014  | 36      | 136.42 (135.41; 137.45) | 630     | 0.0158 (0.0256) | 0.0170 (0.0264) | 0.0312 (0.0414) |
| Muogamarra 2, 2015  | 31      | 113.78 (112.92; 114.64) | 465     | 0.0177 (0.0281) | 0.0193 (0.0295) | 0.0310 (0.0428) |
| Muogamarra 3, 2016  | 21      | 111.25 (109.90; 112.62) | 210     | 0.0216 (0.0303) | 0.0248 (0.0297) | 0.0328 (0.0427) |
| North Head (all)    | 129     | 115.08 (114.79; 115.38) | 8256    | 0.0149 (0.0252) | 0.0162 (0.0261) | 0.0312 (0.0418) |
| North Head 2015     | 25      | 32.72 (32.61; 32.84) | 300     | 0.0219 (0.0422) | 0.0214 (0.0436) | 0.0392 (0.0518) |
| North Head 2016     | 25      | 61.99 (61.63; 62.36) | 300     | 0.0185 (0.0308) | 0.0207 (0.0332) | 0.0261 (0.0389) |
| North Head 2017     | 24      | 123.07 (121.69; 124.47) | 276     | 0.0149 (0.0236) | 0.0162 (0.0247) | 0.0336 (0.0413) |
| North Head 2018     | 25      | 85.24 (84.56; 85.93) | 300     | 0.0165 (0.0284) | 0.0155 (0.0271) | 0.0351 (0.0481) |
| North Head 2019     | 30      | 79.79 (79.33; 80.25) | 435     | 0.0171 (0.0332) | 0.0174 (0.0343) | 0.0314 (0.0455) |

aNumber of individual bush rats sampled.
bN_{50} estimation undertaken with all 9716 SNPs.
cNumber of pairwise combinations over which the kinship standard deviations (SD) were calculated.
dLD-pruned dataset used; maximum number of SNPs comprising each dataset: genomic = 532; genic = 415; exonic = 113.

regions (namely SNPs associated with genes, or more specifically exons), consistent with the smaller sample size of loci (Wright et al., 2019). It would be useful to investigate specific loci associated with local adaptation, especially in the coming years as the North Head population becomes more established, by combining these data with individual fitness data (such as reproductive success) (e.g. following Wright et al., 2020).

The translocation examined here used a large number of individuals (N = 180) from multiple source areas, partly to promote genetic diversity of the bush rat population at North Head. Our genetic analyses showed no substantial loss of genetic diversity during population establishment, confirming that these management considerations were appropriate. Since establishment, ongoing population monitoring has revealed that the bush rat population has grown considerably and recruitment in subsequent generations exceeded 323 individuals in the 36-month period following the initial release (JRA unpubl. data). Such growth has potentially been possible because of concurrent efforts to control invasive red foxes and black rats. The bush rat population may have grown to sufficient numbers to withstand predation pressure or to outcompete black rats (JRA unpubl. data). This demographic success may translate into ecological benefits via the ecosystem role that small mammals, such as bush rats, play in dispersing seeds and pollinating native plants (O’Rourke et al., 2020). However, despite these demographic and potential ecological successes, we observed a declining trend in genetic diversity of North Head-born bush rats over the course of the 5-year study period. As the population approaches its carrying capacity, it will be important to continue monitoring to determine whether genetic drift and inbreeding depression are impacting the genetic viability of the population. Like many small mammals, bush rats have a short generation time, so genetic change can occur rapidly.

Monitoring the population every 1–2 years will be useful to detect loss of diversity and enable timely genetic interventions. An additional approach which can help inform the timing of future management actions is stochastic population modelling, which has previously been used to guide the management of Tasmanian devil Sarcophilus harrisii populations (Grueter et al., 2019). Combining simulation modelling with molecular genetic data could be used to predict the trajectory of genetic change for the North Head bush rat population and help identify an optimal rate of immigration for maintaining genetic diversity. The results of such modelling could also be used to generate an estimate of the expected change in diversity as a further validation of the molecular approach used here. For example, comparing projected and observed patterns of diversity could be used to further validate the empirical data and statistical approaches used for genetic monitoring of urban restoration projects.

In terms of the broader implications of our results, our study confirms that it is possible to translocate a common species and re-establish its population within an urban setting. In Europe, calls have been made to increase rewilding efforts in urban settings because this is where the majority of the human population lives, and where cultural and political futures are shaped and debated. Thus, if rewilding is restricted to more remote settings, conservationists will struggle to influence mainstream culture (Jepson, 2016). This may be particularly pertinent to the Australian biodiversity crisis: the country has recorded half the world’s mammal declines in the last 200 years (Woinarski et al., 2015). At present, conservation initiatives to restore threatened mammal populations in Australia focus on predator-free refuges established in remote areas or on offshore islands (Legge et al., 2018; Ringma et al., 2019). Similar translocations have been a priority in other countries, such as New Zealand (Burns, Innes & Day, 2012; Towns et al., 2016), where many such projects
are undertaken by community groups (Innes et al., 2019). The management of threats is a key determinant of reintroduction success. In areas where the cause of the initial decline can be identified and ameliorated, urban restoration provides new opportunities for the majority of city-dwellers to be more easily exposed to rewilding projects. Conservation initiatives in urban areas could focus on restoring common species as a way to both help restore degraded ecosystems and simultaneously increase public engagement and awareness of the importance of restoring what has been lost. Our analysis demonstrates that this reintroduction has resulted in the establishment of a genetically diverse population. However, even common species may be vulnerable to diversity losses if subject to prolonged population bottlenecks, so it will be important to incorporate genetic monitoring into future translocation efforts in Australia and globally.

**Acknowledgements**

We thank K. Farquharson for bioinformatic advice, and the Applied and Evolutionary Zoology Group for comments on an earlier version of this manuscript. This genetic analysis was funded by a research agreement between the University of Sydney and AWC. The project was funded by Australian Wildlife Conservancy and Sydney Harbour Federation Trust. We also thank two anonymous reviewers whose comments strengthened this work.

**References**

Archer, F.I., Adams, P.E. & Schneiders, B.B. (2017). stratag: an r package for manipulating, summarizing and analysing population genetic data. *Mol. Ecol. Resour.* 17, 5–11.

Banks, P., Cleary, G. & Dickman, C. (2011). Sydney’s bubonic plague outbreak 1900–1910: a disaster for foreshore wildlife? *Aust. Zool.* 35, 1033–1039.

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

Bragg, J.G., Cuneo, P., Sherieff, A. & Rossetto, M. (2020). Optimizing the genetic composition of a translocation population: incorporating constraints and conflicting objectives. *Mol. Ecol. Resour.* 20, 54–65.

Burns, B., Innes, J. & Day, T. (2012). The use and potential of pest-proof fencing for ecosystem restoration and fauna conservation in New Zealand. In *Fencing for conservation: restriction of evolutionary potential or a riposte to threatening processes?:* 65–90. Somers, M.J. & Hayward, M. (Eds). New York: Springer.

Callandar, M. (2018). Translocation and reintroduction of native bush rats (*Rattus fuscipes*) into Sydney Harbour National Park: restoring ecosystem function. Penrith: School of Science and Health, Western Sydney University.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L. & Robinson, G.E. (2011). Patterns of widespread decline in north American bumble bees. *Proc. Natl. Acad. Sci. USA* 108, 662–667.

Cruz, V.M.V., Kilian, A. & Dierig, D.A. (2013). Development of DArT marker platforms and genetic diversity assessment of the U.S. collection of the new oilseed crop Lesquerella and related species. *PLoS One* 8, e64062.

Ewen, J.G., Armstrong, D.P., Parker, K.A. & Seddon, P.J. (2012). Reintroduction biology: integrating science and management. Chichester: Blackwell Publishing.

Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R. & Fenster, C.B. (2011). Predicting the probability of outbreeding depression. *Conserv. Biol.* 25, 465–475.

Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M., Dudash, M.R., Fenster, C.B., Lacy, R.C. & Sunnucks, P. (2017). Genetic management of fragmented animal and plant populations. Oxford: Oxford University Press.

Furlan, E.M., Gruber, B., Attard, C.R.M., Wager, R.N.E., Kerezsy, A., Faulks, L.K., Beheragray, L.B. & Unmack, P.J. (2020). Assessing the benefits and risks of translocations in depauperate species: a theoretical framework with an empirical validation. *J. Appl. Ecol.* 57, 831–841.

Galla, S.J., Forsdick, N.J., Brown, L., Hoeppner, M.P., Knapp, M., Maloney, R.F., Moraga, R., Sature, A.W. & Steeves, T.E. (2019). Reference genomes from distantly related species can be used for discovery of single nucleotide polymorphisms to inform conservation management. *Genes* 10, 9.

Gaston, K.J. (2010). Valuing common species. *Science* 327, 154–155.

Gaston, K.J. & Fuller, R.A. (2008). Commonness, population depletion and conservation biology. *Trends Ecol. Evol.* 23, 14–19.

Gibbs, R.A., Weinstock, G.M., Metzker, M.L., Muzny, D.M., Sodergren, E.J., Scherer, S., Scott, G. et al. (2004). Genome sequence of the brown Norway rat yields insights into mammalian evolution. *Nature* 428, 493–521.

Gruber, B., Unmack, P.J., Berry, O.F. & Georges, A. (2018). DArT: an r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Mol. Ecol. Resour.* 18, 691–699.

Grueber, C.E., Fox, S., McLennan, E.A., Gooley, R.M., Pemberton, D., Hogg, C.J. & Belov, K. (2019). Complex problems need detailed solutions: harnessing multiple data types to inform genetic management in the wild. *Evol. Appl.* 12, 280–291.

Hansen, N., Hughes, N.K., Byrom, A.E. & Banks, P.B. (2020). Population recovery of alien black rats (*Rattus rattus*): a test of reinvasion theory. *Austral Ecol.* 45, 291–304.

Holland, G.J. & Bennett, A.F. (2010). Habitat fragmentation disrupts the demography of a widespread native mammal. *Ecography* 33, 841–853.

Innes, J., Fitzgerald, N., Binny, R., Byrom, A., Pech, R., Watts, C., Gillies, C., Maitland, M., Campbell-Hunt, C. & Burns, B. (2019). New Zealand ecosanctuaries: types, attributes and outcomes. *J. Roy. Soc. NZ* 49, 370–393.
IUCN/SSC. (2013). Guidelines for reintroductions and other conservation translocations version 1.0. Gland, Switzerland: IUCN Species Survival Commission.

Jacobsen, F., Nesje, M., Bachmann, L. & Lifjeld, J.T. (2008). Significant genetic admixture after reintroduction of peregrine falcon (Falco peregrinus) in southern Scandinavia. *Conserv. Genet.* 9, 581–591.

Jamieson, I.G. (2011). Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conserv. Biol.* 25, 115–123.

Jepson, P. (2016). A rewilding agenda for Europe: creating a network of experimental reserves. *Ecography* 39, 117–124.

Karolchik, D., Hinrichs, A.S., Furey, T.S., Roskin, K.M., Sugnet, C.W., Haussler, D. & Kent, W.J. (2004). The UCSC table browser data retrieval tool. *Nucleic Acids Res.* 32, D493–D496.

Legge, S., Woinarski, J.C.Z., Burbidge, A.A., Palmer, R., Ringma, J., Radford, J.Q., Mitchell, N., Bode, M., Wintle, B., Baseler, M., Bentley, J., Copley, P., Dexter, N., Dickman, C.R., Gillespie, G.R., Hill, B., Johnson, C.N., Latch, P., Letnic, M., Manning, A., McCleess, E.E., Menkhorst, P., Morris, K., Moseby, K., Page, M., Pannell, D. & Tuft, K. (2018). Havens for threatened Australian mammals: the contributions of fenced areas and offshore islands to the protection of mammal species susceptible to introduced predators. *Wildl. Res.* 45, 627–644.

Lindenmayer, D., Wood, J., McBurney, L., Macgregor, C., Youngentob, K. & Banks, S. (2011). How to make a common species rare: a case against conservation complacency. *Biol. Conserv.* 144, 1663–1672.

Loth, A.F. & Newton, A.C. (2018). Rewilding as a restoration strategy for lowland agricultural landscapes: stakeholder-assisted multi-criteria analysis in Dorset, UK. *J. Nat. Conserv.* 46, 110–120.

Lott, M.J., Wright, B.R., Kemp, L.F., Johnson, R.N. & Hogg, C.J. (2020). Genetic management of captive and reintroduced bilby populations. *J. Wildl. Manag.* 84, 20–32.

McLennan, E.A., Grueber, C.E., Wise, P., Belov, K. & Hogg, C.J. (2020). Mixing genetic lineages successfully boosts diversity of an endangered carnivore. *Anim. Conserv.* 23, 700–712.

McLennan, E.A., Wright, B.R., Belov, K., Hogg, C.J. & Grueber, C.E. (2019). Too much of a good thing? Finding the most informative genetic data set to answer conservation questions. *Mol. Ecol. Resour.* 19, 659–671.

Milligan, B.G. (2003). Maximum-likelihood estimation of relatedness. *Genetics* 163, 1153–1167.

Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R. & Hohenlohe, P.A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Mol. Ecol.* 22, 2841–2847.

O’Rourke, R.L., Anson, J.R., Saul, A.M. & Banks, P.B. (2020). Limits to alien black rats (Rattus rattus) acting as equivalent pollinators to extinct native small mammals: the influence of stem width on mammal activity at native *Banksia ericifolia* inflorescences. *Biol. Invasions* 22, 329–338.

Parrott, M.L., Ward, S.J., Temple-Smith, P.D. & Selwood, L. (2007). Effects of drought on weight, survival and breeding success of agile antechinus (Antechinus agilis), dusky antechinus (A. swainsonii) and bush rats (Rattus fuscipes). *Wildl. Res.* 34, 437–442.

Peakall, R., Ruibal, M. & Lindenmayer, D.B. (2003). Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution* 57, 1182–1195.

R Core Team. (2021). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/.

Rick, K., Ottewell, K., Lohr, C., Thavornkanlapachai, R., Byrne, M. & Kennington, W.J. (2019). Population genomics of Bettongia lesueur: admixing increases genetic diversity with no evidence of outbreeding depression. *Genes* 10, 851.

Ringma, J., Legge, S., Woinarski, J.C.Z., Radford, J.Q., Wintle, B., Bentley, J., Burbidge, A.A., Copley, P., Dexter, N., Dickman, C.R., Gillespie, G.R., Hill, B., Johnson, C.N., Kanowski, J., Letnic, M., Manning, A., Menkhorst, P., Mitchell, N., Morris, K., Moseby, K., Page, M., Pannell, D. & Tuft, K. (2018). Havens for threatened Australian mammals: the contributions of fenced areas and offshore islands to the protection of mammal species susceptible to introduced predators. *Wildl. Res.* 45, 627–644.

Saul, A.M. (2013). Aliens replacing natives: are black rats effective substitutes for extinct native mammalian pollinators? Camperdown: School of Biological Sciences, The University of Sydney.

Seddon, P.J. (2010). From reintroduction to assisted colonization: moving along the conservation translocation spectrum. *Restor. Ecol.* 18, 796–802.

Spielman, D., Brook, B.W. & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15261–15264.

Stoffel, M.A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., Hoffman, J.I. & Poisot, T. (2016). inbreedR: an R package for the analysis of inbreeding based on genetic markers. *Methods Ecol Evol* 7, 1331–1339.

Sweeney, O.F., Turnbull, J., Jones, M., Letnic, M., Newsome, T.M. & Sharp, A. (2019). An Australian perspective on rewilding. *Conserv. Biol.* 33, 812–820.

Taylor, J.M. & Horner, B.E. (1973). Reproductive characteristics of wild native Australian Rattus (Rodentia: Muridae). *Aust. J. Zool.* 21, 437–475.

Thavornkanlapachai, R., Mills, H.R., Ottewell, K., Dunlop, J., Sims, C., Morris, K., Donaldson, F. & Kennington, W.J. (2019). Mixing genetically and morphologically distinct populations in translocations: asymmetrical introgression in a newly established population of the boodie (Bettongia lesueur). *Genes* 10, 729.

Towns, D.R., Miller, K.A., Nelson, N.J. & Chapple, D.G. (2016). Can translocations to islands reduce extinction risk
for reptiles? Case studies from New Zealand. *Biol. Conserv.* **204**, 120–127.

van Heezik, Y. & Seddon, P.J. (2018). Animal reintroductions in peopled landscapes: moving towards urban-based species restorations in New Zealand. *Pac. Conserv. Biol.* **24**, 349–359.

Walsh, J.R., Carpenter, S.R. & Zanden, M.J.V. (2016). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proc. Natl. Acad. Sci. USA* **113**, 4081–4085.

Wang, K., Li, M. & Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from next-generation sequencing data. *Nucleic Acids Res.* **38**, e164.

Waples, R.S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv. Genet.* **7**, 167–184.

Warneke, R.M. (1971). *Field study of the bush rat (Rattus fuscipes).* Melbourne: Fisheries and Wildlife Department, Victoria.

Watson, D.M. & Watson, M.J. (2015). Wildlife restoration: mainstreaming translocations to keep common species common. *Biol. Conserv.* **191**, 830–838.

Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A. & Hoffmann, A.A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol. Appl.* **4**, 709–725.

Weir, B.S. & Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

White, L.C., Moseby, K.E., Thomson, V.A., Donnellan, S.C. & Austin, J.J. (2018). Long-term genetic consequences of mammal reintroductions into an Australian conservation reserve. *Biol. Conserv.* **219**, 1–11.

Winfree, R., Fox, J.W., Williams, N.M., Reilly, J.R. & Cariveau, D.P. (2015). Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecol. Lett.* **18**, 626–635.

Woinarski, J., Burbidge, A.A. & Harrison, P.L. (2015). Ongoing unraveling of a continental fauna: decline and extinction of Australian mammals since European settlement. *Proc. Natl. Acad. Sci. USA* **112**, 4531–4540.

Wright, B.R., Farquharson, K.A., McLennan, E.A., Belov, K., Hogg, C.J. & Grueber, C.E. (2020). A demonstration of conservation genomics for threatened species management. *Mol. Ecol. Resour.* **20**, 1526–1541.

Wright, B.R., Grueber, C.E., Lott, M.J., Belov, K., Johnson, R.N. & Hogg, C.J. (2019). Impact of reduced-representation sequencing protocols on detecting population structure in a threatened marsupial. *Mol. Biol. Rep.* **46**, 5575–5580.

Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C. & Weir, B.S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* **28**, 3326–3328.

Zou, F., Lee, S., Knowles, M.R. & Wright, F.A. (2010). Quantification of population structure using correlated SNPs by shrinkage principal components. *Hum. Hered.* **70**, 9–22.

**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Alternate SNP datasets used to generate principal coordinate analyses of bush rats sampled from three sites (per legend) in Sydney (N = 250). The analysis illustrated in (a) is based on our full dataset of 9716 mapped reduced-representation SNP loci. (b) Based on an LD-pruned set of 415 genic SNPs and (c) is based on an LD-pruned set of 113 exonic SNPs. Note that PC1 of the ‘exonic’ data has been flipped (multiplied by −1) to orient the output similarly to the other plots and facilitate visual comparisons between analyses. All figures can be compared to Fig. 3a in the main analysis, which includes all 250 samples, and is based on LD-pruned genomic SNPs; x- and y-axes here are set to the same scale as Fig. 3.

**Table S1.** Error rates (genotype mismatches) between intra-plate and inter-plate technical replicates for DArTseq of bush rats. Error rates were calculated prior to stringent filtering, as the results were used to remove any loci with >1 mismatches among replicates; thus, the anticipated error rate of the final dataset is likely far lower than reported here.

**Table S2.** Filtered bush rat DArTseq SNPs mapped to Norway rat *Rattus norvegicus* Rnor6 reference genome (Gibbs et al., 2004).

**Table S3.** Functional categorization of SNPs according to the Rnor6 (Gibbs et al., 2004) reference genome annotation.

**Table S4.** Gene identities of 224 polymorphic exonic positions detected in bush rats, aligned to the Norway rat reference genome Rnor6, with the reference (Ref) and alternate (Alt) alleles indicated.

**Table S5.** Change in individual observed standardized heterozygosity with each supplementation. Each linear model is fitted using ‘population ID’ as the predictor variable (with two levels: source population and North Head sample, as indicated in table), to determine whether the response, heterozygosity, is changed at North Head relative to the source populations after each translocation. To fit each model, the source population ID is the reference category, so that a positive slope estimate corresponds to increased individual heterozygosity in the North Head population.