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Genetic management and population modelling of translocated fauna: Banded hare-wallaby (*Lagostrophus fasciatus*)

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Cover image: Pair of released hare-wallabies. Image: Richard Manning

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Sample data is available from Daniel White on request

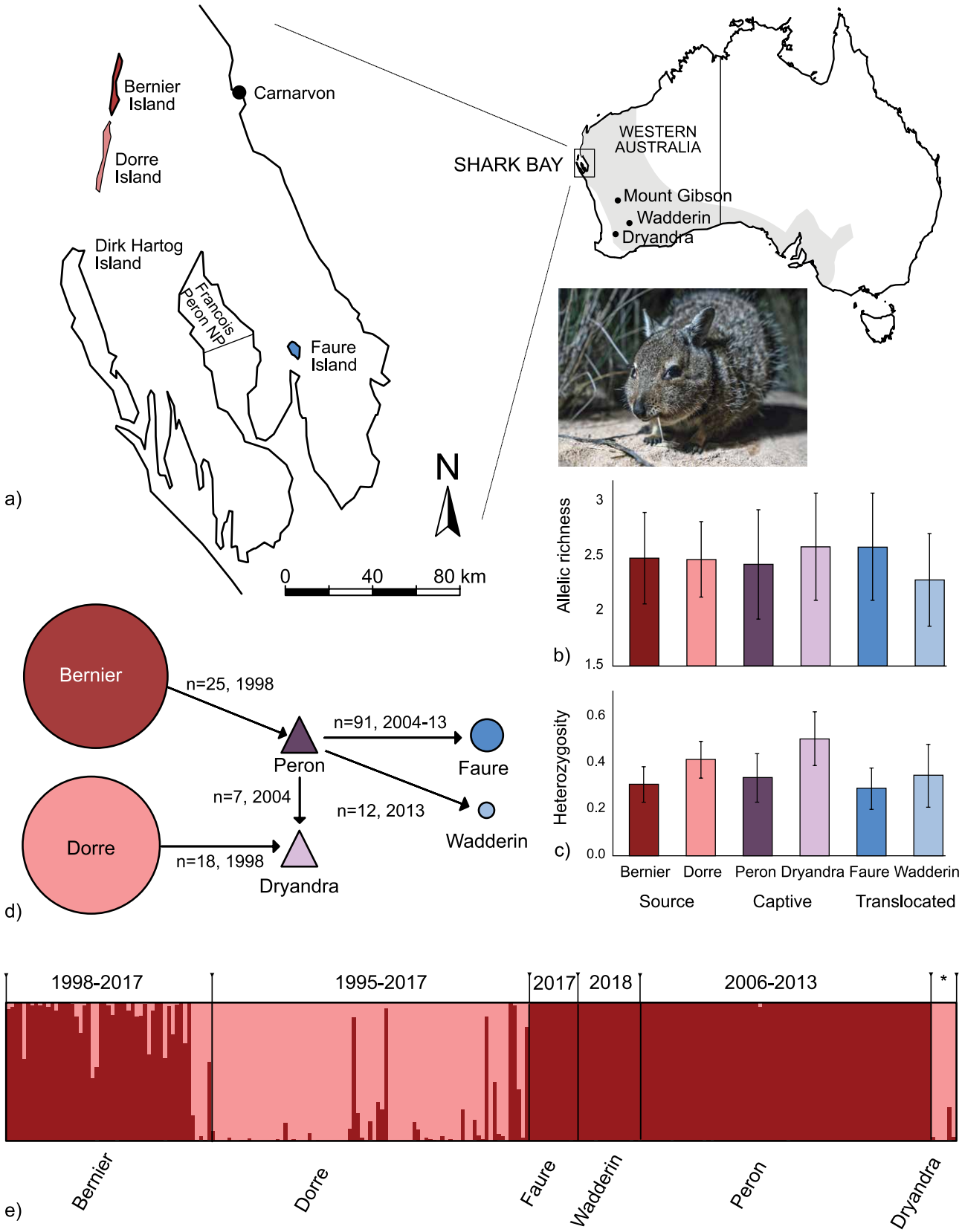

Figure 1. a) Distribution of *Lagostrophus fasciatus* in Western Australia (inset shows the approximate historical distribution); b-c) measures of genetic diversity based on seven microsatellite markers; d) translocation history (failed translocations not shown). Two recent translocations to Mt. Gibson and Dirk Hartog Island have now been completed; e) STRUCTURE analysis of the two remnant wild populations (Bernier and Dorre Islands), two translocated populations (Faure Island and Wadderin) and two historic captive breeding populations (Peron CBC and Dryandra). Sampling periods are indicated at top of plot.
* sampling period for Dryandra is 1999-2002.

Executive summary

Australia has the world's highest rate of mammal extinction. Given major threats to Australia's remaining mammals posed by introduced predators, habitat degradation and climate change, interventions are necessary to secure their continuing diversity. Translocations, the intentional movement of animals from one location to another, is becoming an increasingly essential conservation tool. The banded hare-wallaby, *Lagostrophus fasciatus*, a medium-sized (1,700 g) macropod, had a pre-European range that extended from the coast of central Western Australia to southern South Australia. Remnant wild populations now persist on only two islands in the Shark Bay region of Western Australia, Bernier (ca. 2,790 individuals) and Dorre Islands (ca. 2,440 individuals). This makes the species particularly vulnerable to catastrophic events and continued loss of genetic diversity via genetic drift. These small island populations have therefore been used as source populations for conservation translocations, but harvesting reduces source population sizes and could lead to further reductions in genetic diversity.

This study aimed to determine how the translocation history of the banded hare-wallaby has affected the genetic health of all populations, particularly as serial translocations via intermediary captive populations have led to the possibility of genetic "bottlenecks", or further narrowing of genetic diversity. We used a limited 7-strong microsatellite marker panel to quantify genetic variation and incorporated this data into population viability analysis models, designed to optimise translocation scenarios. Crucially, we included the impacts of regular periods of low rainfall on demographic rates in our models, and tested increases in drought frequency.

A clear trade-off exists in conservation translocation programs between maximising the viability of new populations and minimising the negative impact on critical and precious source populations. Our findings show that the genetic diversity of the banded hare-wallaby was very low compared to that of mainland populations of the rufous hare-wallaby, and that both remnant island populations possibly underwent genetic bottlenecks in the 1990s. This is to be expected of these island populations that are unlikely to migrate and interbreed and are exposed to regular drought-like conditions. One translocated population may have passed through a genetic bottleneck while another is possibly inbred. Empirical data, supported by modelling, showed that when establishing new populations 25 founders may be sufficient to avoid a lasting bottleneck effect under favourable conditions, but that risks multiply for founder sizes of less than 20. Our modelling suggests 100 founders should lead to high survival probabilities and genetic diversity retention in newly translocated populations, and we recommend mixing both source islands as opposed to harvesting from a single source. Increasing drought frequencies, as expected under climate change, strongly impacts both survival and growth of new populations.

Results from this research have already been taken up in the "Dirk Hartog Island: Return to 1616" translocation program run by the Western Australia Department of Biodiversity, Conservation and Attractions, as well as integrated into translocations to Australian Wildlife Conservancy's managed eco-sanctuaries such as Mt Gibson. Our models will be used in future translocations and can be updated and further refined with additional data. We recommend ongoing monitoring, including genetic monitoring, of translocated populations for signs of recruitment and growth, and inbreeding.

Banded hare-wallaby release, Dirk Hartog Island. Image: Peter Nicholas, DBCA

Introduction

The banded hare-wallaby, *Lagostrophus fasciatus*, is a medium-sized (approximately 1,700 g), critical weight range herbivorous and nocturnal macropod, the sole member of the Lagostrophinae sub-family. It is currently listed as Vulnerable by the IUCN and under Australia's environmental legislation (the *Environment Protection and Biodiversity Conservation Act 1999*). Their pre-European range stretched from the coast of central Western Australia to southern South Australia, but the species now survives only in the Shark Bay region of Western Australia on Bernier Island (4,267 ha) and Dorre Island (5,163 ha) (Figure 1a). The last recorded sighting on the mainland was in 1906 (Shortridge 1909). A 2016 survey suggested there were 2,790 individuals on Bernier Island and 2,440 individuals on Dorre Island (Thomas 2018); however, populations cycle through boom and bust phases, triggered by rainfall and drought respectively, and may be reduced by as much as 75% before subsequent recovery (Chapman et al. 2015; Short et al. 1997). These small island populations are expected to have low genetic diversity, which could be further reduced if used as source populations for conservation translocations.

Since 1974, four translocations of *L. fasciatus* have been attempted, and two captive breeding colonies have been established but since discontinued (Figure 1d). The captive colony at Peron Captive Breeding Centre (hereafter Peron CBC) was managed by the Western Australian Department of Biodiversity, Conservation and Attractions (DBCA), and was sourced from Bernier Island. In contrast, a captive breeding colony at Dryandra was sourced from Dorre Island. Two translocation attempts were unsuccessful – specifically the movement of 21 animals from Dorre Island to southern Dirk Hartog Island from 1974 to 1978, and a movement of 18 animals from the Peron CBC to Francois Peron National Park in 2001. The failure of these translocations were attributed to predation by feral cats, drought (Dirk Hartog Island translocation), and the impact of livestock (Hardman et al. 2016; Morris et al. 2004; Short and Turner 1992). The two successful translocations were each founded from the Peron CBC, with 91 individuals released onto Faure Island between 2004 and 2013, and 12 individuals to Wadderin Sanctuary in 2013. Faure Island is a 4,561 ha island in Shark Bay managed by the Australian Wildlife Conservancy (AWC) where all feral predators and livestock have been removed and was reported to support a population of approximately 300 individuals in 2017 (Ruykys et al. 2017). Wadderin Sanctuary on the mainland (Figure 1a) is a 430 ha predator-free enclosure managed by a community group (Short and Hide 2014) and now holds approximately 30 animals (Short 2020).

Currently, two new populations of *L. fasciatus* are being established. From 2017 to 2018, a population of *L. fasciatus* was reintroduced to a 7,832 ha safe haven (a fenced area from which introduced predators have been removed) at Mount Gibson Wildlife Sanctuary. A total of 119 individuals were translocated to Mt Gibson from Bernier (n=40), Faure (n=19) and Dorre (n=60) islands. In 2017, a translocation program began for *L. fasciatus* to Dirk Hartog Island (58,640 ha), forming part of DBCA's "Return to 1616" project following the removal of exotic predators and herbivores (Morris et al. 2017). Fifty-six individuals from both Bernier and Dorre Islands were proposed to be moved over 3 years (total n = 112). In this study we determined how the translocation history of *L. fasciatus* has affected the genetic health of all extant populations, particularly as serial translocations via intermediary captive populations have led to the possibility of genetic bottlenecks. We used genetic and population viability analysis models to explore the impact on precious source populations of concurrent harvesting scenarios and how the retention of genetic diversity can be maximised, and we predicted growth rates and genetic diversity of the new Dirk Hartog Island and Mount Gibson populations. Most analyses were conducted under a baseline assumption of regular drought cycles that reduce reproductive success and survival, and we tested the sensitivity of our results to both reduced and increased drought frequencies, as both scenarios are possible under climate change (Harris et al. 2018).

Context

Australia has the world's highest rate of mammal extinction (Woinarski et al. 2015). Since European colonisation in 1788, 29 species have gone extinct and the western long-beaked echidna (*Zaglossus bruijnii*) is now extinct in Australia, equating to a rate of loss of around 0.13 species per year (Woinarski et al. 2015). Major threats include predation from exotic introduced pests, in particular the European red fox (*Vulpes vulpes*) and feral cat (*Felis catus*), as well as habitat degradation. Habitat degradation includes both the loss of habitat and habitat fragmentation, both of which can exacerbate predation issues. In addition, small to medium-sized mammals within a critical weight range (35 – 5,500 g) are more prone to extinction than those outside this range (Burbidge and McKenzie 1989; Cardillo and Bromham 2001; Chisholm and Taylor 2007; Woinarski 2015). For many of the endemic mammals of Australia that remain, their distributions have contracted to isolated populations, including off-shore islands that remain feral predator–free. To compound matters, climate change increases the risks of starvation, drought stress, and hyperthermia (Molloy et al. 2014; Reckless et al. 2017). Accordingly, it is widely agreed that interventions are essential to secure Australia's remaining mammal diversity (Woinarski et al. 2015).

Translocation, the anthropogenic movement of a group of organisms from one location to another, is an increasingly necessary tool for conservation management (Frankham et al. 2017). Conservation translocations take many forms (reintroductions, reinforcements, genetic rescue, assisted colonisation) and in all cases should be carefully planned, as they are costly and their success is hard to predict (Fischer and Lindenmayer 2000; Germano and Bishop 2009; Ottewell et al. 2014; Perez et al. 2012; Sheean et al. 2012; Weeks et al. 2011). For example in fauna translocations, animals may be naïve to predators at translocation sites (Sheean et al. 2012; Short 2016), and harvesting of source populations may disrupt existing social dynamics (Brashares et al. 2010; IUCN/SSC 2013; Saltz 1998). Nevertheless, translocations are often the only option outside of *ex situ* conservation, to spread demographic risk and prevent extinctions (Holzapfel et al. 2008; Lloyd and Powlesland 1994; Weeks et al. 2017).

As translocated populations often arise from a small number of founders, they risk losing genetic diversity via bottlenecks and/or genetic drift, and inbreeding depression can occur due to mating between related individuals (Frankham 2005). It is therefore important to quantify the genetic diversity in source populations in order to manage these risks and to increase the evolutionary potential of translocated populations (Allendorf 1986; Gilpin and Soule 1986; Lacy 2019; Tracy et al. 2011; Weeks et al. 2011). Individual-based population viability analysis modelling is a powerful tool for making predictions about potential outcomes of various translocation scenarios and can be used to optimise inherent, and often sensitive, trade-offs (Lacy 2019; Pacioni et al. 2019). The incorporation of genetic data into population viability modelling is an important component of conservation decision-making, and there has been a marked uptake of these types of analyses for Australian mammals (Grueber et al. 2019; Kelly and Phillips 2019; Ottewell et al. 2014; Pacioni et al. 2019; Ramalho et al. 2018).

Methodology

2.1 Tissue samples

Ear biopsies were sampled over 19 years, from 1998 to 2017, from six locations: Dorre Island (n = 79), Bernier Island (n = 51), Faure Island (n = 10), the Peron CBC (n = 73), the Return to Dryandra Captive Facility (hereafter Dryandra, n = 6) and Wadderin Sanctuary (n = 17) (Figure 1d). All available *L. fasciatus* samples (n = 236, supplementary material – available on request) were analysed.

2.2 DNA extraction and nuclear microsatellite amplification

Genomic DNA was extracted from ear tissue using a standard "salting out" protocol (Sunnucks and Hales 1996). Ten microsatellite loci were initially amplified using primers derived from the tammar wallaby (*Macropus eugenii*; Me14, Me17; (Taylor and Cooper 1998)), yellow-footed rock-wallaby (*Petrogale xanthopus*; Y105, Y175, Y151, Y148; (Pope et al. 1996)) and allied rock-wallaby (*P. assimilis*; Pa593, Pa297, Pa385, Pa55; (Spencer et al. 1995)). Briefly, for samples prior to 2016, polymerase chain reactions (PCRs) were carried out for individual microsatellite primers in a total volume of 30 µl with ~100 ng DNA, 1X PCR buffer, 400 µM of dNTPs, 2mM MgCl2, 0.2 µM of each primer and 0.825 U Taq or, after 2016, in three PCR multiplexes using the Qiagen Multiplex PCR kit for a total volume of 7.5 µl per multiplex with ~5-10 ng DNA, 1X Qiagen buffer, 0.2 µM primer mix and water. Details of the PCR multiplexes can be found in Table S1. Fluorescently labelled DNA fragments were separated using an ABI373xl capillary sequencer (Applied Biosystems, Foster City, CA, USA) and scored manually with the aid of GENEMARKER software (v1.5, Soft Genetics State College, PA, USA). Allele size was determined by co-running a Genescan500 standard (Applied Biosystems, Melbourne, Australia). Data were checked for input errors and duplicate genotypes using the Excel Microsatellite Toolkit add-in (Park 2001). Deviations from Hardy-Weinberg equilibrium and linkage disequilibria were tested using GENEPOP v4.1.4 (Rousset 2008). For markers with fewer than five alleles, a complete enumeration algorithm was used to estimate the exact p value; and for markers with five or more alleles the Markov chain algorithm of Guo and Thompson (1992) was used to generate an unbiased estimate of the exact p value. The presence of any null alleles was tested with MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004).

2.3 Statistical analyses of genetic data

The program STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to estimate the number of genetically distinct populations and to assign individuals to populations. STRUCTURE uses a Bayesian clustering method to assign individuals to one of k populations and to estimate the degree of inter-population admixture. While some assumptions made by the software will likely not be met by the island populations in our system, such as the distribution of genotypes under Hardy Weinberg Equilibrium (HWE) and marker linkage equilibria, this approach still provides a useful assessment of population genetic divergence. Our models assumed no admixture between locations, but since in some cases locations represent recently sub-sampled source populations, and therefore could have shared allele frequencies, we compared models that assumed either correlated or uncorrelated allele frequencies between groups.

After preliminary assessment of convergence times for the Monte Carlo Markov chain, a burn-in period of 100,000 steps was chosen, followed by 1,000,000 steps of the chain. To estimate k, four replicate runs at each value of k from 1 to 8 were performed, and the most likely value was estimated from the plot of ln Pr (X|k) vs k, as well as from Evanno's method (Evanno et al. 2005) which plots Δ k (a second order rate of change of ln Pr (X|k)) vs k, using CLUMPAK beta version (Kopelman et al. 2015). STRUCTURE figures were generated using DISTRUCT v1.1 (Rosenberg 2004) in CLUMPAK (Kopelman et al. 2015). The level of genetic differentiation among the two source populations was determined by estimating pairwise FST and Jost's D_{est}, both in GENALEX v6.503 (Peakall and Smouse 2012; Peakall and Smouse 2006). Probabilities were calculated as the proportion of times the observed data value was greater than values generated from 999 random permutations.

Standard genetic diversity metrics including mean number of individuals per marker, mean number of alleles per marker, and observed and expected heterozygosities were estimated for each sampling site, in GENALEX v6.503. Allelic richness, a measure of allelic diversity that controls for variable sample size, was estimated in HP-RARE (Kalinowski 2005). Estimates were rarefied to ten individuals, except Dryandra (now extirpated), which only had 6 individuals in the sample. As marker Me17 was monomorphic except for one individual in the Faure population, in which it was homozygous for the alternate allele, allelic richness was calculated with and without this marker. Population inbreeding coefficients were measured using Wright's FIS in GENEPOP v4.1.4 (Rousset 2008). Where sample size permitted, diversity measurements were also made for cohorts separated by year. We estimated the genetic effective population size (Ne) using the linkage disequilibrium method (Hill 1981; Waples 2006; Waples and Do 2010), excluding singleton alleles (those that occur in one copy in one heterozygote) to prevent an upward bias in Ne estimation, as implemented in NeESTIMATOR v2.1 (Do et al. 2014). Since low numbers of polymorphic markers and small sample sizes can lead to large errors in estimates, analyses were restricted to samples with 25 or more individuals (Waples and Do 2010).

To assess whether any of the managed or natural populations underwent a genetic bottleneck we compared the heterozygote distribution for each marker with the number of alleles for each population in BOTTLENECK v1.2 (Piry et al. 1999), using the two-phase substitution model with default proportions of the infinite alleles and single stepwise mutation models (Piry et al. 1999). A one-tailed Wilcoxon sign-rank test was used to estimate the likelihood that the observed data deviates from what is expected under mutation-drift equilibrium, the most appropriate test with less than 20 loci (Cornuet and Luikart 1996; Luikart et al. 1998b). We also reported whether the allele frequency distribution deviated from an L shaped mode, which can be a qualitative assessment of whether a population has passed through a genetic bottleneck (Luikart et al. 1998a).

Queller and Goodnight's (1989) estimator was used to measure the mean pairwise relatedness within each population, as an indicator of genotype diversity and identity by descent within source populations (Queller and Goodnight 1989). Whether the mean pairwise relatedness of a population was statistically different from 0 (defined as mean pairwise relatedness across the entire data set) was estimated by randomly permuting the data in the population pairwise matrix 999 times, and determining the proportion of permutations that gave a relatedness value greater than the observed value, as calculated in GENALEX v6.503.

2.4 Population modelling

To estimate the number of founders needed to retain low-frequency alleles within newly established populations, scenarios were simulated in ALLELERETAIN v1.1 (Weiser et al. 2012; Weiser et al. 2013) implemented in R v3.6.2 (Team 2018). ALLELERETAIN is an individual-based model that simulates population growth using a user-defined suite of parameters based on life-history traits. It estimates the probability of retention of selectively neutral alleles in founder populations over generations, with the starting frequency of these alleles set by the user. We were interested in assessing the impacts of translocating individuals of varying founder group size (N) on allele retention. To do this we tested founder N values from 20 to 200 in increments of 20 individuals with allele frequency set to 0.05. We then repeated each scenario but considered the translocation to have occurred during a drought year. This was simulated by setting the initial survival after translocation to 0.4 and excluding reproduction for the first breeding cycle. For all simulations we assumed that one *L. fasciatus* breeding cycle lasts 9 months (Sims 2018). Details and justification of the demographic parameters used in the model are provided in Tables 1 and S2.

To estimate the impact of current translocation programs on extinction probabilities and heterozygosities of the newly established *L. fasciatus* populations and their source populations, population viability analysis (PVA) simulations were run in VORTEX v10.17.2 (Lacy 1993; Lacy and Pollak 2017). VORTEX is an individual-based model that uses Monte Carlo simulations to estimate how factors intrinsic to individuals within populations alter growth rates, birth rates and extinction probabilities (Lacy and Pollak 2017). Initially, we developed a baseline PVA for a closed population with a starting N of 2,000 individuals and carrying capacity of 3,000, where droughts at an average frequency of 1 in 6.25 calendar years reduced survival and reproduction by 50%, similar to empirical observations (Chapman et al. 2015;

Short et al. 1997). Although there is no empirical evidence for inbreeding depression we included it in our models as there has been as yet no formal effort to detect it and populations do regularly pass through extreme population bottlenecks (Chapman et al. 2015; Short et al. 1997). However, as populations appear to persist through these bottlenecks without obvious signs of depression we halved the default number of lethal equivalents. Other island populations of marsupials are known to survive small effective population sizes and high levels of inbreeding (Eldridge et al. 1999). Baseline model parameters are provided in Tables 1 and S3.

Various translocation scenarios (each of 1,000 replicates) were then simulated that tested the number of founders and source populations needed to maximise genetic diversity and population growth for a translocation program that, due to the logistical cost to the management agency, runs for a maximum of two years (Table 2). Bernier Island was chosen for single source population translocations for two reasons. First, as this population is smaller in size and has lower genetic diversity it represents a conservative, worst case scenario, and second, this island is easier to access due to protected landing beaches. To quantify the impact of drought on translocation success, the best performing of these scenarios was re-run with both increased and decreased drought frequencies, and with no drought. Finally, a case study (scenario 8) was simulated based on the recent movements of *L. fasciatus* from Bernier and Dorre Islands to Dirk Hartog Island, and from Bernier, Dorre and Faure Islands to the Mount Gibson Wildlife Sanctuary. Although the carrying capacity of Faure Island could theoretically be similar to Bernier and Dorre Islands (predicted here to be 3,000) based on land area, there is currently no empirical evidence for this and the census population size remains well below 1,000. We therefore tested two different carrying capacities for Faure Island, 3,000 and a more conservative 1,000. To further assess the impact of drought cycles in translocation planning, both the current and post-drought estimates of source population sizes were used as inputs in case study models.

Table 1. Life history parameters used for *L. fasciatus* population modelling in VORTEX and ALLELERETAIN. Parameter values were obtained from data in (Chapman et al. 2015; Morris et al. 2017; Richards et al. 2001; Short et al. 1997) and refined by Colleen Sims and Jeff Short. Where a parameter is not listed, defaults are used. V: VORTEX; A: ALLELERETAIN; EV: environmental variation; BHW: banded hare-wallaby; DDR: density dependent reproduction function.

Life History Parameter	Value	Used In
Species description		
Inbreeding depression		V
- Lethal equivalents	3.14	V
- % due to recessive lethal	50	V
EV concordance of reproduction & survival	0.5	V
- EV correlation among populations	0.5 – 0.8	V
Reproductive system		
Reproductive system	polygynous	V/A
Duration of breeding cycle in days	274	V/A
Age of first offspring for females/males	9 months/18 months	V/A
Maximum age of reproduction	8 years	V/A
Maximum lifespan	10 years	V/A
Maximum number of broods per breeding cycle	1	V/A
Maximum number of progeny per brood	1	V/A
Mean female number of progeny per breeding cycle	1	A
Mean male lifetime reproductive success (± SD)	7 ± 3	A
Sex ratio at birth	50	V/A
Reproductive rates		
% Adult females breeding	90% with DDR	V

Life History Parameter	Value	Used In
- EV in % breeding	18	V
Distribution of broods per breeding cycle		
- 0 broods	0	V
- 1 broods	100%	V
Number of offspring per female brood		
- 1 offspring	100%	V
Mate monopolisation		
% males in breeding pool	85	V
% males successfully siring offspring	63	V
Mortality rates		
Females		
Mortality Age 0 to 1 (± SD)	40 (± 10)	V/A
Annual mortality after Age 1 (± SD)	10 (± 3)	V/A
Males		
Mortality Age 0 to 1 (± SD)	40 (± 10)	V/A
Annual mortality after Age 1 (± SD)	10 (± 3)	V/A
Catastrophes		
- Number of types of catastrophes	1 (drought)	V
- Frequency	1 in 6.25 calendar years	V
- Severity	50% reduction in survival and reproduction	V
Initial population size		
Bernier	2000	V/A
Dorre	2000	V/A
Faure	300	V/A
Carrying capacity, K (SD due to EV)		
Bernier	3000 (300)	V/A
Dorre	3000 (300)	V/A
Faure	1000 (100) or 3000 (300)	V/A
DHI	10000 (1000)	V/A
Mt. Gibson	5000 (500)	V/A
Genetic management		
Number of neutral loci to be modelled	7 empirical, 1 simulated	V
Initial minor allele frequency	0.05	A
Scenario settings		
No. replicates	1000/100	V/A
No. years	50 calendar years	V/A

Table 2. Hypothetical and case study translocation scenarios* examined using population viability analyses

Target population	Scenario	Description
Dirk Hartog Island	1	One translocation from Bernier Island in year 1
	2	Individuals translocated from Bernier Island only, half in each of first two years
	3	Individuals translocated from Bernier and Dorre islands, half from one island in year 1 and half from other island in year 2
	4	Individuals translocated from Bernier and Dorre islands, half from each island in year 1
	5-7	Best performing scenario from 1 to 4, with drought frequencies of no drought, 1 in 10 years and 1 in 5 years
Dirk Hartog Island & Mount Gibson Wildlife Sanctuary	8	**To Dirk Hartog Island:** 6 individuals from Bernier and 6 from Dorre in BHW year 1; 50 from Bernier in BHW year 2; 50 from Dorre in BHW year 3
		To Mount Gibson: 23 individuals from Bernier, 39 from Dorre and 10 from Faure in BHW year 1; 37 from Bernier, 1 from Dorre and 20 from Faure in BHW year 3

* different founder numbers were trialled for scenarios 1-4, ranging from 60 to 140 every 20 individuals. Scenario 8 is a case study representing current translocations. Unless otherwise stated, a drought is simulated at a frequency of 1 in 6.25 years.

Findings

Genetic diversity

Combining samples from all years, only one source population, Dorre Island, had statistical support (p = 0.039) for passing through a genetic bottleneck (Table 3). However, when Bernier and Dorre Islands were separated into year cohorts, both populations showed evidence of genetic bottlenecks in the earlier cohorts (1990s), and for Dorre Island also in 2013. Of the managed populations, Wadderin was the only population showing evidence of a genetic bottleneck (Table 3). Thirty individuals and 10 polymorphic loci are recommended to achieve power of at least 0.8 with the Wilcoxon signed-rank test however, so these results should be considered with caution. But both Wadderin and Peron CBC had an allele frequency distribution shifted towards more common alleles, consistent with a bottleneck, although this qualitative test requires at least 30 individuals and 10 to 20 polymorphic loci to be reliable (Piry et al. 1999).

Table 3. Results of two tests for a genetic bottleneck of source and translocated populations

Population (time period)	n	Bottleneck test	
		Wilcoxon (1-tailed, H excess)	Mode Shift
Bernier Island (all)	51	0.281	No
Bernier Island (2016/2017)	33	0.500	No
Bernier Island (2010/2011)	9	0.109	Yes
Bernier Island (1998)	6	**0.016**	Yes
Dorre Island (all)	79	**0.039**	No
Dorre Island (2016/2017)	52	0.422	No
Dorre Island (2013)	11	**0.008**	Yes
Dorre Island (1999/2000)	8	**0.008**	Yes
Dorre Island (1995/1996)	7	**0.016**	Yes
Peron CBC (from Bernier; t=25, y=1998)	73	0.219	Yes
Dryandra (from Dorre & Peron CBC; t=25, y=1998)	6	0.578	No
Faure Island (from Peron CBC; t=91, y=2004 to 2013)	10	0.281	No
Wadderin (from Peron CBC; t=12, y=2013)	17	**0.031**	Yes

Integers represent p values, with significant values in bold. n: sample size; t: translocated population size; y: year of translocation. The two-phase mutation model of microsatellite evolution is assumed with a 70%:30% ratio of single stepwise mutation to infinite alleles models. Year cohorts with fewer than 5 individuals were omitted from analyses. Estimates based on sample sizes of fewer than 30 should be considered tentative.

Estimates of effective population sizes in extant populations reflected overall diversity measurements (Table 4), as Dorre Island had the highest Ne in the 2016–17 cohort (Ne = 140, 95% CI 29-∞), which was 71% higher than for Bernier Island *L. fasciatus* sampled in the same period (Ne = 82, 95% CI 12-∞). Confidence intervals were very large for most estimates (Table 4), and a value of infinity was often reported for upper confidence limits. Values of infinity for upper confidence limits arise from a lack of evidence for variation in the genetic characteristic which in this case is likely due to the small sample sizes and the small, homogeneous marker panel (Do et al. 2014).

Structure analysis revealed that the most likely number of genetically distinct clusters of *L. fasciatus* is two (k = 2; Figure 1e), representing the two remnant wild populations on Bernier and Dorre Islands. There was very good agreement in results whether allele frequencies were assumed to be correlated or uncorrelated between locations, and the absolute probability for k = 2 was slightly greater for correlated allele frequencies. Of the contemporary translocated populations, Faure Island and Wadderin are predominantly of Bernier Island origin, with negligible genomic contribution from Dorre Island. Of the extinct captive populations, Peron CBC was an extension of Bernier Island, whereas Dryandra was predominantly of Dorre Island origin. A small amount of potential admixture was detected between Bernier and Dorre Islands; in total there are ten individuals (four from Bernier and six from Dorre) that have an 80% or greater probability of assignment to the other source population. Both pairwise F_{ST} and Jost's D_{est} measurements were similar (F_{ST} = 0.026, $p < 0.001$; D_{est} = 0.029, $p < 0.001$) reflective of weak, significant divergence between the two parental populations. Pairwise relatedness analysis revealed that one source population (Bernier Island, $p < 0.005$), one extinct captive population (Peron CBC, $p < 0.002$) and one contemporary translocated population (Wadderin, $p < 0.014$) have relatedness values statistically greater than zero (Figure S1).

Overall, genetic diversity was low across all populations (mean expected heterozygosity, HE = 0.34 – 0.45). Of the source populations, Dorre Island exhibited higher heterozygosity and lower inbreeding than Bernier Island, although allelic richness was comparable. When the two parental populations were compared only to each other, Dorre Island had four private alleles (alleles present in one population only) and Bernier Island had one. Diversity estimates within year cohorts for both Bernier and Dorre Islands showed a similar trend of higher than expected heterozygosities in the late 1990s, and lower than expected heterozygosities in more recent cohorts. Both expected heterozygosity and allelic richness have declined over the sampling period (Table 4). There were no significant differences in genetic variation between translocated and source populations except for Faure Island which showed a significant decrease in observed heterozygosity compared to Peron CBC using a one-tailed Wilcoxon signed-rank test ($p < 0.05$), and this island also indicated significant inbreeding due to non-random mating. Considering the two parental populations as one remnant *L. fasciatus* metapopulation, Faure has retained 93 % of allelic diversity, while Wadderin has retained 87% (Figure 1c). Calculating allelic richness without marker Me17 increases its value in all populations, but Faure no longer has the highest allelic diversity (Table S4). Caution is needed when interpreting all diversity results, however, as markers had limited polymorphism (Table S5) and sample sizes for diversity measurements were often very low and varied between sites and temporal data.


Hare-wallaby. Image: Richard Manning

Table 4. Population level diversity metrics using seven polymorphic microsatellite markers.

Population		n	HWE[a]	NA	PA		AR (±s.e.)	HE (±s.e.)	HO (±s.e.)	FIS (±s.e.)	Ne (95% CI)
Bernier Island (all)		51	1/6	20	0		2.47 (0.15)	0.36 (0.09)	0.30 (0.08)	0.14 (0.05)	
Bernier (1998)		6						0.38 (0.11)	0.43 (0.15)	-0.06 (0.17)	n/a
Bernier (2010-2011)		9						0.34 (0.10)	0.23 (0.07)	0.31 (0.10)	n/a
Bernier (2016-2017)		33						0.36 (0.08)	0.31 (0.07)	0.11 (0.04)	82 (12, ∞)
Dorre Island (all)		79	2/6	23	1		2.46 (0.10)	0.42 (0.08)	0.41 (0.08)	0.03 (0.07)	
Dorre (1995-1996)		7						0.44 (0.08)	0.61 (0.11)	-0.39 (0.07)	n/a
Dorre (1999-2000)		8						0.45 (0.09)	0.45 (0.09)	0.00 (0.07)	n/a
Dorre (2013)		11						0.42 (0.08)	0.43 (0.09)	0.01 (0.08)	n/a
Dorre (2016-2017)		52						0.40 (0.07)	0.38 (0.08)	0.07 (0.10)	140 (29, ∞)[b]
Faure Island (2017)		10	0/6	18	2		2.57 (0.40)	0.39 (0.08)	0.29 (0.09)	0.36 (0.14)	n/a
Wadderin (2018)		17	1/5	16	0		2.27 (0.27)	0.36 (0.10)	0.34 (0.13)	0.09 (0.20)	n/a
Peron CBC (2006-2013)		73	1/5	19	1		2.41 (0.15)	0.36 (0.10)	0.33 (0.10)	0.08 (0.08)	20 (7, 57)
Dryandra (1999-2002)		6	0/6	18	0		2.57 (0.52)	0.40 (0.08)	0.50 (0.12)	-0.28 (0.15)	n/a
	Σ	236				μ (±s.d.)	2.34 (0.13)	0.39 (0.02)	0.37 (0.08)	0.07 (0.21)	

n: number of samples; HWE: Hardy-Weinberg Equilibrium; NA: total number of alleles; PA: private alleles; AR: allelic richness (rarefied to 10 individuals except Dryandra); HE: expected heterozygosity; HO: observed heterozygosity; FIS: inbreeding coefficient; Ne: effective population size. [a] Proportion of polymorphic markers that violate HWE at $p < 0.05$; [b] value obtained only with no critical minor allele frequency threshold. Heterozygosity metrics and inbreeding coefficients are also calculated for different time periods for Bernier and Dorre islands, when at least five individuals were sampled for that time period.

Population modelling

In the absence of periodic droughts, ALLELERETAIN demonstrated strongest increases in the probability of retaining low-frequency alleles when the founder population size approached 20, and began to plateau after 40 (Figure 2). Sixty founders are needed to retain 90% of alleles at a frequency of 0.05, and 80 founders are needed to retain 95% of alleles (recommended thresholds) at a frequency of 0.05. These numbers rose to 120 and 140 founders respectively when realistic impacts of drought were simulated.

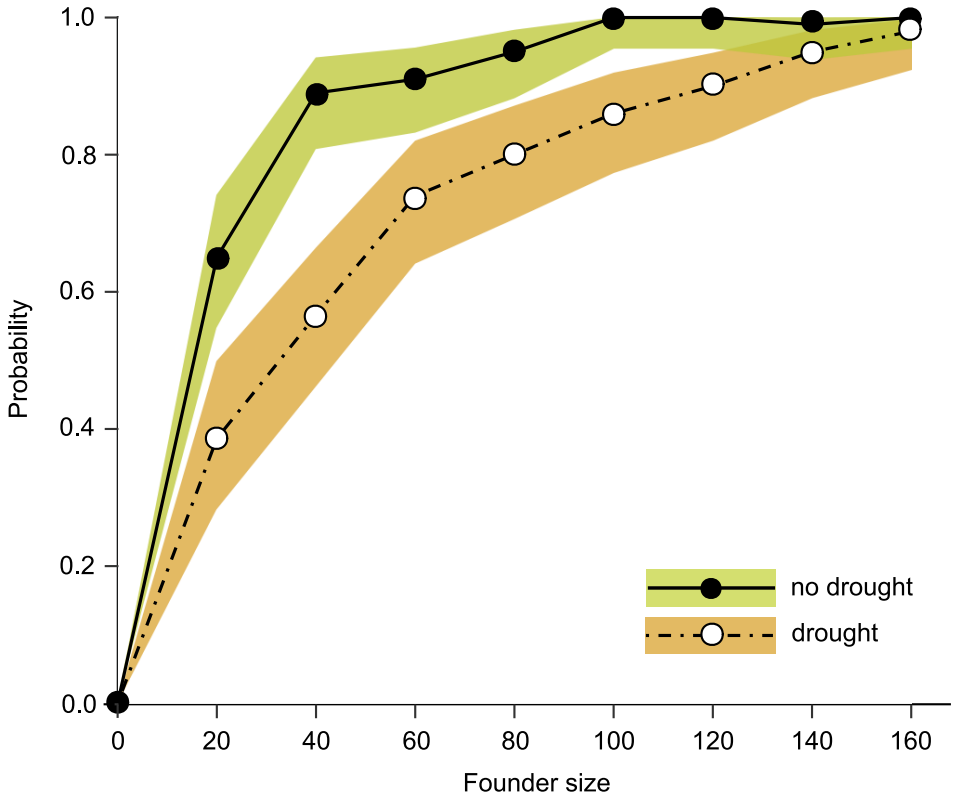

Figure 2. Probability of retaining a selectively neutral allele at a starting population frequency of 0.05 after 50 calendar years for translocated *L. fasciatus* populations with various founder sizes, with and without impacts of drought. Shaded ribbons represent 95% confidence intervals.

Population viability analysis modelling showed that when contrasting founder population sizes between 60 and 140, survival probabilities increase with increasing founder number until this relationship asymptotes from around 120 individuals (Table S6a-d). This level of harvesting also had limited impact on the source populations. Mixing the two source populations of Bernier and Dorre Island increased heterozygosity in the translocated population, relative to only using founders from Bernier Island, and there was minimal impact of moving all individuals in one year versus splitting the translocation over two years (Table 5). Considering the additional logistical cost of a second year of translocation activities, the best scenario in terms of maximising survival probability, genetic diversity (heterozygosity and allelic diversity) and the final population size of the translocated population was to harvest the two source populations in one year (Scenario 4, Table 5).

Table 5. Viability of a translocated *L. fasciatus* population after 50 calendar years testing four scenarios. 100 individuals translocated in one or over two years, including a drought with a mean frequency of one every 6.25 calendar years. Carrying capacity was set at 10,000 and scenarios were run for 1,000 replicates.

Scenario	Description	P (surv)	HE	*N*
1	100 in year 1 from Bernier Island	0.76	0.311	2094
2	50 from Bernier Island in year 1 and year 2	0.79	0.330	2489
3	50 from Bernier Island in year 1, 50 from Dorre Island in year 2	0.80	0.368	2251
4	50 from Bernier Island, 50 from Dorre Island in year 1	0.79	0.362	2277

P (surv): mean probability of survival; HE = mean expected heterozygosity using empirical estimates of allele frequencies to determine starting heterozygosities; *N*: final mean population size of extant populations.

Simulations that varied drought frequency indicated the likelihood of appreciable impact on the survival probability of a translocated population after 50 calendar years, dropping from 100% under the assumption of no drought, to 79% with a realistic drought frequency of 1 in 6.25 years and 58% with a drought frequency of 1 in 5 years (Figure 3). There was also predicted to be a profound effect on the final *N*, as predicted carrying capacity (set to 10,000) was reached with no drought, whereas final *N* was estimated at 2,277 individuals with a 1 in 6.25-year drought and 1,064 at 1 in 5 years (Figure 3).

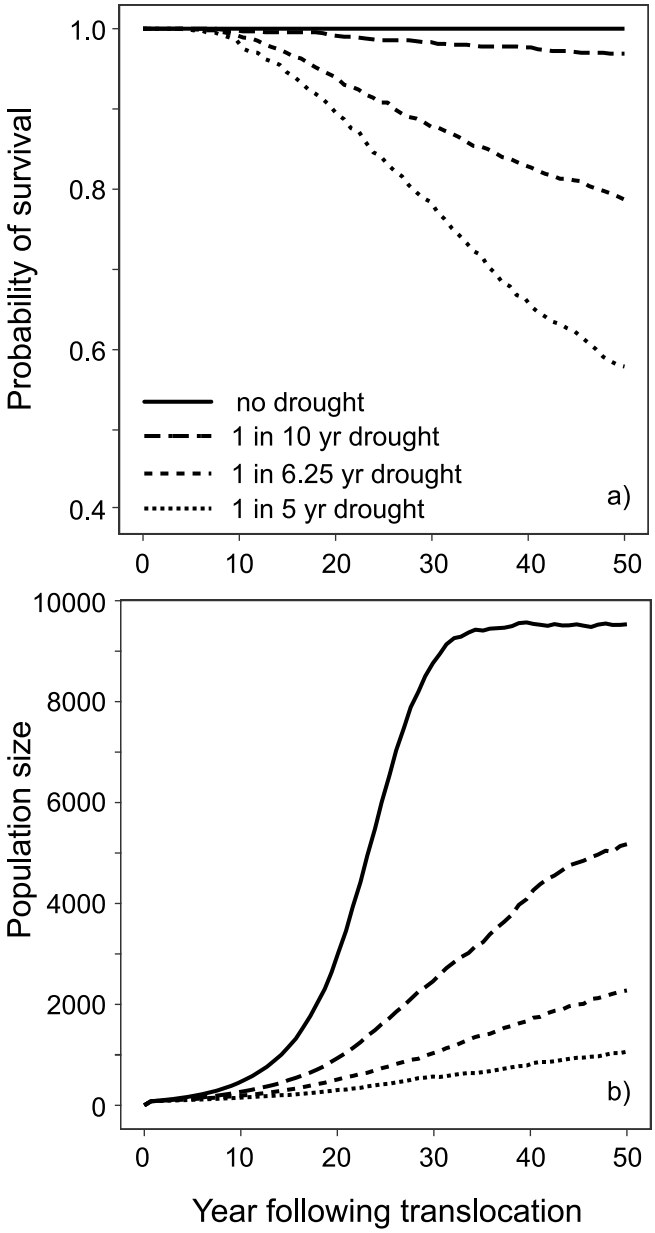

Figure 3. Performance over 50 years for 100 translocated *L. fasciatus* to DHI with drought frequencies of 1 in 5 years, 1 in 6.25 years, 1 in 10 years or no drought. a. Survival probability. b. Population size

Simulation of translocations recently conducted by management agencies between 2017 and 2018 - harvesting Bernier and Dorre Islands for translocation to Dirk Hartog Island, and concurrent harvesting of Bernier, Dorre and Faure Islands for translocation to Mount Gibson - including the impacts of 1 in 6.25-year droughts, predicted good survival probabilities over 50 years for the translocated populations of 83% and 84%, respectfully. This result was also achieved even if harvesting occurred with a reduction in the size of source populations following drought (Table 6). In contrast, the timing of harvesting had a more significant effect on the source populations. Harvesting at current census population sizes (Nc) resulted in high survival probabilities (99%, 98% and 93% for Bernier, Dorre and Faure Islands respectively; Table 6), assuming Faure Island to have a carrying capacity (K) of 1,000. Bernier and Dorre Island population sizes stabilise at ~82% of current estimates, whereas the Faure Island population stabilises after ~65% growth from current estimates to a census size of around 500. If harvesting were to occur immediately after a drought, predicted survival probabilities reduced after 50 calendar years, most markedly to 60% on Faure Island. In addition there was a further reduction in predicted Nc in Bernier and Dorre Island populations to around 74% of current estimates, whereas growth on Faure Island was limited to 29% (Nc ~386). Interestingly, when Faure Island was assumed to have a K of 3,000, there was little improvement on survival probability post-harvest if a drought census size was assumed (63% with K of 3,000 vs. 60% with K of 1,000). However, there was a dramatic increase in predicted Nc as 1,400 was reached when harvesting occurred with a non-drought Nc and 900 with a drought impacted Nc, compared to 495 and 386 with a K of 1,000, respectively.

Table 6. Comparison of PVA outputs after 50 calendar years when harvesting source populations using either current conservative source census sizes, or likely source census sizes following droughts. Models are based on scenario 8, describing recent and ongoing movement of *L. fasciatus*, and assuming an average drought frequency of 1 in 6.25 years.

	Conservative current census sizes of source populations	Census sizes following drought
	$N_{Bernier}$=2000, N_{Dorre}=2000, N_{Faure}=300	$N_{Bernier}$=500, N_{Dorre}=500, N_{Faure}=75
a) Target Populations		
Dirk Hartog Island		
P(surv)	0.83	0.81
N	2363	2436
HE	0.367	0.367
Mount Gibson		
P(surv)	0.84	0.84
N	1600	1565
HE	0.372	0.372
b. Source populations		
Bernier Island		
P(surv)	0.99	0.94
N	1640	1474
HE	0.359	0.353
Dorre Island		
P(surv)	0.98	0.95
N	1647	1465
HE	0.417	0.408
Faure Island*		
P(surv)	0.93/0.93	0.60/0.63
N	495/1401	386/901
HE	0.369/0.374	0.344/0.343

P(surv): mean probability of survival; *N*: final mean population size of extant populations; HE = mean expected heterozygosity.
*Numbers to the left of the slash are for a carrying capacity of 1000, and to the right are for a carrying capacity of 3000.
Scenarios were run for 1000 replicates.

Pair of released hare-wallabies. Image: Richard Manning

Discussion

Genetic diversity in remnant populations

Island populations are expected to lose genetic diversity as a result of genetic drift, especially in the absence of migration (i.e. gene flow) that acts to increase allelic diversity (Frankham 1997; Wright 1931). Here, we found that expected heterozygosity was similar to Shark Bay island populations of rufous hare-wallaby (*Lagorchestes hirsutus*), which was measured using the same markers, and both were less than a mainland rufous hare-wallaby population (Eldridge et al. 2019). Interestingly, genetic diversity (allelic richness and heterozygosity) of *L. fasciatus* on Bernier and Dorre Islands was approximately two-fold lower relative to values reported for mainland populations of other Australian marsupials (Pacioni et al. 2013; Smith and Hughes 2008; and reviewed in Thavornkanlapachai et al. 2019), although markers were not shared between studies and our study consists of only a modest number of lowly polymorphic markers. Nonetheless, an island effect on *L. fasciatus* genetic diversity is suggested that we cannot confirm without a mainland population for comparison. We also identified extremely low effective population sizes for *L. fasciatus* (Table 4) given census sizes of ~2,800 for Bernier Island and ~2,500 for Dorre Island (Chapman et al. 2015; Thomas 2018), leading to good agreement in Ne/Nc ratios of 0.05 and 0.07, respectively. These estimates are several times smaller than those for other mammals, for example, 0.2 to 0.8 reported in Frankham (1995) and Nunney (1996), and a mean of 0.75 reported in Waples et al. (2013).

Bernier and Dorre Islands have been separated from mainland Australia for 8,000 years (around 4,000 generations), and from each other for around 5,000 years (Churchill 1959; Short et al. 1989), providing substantial opportunity for stochastic loss of allelic variation through genetic drift. Intriguingly, Bayesian clustering and population structure analyses indicated that the Bernier and Dorre Island populations of *L. fasciatus* are not as strongly genetically structured as might be predicted from the islands' geological histories, as our structure analysis suggests ongoing or recent gene flow. However, our result necessitates new migrants traversing a 500 m wide sea channel and not interbreeding with the resident population, which are both unlikely. More likely explanations include a lack of discriminatory power in our markers, or there has been a technical issue such as mis-labelling. Further sampling and the use of more powerful markers (e.g. SNP markers generated through next-generation sequencing) are required to confirm genetic structure.

Impact of past translocations on genetic diversity

Despite describing two examples of serial translocations occurring from a single source population via a captive breeding program, and hence providing opportunity for two bottleneck events, in only one case did we find evidence for a bottleneck – the Wadderin population translocated in 2013 with 12 founders. Wadderin was founded from the Peron CBC population, which itself was founded from Bernier Island (Figure 1d), and each translocation contributed a 2.4% and 5.7% loss of allelic diversity, cumulating in a total loss from the Bernier source population of 8.1%. In contrast, Faure Island was founded by more individuals from the Peron CBC (91 versus 12), and although it also had two bottleneck opportunities, neither were realised. In fact, we found that Faure Island had higher allelic richness than Peron CBC and Bernier Island, although these differences were not statistically significant using either a paired t-test or a Wilcoxon signed-rank test.

While we were not able to detect a genetic signal of a bottleneck in the Peron CBC population, this does not preclude the possibility that a bottleneck has occurred from which the population later recovered. However, it does suggest that 25 founders may be sufficient to avoid a lasting bottleneck effect under good growth conditions – a result supported by our modelling which shows that a 70% probability of retaining a low frequency allele is maintained with a founder size of 25, but that retention probabilities drop dramatically with founder sizes less than 20. Similarly, releasing 91 individuals onto Faure Island over a ten-year period appears to have avoided a genetic bottleneck in this population. The high allelic diversity in Faure is surprising, considering its demographic history, and we found it to be driven by the discovery of an additional allele (marker Me17), present in one individual in homozygous form. While this allele could have arisen in the translocated Faure Island population, its independent verification would benefit from expanding sampling of Faure and Bernier Islands.

The apparently low power to detect genetic bottlenecks in translocated *L. fasciatus* populations could reflect relatively small changes in allelic diversity between source and founder populations (due to low effective population sizes and genetic variation in the source), compounded by our small panel of mostly lowly polymorphic markers. Further, the relatively rapid demographic recovery inherent to *L. fasciatus* during the boom phase of natural population cycles could mask any bottleneck signal that depends on heterozygosity excess (Cornuet and Luikart 1996). Therefore, ongoing monitoring, including genetic monitoring, of translocated populations during their establishment would provide useful data with which to detect any detrimental genetic effects that might be initially masked.

Since translocations began to Faure Island, heterozygosity appears to have decreased below equilibrium expectations and inbreeding coefficients have increased, possibly due to limited dispersal and increased breeding between related animals post-translocation. Another possibility is an increase in mating success heterogeneity (Thavornkanlapachai et al. 2019). These factors would also lower effective population size estimates. This is of concern, as small populations are prone to higher rates of loss of genetic variation via genetic drift, especially if inbreeding depression leads to lower rates of reproduction and further reductions in population size (Gilpin and Soule 1986). However, the apparently high inbreeding does not appear to be associated with poor health of the Faure Island population, as it is well established with ongoing recruitment (Smith 2020). One explanation for this apparent paradox is that the boom and bust population cycles characteristic of Shark Bay *L. fasciatus* populations have led to a purging of lethal alleles, negating any detrimental effect of inbreeding (Short et al. 1997). A more prosaic explanation is that the Faure Island sampling cohort was both small (n = 10) and consists of a disproportionately high number of related individuals, leading to an overestimate of the inbreeding coefficient. A more comprehensive genetic analysis and a future study of the social dynamics of the Faure Island population could resolve this uncertainty.

Limitations of genetic analysis

The low number and informativeness of the markers used for this study means that measures of genetic diversity (e.g., heterozygosity) and measurements dependent on genetic diversity (e.g., Ne and probabilities of bottlenecks) are prone to error. For populations with very small sample sizes of 10 or less this effect is compounded further. As such, results should be considered indicative and await confirmation by increasing sample sizes and using more powerful markers (e.g. SNPs).

Applications of the research

One way to increase genetic diversity of species that exist as only small and genetically depauperate remnants is to manage the species as a metapopulation (Pacioni et al. 2018). However, whether to combine genetically distinct populations is contentious (Allendorf et al. 2001; Edmands 2007; Frankham et al. 2011; Frankham et al. 2017; Thavornkanlapachai et al. 2019; Weeks et al. 2011). While there is an immediate and obvious benefit of maximising genetic diversity and heterozygosity, thereby reducing the chance of inbreeding depression, mixing comes with risks, including outbreeding depression (Armbruster et al. 1999; Edmands 1999; Frankham et al. 2017; Marr et al. 2002; Tymchuk et al. 2007). Other risks include competition for resources when there are morphological and size differences between source populations, and behavioural differences could affect mate choice potentially reducing Ne and leading to a bias in representation of future generations (e.g., as occurs in the boodie *Bettongia lesueur*, Thavornkanlapachai et al. 2019). Here, however, the habitats of the two adjacent *L. fasciatus* source populations have been similar across the 4,000–5,000 years (approximately 2,000 generations) of their separation (Smith and Hughes 2008). Further, there are no known differences in size, morphology or mating behavior between islands, suggesting a low probability of outbreeding depression (Frankham et al. 2011). Considering the overall low genetic divergence between source populations and low genetic diversity within this species – and by mixing we achieve a 10–12% increase in HE of the recipient population compared to using only Bernier Island as a source population – we advocate mixing the two source island populations in future translocations and predict a low risk of any adverse consequences.

A clear trade-off exists in conservation translocation programs between maximising viability of a new population and minimising the negative impact on critical source populations (Easton et al. 2019). For example, sufficient founders are required to ensure a translocated population is buffered from some post-establishment mortality, as well as to retain rare alleles that bolster evolutionary potential (Easton et al. 2019; Weiser et al. 2013). Smaller founder numbers, on the other hand, reduce any negative impacts on source populations, but translocated populations will be more sensitive to mortality and the loss of rare alleles. Our PVA models for *L. fasciatus* show that for newly translocated populations, survival probabilities increase with increasing founder number until this relationship asymptotes at around 120 individuals, and release of 100 individuals ensures good growth and survival in a hypothetical haven (island or fenced sanctuary with a carrying capacity of 10,000). That carrying capacity did not significantly impact 50-year predicted survival probabilities for the Faure Island population when using reduced drought-influenced census sizes, indicates the sensitivity of successful establishment of *L. fasciatus* translocated populations to the founder number, and suggests that sufficient numbers are required to withstand the regular population reductions caused by stochastic events. Importantly, harvesting the numbers recommended here has no apparent impact on the critical source populations, either in terms of population growth or survival probability over a 50-year period.

Our allele retention models predict that 80 founders are needed to retain 95% of low-frequency alleles, noting that retaining 90 or 95% genetic variation are recommended minimum thresholds for maintaining the evolutionary potential of populations (Allendorf and Ryman 2002; Gilpin and Soule 1986). Hence, aggregating the results of both modelling processes suggest that at least 100 individual founder animals are needed to maximise viability and retention of allelic diversity, a comparable figure to other taxa (e.g. 120 in frogs (Easton et al. 2019), 60 to 120 in passerines (Tracy et al. 2011)). Further, by simulating the effects of periodic drought on population growth and survival, we have provided predictions about when best to translocate. For example, if harvesting were to occur immediately after a drought, we predict a 10–22% reduction in the census size of the source population after 50 years, relative to harvesting when populations are at their peak. In turn, we predicted that 140 founders would be required to retain 95% of alleles with a population frequency of 0.05 in a new population under drought conditions (as opposed to 80 founders under no drought); a 75% increase in the number harvested from the critical source populations. Therefore, climatic cycles appear to be an important consideration in translocation programs, and we advise that the movement of animals should occur outside periods of low rainfall and when populations have recovered demographically from their impacts.

Impact of the research

Results from this research have already been taken up by the "Dirk Hartog Island: Return to 1616" translocation program managed by DBCA as well as integrated into translocations to AWC's managed eco-sanctuaries such as Mt. Gibson. Both the data generated and models developed here will be used in future translocations and can be further refined and validated by incorporating additional empirical data from ongoing population monitoring and making comparisons against model predictions.

Broader implications

As the remnant range of *L. fasciatus* consists of just two adjacent offshore islands, the species-level impact of detrimental stochastic events affecting one or both islands could be profound. Establishing insurance populations and restoring the species to other suitable habitats free from introduced predators is a fundamental management objective, as is the retention of remnant genetic diversity to maintain adaptive capacity. Here we provide evidence that suggests both remnant island populations underwent genetic bottlenecks in the mid to late 1990s, potentially linked to the boom and bust cycles of *L. fasciatus* that occur in response to periodic droughts and thus a marked reduction in primary productivity (Chapman et al. 2015; Short et al. 1997). Further, we show that impact of past conservation actions (captive breeding and translocations) have possibly manifested in different ways in the two surviving translocated populations (Faure Island and Wadderin). Based on the limited samples available, Faure Island may be inbred due to non-random breeding but has relatively high allelic diversity, whereas Wadderin does not show signs of genetic inbreeding but may have passed through two selectively stronger bottlenecks (Bernier Island to Peron CBC, and Peron CBC to Wadderin). Our population viability and genetic modelling has revealed the importance of considering periodic fluctuations in population size when planning translocations and informed on the number of founders needed to avoid genetic bottlenecks. Taken together, these lines of evidence point to a critical need for both genetic and demographic guidance in future translocations of other threatened marsupials across arid Australia that endure similar pressures.

Future research priorities

Ongoing monitoring of translocated populations should be a priority. First, continued monitoring of both established translocated populations at Faure Island and Wadderin sanctuary to determine if there is ongoing recruitment and growth. As we provide evidence for genetic consequences of their demographic histories, genetic monitoring to determine if inbreeding is occurring and formally investigating whether there is inbreeding depression would be very useful. Second, the ongoing and regular monitoring of the newly translocated populations at Dirk Hartog Island and Mt Gibson. This would confirm whether these new populations have successfully established and are maintaining genetic diversity. This would also allow a comparison of population performance to expectations under the models developed here, providing a timely validation of the PVA approach to conservation management. Finally, to mitigate for the possibility of translocations having to occur during drought periods, the likelihood of which increases with increasing drought frequency, the benefits of *in situ* tools for assisting population growth such as water or feeding stations could be explored.

Data sets

Microsatellite PCR multiplex reactions, microsatellite genotype data and PVA models are available upon request.

Recommendations

We recommend that:

- Ideally 120 founder individuals (and no fewer than 100) are used to establish new *L. fasciatus* populations with high survivorship and genetic diversity
- Ideally this would include 60 individuals from each of the remaining remnant island populations, Bernier Island and Dorre Island
- Faure Island could be used as a surrogate for Bernier Island, thereby relieving demographic pressure on this source population, but only after projections of the impact of harvesting be modelled using the resource developed here
- Harvests should not occur during periods of drought or recovery from recent droughts – particularly if source populations have census population sizes less than 500
- Ongoing monitoring is done on all translocated populations to confirm whether there is recruitment and growth, that genetic diversity is maintained and inbreeding depression avoided
- Monitoring data is incoporated into PVA at regular intervals to evaluate translocation outcomes against model predictions
- To bolster genetic diversity in Faure Island and Wadderin populations, supplementation should come from Dorre Island and impacts on target and source populations could be projected using the tools developed here

Conclusion

Our work demonstrates how an integrated analysis of genetics and population modelling can be used to inform management planning by simulating sensitive trade-offs involved in translocating threatened species. While much work has been done on the numbers of founders needed to establish new populations, less is known about the impact of harvesting for translocations on source populations (Easton et al. 2019), which is highly relevant for species with few remnant populations remaining. Our results reveal the importance of founder number to successful *L. fasciatus* translocations. We suggest that the optimal translocation protocol is to mix the Bernier and Dorre Island populations in the same year, harvesting 60 individuals from each island source population (total n = 120). This gives a good probability of retaining low-frequency private alleles from each island, maximises survival probability and heterozygosity of translocated populations, limits logistical cost and, crucially, has no major impact on the source populations. As the translocations currently underway to Dirk Hartog Island and Mt Gibson approximate these recommendations, it will be valuable to monitor these populations over time to compare performance to model predictions. It is also worth considering Faure Island as an alternative source population to Bernier Island for future translocations, as was done with the Mt Gibson translocation, to relieve pressure on one of the two remnant wild populations. However, in addition to possible inbreeding, our modelling shows Faure Island is more sensitive to stochastic events due to a smaller census population further compounded if its carrying capacity is less than Bernier and Dorre Islands, and so the PVA models developed here should be used to assess the impact of any future harvesting. Wherever feasible, and after consideration of potential over-harvesting, future supplementations of the established translocated populations on Faure Island and Wadderin Sanctuary should derive from Dorre Island which, as a genetic mixing event, would be expected to increase gene diversity of the recipient population. Further, Dorre Island has a higher heterozygosity, more private alleles and lower inbreeding than Bernier Island. Finally, while we show that *L. fasciatus* seems resilient to harvesting and is well suited to translocation programs, including multiple harvests from source populations, regular droughts and limited carrying capacity have a substantial impact on population viability, particularly for populations with census sizes less than 500. We recommend that translocations are avoided during extended periods of drought and subsequent demographic recovery, due to a notably lower growth rate of the source populations after harvesting, and reduced capacity of new populations to retain allelic diversity.

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Ethics statement

Samples used in this study have been collected under appropriate ethics approvals and under the guidance of state government conservation agencies.

Release of two hare-wallabies. Image: Richard Manning

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Scientist Keith Morris releasing hare-wallaby. Image: Claudia Buters

Appendices

Table S1. PCR multiplexes for *Lagostrophus fasciatus*.

Multiplex	Primer	Fluorescent label	Size Range
1	Pa593	FAM	93-107
1	Me14	NED	155-159
1	Y105	FAM	219-235
2	Pa385	FAM	155
2	Pa297	VIC	115-119
2	Y175	VIC	268-274
2	Pa55	PET	145
3	Y151	FAM	142
3	Me17	VIC	99-101
3	Y148	NED	154-160

Table S2. Parameters used for modelling rare allele retention using ALLELERETAIN in non-drought years. Where a parameter is not listed, defaults are used. Parameter values were obtained from data in Short et al. (1997), Richards et al. (2001) and the knowledge of Colleen Sims and Jeff Short. *A banded hare wallaby year is assumed to be 9 months.

Parameter	Value
Mating system	polygyny
Maximum lifespan	13 BHW years*
Age at maturity	1 BHW year
Mean young per adult female per BHW year	1
Mean young per young female per BHW year	1
SD annual young per adult female	0.3
Maximum young per adult female per breeding period (months)	1
Male breeding ages	2-11 BHW years (1.5-8 calendar years)
Mean male lifetime reproductive success (MLRS)	7
SD MLRS	3
Juvenile survival	0.6
Juvenile survival at K	0.6
Nonbreeder annual survival	0.9
Nonbreeder annual survival at K	0.9
Adult female annual survival	0.9
Adult male annual survival	0.9
Carrying capacity (K)	10000
Number of years pop held at or below initial size	1
Initial sex ratio	0.5

Table S3. Parameters used for population viability analyses of banded hare wallaby. Additional parameters were extracted from [3] and [4]. BHW: banded hare wallaby; DDR: density dependent reproduction function.

Vortex Parameter	Value
Scenario settings	
No. iterations	1000
No. "years"	50 years
Duration of each "year" in days	274
Extinction definition	only 1 sex remains
Number of populations	5 (Bernier, Dorre, Faure, DHI, Mt. Gibson)
Species description	
Inbreeding depression	
- Lethal equivalents	3.14
- % due to recessive lethal	50
EV concordance of reproduction & survival	
- EV correlation among populations	0.5 (default)
Catastrophes	
- Number of types of catastrophes	1 (drought)
- Frequency	12% (1 in 6.25 calendar years)
- Severity	50% reduction in survival and reproduction
Reproductive system	
Reproductive system	polygynous
Age of first offspring for Females/Males	9 months/18 months
Maximum age of reproduction	8 years
Maximum lifespan	10 years
Maximum number of broods/year	1
Maximum number of progeny per brood	1
Sex ratio at birth	50
Reproductive rates	
% Adult females breeding	90% with DDR
- EV in % breeding	18
Distribution of broods per year	
- 0 broods	0
- 1 broods	100
Number of offspring per female brood	
- 1 offspring	100
Mortality rates	
Females	
Mortality Age 0 to 1 (± SD)	40 ± 10
Annual mortality after Age 1 (± SD)	10 ± 3
Males	
Mortality Age 0 to 1 (± SD)	40 ± 10
Annual mortality after Age 1 (± SD)	10 ± 3

Vortex Parameter	Value
Mate monopolisation	
% males in breeding pool	85
% males successfully siring offspring	63
Initial population size	
Bernier	2000
Dorre	2000
Faure	300
Carrying capacity, K (SD due to EV)	
Bernier	3000 (300)
Dorre	3000 (300)
Faure	1000 (100)
DHI	1000 (100)
Mt. Gibson	500 (50)
Genetic management	
Number of neutral loci to be modelled	7 empirical, 1 simulated
Read starting allele frequencies from file	yes

Table S4. Comparing allelic richness with and without marker Me17.

Population		N		AR with marker Me17 (±s.e.)	AR without marker Me17 (±s.e.)
Bernier Island (all)		51		2.47 (0.15)	2.71 (0.13)
Bernier (1998)		6			
Bernier (2010-2011)		9			
Bernier (2016-2017)		33			
Dorre Island (all)		79		2.46 (0.10)	2.70 (0.08)
Dorre (1995-1996)		7			
Dorre (1999-2000)		8			
Dorre (2013)		11			
Dorre (2016-2017)		52			
Faure Island (2017)		10		2.57 (0.40)	2.67 (0.43)
Wadderin (2018)		17		2.27 (0.27)	2.48 (0.25)
Peron CBC (2006-2013)		73		2.41 (0.15)	2.65 (0.15)
Dryandra (1999-2002)		6		2.57 (0.52)	2.83 (0.48)
	Σ	236	μ (±s.d.)	2.34 (0.13)	2.67 (0.11)

N: number of samples; AR: allelic richness (rarefied to 10 individuals except Dryandra).

Table S5. Performance of the seven polymorphic microsatellite markers for *Lagostrophus fasciatus*.

Marker Name	NA	HE	HWE*
Pa593	8	0.656	1/6
Me14	3	0.414	0/6
Y105	5	0.509	0/6
Pa297	3	0.145	1/3
Y175	2	0.460	3/6
Me17	2	0.030	0/1
Y148	4	0.463	0/6
Mean (± s.d.)	3.86 (± 2.12)	0.382 (± 0.218)	

NA: total number of alleles per marker; HE: expected heterozygosity; HWE: Hardy-Weinberg Equilibrium. *No. populations in which marker is polymorphic and HWE violated at $p < 0.05$.

Table S6. Summary of PVA performance metrics after 50 calendar years for various translocation scenarios including a drought with probability of 12% (or one every 6.25 years). Metrics include survival probabilities of the translocation and source populations, and final average population size, genetic diversity and inbreeding coefficient of the translocated populations.

a. Scenario 1. To Dirk Hartog Island from Bernier Island in one year.

Metric	Starting N				
	60	80	100	120	140
Prob Surv DHI	0.66	0.73	0.79	0.83	0.85
Final N DHI	1653	2082	2499	2628	2892
GD DHI	0.316	0.325	0.330	0.336	0.338
Inbreeding DHI	0.677	0.671	0.665	0.659	0.658
Prob Surv Bernier	0.99	0.99	1.00	0.99	0.99

b. Scenario 2. To Dirk Hartog Island from Bernier Island over two years, with half in each year.

Metric	Starting N				
	60	80	100	120	140
Prob Surv DHI	0.66	0.72	0.79	0.82	0.83
Final N DHI	1714	2133	2489	2477	2819
GD DHI	0.312	0.325	0.330	0.333	0.337
Inbreeding DHI	0.681	0.671	0.665	0.663	0.658
Prob Surv Bernier	1.00	1.00	0.99	1.00	1.00

c. Scenario 3. To Dirk Hartog Island over two years, with half in year one from Bernier and half in year two from Dorre.

Metric	Starting N				
	60	80	100	120	140
Prob Surv DHI	0.65	0.74	0.80	0.82	0.85
Final N DHI	1726	1935	2251	2383	2789
GD DHI	0.353	0.358	0.368	0.368	0.374
Inbreeding DHI	0.642	0.637	0.627	0.627	0.623
Prob Surv Dorre	0.99	0.99	0.99	1.00	0.99
Prob Surv Bernier	0.99	0.99	0.99	0.99	0.99

d. Scenario 4. To Dirk Hartog Island in one year, with half from Bernier and half from Dorre.

Metric	Starting N				
	60	80	100	120	140
Prob Surv DHI	0.66	0.71	0.79	0.82	0.83
Final N DHI	1622	2029	2277	2459	2684
GD DHI	0.349	0.359	0.362	0.366	0.374
Inbreeding DHI	0.644	0.635	0.632	0.629	0.622
Prob Surv Dorre	0.99	0.99	0.99	0.99	1.00
Prob Surv Bernier	0.99	1.00	0.99	0.99	0.99

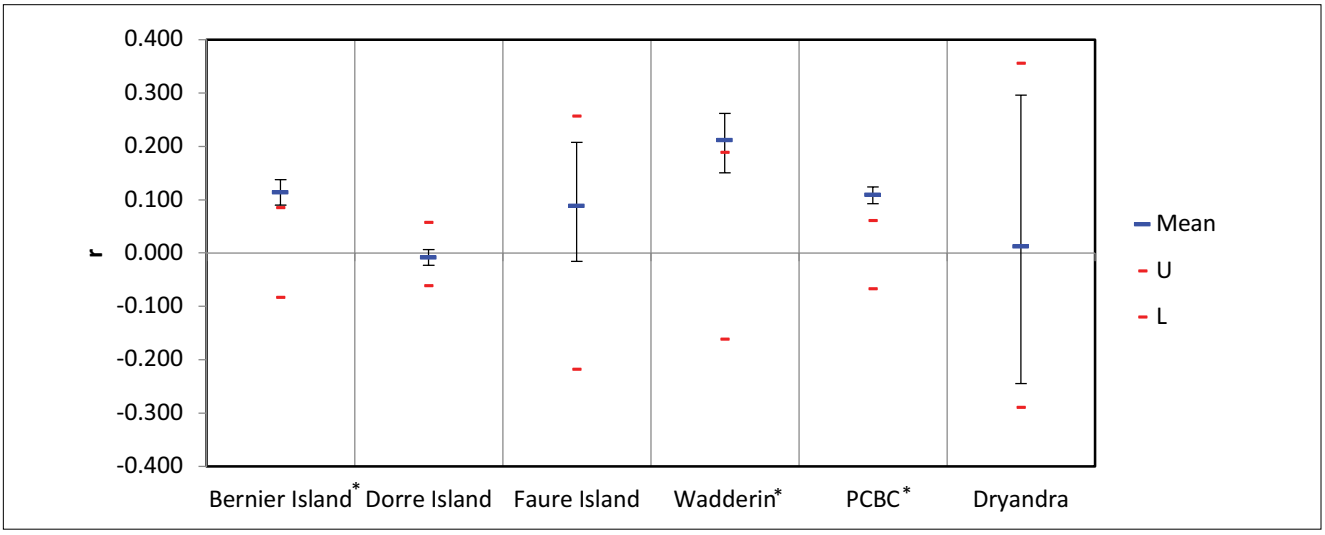

Figure S1. Mean pairwise relatedness using the Queller and Goodnight estimator within two remnant wild populations of (Bernier and Dorre Islands), two contemporary translocated populations (Faure Island and Wadderin), and two historic translocated populations (Peron CBC and Dryandra). *statistically significant p values (Bernier = 0.005, Wadderin = 0.014, Peron CBC = 0.002)

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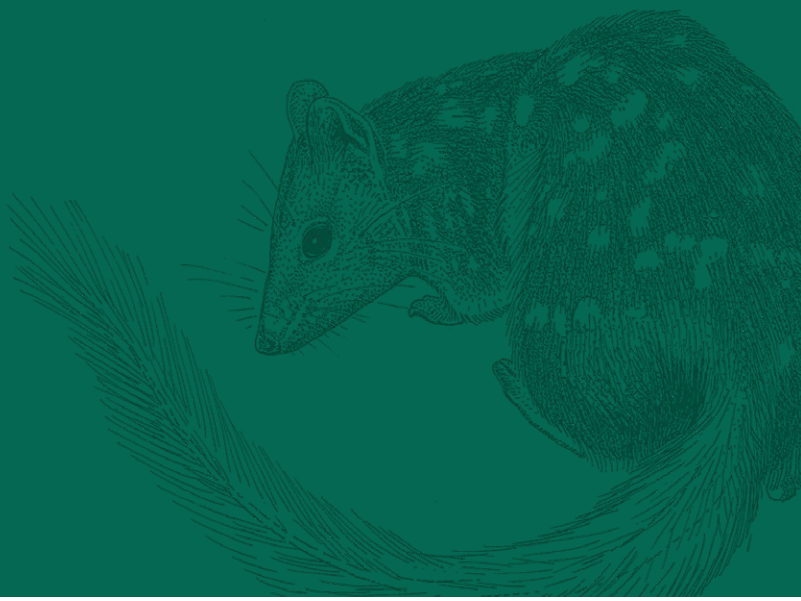
Sample data and population models are available from DBCA on request.

Further information:
http://www.nespthreatenedspecies.edu.au



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