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Article

Augmenting the conservation value of rehabilitated wildlife by integrating genetics and population modeling in the post-rehabilitation decision process

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Abstract

Insular populations are particularly vulnerable to the effects of stochastic events, epidemics, and loss of genetic diversity due to inbreeding and genetic drift. The development of successful management options will require accurate baseline data, establishment of clear objectives, and finally monitoring and implementation of corrective measures, if and when required. This study assessed management options for the genetic rehabilitation of highly inbred woylies obtained from wildlife rehabilitation centers. The study generated genetic data for the woylie *Bettongia penicillata* from a conservation reserve and calculated measures of genetic diversity and individual relatedness. These data were fed into a population viability analysis (PVA) to test genetic outcomes in relation to different management actions. We demonstrated that a careful selection of the founder cohort produced a population with an expected heterozygosity of ~70% for a window of approximately 10 years. A proposal to increase the size of the reserve available to the colony was shown to almost double the time at which the colony would retain heterozygosity levels of ≥70%. Additionally, developing a regular program of supplementation of unrelated woylies would result in a further improvement in their genetic value. This study demonstrated how the application of molecular techniques in combination with PVA can be beneficial for the management of rehabilitated wildlife otherwise considered of little conservation value. This approach can be applied to the management of breeding programs, but also to small, closed populations such as those found on islands, fenced enclosures, insurance populations, and in zoological collections.

Key words: *Bettongia penicillata*, conservation genetics, microsatellite loci, rescue centres, Vortex, vortexR.

The ultimate goal of the wildlife rehabilitation is to release individuals back into the wild in order to mitigate the effects of declining wild populations (Conway 2011). There are a number of factors that contribute to preventing successful reintroductions, including concerns about the genetic suitability of the rehabilitated individuals. For example, when the origin of animals is unknown, it may be difficult to identify a suitable recipient population for their release. Specific local adaptations can be advantageous for resident animals (Edmands 1999; Tallmon et al. 2004), while rehabilitated, introduced individuals from different locations may lack these specific physical or physiological characteristics that would increase their chances of survival or successful breeding at the relocation site. In

other instances, when a small number of rehabilitated animals interbreed within the rehabilitation center facilities, the concern may be related to the level of inbreeding. Inbred animals can have a reduced fitness and deleterious alleles are expressed in homozygous form (Frankham 1995). The situation may be further compromised when proper records are unavailable. In this case, identifying wild-caught individuals from inbred offspring may be a challenge. While alternative post-rehabilitation pathways do exist (e.g. display for education purposes only) these are suboptimal, especially when the species in question is a critically endangered taxon.

Population genetic tools, in combination with population viability analysis (PVA), can provide the means to redirect these "genetically compromised" animals toward immediate wildlife conservation actions. An additional situation where the post-rehabilitation decision process can be helped by these techniques is when rehabilitated animals cannot be moved back into the wild because of logistical or other conservation-related reasons, for example, when a disease outbreak has caused a total halt of animal movements (Hollings et al. 2013). In these situations, the individuals that are in rehabilitation facilities need to be managed in order to maximize their chances (or their offspring's chances) of being released back into the wild when the opportunity presents itself. As opposed to captive breeding facilities, where breeding individuals are carefully selected and reproduction is closely monitored and managed, rehabilitation centers typically have no or little control over which animals are admitted and often lack the facilities and expertise to manage breeding to maintain or increase the genetic value of their offspring.

In this study, we demonstrate how the application of population genetics in combination with PVA has enabled the management of rehabilitated woylies *Bettongia penicillata*, to maximize the conservation outcome of individuals otherwise considered of little conservation value. While there are examples in the literature using similar tools (e.g. Russello et al. 2007; Ivy et al. 2009; Ottewell et al. 2014), this study is unique because we show how in highly inbred individuals from unknown locations and over time augment genetic diversity of previously unmanaged captive populations.

The woylie is a small Australian marsupial that has undergone a dramatic decline. It is currently classified as Critically Endangered (Wayne et al. 2008; Wayne et al. 2013) because the species went from more than 200,000 individuals in 1999 to around 15,000 in 2010 (Wayne et al. 2013). Although never identified, a disease outbreak was suspected to be either directly or indirectly responsible for the decline (Wayne et al. 2015). As a result of a perceived pandemic, all woylie translocations were halted for several years, including the release of rehabilitated animals into the wild. During this time, animals held in rehabilitation facilities (either within rehabilitation centers or with single, licensed wildlife rehabilitators) produced offspring. In most cases, there were no good records of the parental contributors (e.g. identification of founders and offspring). Government authorities deemed these captive colonies unsuitable for release due to the high likelihood of inbreeding.

Previous genetic work identified four genetically distinct wild woylie populations in Western Australia: Dryandra, Perup, Kingston, and Tutanning (Figure 1) (Pacioni et al. 2011). However, the separation of these populations is likely to be the result of recent habitat fragmentation. In fact, analysis of historical samples confirmed that all current Australian wild woylie populations belong to the same evolutionarily significant unit (Pacioni et al. 2015). During the late 1990s several translocated populations were established as part of the woylie management plan, including Karakamia, a fenced wildlife sanctuary near Perth, Dwellingup, in the northern Jarrah forest, and Batalling, in the Batalling state forest, ~70 km east of Bunbury. Karakamia and Dwellingup were established using individuals from Dryandra, while Batalling founders were sourced from Perup (Pacioni et al. 2013).

In 2010, a decision was made to establish a woylie colony at a feral predator-free enclosed area at Whiteman Park, a conservation reserve north-east of Perth, Western Australia. In this study we focused on identifying the best management actions for this newly established woylie colony at Whiteman Park that received rehabilitated woylies from Chidlow Marsupial Hospital (Chidlow, Western Australia) and from a wildlife carer in Wellard (Western Australia). The woylies at the Chidlow Marsupial Hospital were believed to have been sourced from Karakamia Sanctuary, while the origin of the animals from the wildlife carer in Wellard were of unclear origin, but were likely from Dwellingup (a translocated population from Dryandra) or Batalling (a translocated population from Perup) stock (Figure 2).

Small populations are exposed to a higher rate of genetic drift and therefore, genetic diversity of enclosed (small) populations should be closely monitored and managed. Management actions should ideally aim to maintain genetic diversity at similar levels to wild populations. This aim was going to be a challenge for Whiteman Park managers given the highly inbred cohort they had available to establish the woylie colony. However, not meeting this target would mean that this colony would have very little (if any) conservation value other than serving as education display. Therefore, restoring genetic diversity to the newly established colony was deemed to be a high management priority. The specific aims of this study were to:

1. assess the genetic diversity and relatedness between individual pairs of the population established at Whiteman Park and quantify their difference from naturally occurring populations;
2. identify founders' populations of origin (if wild caught) or their ancestors (if bred in captivity);
3. evaluate long-term effect of different management actions aimed to maintain or improve current levels of genetic diversity and limit genetic drift.

Materials and Methods

Individuals from the rehabilitation center and the wildlife carer (Chidlow and Wellard) were managed in Whiteman Park as 2 different stocks: animals from Chidlow ($n = 21$) were housed in a "soft-release" enclosure (~1 ha) and animals from Wellard ($n = 11$) in a Woodland Reserve (~52 ha). We conducted an assessment of the colony's genetic diversity, identified the source population through assignment tests, estimated the relatedness between individuals and conducted a simulation study to evaluate management options as described below. Individuals were identified using a microchip implant and ear tags. Blood samples from 30 animals were collected in commercial EDTA tubes as part of a health screening. DNA was extracted using QIAGEN kit following manufacturer's instructions, using 100 μL of whole blood. Woylies were genotyped at 12 microsatellite loci (summarized in Supplementary Appendix 1) following protocols described in Pacioni and Spencer (2010). Reference samples from previously genotyped wild populations (Pacioni et al. 2011; Pacioni et al. 2013) were included to ensure consistency of allele scoring.

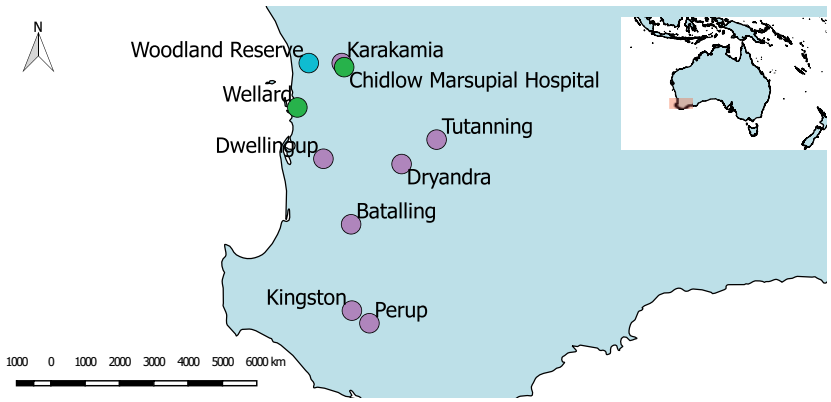

Figure 1. Map of woylie population locations. In cyan, the Woodland Reserve at Whiteman Park. In green, the Chidlow Marsupial Hospital and Wellard. In purple other woylie populations.

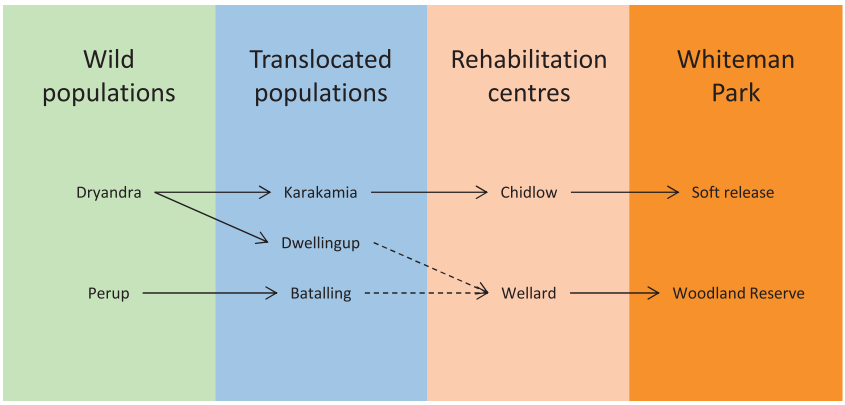

Figure 2. Origin of the woylies (*Bettongia penicillata*) relocated to Whiteman Park. Dashed arrows indicate unconfirmed origins as reported by the rehabilitation center(later to be confirmed as Dwellingup by the genetic analysis).

Genetic diversity

Descriptive measures of population genetic diversity were all calculated using GENALEX 6.4 (Peakall and Smouse 2006) and included estimates of genetic diversity within populations: observed (H_o) and expected heterozygosity (H_E); and mean number of observed (N_A), expected (N_E) and private alleles (PA). In order to further enable the comparison of the genetic variability among populations, we calculated the mean allelic richness (N_{AR}) based on 14 diploid individuals using the rarefaction method implemented in HP-RARE (Kalinowski, 2005), which compensated for differences in sample size producing unbiased estimates of allelic richness.

Population assignment tests

Data for indigenous and translocated populations were obtained from Pacioni et al. (2013; 2011) and used to identify the source populations of the Chidlow and Wellard stock by performing a Bayesian assignment test using the program STRUCTURE 2.2 (Pritchard et al. 2000). Analysis of the data was carried out with the admixture model with correlated allele frequencies (Pritchard et al. 2000). Information on the geographic origin was included for the reference data in the prior probability (i.e. potential source populations), while it was ignored for the individuals that had to be tested (i.e. woylies at Whiteman Park). STRUCTURE results were based on 10 independent runs, using a "burn-in" period of 10,000 iterations followed by 100,000 iterations of a Markov Chain MonteCarlo. Population differentiation was estimated by calculating $G"_{ST}$ (Meirmans and Hedrick 2011) in GENALEX 6.4 (Peakall and Smouse 2006) using 1,000 permutations to test significant difference from zero. We also reported Nei's F_{ST} (Nei, 1973), calculated under the AMOVA (analysis of molecular variance) framework for comparative purposes as recommended by Meirmans and Hedrick (2011).

Parentage and relatedness analyses

Two independent analyses were undertaken in order to examine woylie relatedness at Whiteman Park. First, Queller and Goodnight's (1989) pairwise relatedness (r) was calculated for each pair. The within-group mean (QGM) was then calculated along with 95% confidence interval via bootstrapping (1,000 iterations) and compared with the null hypothesis of no relatedness among individuals within groups by testing 1,000 random permutation of the dataset. As a number of offspring from the colony established in the soft-release enclosure were already sampled, parentage testing was only carried out for this colony, using CERVUS (Kalinowski et al. 2007). Allele frequencies for analysis were calculated and adjusted for null allele, after merging the dataset of the soft-release enclosure genotypes with the data of the source population (as identified by STRUCTURE). Additional details of CERVUS analysis are reported in Appendix 2.

Population modeling

A modification of the baseline PVA model developed by Pacioni (2017b) was implemented in VORTEX v9.99 b and used to test

possible management actions. Based on expert opinion (Chris Rafferty and Manda Page, pers. comm.), carrying capacity was set to 15 woylies for the soft-release enclosure (note that Whiteman Park supplements food to woylies in this enclosure) and 70 woylies for Woodland Reserve. Whiteman Park plans to expand the Woodland Reserve by a further 108 ha (giving a total of ~160 ha with an estimated carrying capacity of 200 woylies). Allele frequencies data for the first 9 of the 12 neutral microsatellite loci described above were monitored during the simulations. Two possible management scenarios that would have been implemented without the supportive information generated from this genetic study were investigated with this PVA model. A third scenario represents possible management actions that make use of the genetic data available from this study.

Scenario 1. *The two sub-units (Chidlow, n = 17, and Wellard, n = 11) are managed as separate colonies, i.e. the soft-release and Woodland enclosure, respectively.* For Wellard stock only, the effect of changes in carrying capacity (K) to reflect the hypothesis that woylies may use the whole reserve after the first year (i.e. the area would increase from 52 ha to 160 ha) is also included in the model.

Scenario 2. *Surplus from Chidlow (i.e. the proportion of the population above the carrying capacity of the soft-release enclosure) is moved, once a year, into the Woodland Reserve colony*. More specifically, based on previous trapping data, we predict that ~6 woylies a year (with a male sex bias of 3:2) would be relocated to Woodland Reserve from the soft-release enclosure once the size of the colony reaches 15 individuals.

Scenario 3. *In order to include all individuals with the highest heterozygosity (n = 16) and limit the co-presence of highly related individuals the founding cohort in the Woodland Reserve colony is selected using genetic data*. The effect of changes in K to reflect the hypothesis that woylies may use the whole reserve after the first year (i.e. the area would increase from 52 ha to 160 ha) is also taken into consideration.

We also investigated whether implementing seasonal breeding—as observed in Karakamia Sanctuary (Ward et al. 2008) as opposed to continuous breeding implemented in Pacioni's et al. (2017b) baseline model—in Scenario 3 would cause any significant difference in the projected trajectories. This was achieved by using the following formula as reproductive rate:

$$\left\{\frac{\left[89.3-(89.3-57)\times\left(\frac{N}{K}\right)^{16}\right]\times\left(\frac{N}{0.1+N}\right)-4}{2}+4\right\}+\frac{\left[89.3-(89.3-57)\times\left(\frac{N}{K}\right)^{16}\right]\times\left(\frac{N}{0.1+N}\right)-4}{2}\times\frac{sin(\pi\times Y)}{2}$$

where 83.9 and 57 represent the breeding rate at low and high density, respectively (see Pacioni et al. 2017b for details); N = population size; K = carrying capacity; 4 is the maximum percentage of breeding females in the non-breeding season (Ward et al. 2008); *sin* = sine and Y is the time-unit of the simulation. Note that in Pacioni et al. (2017b) model, the reproductive rate is density dependent. This feature was maintained in the present study.

Lastly, we also modeled the supplementation of the colony in Woodland Reserve with 10 woylies (6 males and 4 females, randomly chosen) from wild populations (either indigenous or translocated) in Scenarios 2 and 3 to reflect the hypothesis that the colony would receive additional individuals from other rehabilitation centers. It has to be stressed that the so obtained scenario is a particularly optimistic situation. The model assumes that supplemented animals are unrelated, and they can have any possible combination of alleles found in the wild populations (proportionally to their frequencies). These conditions may not be met. However, it is not possible to predict in advance what genetic stock will be in care of rehabilitation centers in the future and this scenario has to be considered for demonstrative purpose only. Additionally, the model also assumes that supplement survival is not reduced compared to "resident" woylies and they will successfully integrate in the breeding pool.

Projections of two genetic parameters, H_E and N_A, were evaluated. Pairwise statistical comparisons between scenarios of similar conditions (e.g. same carrying capacity) were carried out calculating the strictly standardized mean difference (SSMD, Zhang, 2007) between two scenarios considering 2 time horizons: 5 years and 100 years since the beginning of the simulations. To evaluate whether a general trend was detectable, we ranked each scenario based on its strictly standardized mean difference for each genetic parameters and each time horizon using the non-action scenario (Scenario 1) as a reference and then tested for concordance of ranking using the Kendall's coefficient of concordance W. The probability of extinction was calculated as the proportion of iterations where the population went extinct over the total number of iterations run. All statistical analysis and plots were conducted using vortexR (Pacioni and Mayer 2017a) in R 3.1.0 (R Core Team, 2016).

Results

Genetic assessment

Each enclosure had a relatively low genetic variability (Table 1). Individual heterozygosity ranged from 27.3% to 91.7% (Supplementary Appendix 3), suggesting a high level of inbreeding in some individuals. The selection of breeders based on the genetic data generated from this study (Scenario 3) proved to be an advantageous strategy. The starting expected heterozygosity and average numbers of alleles of this colony would be around 73.4% (95% confidence interval 73.1–73.7%) and 6 (95% confidence interval 5.96–6.04), respectively. An improvement of ~20% and ~50% of the two parameters (H_E and N_A) compared with either of the existing colonies ($P \leq 0.001$).

The source populations were identified with high level of confidence (Supplementary Appendix 3). In fact, the minimum probability at which an individual was assigned to a population was 94%. All woylies from Chidlow (soft release) were identified as sourced from Karakamia (Dryandra stock), while all individuals from Wellard (Woodland Reserve) were sourced from Dryandra. It should be noted that the genetic profile of the population at Dwellingup was not available for analysis. However, since woylies in the northern Jarrah forest (Dwellingup) were originally sourced from Dryandra, it would be expected that the latter is the most similar population to the source population of Wellard stock (if actually from Dwellingup rather than Batalling). Therefore, it is inferred that "Wellard" individuals were actually sourced from Dwellingup, but the analysis assigned them to the most similar (available) population. The Bayesian assignment results were also supported by the overall low differentiation (calculated as G''_{ST}) of Whiteman Park woylies from the two source populations (Table 2).

On average, the relatedness of woylies within each colony was equivalent of half-sibs or above (i.e. QGM ≥ 0.25), confirming the expected high level of inbreeding ($P \leq 0.001$, Figure 3). In the soft release enclosure, the paternity analysis identified, with 95% confidence, the father of 12 of the 14 woylies that were born at

Table 1. Genetic diversity parameters from this study (bold) and natural or translocated woylie populations

Sampling locations	*n*	N_A (SE)	N_E (SE)	N_{AR} (SD)	H_E (SE) %	H_o (SE) %	PA (SE)
Whiteman Park[t]	**28**	**6.5 (± 0.8)**	**4 (± 0.5)**	**5.8 (± 2.2)**	**70.5 (± 4)**	**68.4 (± 4)**	**0.1 (± 0.1)**
Chidlow MH[r]	**17**	**4.3 (± 0.5)**	**2.9 (± 0.3)**	**3 (± 0.8)**	**61.8 (± 4)**	**71.1 (± 6)**	**na**
Wellard[r]	**11**	**3.8 (± 0.4)**	**2.8 (± 0.2)**	**2.9 (± 0.8)**	**58.9 (± 6)**	**62.2 (± 7)**	**na**
Dryandra[a,n]	28	8.9 (± 0.9)	5.8 (± 0.7)	7.8 (± 2.3)	79.6 (± 3)	73.1 (± 5)	0.3 (± 0.2)
Karakamia[b, t]	29	7.5 (± 0.8)	4.9 (± 0.7)	6.7 (± 2.3)	74.5 (± 4)	66.1 (± 7)	0.2 (± 0.1)
Tutanning[a, n]	32	5.5 (± 0.6)	3.2 (± 0.3)	4.8 (± 1.5)	64 (± 5)	64.5 (± 8)	0.6 (± 0.3)
Kingston[a, n]	69	12.1 (± 1.4)	5.9 (± 0.6)	8.2 (± 2.5)	78.8 (± 4)	70.6 (± 6)	1.1 (± 0.4)
Perup[a, n]	102	15 (± 1.8)	7.6 (± 0.9)	9.7 (± 2.7)	83.6 (± 3)	74.6 (± 4)	1.7 (± 0.7)
Batalling[b, t]	35	7.3 (± 0.6)	4.1 (± 0.4)	6.4 (± 1.6)	72.1 (± 4)	71.7 (± 5)	0.2 (± 0.1)

n = number of individuals genotyped at microsatellite loci. N_A=average number of alleles. N_E=average effective number of alleles. N_{AR}=average allelic richness. H_o=observed heterozygosity. *PA* = average private alleles . *SE* = standard error. *SD* = standard deviation., [a] (Pacioni et al. 2011)., [b] (Pacioni et al. 2013)., [n] Natural population., [r] Rehabilitation center., [t] Translocated population.

Table 2. Pairwise G''_{ST} (above diagonal) and F_{ST} (below diagonal) values (all *P* values = 0.001) between this study (bold) and natural or translocated woylie populations

	Batalling	Dryandra	Karakamia	Tutanning	Perup	Kingston	Whiteman Park
Batalling[t]	–	0.530	0.578	0.642	0.318	0.429	**0.482**
Dryandra[n]	0.111	–	0.213	0.606	0.385	0.482	**0.321**
Karakamia[t]	0.130	0.046	–	0.651	0.506	0.518	**0.311**
Tutanning[n]	0.183	0.152	0.175	–	0.620	0.665	**0.721**
Perup[n]	0.065	0.061	0.096	0.137	–	0.316	**0.475**
Kingston[n]	0.096	0.089	0.109	0.164	0.056	–	**0.463**
Whiteman Park[t]	**0.131**	**0.074**	**0.084**	**0.214**	**0.094**	**0.104**	–

[n] Natural population., [t] Translocated population.

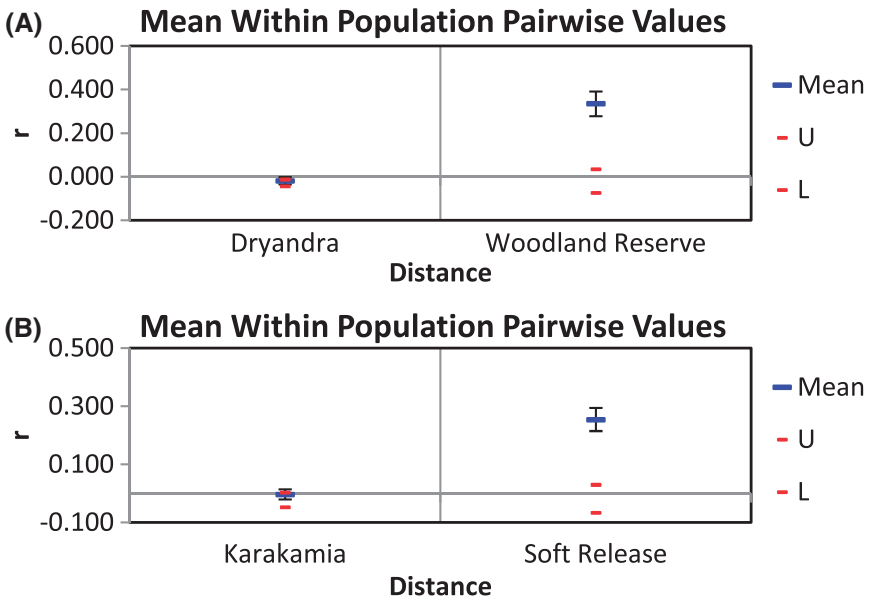

Figure 3. Mean pairwise Queller and Goodnight (with 95% confidence interval bars in black) within each Whiteman Park woylie colony and respective source populations. Red marks represent 95% confidence interval of the null hypothesis of no relatedness between two randomly chosen individuals calculated by performing 1,000 permutations.

Whiteman Park (Table 3). Of the 3 genotyped adult males, the data indicates that 1 male, supposedly dominant, sired 64% of the offspring, with the other two candidates successfully siring 21% and 7% of the offspring as expected given the polygamous mating system of this species.

Population modeling

None of the genetic diversity indices were statistically different ($P > 0.05$) when modeling the reproduction as seasonal or continuous (see Table 4 for a brief scenario summary). Therefore, these simulations are not discussed further.

Scenario 3 (the "genetic management" strategy) had higher H_E and N_A than Scenario 1 (the 2 colonies in Whiteman Park are managed separately).When the genetic management (Scenario 3) was associated with the planned expansion of the Woodland Reserve, this strategy was significantly more efficient in improving genetic diversity than Scenario 1 both in the short term (i.e. Year 5) and the long term (i.e. Year 100) (Figure 4, Supplementary Appendix 4). When compared with Scenario 2, Scenario 3 had overall higher mean values. However, only the heterozygosity at Year 5 was significantly improved when the selection of founders was associated with an extension of the reserve without implementing any supplementations (Figure 4, Supplementary Appendix 4).

When comparing the effect of an increased carrying capacity (i.e. reserve expansion) on genetic diversity parameters, increasing the carrying capacity generally resulted in a higher N_A and, depending on the scenario, in a higher H_E (Figure 4, Supplementary Appendix 4). Simulations that included supplementation had significantly higher H_E and N_A compared to simulations without supplementation. However, there were no significant differences between Scenario 3 and Scenario 2 when the supplementation program was implemented, except for the heterozygosity at Year 5 when carrying capacity was increased (Figure 4, Supplementary Appendix 4).

Table 3. Paternity analysis of offspring in the soft-release enclosure

Offspring ID	First parent non-exclusion probability (%)	Candidate father ID	Pair confidence
6B3C6A2	0.7	6B3E96C	*
6B3DA2B	0.7	6B3B6F4	*
6B3A0AF	0.2	6B3E4B4	*
6B3A0AF	0.2	6B3E96C	
6B3D2E3	0.1	6B3E96C	*
6B39FF3	0.7	6B3E96C	*
6B39264	0.3	6B3B6F4	*
6B3A317	0.1	6B3E96C	*
6B3A6B0	0.1	6B3E96C	*
6B3B485	0.2	6B3E96C	*
6B3BBBA	0.5	6B3B6F4	*
6B3E554	0.0		
6B3AF33	3.5	6B3E96C	*
6B39AD7	0.1	6B3E96C	*

*indicates 95% confidence interval.

We ranked all scenarios using the strictly standardized mean difference against Wellard projections from Scenario 1, with or without increase of the carrying capacity. A significant concordance between ranking was found ($P = 0.0001$ and $P = 0.001$, respectively). Simulations from Scenario 3 ranked higher than relative counterparts and simulations that included supplementation ranked consistently as the top four. None of the analysed scenarios had a probability of extinction (P_E) of more than 2%, except the soft-release colony under the Scenario 1 and Scenario 2 where P_E was equal or more than 50%.

Discussion

In this study, we evaluated how the use of population genetics in association with PVA can assist in the management of rehabilitated wildlife. We provided evidence that rehabilitated wildlife that is not immediately releasable due to genetic concerns can be part of a wider species conservation plan with adequate long-term management.

The 2 Whiteman Park colonies established from woylies that bred unmanaged in rehabilitation settings had, as expected, low genetic diversity, since only few animals were wild-caught and some of the founders of the colonies were offspring born in captivity. An H_E of ~70% is generally considered the minimum acceptable level in macropods (Pope et al. 2000) and the only modeled scenario where a woylie colony at Whiteman Park was above this threshold from early on in the simulations was in the Scenario 3. This demonstrates the value and the need for the genetic management of this colony. The identified levels of H_E were comparable to the lower end of those found in other wild populations (e.g. Tutanning or Batalling) and, broadly speaking, comparable to what is commonly found in other macropods (Pope et al. 2000). It should be stressed that previous translocations indicated that woylie mortality rates are extremely low when moved to fenced, predator-free areas. Consequently, the risk of losing genetically valuable individuals when creating the founding stock in Scenario 3 was considered minimal. Additionally, there was no interest in managing the genetic variability in the soft release enclosure, as the holding area was too small to maintain high genetic variability in the long term.

In the long term, the planned reserve expansion would slow down the rate at which genetic diversity is lost due to genetic drift, making it a very effective conservation strategy. N_A is the genetic index that is mostly influenced by this option. Most importantly, the

Table 4. Abbreviations of PVA scenarios and their brief description

Scenario	Description
Chidlow MH	Scenario 1 Chidlow stock
Wellard K70	Scenario 1 Wellard stock
Wellard incK(200)	Scenario 1 Wellard stock and K increases to 200 after the first year
Chid_Well_disp	Scenario 2
Chid_Well_disp incK(200)	Scenario 2 and K increases to 200 after the first year
Chid_Well_disp Suppl	Scenario 2 plus the supplementation of 10 individuals once a year
Chid_Well_disp Suppl incK(200)	Scenario 2 plus K increases to 200 after the first year and the supplementation of ten individuals once a year
Woodland Sel	Scenario 3 Selection of breeders in the Woodland Reserve
Woodland Sel incK(200)	Scenario 3 Selection of breeders in the Woodland Reserve. K increases to 200 after the first year
Woodland Sel Seas	As for Woodland Sel, but reproduction is seasonal
Woodland Sel Seas incK(200)	As for Woodland Sel, but reproduction is seasonal and K increases to 200 after the first year
Woodland Sel Suppl	As for Woodland Sel, but the colony is supplemented with 10 individuals once a year
Woodland Sel Suppl incK(200)	As for Woodland Sel, but the colony is supplemented with 10 individuals once a year and K increases to 200 after the first year

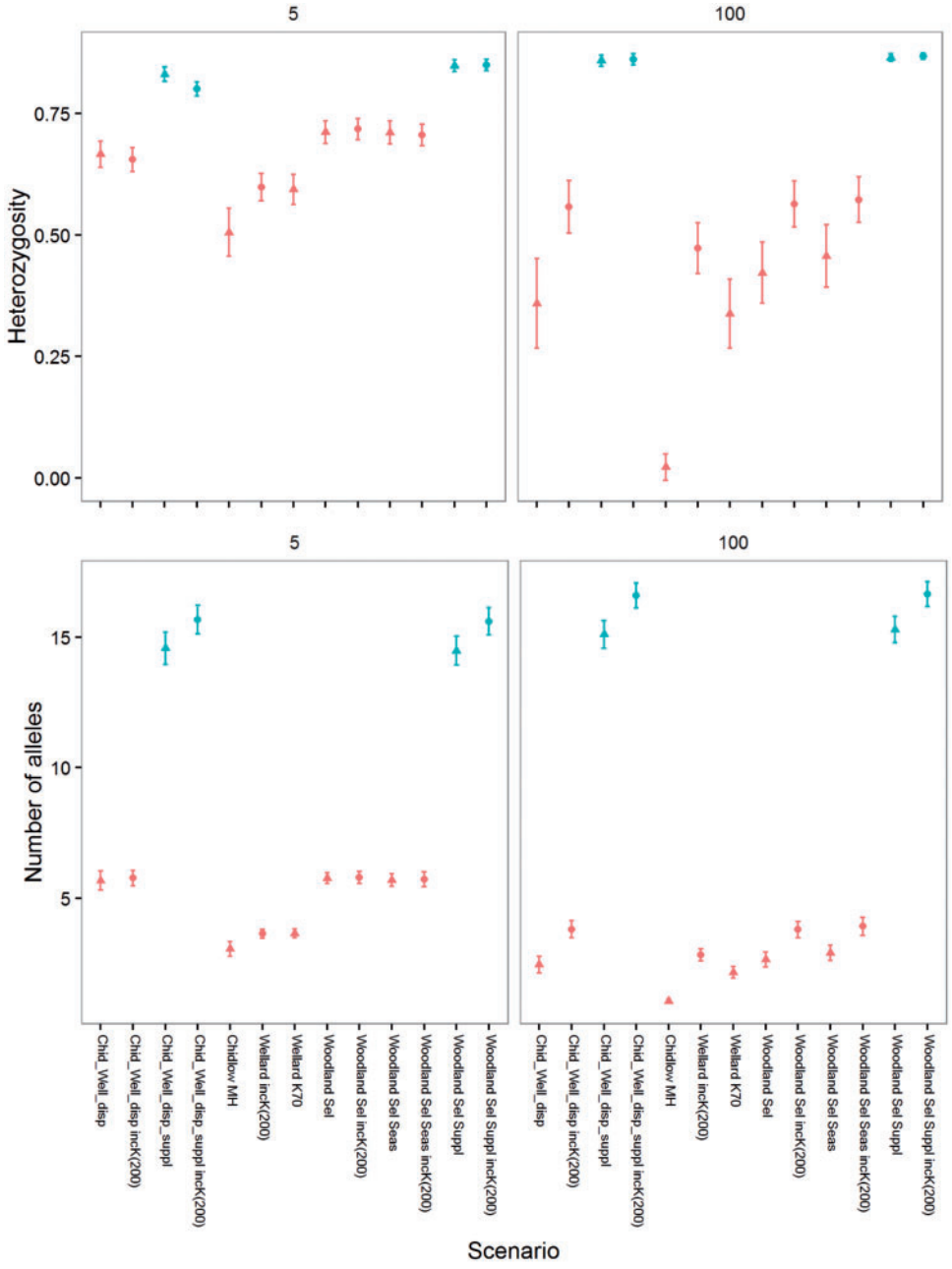

Figure 4. Dot plot showing mean and SD of genetic diversity of the woylie colony in the Woodland Reserve at Whiteman Park at year five (left plots) and Year 100 (right plots) in different PVA scenarios. In blue scenarios where supplementation is implemented. Scenarios with increase in the carrying capacity of the reserve are indicated with circles. Scenario names are listed in Table 4.

simulations demonstrated that supplementing 10 animals once a year is the management action that would substantially improve the genetic profile of the colony and counteract the effect of genetic drift in both short term (5 years) and long term (100 years). A combination of supplementation and increased carrying capacity would boost the genetic diversity of the Whiteman Park woylie colony to a level comparable to that of large wild populations (e.g. Perup). In this case, the role of the woylie colony at Whiteman Park would change from being an education display colony to a potential source population for other translocations, either to establish new populations or supplement existing wild and captive populations. Of course, the conservation value of the Whiteman Park woylie colony will be related to the supplementation with genetically valuable individuals, as well as on their successful settlement in the colony.

Scenario 2 offers similar benefits to Scenario 3, but the obtained information on the genetic makeup of the founding colony will allow managers to make informed, and therefore more targeted, decisions when supplementing animals from other sites. Without genetic management (Scenario 2) individuals with lower genetic and conservation value could utilize resources that could be made available to more valuable individuals (including possible external supplementations), while in Scenario 3 every supplementation is directed toward an increase in genetic variability. Additionally, although the difference was not statistically significant, Scenario 3

options ranked better than the Scenario 2 counterparts. Considering the lowering costs of genetic analyses and the clear advantages when managing inbred populations of unknown sources, we recommend Scenario 3 as optimal management action, and ideally in association with the supplementation of woylies from other sources.

Additionally, we recommend ongoing genetic monitoring to verify the modeled changes in the population. Possible concerns may arise if a small number of males dominate the reproductive output, the projected genetic diversity and fitness of the colony may be negatively affected. While logistic reasons prevent Whiteman Park from directly managing family groups (equalizing family sizes), other management options (e.g. removal of dominant males) can help achieve the targeted conservation goals.

The PVA model did not incorporate any stochastic events such as fire, introduced predators (fox or cat incursions), or epidemics. Consequently, genetic trends and the probability of extinction generated by the analyses depend only on the "natural" fluctuation of the survival, reproductive rates, and environmental variability of the carrying capacity (which were all generally modeled with a standard deviation of 10% of the mean value, see Pacioni et al. 2017b). Increased genetic variability, in association with a large population size, is considered protective toward stochastic events (Maschinski et al. 2013) and it is possible to formally test the effect of these stochastic incidents within a PVA should this be required. Regardless, adequate steps toward prevention from these events should be undertaken given the potential impact on the viability of the colony, due to their relatively small population size and captive environment.

We argue that our approach and methodology have general applicability when evaluating possible destinations for any species of rehabilitated wildlife. This information, in combination with already available frameworks (Frankham et al. 2011; Weeks et al. 2011) would be of great help to make informed management decisions. Similarly, our approach is suitable for the development of management plans for captive breeding colonies or zoological collections, where founders are generally assumed to be unrelated. However, we warn the readers that a number of factors played in "favour" of the woylie and would like to stress that by presenting the possible applications of genetic and population modeling techniques described in this article for rehabilitated wildlife, we by no means intend to promote uncontrolled breeding of wildlife in care. Previous studies had confirmed that all current Australian wild woylie populations belong to the same evolutionarily significant unit (Pacioni et al. 2011; Pacioni et al. 2015) and admixing of individuals from different sources would not be expected to pose an outbreeding depression risk for the colony. Historically woylies have had a wide distribution and therefore assumed to be highly adapted to different environments (from arid and semi-arid coastal and inland habitats to Mediterranean and temperate forests) and it is not expected that the genetic profile of individuals will influence their capacity to successfully settle in the colony at Whiteman Park or in future releases in the wild, should these being attempted. More than 3,400 woylies were translocated to more than 61 sites between 1977 and 2006 (Orell 2004; Groom 2010) and, regardless of the genetic stock used, inadequate feral predator control was the primary cause of failure in this species (Finlayson et al. 2010; Short et al. 1992).

In our study, the main purpose of the genetic analyses were to identify the founders' genetic makeup, in order to prioritise supplementations from different populations that would maximise genetic diversity at a species level as recommended by the woylie recovery team. This is in contrast with the most common situation where the population of origin is identified to minimize the risk of outbreeding depression. Additionally, adaptation to captivity (Frankham 2007) is a serious issue that needs to be considered and when possible the use of wild-caught individuals has been recommended as the preferred option when carrying out translocations (Pacioni et al. 2013). In our cases, most individuals have been kept in captivity for only 2–3 generations prior to being released in Woodland Reserve, while most of the concerns related to captivity adaptation are associated with long-term captive breeding (Frankham 2007).

Our recommendations rely on the assumption that the projected trajectories of our simulations are accurate and effectively predict the population responses to different management options. Future research will need to evaluate how reliable these conclusions are and inform management on possible needed adjustments in order to meet the established targets. The focus of future studies should aim to monitor genetic diversity over time, quantify uncertain simulation parameters, like supplement survival rates, to further improve accuracy of the models, and, critically, compare predictions with actual data to verify the appropriateness of the developed models for making management decisions.

To conclude, while wildlife captive breeding should always be planned and coordinated in consultation with conservation agencies and relevant authorities, and be accompanied by as accurate records as possible, we demonstrated that by using a combination of population genetic tools and population modeling rehabilitated animals can be used to achieve targeted genetic variability goals over time even with suboptimal founders. Our approach has wide application for different species and in widely differing demographic and management contexts, where ultimately, the goal is to maximize the potential conservation outcome of any rehabilitation process.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

References

Conway WG, 2011. Buying time for wild animals with zoos. *Zoo Biol* **30**:1–8.

Edmands S, 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**:1757–1768.

Finlayson GR, Finlayson ST, Dickman CR, 2010. Returning the rat-kangaroos: a review of translocation attempts in the Family Potoroidae (Superfamily Macropodoidea) and recommendations for future conservation efforts. In: Coulson GM, Eldridge MDB, editors. *Macropods: Biology of Kangaroos, Wallabies and Rat-kangaroos*. Melbourne: CSIRO Publishing. 245–262.

Frankham R, 1995. Inbreeding and extinction: a threshold effect. *Conserv Biol* **9**:792–799.

Frankham R, 2007. Genetic adaptation to captivity in species conservation programs. *MolEcol* **17**:325–333.

Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K et al., 2011. Predicting the probability of outbreeding depression. *Conserv Biol* **25**: 465–475.

Groom C, 2010. Justification for continued conservation efforts following the delisting of a threatened species: a case study of the woylie *Bettongia penicillata ogilbyi* (Marsupialia: Potoroidae). *Wildl Res* **37**:183–193.

Hollings T, Jones M, Mooney N, McCallum H, 2013. Wildlife disease ecology in changing landscapes: mesopredator release and toxoplasmosis. *Int J Parasitol Parasites Wildl* **2**:110–118.

Ivy JA, Miller A, Lacy RC, DeWoody JA, 2009. Methods and prospects for using molecular data in captive breeding programs: an empirical example using parma wallabies *Macropus parma*. *Jof Hered* **100**:441–454.

Kalinowski ST, 2005. Hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol Ecol Notes* **5**:187–189.

Kalinowski ST, Taper ML, Marshall TC, 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Mol Ecol* **16**:1099–1106.

Maschinski J, Wright SJ, Koptur S, Pinto-Torres EC, 2013. When is local the best paradigm? Breeding history influences conservation reintroduction survival and population trajectories in times of extreme climate events. *Biol Conserv* **159**:277–284.

Meirmans PG, Hedrick PW, 2011. Assessing population structure: F_{st} and related measures. *Mol Ecol Resour* **11**:5–18.

Nei M, 1973. Analysis of gene diversity in subdiveded populations. *Proc Natl Acad Sci U S A* **70**:3321–3323.

Orell P, 2004. Fauna monitoring and staff training: Western Shield review—February 2003. *Conserv Sci W Aust* **5**:51–95.

Ottewell K, Dunlop J, Thomas N, Morris K, Coates D et al., 2014. Evaluating success of translocations in maintaining genetic diversity in a threatened mammal. *Biol Conserv* **171**:209–219.

Pacioni C, Spencer P, 2010. Capturing genetic information using non-target species markers in a species that has undergone a population crash. *Aust Mammal* **32**:33–38.

Pacioni C, Wayne AF, Spencer PBS, 2011. Effects of habitat fragmentation on population structure and long distance gene flow in an endangered marsupial: the woylie. *JZool* **283**:98–107.

Pacioni C, Wayne AF, Spencer P, 2013. Genetic outcomes from the translocations of the critically endangered woylie. *Curr Zool* **59**:294–310.

Pacioni C, Hunt H, Allentoft ME, Vaughan TG, Wayne AF et al., 2015. Genetic diversity loss in a biodiversity hotspot: ancient DNA quantifies genetic decline and former connectivity in a critically endangered marsupial. *Mol Ecol* **24**:5813–5828.

Pacioni C, Mayer F. 2017a. vortexR: an R package for post Vortex simulation analysis. *Methods in Ecology and Evolution* **8**:1477–1481.

Pacioni C, Williams M, Lacy RC, Spencer PBS, Wayne AF. 2017b. Predators and genetic fitness: key threatening factors for the conservation of bettong species. *Pacific Conservation Biology*, **23**:200–212.

Peakall ROD, Smouse PE, 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**: 288–295.

Pope LC, Estoup A, Moritz C, 2000. Phylogeography and population structure of an ecotonal marsupial *Bettongia tropica* determined using mtDNA and microsatellites. *Mol Ecol* **9**:2041–2053.

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.

Queller DC, Goodnight KF, 1989. Estimating relatedness using genetic markers. *Evolution* **43**:258–275.

R Core Team, 2016. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for statistical computing.

Russello MA, Hyseni C, Gibbs JP, Cruz S, Marquez C et al., 2007. Lineage identification of Galápagos tortoises in captivity worldwide. *Anim Conserv* **10**:304–311.

Short J, Bradshaw SD, Giles J, Prince RIT, Wilson GR, 1992. Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia: a review. *Biol Conserv* **62**:189–204.

Tallmon DA, Luikart G, Waples RS, 2004. The alluring simplicity and complex reality of genetic rescue. *Trends Ecol Evol* **19**:489–496.

Ward C, Wayne AF, Maxwell M, Vellios C, Williams J, et al., 2008. Demographics. In: DEC Science Division, editor. Diagnosis of Recent Woylie *Bettongia penicillata ogilbyi* Declines in South-western Australia. *Progress Report of the Woylie Conservation Research Project: A Report to the Department of Environment and Conservation Corporate Executive*. Perth: Department of Environment and Conservation, Science Division, 134–146.

Wayne AF, Friend T, Burbidge AA, Morris K, Van Weenen J, 2008. *Bettongia penicillata* IUCN Red List of Threatened Species. Available at: http://www.iucnredlist.org/details/2785/0.

Wayne AF, Maxwell M, Ward C, Vellios C, Ward B et al., 2013. The importance of getting the numbers right: quantifying the rapid and substantial decline of an abundant marsupial *Bettongia penicillata*. *Wildl Res* **40**: 169–183.

Wayne AF, Maxwell M, Ward C, Vellios C, Wilson I et al., 2015. Sudden and rapid decline of theabundant marsupial *Bettongia penicillata* in Australia. *Oryx* **49**:175–185.

Weeks AR, Sgro CM, Young AG, Frankham R, Mitchell NJ, et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol Appl* **4**:709–725.

Zhang XD, 2007. A pair of new statistical parameters for quality control in RNA interference high-through put screening assays. *Genomics* **89**:552–561.