Evaluating success of translocations in maintaining genetic diversity in a threatened mammal

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Abstract

The effectiveness of fauna reintroduction programs has been limited by the availability of source animals and the lack of follow up monitoring to assess whether viable populations have been successfully established, particularly in terms of conserving genetic diversity. Here we present genetic assessment of the translocation of golden bandicoots (*Isoodon auratus*) from a large source population on Barrow Island off the north-west coast of Western Australia to two other island sites and a mainland fenced enclosure. We assessed the genetic diversity of animals translocated to each site and their wild-born progeny, and whether wild-born animals showed evidence of genetic bottlenecks or genetic drift from the source population. Encouragingly, we found no significant loss of genetic diversity in any of the wild-born populations compared to the source population and no significant increase in inbreeding or relatedness amongst wild-born individuals compared to founder populations two years post-translocation. However, we detected an approximately 10-fold reduction in effective population size between founding and wild-born populations. We found no apparent differentiation between wild-born populations and the original source population, or between wild-born animals and their respective founders. Population viability modeling predicts that each of the translocated populations is susceptible to loss of genetic diversity over time. Taken together these results suggest that the golden bandicoot reintroduction program has been initially successful as a result of large founding sizes and high reproductive rates; however, ongoing augmentation will be required to prevent genetic erosion and maintain evolutionary potential in the long-term.

1. Introduction

The reintroduction of extirpated fauna to parts of their former native range is a key conservation tool used by wildlife managers to increase effective population size and spatial representation of threatened species, and reduce the risk of extinction. Reintroductions are also expected to have a greater benefit on ecosystems by restoring some level of ecosystem function in the species' former habitat; for example, bandicoots are considered 'ecosystem engineers', having an important role in soil turnover and nutrient cycling (Valentine et al., 2012). The ultimate aim of reintroduction programs is to establish viable, self-sustaining populations (IUCN, 2012), though the criteria used to judge their success or failure are often not clearly defined, such that there is still no clear agreement on what constitutes a successful reintroduction (Moseby et al., 2011; Seddon, 1999). For example, Bajomi (2010) summarises four different definitions of success, including breeding of the first wild-born generation, positive population growth rate over three generations or 10 years, the use of population viability analysis indicating a self-sustaining population and population persistence over a defined period of time.

Globally, reintroductions have been attempted for a large number of vertebrate species (primarily mammals and birds) but have had only what is viewed as limited success in establishing viable, self-sustaining populations in the medium to long-term (Fischer and Lindenmayer, 2000; Sheean et al., 2012; Short, 2009). Habitat suitability and quality, and the failure to control or remove threatening processes (such as predation) are frequently identified as the reasons for the failure of reintroduced populations (Moseby et al., 2011; Sheean et al., 2012), though others may include naivety of captive-reared individuals, too few individuals released or disease (Short 2009). Whether successful or not, the genetic viability of reintroduced populations is rarely investigated despite widespread recognition of the negative impacts of inbreeding and genetic drift in small populations (but see Jamieson, 2011; Mock et al., 2004; Reynolds et al., 2013; Weiser et al., 2013). To avoid or ameliorate these effects it is important that genetic issues be incorporated early in the design of reintroduction strategies, and also in the post-release monitoring, since they are key aspects of both short-term (e.g. inbreeding depression) and long term (e.g. erosion of genetic diversity) population sustainability. These issues have been highlighted recently, with consideration of the selection of founders, maintaining genetic diversity and monitoring genetic diversity in reintroduced populations included within the IUCN species reintroduction guidelines (IUCN, 2012).

Typically, reintroduced populations are established from small numbers of founder individuals due to the rarity of wild populations and the high costs associated with translocation and captive breeding programs, leading to a founding population of small effective size that may be genetically bottlenecked (Fischer and Lindenmayer, 2000; Jamieson, 2011; Tracy et al., 2011). Further, it is becoming more commonplace to establish conservation sites that physically separate vulnerable species from their threatening processes, such as on predator- or disease-free islands or in fenced enclosures (Abbott, 2000; Hayward and Kerley, 2009), resulting in the isolation of these populations from extant ones. Small, isolated populations such as these are likely to be highly susceptible to the loss of genetic variation through random genetic drift and inbreeding, which can impact on long-term population adaptation and persistence (Brook et al., 2002; Frankham, 2005; Jamieson et al., 2006). In addition, in the shorter term, inbreeding depression resulting in lower survival or fitness of offspring may further reduce demographic population sizes contributing to population decline or failure (Gilpin and Soule, 1986). The rate of inbreeding is likely to be

affected by the mating patterns and dispersal behavior of the species, which determines the within-population spatial genetic structure. Low density of founding populations may contribute to non-random mating if animals have low dispersal and mate more frequently with closely-located individuals. Thus, ideally, founding populations should be large and genetically diverse to overcome small population inbreeding effects and to retain longer-term adaptive capacity.

There have been extensive declines in the mammal fauna of Australia since European settlement began in 1788 (Burbidge et al., 2008a), with 94 species currently listed as critically endangered, endangered or vulnerable under Australian legislation (*Environment Protection and Biodiversity Conservation (EPBC) Act* 1999). Today, several of these species persist only on islands or in remote areas where exotic predators (foxes, cats, rats) or competitors (goats, rabbits) are absent. Barrow Island is a large island (23,400ha) approximately 70km off the north-western coast of Western Australia that is free of exotic predators. This important nature reserve supports 13 native mammal species that are extinct or rare on the mainland, including the golden bandicoot (*Isoodon auratus*) that is currently listed as Vulnerable under the *EPBC* Act. Golden bandicoots were once widespread across Australia's arid zones prior to European arrival but have suffered severe declines throughout much of their range as a result of predation by exotic predators and loss of habitat (Burbidge et al. 2008b). In Western Australia, golden bandicoots occur in large numbers (estimated population size of 20,000-50,000) on Barrow Island, with smaller and sparser populations on the mainland and coastal islands of the Kimberley region (McKenzie et al. 2008). The Barrow Island population was used as the source population for translocation of golden bandicoots to three conservation sites: to two nearby islands, Doole and Hermite Island, and to the Australian mainland within a fenced enclosure at Lorna Glen proposed conservation reserve (DEC, 2010; 2011). The translocations to Hermite Island and Lorna Glen are considered reintroductions as there is sub-fossil evidence of golden bandicoots at these locations in the recent past (Baynes, 2006; Montague, 1914); however, golden bandicoots are not known from Doole Island and this site is considered a conservation introduction. Due to the large size of the Barrow Island population, large numbers of animals (92-165 animals) were able to be sourced and released to each translocation site; greater numbers than are typically used in threatened species reintroduction programs (Fischer and Lindenmayer, 2000; Short, 2009).

This operational scale translocation of golden bandicoots from a large population to two smaller islands and a fenced reintroduction site provides an ideal opportunity to explore the interacting effects of founder population size on maintenance of genetic diversity and long-term persistence in effectively closed populations. We surveyed the genetic diversity of source and reintroduced populations of the golden bandicoot to determine whether a large founder size contributed to the initial success of reintroductions and to predict future patterns of genetic diversity. Specifically our aims were to: (1) compare the genetic diversity of founding and wild-born offspring at each translocation site to assess how diversity was conserved during the establishment phase; (2) determine whether there was any evidence for inbreeding in the established populations, which may lead to a reduction in fitness in the longer term; (3) assess effective population size of source and reintroduced populations and whether there is any evidence of genetic drift amongst populations; and (4) use modeling approaches to determine whether founding numbers were sufficient to maintain genetic diversity over time or whether further intervention (genetic augmentation) is required to maintain genetic diversity in these populations.

2. Material and Methods

2.1 Study species and location

Two subspecies of the golden bandicoot *I. auratus* have previously been recognized (McKenzie et al., 2008). *Isoodon a. auratus* is currently restricted to four islands and several mainland sites along the north-west Kimberley coast of Western Australia, and Marchinbar Island in the Northern Territory. The Barrow Island subspecies, *I. a. barrowensis*, was until recently restricted to Barrow and Middle Islands off the Pilbara coast of Western Australia. The two sub-species are differentiated on morphological grounds though there appears to be little genetic support for the division (Westerman and Krajewski, 2000). *Isoodon a. barrowensis* is slightly smaller and has slightly darker fur than the mainland subspecies, and weighs between 250 and 600 g when mature (McKenzie et al., 2008). The species is mainly solitary, although home ranges overlap and may alter by seasons, usually increasing in size in drier seasons (McKenzie et al., 2008). Females give birth throughout the year, with up to five pouch young possible, though typically only one to two young may survive to weaning (J. Dunlop, pers. comm.).

2.2 Translocation history

The Western Australian Department of Parks and Wildlife (DPaW) have successfully established populations of *I. a. barrowensis* at three locations within their former range that are free of exotic predators: Lorna Glen proposed conservation reserve, Hermite Island and Doole Island (Figure 1). Animals were sourced from the large Barrow Island population (BI, 20°51’ S, 115°24’ E) by trapping several areas on the island over a four week period. Animals were sexed, weighed and measured before being transported to each translocation site via helicopter and fixed wing aircraft, and released within 24 hours of capture. All individuals were tagged with a unique identifier PIT (passive implant transponder) tag. Bandicoots were released at Lorna Glen proposed conservation reserve in central Western Australia (LG, 26°13’ S, 121°33’ E), a 244 000 ha ex-pastoral lease now managed by DPaW in partnership with the Martu people from the Wiluna Aboriginal community, and the site of the Rangelands Restoration project. In this translocation, 160 animals (78 males/82 females) were released into an 1100 ha predator proof enclosure in February 2010. The enclosure consists of sandy spinifex grassland (*Triodia basedowii*) and open mulga (*Acacia aneura*) woodlands with a rocky clay substrate. Hermite Island (HI, 20°29 S, 115°31 E) is the largest island in the Montebello group located approximately100km off the Pilbara coast and 25 km north of BI. The island is 1020 ha in size, from which cats and black rats have been eradicated (Burbidge, 2004) and consists of dense spinifex (*T. wiseana* and *T. augusta*) on a rocky limestone substrate. At HI, 165 *I. a. barrowensis* were released in February 2010 (82 males/83 females). The third translocation site was Doole Island (DI, 22°27’ S, 114°09’ E), a 261 ha island in Exmouth Gulf, approximately 214 km southwest of BI. Ninety-two *I. a. barrowensis* (49 males/43 females) were released onto DI in July 2011. No exotic predators are known on DI. Using mark-recapture analysis, the current population size is estimated at 249 animals within the fenced enclosure at LG (J. Dunlop, unpubl. data) but total population sizes are not known for HI or DI. At HI, mark-recapture analysis of data from two trap lines close to the release site (estimated to represent ~20% of the island’s area) estimate population size to be 280 animals (N. Thomas, unpubl. data).

2.3 DNA sampling and microsatellite genotyping

Ear punch biopsies were taken from animals caught in standard cage (Sheffield Wire Products, Welshpool, WA) or Elliott (Elliott Scientific, Upwey, Victoria) traps using a sterilized commercial 1-2mm ear punch tool during regular monitoring surveys at each location. Samples were obtained from 57 founders (released February 2010) and 67 progeny (trapped 2010-2012) at LG; 38 founders (released February 2010) and 44 progeny (2010-2012) at HI and 49 founders (released July 2011) and 39 progeny (2012) at DI. Ear biopsies were stored in 80-100% ethanol until DNA extraction. We extracted genomic DNA from biopsy samples using a standard 'salting out' extraction procedure. Polymerase Chain Reaction (PCR) amplification was conducted for 12 microsatellite loci sourced from previously published studies (Li et al., 2012; Zenger and Johnston, 2001) (details in Appendix A) using the Qiagen Multiplex Kit, following reaction conditions specified by the manufacturers with an annealing temperature of 58°C. Amplification products were separated on an ABI PRISM 3100 capillary sequencer using a commercial service (WA State Agricultural Biotechnology Centre) and fragment sizes determined using an internal size standard (LIZ500) in the program GENEMAPPER (Applied Biosystems). We genotyped approximately 10% of samples twice to calculate genotyping error rates.

2.4 Genetic data analysis

Genotyping data quality was assessed by calculating the allele-specific and locus-specific genotyping error rates (Pompanon et al., 2005) and conducting null allele analysis in MICROCHECKER (Van Oosterhout et al., 2004). We tested for Hardy-Weinberg equilibrium (HWE) in each population/locus combination using GENALEX v6.5 (Peakall and Smouse, 2012) and used corrected alpha values determined by False Discovery Rate (FDR) analysis using the online calculator available at http://users.ox.ac.uk/~npike/fdr/ (Pike, 2011). Since we found significant disequilibrium at locus Ioo2 (see Results) we removed this locus from the following genetic diversity and differentiation analyses. Using the remaining 11 loci, we calculated standard population genetic parameters using GENALEX (N_a, number of alleles per locus; A_e, effective number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{is}, inbreeding coefficient) for both the original source population (BI, by pooling data for each of the founding translocated populations, n=144) and the founding and wild-born populations (separately) at each translocation site. We calculated allelic richness (A_R) in HP-RARE (Kalinowski, 2005) standardized to the smallest population size (n=38). We tested for significant differences in these descriptive population statistics between the source population and all wild-born populations, and the founding and wild-born populations at each translocation site, using a randomized block design ANOVA (with locus as the blocking factor) with acrsine transformed data where appropriate (H_O, H_E). Mean population pairwise relatedness was calculated in GENALEX using the Lynch and Ritland (1999) estimator of *r*. We assessed the homogeneity of allele frequencies between founding and wild-born populations within and between translocation sites using exact *G* tests for genic differentiation in GENEPOP (Raymond and Rousset, 1995).

We tested for genetic differentiation between the source population (BI) and wild-born populations, and between the founding and wild-born populations at each translocation site using two metrics (F_{ST} and D_{est}) calculated within GENALEX. The significance of observed values was tested using permutation testing (n=999). We used the single-sample estimator implemented in the software package LDNE (Waples and Do, 2008) to estimate effective population size (N_e) for the source population and each of the translocated populations with founding and wild-born populations combined. All of our population samples consist of

overlapping generations. We used a random mating model and estimated linkage disequilibrium amongst alleles using only alleles with frequencies > 2%, as this is expected to give the best balance between precision and bias in the N_e estimator (Waples and Do, 2010).

We calculated allele rarefaction curves for the BI source population using the package PopGenKit v1.0 (Paquette, 2012) implemented in R v2.15.1 (R Development Core Team, 2011). Allele rarefaction curves were estimated for each of the 11 individual microsatellite loci using 1) all rare and common alleles and 2) only common alleles with >5% frequency. From each of these curves we determined, per locus, the number of individuals that would need to be translocated to capture 95% of allelic diversity present in the BI source population and present the mean and range of these estimates.

2.5 Population viability analysis

Demographic and genetic models of population viability for the LG and HI translocated populations were constructed using the software Vortex v9.99c (Lacy and Pollak, 2012). We ran the models with 1000 iterations and for 100 years using the default model of population growth and our site-specific genetic and demographic parameters as input. Since locus Ioo2 was in HWE in the LG and HI founder populations from which genetic input parameters were taken, this locus was retained for analysis. Demographic parameters on reproductive rates were derived from unpublished field studies conducted at LG (three years data, J. Dunlop), HI (three years data, N. Thomas) and BI (10 years data, K. Morris) (Table 1, Appendix B). Data is not currently available for the DI population and we did not develop a model for this site. We modeled genetic diversity change firstly using the initial number of founders at each site (LG = 160, HI = 165) and using the mean number of founders required to capture 95% of allelic diversity of the source population estimated from the allele rarefaction analysis (N = 75). We modeled several management scenarios to maintain heterozygosity and allelic richness in the LG and HI translocated populations including, a) increasing fence size at LG to increase carrying capacity (K=600-1000); b) supplementation at periodic intervals to maintain genetic diversity in LG and HI populations (augment with 20/30/50 animals every 5/10 years); and c) follow-up supplementations at HI to boost initial population growth (augment with 80-160 animals at 5 and 10 years). Full details of the demographic parameters, management scenarios and assumptions made in the model are provided in Appendix B.

3. Results

3.1 Microsatellite genotyping quality

Across the *I. a. barrowensis* genetic dataset the allele-specific and locus-specific genotyping error rates were 0.001 and 0.017, respectively. Overall, the 12 microsatellite loci were variable across the study population with the number of alleles ranging from 3-14 alleles per locus and mean observed heterozygosity from 0.11-0.85 (Appendix A). All loci were in Hardy-Weinberg equilibrium when analysed across the entire data set, with the exception of Ioo2. Tests of HWE per population/locus combination resulted in significant deviations from HWE in 11 out of 78 tests. Once FDR analysis was applied to correct for multiple tests only five population/locus combinations remained significant, three of these involving locus Ioo2. Indeed, null allele analysis in MICROCHECKER suggested null alleles were present (significant excess of homozygotes) at locus Ioo2 in two of the wild-born populations (Hermite Island,

Lorna Glen) and one of the founder populations (Doole Island). For this reason Ioo2 was excluded from the following genetic analyses.

3.2 Genetic diversity in founder and wild-born populations

Overall, genetic diversity was moderately high and similar in all founder and wild-born populations of *I. a. barrowensis* at each of the translocation sites (Table 2). When animals translocated to the three different locations (founder populations) were pooled into a single 'population' representative of the source, BI (n=144), a significantly lower number of alleles were detected in individuals sampled from each of the wild-born translocated populations than the original source population (Table 2, ANOVA F=8.11, df=3, $p<0.001$). However, when allelic richness was standardized for differences in sample size (A_R), there were no significant differences between source and wild-born populations for rarefied allelic richness or any other measure of genetic diversity (A_e, H_O, H_E).

When analysed per translocation site, expected heterozygosity of animals ranged from 0.64 – 0.67 and allelic richness from 7.13 - 7.99 (Table 2). Slightly higher, but not significantly different (ANOVA, $p>0.05$), levels of genetic diversity (N_a, A_r, A_e) were detected in founder compared to wild-born populations of *I. a. barrowensis* at all locations (Table 2). Expected and observed heterozygosity were not significantly different in all founder and wild-born populations also. The inbreeding coefficient (F_{is}) was near zero in all populations, with the exception that inbreeding was significantly higher in wild-born HI animals than wild-born LG animals (ANOVA, F=3.46, df=3, $p=0.028$). Estimates of pairwise relatedness amongst individuals in each population showed individuals on average were unrelated in each of the founder populations, but were more related than random in each of the wild-born populations (DI $p=0.041$; HI $p=0.016$; LG $p=0.028$, Table 3). There was a non-significant trend for higher relatedness amongst wild-born animals compared to founders at each of these sites (standard errors overlap).

3.3 How representative are wild-born populations of their source?

Allele frequencies were mostly consistent between the sample of animals from the initial source population (BI) and each of the wild-born populations at the different translocation sites. Exact tests for differentiation of allele frequencies between populations detected significant variation in allele frequencies at only one locus, Ioo10, whilst allele frequencies were homogeneous across the remaining loci (Appendix C). Similarly, genetic differentiation between the source population, BI, and wild-born populations of *I. a. barrowensis* was low (F_{ST} = 0.003-0.008) (Table 4A), although wild-born animals from HI showed significant differentiation from wild-born LG animals (F_{ST} = 0.007, $p=0.043$). When populations were analysed as founder and wild-born populations at each location, we also found that DI founders were significantly differentiated from the LG founder population (F_{ST} = 0.007, $p=0.044$), though wild born animals on DI were not differentiated from LG founder or wild-born animals (Table 4B).

3.4 Conserving genetic diversity

A total of 100 alleles were observed in the 144 adult animals translocated from BI; of these, 58 alleles were present in frequencies above 5% and considered "common" alleles (Table 5; Appendix C). Using rarefaction analysis of allelic diversity including all rare and common alleles, we found it would be necessary to source between 39 and 121 animals to capture 95%

of the allelic diversity present in our sample of the source population, BI (Mean ± SE = 74.9 ± 8.8 individuals; Table 5). When rare alleles (frequency <5%) were removed from the dataset, significantly fewer animals would be required to conserve 95% of allelic diversity (Mean ± SE = 14.3 ± 1.4 individuals; Range 5-21).

3.5 Effective population size (N_e)

Based on the single sample estimator of Waples and Do (2008), we found that N_e was greatest in the source population, BI (N_e = 1124) (Table 6). The program LDNE was unable to resolve the upper confidence limit on this estimate, which suggests N_e may be even larger. In contrast, each of the translocated populations experienced approximately 5-10 fold reduction in N_e, with estimates ranging from approximately 100 individuals on DI (the smallest translocation site) and LG, to 212 individuals on HI.

3.6 Population modeling

Demographic modeling suggests that the population of golden bandicoots at LG reached carrying capacity very quickly (r=0.213, λ = 1.237), within two years. Despite rapid population growth, both expected heterozygosity and the mean number of alleles declined at a linear rate when the population had reached and was maintained at carrying capacity (Figure 2a, b). After running the model for 100 years, mean H_e declined from 0.65 to 0.54 (27% decline) and the mean number of alleles declined from 7.6 to 4.3 (43% decline). Rare alleles were lost at a higher rate than common alleles (mean probability of retention for alleles <1% frequency = 0.08; 1-5% frequency = 0.28; 6-10% frequency = 0.58). We found that if we had used an initial founding population of 75 individuals as predicted from the allelic richness analysis above (i.e. the number of individuals required to capture 95% of allelic diversity), the rate of decline of genetic diversity would have been similar (Figure 2a,b). A proposal to double the size of the enclosure at LG to increase carrying capacity (K=600-1000) leads to a much slower decline in genetic diversity with time; when K=1000, H_e is maintained at >95% without supplementation, although the number of alleles still declines to approximately 80% of the number in the founding population (Figure 2a, b). Loss of genetic diversity can also be ameliorated by supplementing the population with more animals. Through modeling we found that supplementing the population with larger numbers of individuals (30-50 animals) at greater intervals (10 years) was more effective than smaller numbers of individuals (20 animals) at more frequent intervals (five years). We also found that the number of alleles declined at a greater rate than heterozygosity and that the addition of even larger numbers of animals (50 animals/10 years) would be required to maintain 95% of the allelic diversity present in the founding population, compared to 20 animals/10 years for expected heterozygosity (Figure 2c, d).

The translocated population on HI had lower reproductive rates than LG and a slower rate of intrinsic population growth (r=0.024, λ = 1.024). The HI population did not reach carrying capacity within the 100 years modeled without supplementation (Figure 2e). In this scenario, the population grew to approximately 900 individuals but genetic diversity declined from H_e = 0.66 to 0.60 (10% decline) over the 100 year period and N_a declined from 7.7 to 5.61 (27% decline). Over this time there was also a 16% probability of extinction. Though not shown, we modeled a scenario of using an initial population size of 75 individuals to capture 95% of BI allelic diversity, which resulted in a high probability of extinction (82%). We found that supplementing the populations early (at 5 and 10 years) was sufficient to increase population sizes and to maintain genetic diversity above 95% (Figure 2e, f). Alternatively,

supplementing small numbers of animals frequently (20 animals/5 years) or a slightly larger number of animals less frequently (30 animals/10 years) was sufficient to maintain genetic diversity (Figure 2g, h).

4. Discussion

The assessment of both demographic and genetic data has demonstrated that the translocation of golden bandicoots to three secure conservation sites in Western Australia has resulted in the successful establishment of populations with positive trends in population recruitment and persistence. Use of large founder sizes has led to maintenance of genetic diversity in wild-born populations of *I. a. barrowensis* over six generations. However, modelling shows management intervention through periodical supplementation of animals is likely to be required to maintain genetic diversity over longer time-frames. Importantly, our study highlights the benefit of evaluating long term success of translocated populations through monitoring genetic diversity change in source and reintroduced populations. It also makes the case for incorporating genetics in population viability analysis to explore factors that affect success of translocation strategies and management interventions on both short and long term extinction risk for threatened species. To date, relatively few studies have incorporated genetic diversity assessment in PVAs (Allendorf and Ryman, 2002; but see Haig et al., 1993; Jamieson, 2011, Weiser et al., 2013).

4.1 Genetic diversity in translocated populations

Interestingly, the source population of golden bandicoots on Barrow Island retains moderately high levels of genetic diversity, in contrast to what has been noted for numerous other island populations of animals (Boessenkool et al., 2006; Eldridge et al., 1999; Eldridge et al., 2004). It is likely that the large population size of golden bandicoots on Barrow Island has buffered this population from genetic diversity loss following isolation from mainland populations. We found that there was no significant loss of genetic diversity between wild-born animals and their founding populations at each of the translocation sites up to two years post-translocation. Indeed, each of the wild-born populations remain genetically representative of the initial source population on Barrow Island, in terms of both allelic diversity and allele frequencies, with the exception of the Hermite Island wild-born population which showed low but significant genetic differentiation from the Lorna Glen population. The wild-born populations appeared to effectively result from random mating, with little evidence for inbreeding in the newly-established populations. Despite maintaining high levels of genetic diversity in the wild-born golden bandicoot populations, there was a five- to 10-fold reduction in effective population size within each translocated population compared to the larger source population on Barrow Island reflecting the reduction in the number of animals contributing to breeding at the newly established sites. At both the smaller translocation sites, Lorna Glen and Doole Island, effective population size was estimated at ~100 individuals, compared to 212 individuals on Hermite Island and >1000 individuals on Barrow Island. These results indicate that the reintroduction process has left a genetic signature of a population bottleneck and that these populations will be susceptible to loss of genetic diversity over time if population sizes remain small and the populations remain effectively isolated.

Over the longer term, population persistence is obviously one measure of translocation success but several authors have suggested genetic criteria also be used in evaluating the viability of populations, most commonly with the goal of retaining at least 90-95% of

heterozygosity over 100-200 years (Allendorf and Ryman, 2002; Soule et al., 1986). Since each of the golden bandicoot translocation sites are effectively closed populations, we predicted they would be susceptible to genetic erosion with time as a result of genetic drift. Indeed, using the demographic parameters gained from field monitoring at two of the translocation sites, Hermite Island and Lorna Glen, the population viability models showed that despite maintenance of genetic diversity in the initial reintroduction stages, both populations lost genetic diversity over time. The modeled rate of loss was highly dependent on population size, with Lorna Glen suffering greater declines in expected heterozygosity and number of alleles over 100 years than the larger Hermite Island population. Increasing demographic population size through expansion of the enclosure (Lorna Glen) or through low levels of population supplementation (Hermite Island), led to minimal rates of genetic diversity loss over the time frame modeled. Modeling predicted that if the current small size of the enclosure at Lorna Glen is maintained, frequent periodic supplementation of animals will be required to avoid the sustained erosion of genetic diversity. Our findings have implications not only for golden bandicoot translocations, but for other translocations that involve effectively closed populations that might be susceptible to genetic erosion and indicate that periodic supplementation of animals may be an ongoing action that is a necessary part of those translocation programs (Jamieson, 2011; Jamieson and Lacy, 2012; Weiser et al., 2013).

Consistent with population genetic theory, we found that allelic richness declined in our translocated populations at a more rapid rate than heterozygosity and that this was primarily due to the loss of rare alleles (alleles <5% frequency had a much lower rate of retention than common alleles). Arguably it is allelic richness rather than heterozygosity that reflects the long-term evolutionary potential of a population, since it is the number of genetic variants in a population that determines the material available for selection to act upon (Allendorf, 1986; Tracy et al., 2011; Weiser et al., 2013). In addition, whereas heterozygosity can be recovered following a population bottleneck, novel alleles can only arise through mutation or migration. Recent meta-analyses have shown that the loss of diversity at neutral loci following a population bottleneck is often accompanied by an equivalent or greater loss of diversity at other highly variable adaptive genetic loci, such as major histocompatibility complex (MHC) genes that are important in disease resistance (Radwan et al., 2010; Sutton et al., 2011). If this is the case, our results suggest managers will have to make concerted efforts to maintain allelic richness in translocated populations to promote their resilience to future environmental change or novel disease risk and ensure their long-term viability.

4.2 Retrospective analysis on the number of animals for translocation

Analytical reviews of reintroduction success rates have suggested that translocations employing release group sizes of n>100 leads to greater establishment success (Fischer and Lindenmayer, 2000; Short, 2009). Large numbers of golden bandicoots were released in the translocation investigated in this study (DI n = 92; HI n = 165, LG n = 160) and a mean sample size of 75 animals was sufficient to capture 95% of total allelic diversity (at the 11 microsatellite loci used in this study) of the Barrow Island population. At Lorna Glen there was no difference in the rate of genetic diversity decline between a founder group of 75 compared to 160 animals, potentially a result of buffering due to random mating and the high reproductive rate at this site. This implies that a cost-saving could potentially be made in future reintroductions by reducing the number of founders, as long as it was to be reasonably expected that mortality rates of the released animals would not be excessive and that the population size would increase quickly so that a large population size can be established and

maintained. The effect of inadequate founder size was demonstrated in our Hermite Island population model where a lower reproductive rate led to a higher probability of population extinction when the smaller founder size was used. In this situation, larger founder sizes were required to ensure population persistence.

4.3 Genetic viability and management of translocated populations

Each of the translocated populations in this study is considered a closed population as they are either on islands or in a fenced enclosure. With no migration, our population viability modeling showed a signature of genetic diversity decline over time unless population numbers were high. Loss of genetic diversity can be mitigated through population supplementation at regular intervals. Modeling found that the small population of golden bandicoots in the fenced enclosure at Lorna Glen required the most intensive management but that genetic diversity loss could be ameliorated through regular translocations of ~50 animals every 10 years. It is expected that the enclosure size at Lorna Glen will be expanded to approximately 5000 ha within the next decade, which clearly will benefit the long-term management of golden bandicoots if population sizes expand accordingly to >1000 animals. Genetic augmentation is not likely to be required if population size is increased and sustained at >1000 animals.

Reproductive rates of golden bandicoots were lower on Hermite Island than on the mainland and consequently population size was slow to increase. In this case, either early supplementation of a large number of individuals (>100) or supplementation of only a small number of individuals at regular intervals (30 animals/10 years) was required to increase population sizes and maintain genetic diversity at 95% of the founders. We could expect that once Hermite Island reaches carrying capacity (K=~1500) the population should be self-sustaining in the longer-term. While we did not model changes in genetic diversity at the second island translocation site, Doole Island, we would expect that if the population was sustained at less than ~1000 animals, as is likely due to the small size of the island, ongoing genetic augmentation will be required.

The population viability models as we have presented here provide insights into the management of translocated populations to maintain genetic diversity. However, the models were paramaterised with limited information on survival rates and with little knowledge of the true rate of inbreeding depression (though we have used a higher rate than commonly applied; O'Grady et al., 2006) or environmental variation in carrying capacity and how this changes with time. Recent experience has shown that translocated populations may achieve high population growth in initial stages but decline sharply some years after (e.g. Pearson, 2012; Smith et al., 2008). Additionally, we have not modeled for demographic changes that may result from catastrophes, such as fire, disease outbreak or predator invasion. Incorporating this type of information could provide a more realistic model of the long-term demographic and genetic viability of these populations, particularly as closed or island populations may be especially vulnerable to deterministic and stochastic phenomena.

4.4 Effective population size

Populations with a large effective population size are resistant to loss of genetic diversity through genetic drift and inbreeding, thus it is important in conservation to establish populations that are capable of growing and maintaining a large population size. In addition,

inbreeding depression may occur in small newly established populations in the short term, so that initial population size may be important even if the population increases rapidly. It is estimated that minimum viable population sizes should be ~5000 to prevent species extinction in the long-term (i.e. over a time-frame of several hundred to 1000 years) (Clements et al., 2011; Traill et al., 2010). In the immediate term, for management of recovering populations it may be appropriate to follow the 50/500 rule instead, where effective population size should not be less than 50 in the short term and should be greater than 500 in the long term to maintain genetic diversity (Franklin, 1980; Jamieson and Allendorf, 2012). If we use these numbers as a guide, the initial reintroduction of golden bandicoots to mainland and island sites has been successful in maintaining an effective population size greater than 50 at all sites. With the short generation time and high reproductive rate of golden bandicoots, it is likely that population expansion will occur rapidly (in the absence of catastrophic events or artificial constraints to population growth) to maintain an effective population size >500 at Lorna Glen and Hermite Island, at least.

4.5 Conclusions

Studies have shown that many reintroduced populations show reduced genetic diversity compared to source populations (Maudet et al., 2002; Mock et al., 2004; Sigg, 2006; Vernesi et al., 2003), leading to problems with genetic drift and increased inbreeding in the establishing populations, even when large numbers of founders are used (e.g. Stockwell et al., 1996). However, we found here that using a large founder size of 92-165 animals contributed to successful conservation of genetic diversity between the source population of golden bandicoots and wild-born progeny approximately six generations post-translocation at each of the conservation sites. Nonetheless, population models predicted that these populations would be susceptible to erosion of genetic diversity over time with no immigration, particularly the smaller populations in the Lorna Glen fenced enclosure and presumably the small Doole Island site, though this was not modeled. A program of periodic genetic augmentation is required to prevent the loss of genetic diversity over time if translocated population sizes remain at less than ~1000 animals. Supplementation of animals to maintain evolutionary potential is typically not explicitly included, or budgeted for, in reintroduction plans (Jamieson and Lacy, 2012). Thus, we highlight the use of genetic diversity assessment and incorporation into PVA to determine the interacting factors contributing to population persistence, and the evaluation of potential actions required to ensure viable populations to greater assist in conservation planning. The incorporation of genetic information into the reintroduction process at an early stage is a critical aspect in evaluation of translocation success beyond monitoring of demographic parameters, especially since early intervention to address problems is more likely to contribute to long-term success.

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795

Figure Legends

Figure 1: Map showing location of *Isoodon auratus barrowensis* source population (Barrow Island) and translocation sites (Hermite Island, Doole Island and Lorna Glen proposed conservation reserve)

Figure 2: Change in expected heterozygosity and number of alleles of *Isoodon auratus barrowensis* translocated populations modeled over 100 years using population viability analysis with input parameters as described in Appendix B. (a-b) Lorna Glen, change in H_e and N_a with initial founding population N=160; with founding population N=75 to capture 95% allelic diversity (from rarefaction analysis); with increased carrying capacity K= 600 – 1000 as a result of expansion of enclosure. (c-d) Lorna Glen, change in H_e and N_a with different supplementation strategies (20, 30, 50 animals at continuous 5 or 10 year intervals) to prevent genetic diversity loss. (e) Hermite Island, change in population size with initial population size N=165 and different supplementation strategies (small numbers of animals at continuous intervals or larger numbers of animals as early supplementations). (f) Hermite Island, change in H_e with early supplementation strategies. (g-h) Hermite Island, change in H_e and N_a with initial founding population N=165 and with different continuous periodic supplementation strategies. Horizontal dotted lines represent 95% H_e or N_a.

Table Legends

Table 1: Demographic and life history parameters used in population viability models of *Isoodon auratus barrowensis* translocated populations at Lorna Glen and Hermite Island, and the sources of data used. Full details and justification of the parameters used is provided in Appendix B.

Table 2: Mean and standard error of genetic diversity measures from the source population at Barrow Island, and founder and wild-born populations of *Isoodon auratus barrowensis* at each of three translocation sites at Doole Island, Hermite Island and Lorna Glen proposed conservation reserve. N = Number of individuals genotyped; N_a = Number of alleles per locus; A_R = Allelic richness (rarefied to n=38); A_e = Number of effective alleles per locus; P_A = Number of private alleles; H_o = Observed heterozygosity; H_e = Expected heterozygosity; F = Wright's inbreeding coefficient. Superscripts denote significantly different ($p < 0.05$) groups determined by Tukey's post-hoc multiple comparisons test following ANOVA testing for differences amongst 1) the Barrow Island source population and wild-born animals and 2) all founding and wild-born populations.

Table 3: Mean and standard error of estimates of pairwise relatedness (*r*) amongst founder and wild-born individuals of *Isoodon auratus barrowensis* at three translocation sites at Doole Island, Hermite Island and Lorna Glen proposed conservation reserve.

Table 4: Pairwise genetic differentiation between (a) source and wild-born populations of *Isoodon auratus barrowensis*, and (b) founder and wild-born populations at each location. F_{ST} values below the diagonal and D_{est} values above; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5: The number of individuals required to capture 95% of allelic diversity at each locus when (a) all rare and common alleles are included and (b) only common alleles occurring at greater than 5% frequency are included.

Table 6: Effective population size (N_e) estimated from genetic data from the source population of *Isoodon auratus barrowensis* on Barrow Island and at each of three translocation sites, Doole Island, Hermite Island and Lorna Glen proposed conservation reserve

Table 1

Parameter	Lorna Glen	Hermite Is
Breeding system	Polygynous [A]	
Inbreeding Depression	Recessive Lethals (8 Lethal equivalents) [B]	
Adult males in breeding pool	97.8% [C]	
% males successful	50% [C]	
Mean no. mates per male	1.4 [C]	
Age of first reproduction (Females)	4 months [D]	
Age of first reproduction (Males)	4 months [E]	
Max. age of reproduction	5 years [D]	
No. litters/yr	3 [E]	
Sex ratio at birth (in % males)	50% [E]	
Max. no. progeny/litter	5 [D]	5 [D]
% Adult females producing:		
0 young	31% [D]	48% [D]
1 young	11%	19%
2 young	35%	31%
3 young	20%	1%
4 young	3%	0.5%
5 young	1%	0.5%
Mortality of females & males		
0-1 years of age	50% [E]	
>1 years of age	10% p.a. [E]	
Population carrying capacity (K)	300 [E]	1500 [E]
Dispersal between pops	30% juveniles disperse outside fence p.a.	None, closed pop
Initial population size	160	165
Years modeled	100	
No. iterations	1000	

[A] McKenzie et al., 2008
[B] O'Grady et al., 2006
[C] Data from congener, *I. obesulus*
[D] J. Dunlop, N. Thomas, K. Morris, unpublished data
[E] Assumption made – see Appendix B for rationale

Table 2

Population	N	N_a	A_R	A_e	P_A	H_o	H_e	F
Source population	142.5 ± 0.76	9.09 ± 1.04 [a]	7.76 ± 0.91	4.40 ± 0.27	0.818	0.655 ± 0.080	0.663 ± 0.083	0.010 ± 0.012 [ab]
Doole Island								
Founder	47.5 ± 0.76	8.18 ± 0.99	7.99 ± 0.97	4.32 ± 0.59	0.273	0.638 ± 0.078	0.662 ± 0.081	0.028 ± 0.018
Wild-born	38.8 ± 0.12	7.27 ± 0.92 [b]	7.25 ± 0.91	4.14 ± 0.59	0.000	0.657 ± 0.080	0.654 ± 0.080	-0.016 ± 0.022 [ab]
Hermite Island								
Founder	38.0 ± 0.00	7.82 ± 1.09	7.82 ± 1.09	4.27 ± 0.57	0.091	0.672 ± 0.078	0.667 ± 0.077	-0.005 ± 0.031
Wild-born	42.6 ± 0.43	7.36 ± 0.80 [b]	7.27 ± 0.79	4.09 ± 0.58	0.182	0.631 ± 0.079	0.651 ± 0.079	0.045 ± 0.031 [a]
Lorna Glen								
Founder	57.0 ± 0.00	7.91 ± 0.86	7.42 ± 0.81	4.22 ± 0.60	0.273	0.659 ± 0.087	0.648 ± 0.086	-0.024 ± 0.023
Wild-born	66.6 ± 0.20	7.55 ± 1.00 [b]	7.13 ± 0.94	4.15 ± 0.61	0.000	0.665 ± 0.088	0.643 ± 0.086	-0.040 ± 0.018 [b]

Table 3

Population	Founder	Wild-born
Doole Island	-0.002 ± 0.002	0.002 ± 0.003
Hermite Island	-0.004 ± 0.003	0.002 ± 0.002
Lorna Glen	0.000 ± 0.001	0.001 ± 0.001

Table 4

a)

	Barrow Is. (source)	Doole Is. wild-born	Hermite Is. wild-born	Lorna Glen wild-born
Barrow Is. (source)	-	0.000	0.000	0.000
Doole Is. wild-born	0.004	-	0.008	0.007
Hermite Is. wild-born	0.004	0.008	-	0.008 *
Lorna Glen wild-born	0.003	0.007	0.007 *	-

b)

		Doole Is.		Hermite Is.		Lorna Glen	
		Founder	**Wild-born**	**Founder**	**Wild-born**	**Founder**	**Wild-born**
Doole Is.	**Founder**	-	-0.007	0.001	0.002	0.007 *	0.006
	Wild-born	0.004	-	0.004	0.008	0.007	0.007
Hermite Is.	**Founder**	0.008	0.006	-	-0.011	0.002	0.001
	Wild-born	0.007	0.006	0.004	-	0.010 *	0.008 *
Lorna Glen	**Founder**	0.007 *	0.006	0.007	0.005 *	-	-0.002
	Wild-born	0.007	0.007	0.008	0.006 *	0.003	-

Table 5

Locus	No. rare and common alleles	No. Individuals	Common alleles >5% frequency	No. Individuals
B7-2	9	62	6	15
Ioo8	9	44	5	11
B34-2	11	101	7	21
Ioo6	7	121	4	15
B20-5	13	52	7	20
B3-2	4	116	2	10
Ioo10	14	95	5	16
Ioo4	11	70	7	18
B34-1	11	74	7	14
Ioo16	3	39	1	5
Ioo7	8	50	7	12
Mean ± SE	**9.1 ± 1.0**	**74.9 ± 8.8**	**5.3 ± 0.6**	**14.3 ± 1.4**

Table 6

	Harmonic mean no. individuals	**No. independent comparisons**	**Estimated N_e**	**95% CI (jackknifing loci)**
Barrow Is. (source)	141.0	2381	1124	322-Infinite
Doole Is.	84.9	2242	91	67 – 134
Hermite Is.	79.6	2110	212	119 – 659
Lorna Glen	123.3	2257	108	73 – 179

Figure 1

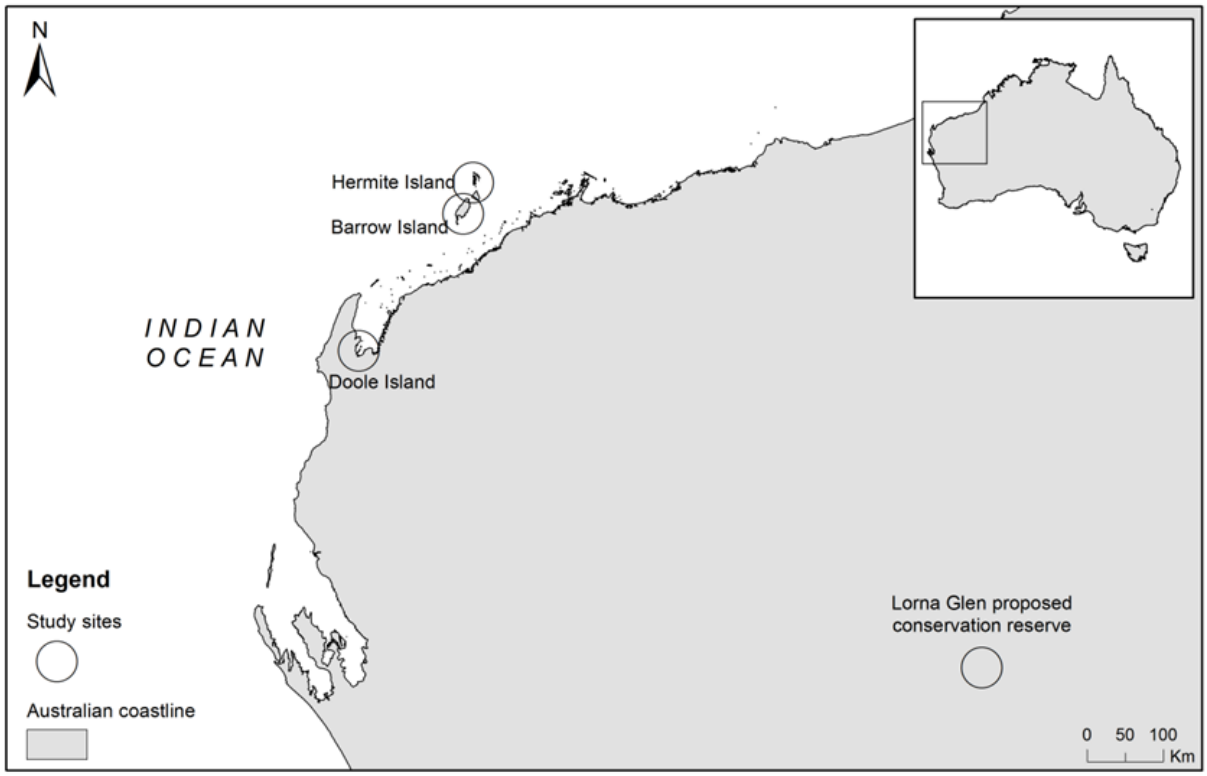
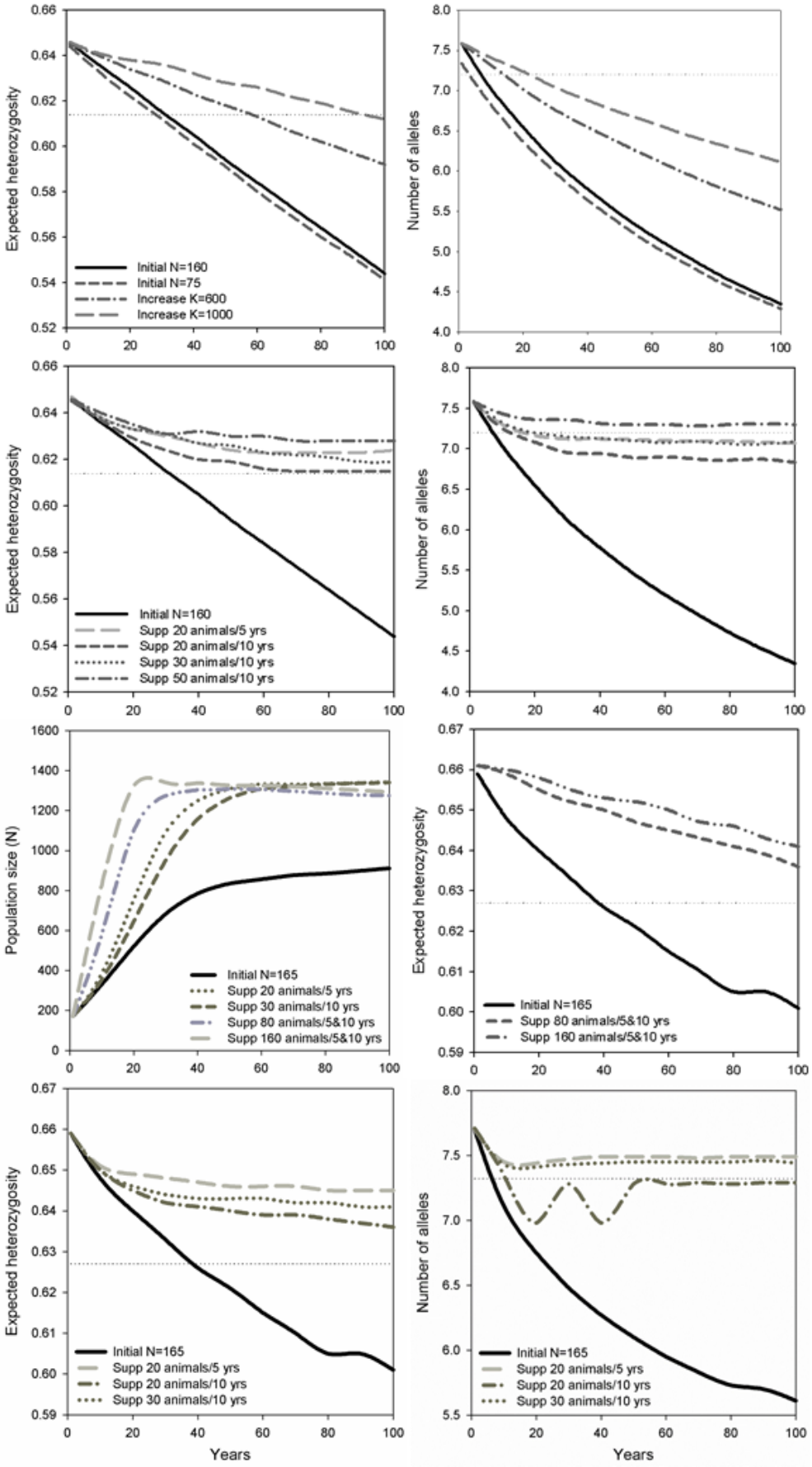

N
Hermite Island
Barrow Island
INDIAN
OCEAN
Doole Island
Legend
Study sites
Australian coastline
Lorna Glen proposed
conservation reserve
0 50 100
Km

Figure 2


Expected heterozygosity
Number of alleles
Initial N=160
Initial N=75
Increase K=600
Increase K=1000
Initial N=160
Supp 20 animals/5 yrs
Supp 20 animals/10 yrs
Supp 30 animals/10 yrs
Supp 50 animals/10 yrs
Population size (N)
Initial N=165
Supp 20 animals/5 yrs
Supp 30 animals/10 yrs
Supp 80 animals/5&10 yrs
Supp 160 animals/5&10 yrs
Initial N=165
Supp 80 animals/5&10 yrs
Supp 160 animals/5&10 yrs
Initial N=165
Supp 20 animals/5 yrs
Supp 20 animals/10 yrs
Supp 30 animals/10 yrs
Years
Years