Effects of habitat fragmentation on population structure and long distance gene flow.

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Abstract:

A deep understanding of population structures and of the relationships among populations is fundamental to guarantee adequate management of endangered species. We used a molecular approach (12 microsatellite loci and mitochondrial DNA) to investigate these aspects in the woylie or brush-tailed bettong (*Bettongia penicillata ogilbyi*). Four distinct indigenous populations were identified in this study (i.e. Dryandra woodland and Tutanning nature reserve in the wheatbelt region and two discrete populations in the Upper Warren in the south-west forests of Western Australia). Additionally, previously undisclosed modern and historical connections between these units became evident, such as the historical connection between populations at 150 km distance (Dryandra and Upper Warren) and the contemporary gene flow between the two populations in Upper Warren (up to 60 km).

Genetic attributes of the four populations were analysed and the evidence of unique genetic material in each of these populations indicated that conservation effort should aim towards the preservation of all these units. Additionally, the lower genetic diversity of the woylie population in Tutanning nature reserve prompted the need for the investigation of factors that are limiting the demographic growth of this population. This study enhances not only our knowledge about the ecology of woylies, but also the genetic consequences of habitat fragmentation and reiterates the strength and pertinence of molecular techniques in similar investigations.

Keywords: Woylie, brush-tailed bettong, *Bettongia penicillata*, Potoroidae, microsatellites, mtDNA, population structure, connectivity.

Introduction

Our understanding of the ecology of a species is considerably improved by knowledge about its population structure and level of connection among units (Paetkau *et al.* 1995). Habitat fragmentation effects on population dynamics also need to be understood to thoroughly assess the risks posed by processes that could threaten the conservation of many species (e.g. reduced dispersal, smaller effective population size and increase inbreeding, Banks *et al.* 2005). The identification of management units and evolutionarily significant units (Moritz *et al.* 1994; Moritz 1999) is also fundamental to correctly manage endangered wild populations (Zenger *et al.* 2003).

For example, knowledge of genetic attributes is needed for the effective recovery of woylie or brush-tailed bettong (*Bettongia penicillata ogilbyi*), which has recently been listed as critically endangered. So far it has declined by about 80% between 2000 and 2006 (Wayne *et al.* 2009). Less than 2000 individuals remain in the three localities where indigenous populations persist Upper Warren region, Dryandra woodland and Tutanning nature reserve, all in southwestern Australia (Fig. 1). The woylie continues to decline. It has been hypothesized that predators and/or a disease may be a concomitant cause if not the primary cause(s) of the decline based on available associative evidence (DEC Science Division 2008).

Information on the population structure and movements between populations is important to assess the direct disease transmission risks and to help determine an effective conservation strategy for the species. Considering the small home ranges of the woylie (19.6-34.8 ha) and the short distance commonly observed in dispersal events (Sampson 1971; Christensen 1980), we would predict that the degree of genetic divergence among populations should be proportional to their geographic distance. The locations we describe have been effectively isolated from each other since the 1920s-1960s. Consequently, the three localities should

represent at least three discrete populations. In the closely related northern bettong (*B. tropica*), populations at more than 12 km of distance were genetically distinct despite being geographically connected by continuous habitat (Pope *et al.* 2000). Thus, it is possible that woylie populations are further structured within each of the three localities.

This study focused on the extant indigenous woylie populations and used mitochondrial DNA (mtDNA) and microsatellite loci (MS) to: 1) identify genetically distinct populations and possible sub structuring, 2) establish population relationships both historically and contemporarily, 3) determine the overall genetic variability and differences, within and among populations, 4) provide an indication of long term genetic viability and suggest management directions to prevent the potential loss of unique genetic material.

Methods

Samples collection

A total of 231 tissue samples were collected between 2006 and 2008 (Table 1) from the three locations where naturally occurring (indigenous) woylie populations are known to exist [Upper Warren in the south-west forests, Dryandra woodland (Dryandra) and Tutanning nature reserve (Tutanning) in the wheatbelt region of Western Australia (Fig. 1)]. In Upper Warren, woylies were trapped using standard monitoring techniques from 11 forest blocks (Fig. 2) conducted by the Department of Environment and Conservation (DEC) using live-cage trapping transects (50 cages per transect spaced 200 m apart) (Orell 2004); no woylies were trapped in two of these areas where previously they had been abundant (Yackelup and Camelar). In the wheatbelt populations (Dryandra and Tutanning), in addition to a standardized transect, woylies were also trapped with opportunistic traps throughout the areas. Small tissue samples from the ear (skin biopsies) were stored in 70% ethanol. In

order to detect possible errors of origin (e.g. mislabelling), samples were bagged separately for each trapping session and each forest block or location.

DNA extraction and amplification

Complete genotypes at 12 microsatellite loci were determined for 231 adult woylies using methods described in Pacioni & Spencer (2010) and are listed in the Appendix S1. In addition, a partial (~600bp) section of the tRNA Proline-end of the control region (or D-loop) was amplified by means of the polymerase chain reaction (PCR) using the primers H15999M and L16498M and reaction concentrations described by Fumagalli *et al.* (1997) on a subset of 152 samples (Table 1). Reaction conditions were slightly modified using a preliminary denaturation step at 95°C for 3 min, followed by 40 cycles of: 95°C for 45 s, 53°C for 45 s, 72°C for 90 s. This was followed by a final extension step at 72°C for 5 min.

PCR products were sequenced using dye terminator cycle sequencing chemistry (3730xl sequencer; Applied Biosystems). The DNA sequences were compared to those in the Genbank database using the basic local alignment tool (http://www.ncbi.nlm.nih.gov/BLAST/) to confirm that the correct product was amplified. Sequences were aligned using the progressive pair-wise alignment algorithm (Drummond *et al.* 2007) incorporated in Geneious Pro 3.8 (Biomatters) and then the alignment was checked manually.

Phylogenetic analysis

The software MEGA v.4 (Tamura *et al.* 2007) was used to reconstruct phylogeny history using the neighbour-joining (NJ) and minimum evolution (ME) tree-searching methods under the Maximum Composite Likelihood substitution model (Tamura *et al.* 2004) with complete deletion of sites with gaps or missing data, leaving 563 sites for analysis. The rate of variation among sites was estimated in PAUP 4.0 (Swofford 2005) and finally modelled with a gamma distribution (shape parameter = 0.6991). The northern bettong (*B. tropica*) was used to root the tree due to its position as a sister taxon to *B. penicillata* and reliability

estimated from 1000 bootstrap replications. Under the same setting, we used MEGA (Tamura et al. 2007) to compute the mean evolutionary distance between haplotypes. MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was used to perform a Bayesian analysis. Briefly, the analysis was conducted with flat priors, carrying out two runs of four chains each of 6,000,000 generations with sampling every 100 generations. PAUP 4.0 (Swofford 2005) was used to estimate parameters for 56 substitution models based on the neighbour joining tree of Jukes-Cantor distances and then Modeltest version 3.7 (Posada & Crandall 1998) was used to select an appropriate substitution model using the corrected Akaike information criterion (AIC) as suggested by Posada and Buckley (2004). Parameter estimates derived from Modeltest were successively implemented in the MrBayes analysis. We used the MrBayes 'convergence diagnostic' function to determine whether the two runs had converged (average standard deviation <0.2) and discarded the first 15,000 trees as a 'burn-in' to obtain a 50% consensus tree. Additionally, we verified the likelihood profile in TRACER version 1.1 (Rambaut & Drummond 2007) to ensure that the 'burn-in' was sufficient.

Microsatellites analysis

Genotype data were initially manipulated using Microsoft Excel, and were checked for errors, Input files were created for other programs using the export function in GENALEX 6.2 (Peakall & Smouse 2006). The population structure was investigated between the sampling units using assignment tests to identify genetic structure and to assign individuals to their likely population of origin with STRUCTURE 2.2 (Pritchard *et al.* 2000). The analysis was repeated with and without the marker Y151 because of the surprising size range of this marker in this species (Pacioni & Spencer 2010). STRUCTURE uses a Bayesian assignment approach to determine the most likely number of *inferred* populations (*K*), based on the observed genotypes, and determine the extent of the contribution from each inferred population to each animal's genotype. A putative population is a group of samples obtained

from a discrete geographic area and an inferred population is a collection of samples that clustered together according to the assignment results. To determine the most likely number of populations, we analysed the posterior probability of the data given K [log Pr(X/K)] (Pritchard et al. 2000) and the second rate of change of the likelihood distribution, ΔK (Evanno *et al.* 2005). STRUCTURE assumes that the distribution of alleles conforms to Hardy-Weinberg equilibrium, that there is no linkage between markers (Pritchard et al. 2000) and allows analysis assuming that individuals originated from a population that had common ancestors (admixture) or alternatively that they are from genetically independent populations. Tests assuming each scenario were carried out. Also, under the admixture model, the hypothesis that the allele frequencies were correlated or alternatively that they were not, have been tested. Lastly, the software allowed the inclusion of the geographic sampling locations of individuals (i.e. individuals are initially assigned to the putative populations) in 'the prior probability' of the model. In case this information was wrong the analysis would overcome these 'mis-assignments' if the genetic signal was strong. Tests with 'blind' and two different 'informed' prior settings were carried out as follows. A first analysis was performed assuming as putative populations each forest block in Upper Warren. Successively, the analysis was repeated with the dataset organized according to the preliminary results, to confirm migrant animals under a more restricted model. Each STRUCTURE result was based on 20 independent runs from one to 25 (K = 1-25) inferred populations, using a 'burn-in' period of 100,000 iterations followed by 10^6 iterations of a Markov Chain Monte Carlo. When the dataset was reorganised according to the preliminary results, K-values were limited to 10, with no changes in all the other conditions.

Genetic diversity

The quality of the data and early detection of null-alleles and allelic dropout was checked with MICRO-CHECKER (Van Oosterhout *et al.* 2004). Hardy-Weinberg equilibrium and linkage disequilibrium were tested with HW-QUICKCHECK (Kalinowski 2006) and

GENEPOP 4.0 (Rousset 2008), respectively. Descriptive measures of population genetic diversity were all calculated using GENALEX 6.2 (Peakall & Smouse 2006) and included measures of observed (H_o) and expected heterozygosity (H_E) (Hartl & Clark 1997), observed (N_A) and expected numbers of alleles (N_E) (Brown & Weir 1983) and average number of private alleles (*PA*) (Maguire *et al.* 2002). To further enable the comparison of the genetic variability among populations, we calculated the average allelic richness (N_{AR}) and average private allelic richness (PA_R). These parameters were based on 28 diploid individuals using the rarefaction method implemented in HP-RARE (Kalinowski 2005), which compensates for differences in sample size producing unbiased estimates of allelic richness and then compared N_{AR} with the non-parametric Wilcoxon signed-rank test using SPSS v.15. The hierarchical population structure was further defined by calculating the estimator of genetic differentiation, F_{ST} (Peakall *et al.* 1995) in GENALEX 6.2 (Peakall & Smouse 2006) under the AMOVA framework using 1000 permutations to test significant difference from zero.

Gene flow

We estimated migration using both direct and indirect methods. The term migration is classically used to indicate displacement of an individual from one genetic population to another (Allendorf & Luikart 2007). However, in ecological studies it is more frequently referred to as a dispersal event and the term migration is reserved to indicate common movements of a species during different seasons or their life cycle (Allendorf & Luikart 2007). Here, the terms migration and dispersal are used interchangeably.

Direct estimates

Direct dispersal estimates are usually based on ecological approaches (e.g. mark-recapture or radio tracking studies). A limitation of these methods is the difficulties to detect occasional or irregular dispersal events. Molecular ecology can provide a valid contribution in that it is possible to assign an individual to its most likely source population according to its genotype.

Using the information generated by STRUCTURE under the admixture model with correlated allele frequencies and including in the prior the information of the putative populations, we identified probable recent (F_0, F_1 or F_2 generation) migrants between wild populations and calculated N_m estimates weighting for the generation in which the dispersal event occurred. That is, F_0 accounts for 1, F_1 for 0.5 and F_2 for 0.25. DNA of migrants was re-extracted and their profiles were re-genotyped to ensure that no genotyping errors occurred.

Indirect estimates

The effective number of migrants between any two inferred populations per generation (N_m) was estimated using F_{ST} values according to Peakall et al (1995), based on allele frequencies methods, using the relationship of N_m=(1/ F_{ST} -1)/4 (Slatkin 1985). We used F_{ST} to obtain indirect measures of gene flow (N_m) between populations because this estimate is based on historical rates of gene flow and it is considered a better estimator of the migration rate than R_{ST}, for studies such as the present one, involving low numbers of loci being scored (n<20; Gaggiotti *et al.* 1999). The limitations of this approach are mainly related to the assumptions implied that are rarely met in natural populations (Hardy-Weinberg equilibrium, populations of equal and constant size, constant and symmetric migration rates, neutral selection, no or negligible mutation, migration-drift equilibrium, same probability of reproduction for migrant and resident individuals). Moreover, this approach has been developed under the island model of migration (Slatkin 1985), which may not always be the real scenario.

An alternative approach is to estimate migration using the 'private allele' method (see Barton & Slatkin 1986) and we used GENEPOP 4.0 (Rousset 2008) for this purpose. This approach also assumes the island model of migration. Another constraining factor in the assumptions is that the migration has to be much higher than mutation rate because the model does not take into account mutation. Despite the limitations of these two methods, they are still widely used and can provide an indication of the general trend (e.g. low versus high migration rates) rather than the exact number of migrants (Allendorf & Luikart 2007).

Results

Phylogenetic trees had similar topology for all the methods used. The nucleotide composition was highly biased towards thiamine (T: 40.3 C: 5.9 A: 26.7 G: 27.1) and the overall transition/transversion bias was R = 6.645. Fifteen haplotypes (GenBank accession number: HQ141321-HQ141335) were identified and their geographic distribution is shown in Fig. 3. The average genetic distance of the 15 haplotypes was 0.02196 (se= 0.0047).

STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003) identified the existence of four genetically distinct groups (i.e. K = 4). No difference in this result was obtained when marker Y151 was removed from the dataset. Two of these clusters, Dryandra and Tutanning, are consistent with their geographic separation, while Upper Warren was divided into two different populations: one that comprises the western blocks (from now on identified as Kingston) and the second that includes the remaining blocks in the east (Perup. Fig. 2). All the analyses that included the geographic information in the prior, generated a mode at K=4 for both distributions: the posterior probability of the data given K (log Pr(X/K)) (Pritchard et al. 2000) and the second rate of change of the likelihood function, ΔK (Evanno et al. 2005). Interestingly, with the no-admixture model and no-correlated allele frequencies under the admixture model (and no geographic information in the prior), the distribution of ΔK showed a mode at K=3, with Dryandra and Perup grouped together while the log Pr(X/K) constantly showed higher values at K=4. The difference between ΔK and the log Pr(X/K) is a result of the reduction of the variance from K=3 onwards, which affects the calculation of the former parameter. This can be interpreted as an indication of the relative similarity between these two populations and the importance of including the sampling locations in the prior to improve the resolution of the analysis.

Several animals were identified as migrants between various populations. Table 2 reports details of these individuals based on the most conservative model. Running the admixture model without using any information about the putative populations, the number of animals, whose fraction of genotype derived from other populations, increases. Interestingly, another four animals appear to be related to Dryandra while being trapped in Upper Warren and two additional woylies for each side of Upper Warren appear to be migrants from the opposite side (data not shown).

Of the 48 tests carried out to verify Hardy-Weinberg proportions, six were significant after Bonferroni's correction. However, no consistent pattern was evident across populations, or loci, and consequently we judged that the results depended more on the single population rather than a problem with these particular loci.

All populations had expected levels of heterozygosity (H_E) of around 0.8 except for Tutanning with 0.64 (Table 1). Not surprisingly, Tutanning also had the lowest N_A 5.5 (± 0.61), N_E 3.23 (± 0.31), *PA* 0.67 (± 0.41) and PA_R 0.86 (± 1.32) (Table 1). Furthermore, N_{AR} was significantly lower at Tutanning than the other populations (P=0.002). Perup showed the highest N_{AR} when compared with the other populations (P<0.005). The analysis of molecular variance among populations was significantly different from zero (Table 3) with values ranging from 0.056 to 0.164. The population in Tutanning showed the highest level of differentiation when compared with the other populations sampled.

Direct migration estimates (N_m) were 2.5 migrants from Kingston to Perup and 0.5 migrants from Perup to Kingston, while only one animal was consistently detected as F_2 migrant from Dryandra to Kingston (N_m=0.25). N_m estimated with the allele frequencies and private alleles approaches were very low and slightly different (Table 4). Given the previously mentioned limitations of these methods, we also show the relative rates of these measures (Table 4).

To illustrate this we used as a unit the *rate* between Dryandra and Tutanning and weighted the other values. In this way, it was evident that with both methods the connection between Dryandra and Upper Warren was about twice as strong as the one between Dryandra and Tutanning and the connection between the two Upper Warren populations was around three times as strong.

Discussion

Four distinct indigenous woylie populations were identified in this study. Whereas previously the woylies in the Upper Warren were considered to form a single population and managed as effectively, it is now clearly and genetically evident that two exist – approximately separated by the Perup River and the associated farmland, these are nominally called the 'Kingston' population in the west and 'Perup' population in the east (Fig. 2). Whether this distinction was historic or a more recent result of habitat fragmentation since settlement and agricultural development by Europeans (post 1829), remains to be unequivocally determined however, for reasons elaborated later, the latter is most likely. Possible limitations of STRUCTURE analysis are related to the limited sample size obtained from the southern blocks (consequences of low density in these areas) and the fact that if a genetic gradient is present, STRUCTURE may tend to artificially increase the number of inferred populations (Schwartz & McKelvey 2009). Nevertheless, the concordance of result obtained from the mtDNA analysis and F_{ST} values lends support to STRUCTURE results.

Perhaps the most striking findings from this study relate to the differences between the Tutanning and other indigenous populations. Tutanning woylies were genetically distinct as demonstrated by the monophyletic clade resulting from the phylogenetic analyses of mtDNA haplotypes (Fig. 3). The limited number of mtDNA obtained from woylies of this population may have prevented the sampling of additional haplotypes. However, the consistent results obtained from STRUCTURE analyses and N_m estimates provided supporting evidence for

the lack of connectivity between Tutanning and the other indigenous populations, which is also likely to be a consequence of lack of continuous suitable habitat. The genetic consequences of habitat fragmentation have been well demonstrated in other species (e.g. Bowyer *et al.* 2002; Banks *et al.* 2005). These together with other ecological and biological consequences of habitat fragmentation present particular challenges to the conservation and management of species and communities (Heinsohn *et al.* 2004; Wayne *et al.* 2006; Fischer & Lindenmayer 2007), which are likely to be relevant to the woylie.

While distinctions exist among the four populations, it is also clear that there is genetic mixing, particularly between the two Upper Warren populations. The phylogenetic analyses also indicate that the mtDNA haplotypes in the Dryandra population are closely related to those in the Upper Warren. Therefore the three populations (Dryandra, Perup and Kingston) were historically connected. As such, these populations may be considered as being part of the same evolutionarily significant unit (Moritz 1999). In other non-vagile macropods there are examples of gene flow across similar spatial scales. Admixture of haplotypes in the yellow-footed rock wallaby (*Petrogale xanthopus*) from locations up to 70 km apart was considered evidence of historical connections between sites (Pope *et al.* 1996). Long distance migrations maintaining high gene flow across large areas have also been demonstrated in other small marsupials, such as the Western Pygmy Possum (*Cercartetus concinnus*) across South Australia and Western Australia (Pestell *et al.* 2008).

Significant gene flow between the two Upper Warren populations (Kingston and Perup) is also evident with remarkable concordance in the N_m estimates derived by three independent methods (direct estimates, allele frequencies and private alleles). Using the most conservative approach, between 2 and 3% of woylies from these populations were estimated to be migrants. With due consideration of the limitations to make direct comparisons because of the differences in methodology, these values are similar to those observed in

other macropods. For example, based on assignment tests, 12% (n=17) of northern bettongs were identified as migrants (Pope et al. 2000) and 5% in the brush-tailed rock-wallaby (Piggott *et al.* 2006). A 6% dispersal rate in juvenile yellow-footed rock-wallaby has also been observed in the field (Sharp 1997).

Contrary to expectation, the degree of genetic divergence among indigenous populations was not related to their geographic distances. Despite the close proximity between Tutanning and Dryandra (< 40 km), the former is notably divergent from the other indigenous populations. Additionally, the two large Upper Warren populations did not show further genetic substructure. The potential for large-scale woylie movements that might prevent substructuring is demonstrated by the evidence of gene flow across the Upper Warren (e.g. N_m) and experimental as well as field observations of individuals moving 3-9 km (Christensen 1980; Pacioni *et al.* In preparation). This is in contrast with the northern bettong where, in seemingly continuous habitat, populations as close as 12 km were genetically distinct (Pope et al. 2000). However, male boodies (burrowing bettong, *B. lesueur*) were capable of dispersing up to 6 km (Parsons *et al.* 2002) and substantial migration in the rufous bettong (*Aepyprymnus rufscens*) has been demonstrated, in spite of significant genetic divergence among populations within a 6.5 km radius (Pope *et al.* 2005).

It is extremely unlikely that the historically evident movements between Dryandra and Upper Warren are still naturally occurring given the extent of habitat fragmentation and patchiness of remnant woylie populations. However, one individual in Upper Warren was constantly assigned as having ancestors (within the last two generations) from Dryandra. This is possibly the result of a human-assisted movement. Woylie joeys commonly come into the care of humans and are then transported throughout southwest Western Australia. It is therefore plausible that one or a number of these animals (or their offspring) have been subsequently released into the wild and effectively translocated to another population.

Furthermore, there is no record of animals moving between Dryandra and Upper Warren despite intensive and regular trapping in these areas over the last 35 years. It is also possible that this seemingly spurious case could be an artefact of historical connectivity and that this individual may represent an 'echo' of past gene flow between these two populations.

Relatively high genetic variability in Dryandra, Kingston and Perup woylie populations were repeatedly demonstrated (e.g. H_E, N_A, N_E, N_{AR}, PA and PA_R). These populations are therefore likely to have reasonable potential medium-long term genetic viability. H_E in these populations (0.78-0.83) was slightly higher than other *Bettongia* (*B. tropica;* H_E: 0.65-0.75, Pope *et al.* 2000; *B. lesueur;* H_E: 0.68-0.7, Donaldson & Vercoe 2008) and some other potorines (*Potoroos longipes;* H_E: 0.556, Luikart *et al.* 1997; *P. gilbertii;* H_E: 0.457, Sinclair *et al.* 2002; but *Aepyprymnus rufescens;* H_E: 0.83, Pope *et al.* 2005).

Historically, the genetic variability at Tutanning was most likely similar to that found in the other indigenous populations. Therefore, it can be considered substantially reduced (H_E = 0.63). This finding is likely to be the result of genetic drift, probably as a consequence of small population size being sustained over an extended period (i.e. c 300 animals for at least the last 40 years). It also suggests that the long term viability of this population could be compromised and potentially at risk of inbreeding depression. Sampson (1971) stated that unless management actions were taken to restore habitat quality and continuity in the reserve to facilitate an increase in woylie population size and connectivity, Tutanning would effectively function as an island population. Interestingly, the H_E, N_A and N_E values at Tutanning were very similar to the woylie population on Saint Peter Island, South Australia, which is of comparable size to Tutanning (our unpublished data). H_E (0.63) at Tutanning was also lower than the H_E in boodies on Dorre Island (H_E: 0.68, Donaldson & Vercoe 2008) in Shark Bay (Western Australia), an arid island of about twice the size of Tutanning.

Ensuring the persistence at relatively abundant levels of all four indigenous woylie populations remains fundamental to the long-term conservation of this species. Each one of these populations retains unique genetic material (e.g. PA_R= ranging from 0.89 to 1.99) and each can be regarded as a discrete management unit. Genetic diversity within the indigenous populations was relatively high at the time of sampling, except for Tutanning. The continuation of the woylie population declines throughout the south-western Australia and elsewhere should be of significant concern. Both the extent of the declines and particularly the duration to which a recovery may be delayed will result in increasing likelihood of genetic loss and associated consequences.

The causes and nature of the divergence of the Tutanning population could be resolved by further studies including the use of more conserved genome regions (e.g. cyt b) and an analysis of historic material available at museums and elsewhere. This would also ensure that the correct management decisions are implemented (e.g. admixture of individuals from Tutanning with other indigenous populations) should an insurance population be established. Monitoring the Tutanning population for signs of inbreeding depression would also be recommended.

In terms of disease transmission risks, this study indicates that woylies, at least within Upper Warren, are able to directly carry and transmit disease across the entire area. Evidence of possibly recent animal movement from Dryandra to Upper Warren (regardless whether human-assisted or not) also raises the potential for disease transmission across greater distances.

Lastly, we recommend including regular genetic monitoring, e.g. every three to six generations (i.e. 6-12 years), in the management plan for the woylie. This would directly enhance conservation prospects for this species but also presents a significant and unique

opportunity to improve our understanding of genetic dynamics during population declines, which may be relevant to species conservation more broadly. The exceptional value of the woylie as a potential 'model' for developing our understanding is accentuated by the well-documented history of woylie declines, translocations and past recovery during the 20th Century (Orell 2004), the depth and breadth of associated ecological and demographic baseline data available (particularly over the last 35 years, e.g. Burrows & Christensen 2002) and the foundations established by this study.

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TABLES

Table 1. Summary of the samples collected in each sampling location, measures of microsatellite variability and genetic contribution (given as a proportion) of each of the four inferred population clusters.

	1. Dryandra	2. Tutanning	3. Kingston	4. Perup
n(MS)[a]	28	32	69	102
n(mtDNA)[b]	8	13	48	83
N_A[c] *(SE)*	8.92 (± 0.92)	5.5 (± 0.61)	12 (± 1.33)	15 (± 1.81)
N_E[d] *(SE)*	5.76 (± 0.66)	3.23 (± 0.31)	5.91 (± 0.62)	7.55 (± 0.88)
N_{AR}[e] *(SD)*	8.92 (± 3.07)	5.4 (± 1.92)	10.05 (± 3.37)	12.03 (± 3.89)
H_E[f] *(SE)*	0.796 (± 0.03)	0.64 (± 0.05)	0.788 (± 0.04)	0.835 (± 0.03)
H_o[g] *(SE)*	0.731 (± 0.046)	0.645 (± 0.078)	0.706 (± 0.058)	0.746 (± 0.039)
PA[h] *(SE)*	0.92 (± 0.31)	0.67 (± 0.41)	1.17 (± 0.42)	2.42 (± 0.89)
PA_R[i] *(SD)*	1.42 (± 1.61)	0.86 (± 1.32)	1.47 (± 1.43)	1.99 (± 2.18)
Cluster 1	**0.998**	0.001	0.005	0.002
Cluster 2	0.001	**0.998**	0.001	0.001
Cluster 3	0.001	0	**0.987**	0.025
Cluster 4	0.001	0.001	0.008	**0.972**

[a] number of individuals genotyped at microsatellite loci.

[b] number of DNA sequences generated at the mitochondrial DNA control region.

[c] average number of alleles.

[d] average effective number of alleles.

[e] average allelic richness.

[f] expected heterozygosity.

[g] observed heterozygosity.

[h] average private alleles.

[i] average private allelic richness.

Table 2. Details of woylie individuals indentified as migrants between populations by STRUCTURE under the admixture model with correlated allele frequencies and using geographic information.

ID	Sex	(%Miss)[a]	Into	From	Generation	P(As)[b]	P(Anc)[c]
07-366	M	0	Kingston	Perup	F2	0.069	0.929
07-370	M	0	Kingston	Dryandra	F2	0.01	0.988
07-381	F	0	Kingston	Perup	F2	0.546	0.434
07-341	F	0	Perup	Kingston	F1	0.04	0.951
07-389	M	0	Perup	Kingston	F0	0	1
07-585	M	0	Perup	Kingston	F0	0	1

[a] percentage of genetic data missing.

[b] probability of being assigned to the putative population.

[c] probability of having ancestors in the inferred population.

Table 3. Pairwise estimates of F_{ST}. Probability values based on 1000 permutations are shown above diagonal.

	Dryandra	Tutanning	Perup	Kingston
Dryandra		*0.001*	*0.001*	*0.001*
Tutanning	0.152		*0.001*	*0.001*
Perup	0.061	0.137		*0.001*
Kingston	0.089	0.164	0.056	

Table 4. Number of migrants calculated with allele frequencies (first line) and private allele methods (second line). Between parentheses are the relative migration values, using the value between Dryandra and Tutanning as unit (equal to 1).

	Dryandra	Tutanning	Kingston
Tutanning	1.39 (1)		
	0.54 (1)		
Kingston	2.53 (1.82)	1.28 (0.92)	
	0.98 (1.82)	0.75 (1.39)	
Perup	3.91 (2.8)	1.59 (1.14)	4.31 (3.09)
	1.11 (2.06)	0.85 (1.58)	1.4 (2.6)

Appendix 1 Details of the microsatellite loci amplified in the woylie (Bettongia penicillata ogilbyi) including the species where these were first developed.

Source species	Locus	Reference
Bettongia tropica	Bt64	(Pope *et al.* 2000)
Bettongia tropica	Bt76	(Pope *et al.* 2000)
Bettongia tropica	Bt80	(Pope *et al.* 2000)
Petrogale assimilis	Pa593	(Spencer *et al.* 1995)
Petrogale xanthopus	Y105	(Zenger *et al.* 2002)
Petrogale xanthopus	Y112	(Zenger *et al.* 2002)
Petrogale xanthopus	Y151	(Pope *et al.* 1996)
Petrogale xanthopus	Y170	(Pope *et al.* 1996)
Petrogale xanthopus	Y175	(Zenger *et al.* 2002)
Potorous longipedis	Pl2	(Luikart *et al.* 1997)
Potorous longipedis	Pl26	(Luikart *et al.* 1997)
Macropus eugenii	T17-2	(Zenger & Cooper 2001)

Figure Legends

Figure 1. Large woylie populations (arrows) and towns (dots) in Western Australia.

Figure 2. Woylie populations and sampling sites within the Upper Warren region.

Figure 3. Phylogenetic tree of the control region of mtDNA based on around 600 bp amplified from naturally occurring woylie populations. More than 0.5 Bayesian posterior probabilities are reported internally to the nodes. More than 50% bootstrap support values were reported above the branches (NJ/ME). Numbers in squares represent the number of different haplotypes. If more than one sample from the same location had the same haplotype the total number of samples is indicated between brackets. Branches of equal length and grouped by a curly bracket represent different populations that had the same haplotype.