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CONSERVATION CONUNDRUM: THE POPULATION DYNAMICS ASSOCIATED WITH RECENT DECLINE OF WOYLIES (*BETTONGIA PENICILLATA*) IN AUSTRALIA.

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Our environment, our future

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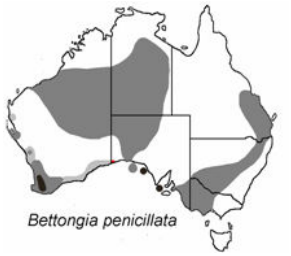
NAME OF PROJECT

CONSERVATION CONUNDRUM: THE POPULATION DYNAMICS ASSOCIATED WITH RECENT DECLINE OF WOYLIES (*BETTONGIA PENICILLATA*) IN AUSTRALIA.

BACKGROUND

The recent plight of the woylie (Bettongia penicillata) presents new challenges for the conservation of the species that was Conservation Dependent and is now seriously threatened. It provides a unique and extremely valuable learning opportunity relevant to conservation biology and species recoveries in general. Similar to many other Australian mammal representatives, the species had a distribution across much of Australia (Fig. 1). At the time of settlement by Europeans, the woylie was reduced to three remnant and isolated populations in the south-west of Australia – (i) Perup (and surrounding Upper Warren River Catchment), (ii) Dryandra and (iii) Tutanning regions. In 1998 the woylie became the first Australian mammal to be downgraded from the threatened species list. For conservation biologists it achieved iconic status on the basis of its robust recovery in response to broad-scale fox control and an extensive reintroduction program as part of the 'Western Shield' project and preceding work (CALM, now DEC). More recently, however, the woylie has undergone substantial and rapid declines that have particularly affected the natural remnant populations and the largest of the reintroduced populations.


Bettongia penicillata

Conservative measures of the population decline (Wayne 2006) show that the woylie population at Dryandra has declined by 88% since 2001. The Perup (and surrounding Upper Warren) region constituted the largest and most extensive remnant woylie population during the last decades of the 20th Century but has declined by 90% since 2002. Similarly, woylies at Batalling (a 1982 reintroduction site east of Collie) have declined by 70%. A number of other Western Shield monitoring sites also indicate declines throughout south-western Australia, all of which remain at low to undetectable densities. The small remnant population at Tutanning persists at low densities. Based on the patterns of decline, the last two forest blocks (Keninup and Warrup) remain at a moderate density of woylies in Upper Warren, but are expected to reach 0% to 10% of their former densities during 2006-2007. The reintroduced and fenced woylie population at Karakamia Wildlife Sanctuary (Australian Wildlife Conservancy), east of Perth, remains the Western Australian exception with densities remaining high. Established translocated woylie populations in South Australia include three islands (St Peters, Wedge and Venus Bay Island) and a fenced mainland peninsula (Venus Bay Peninsula). The fenced Scotia sanctuary (AWC) in New South Wales also supports a moderate woylie population. Very recent reports indicate that rapid and substantial declines are now occurring at Venus Bay Peninsula (P. Copley and J. Van Weenen, pers. comm.) and moderate declines have been observed at Scotia (G. Finlayson, pers. comm.).

A major collaborative research initiative is currently underway that aims to diagnose the recent woylie declines for the purposes of ensuring the immediate and long-term conservation of this ecologically important species (Wayne et al. 2006). Key collaborators in the existing project include the Western

Australian Government Department of Environment and Conservation, Murdoch University, Australian Wildlife Conservancy and Perth Zoo. A vital complement to the existing research and conservation endeavours is a sound understanding of the genetics of this species, and forms this study – funded by the Australian Academy of Sciences. As an integral component of the diagnosis of woylie declines this specific project proposes to characterise woylie genetics directly relevant to conservation and recovery of the species and to relate these findings to the epidemiology and demographics of declining populations.

PROJECT AIMS AND OBJECTIVES

This study represented a unique situation in fauna conservation, as *Bettongia penicillata* appears to have undergone two (recent) demographic bottlenecks. One occurred concurrent with European settlement and another originated as recently as four years ago (Wayne et al. 2006). A well-known outcome of this phenomenon would be the decreased capacity of the population to cope with changes as a result of the loss of genetic diversity. The end result of the loss of genetic information is well understood (theoretically) and it may have already impacted on the population. Therefore, this issue was considered as an important factor for investigation in the recent woylie decline.

As a result of the more recent decline on populations of the woylie, in this study we aimed to;

1. Characterise the historical genetic profile of the woylie species across its former range since the settlement of Australia by Europeans using ancient DNA approaches.
2. Characterise the genetic profiles of remaining contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning). This will include quantifying to the levels of genetic variability and heterozygosity, estimating the effective population sizes within and among extant remnants.
3. Investigate the genetic consequences of previous population declines and range contractions by comparing available historical material with contemporary material from remaining indigenous populations (see above).
4. Model and quantify the genetic responses within translocated populations in Western Australia and South Australia based on the characteristics of the founding cohort (e.g. size, genetic diversity, etc), the characteristics of the translocation population establishment (e.g. rate of increase, population size, density, time since establishment, etc) and the effect of post-establishment animal supplementation (e.g. introduction of additional animals post-establishment for the purposes of increasing genetic diversity). Genetic responses include correlating & assessing the reproductive success of translocated animals; estimating the effective size of the founding population; and, quantifying the extent of loss of genetic diversity.
5. Based on the findings of the above objectives, predict the genetic implications of recent woylie declines, and the likely consequences for future species recovery and conservation. This may allow specific populations to be identified as high priority for conservation efforts
6. Investigate whether there are any relationships between woylie population declines and the genetic attributes of affected and unaffected populations.

1. *Characterise the historical genetic profile of the woylie species across its former range since the settlement of Australia by Europeans using ancient DNA approaches.*

We were able to locate 10 historical samples from WA museum and extract DNA from eight of these in addition to a sub-fossil individual from Tunnel cave (SW of WA). Mitochondrial DNA was amplified from all 8 of these samples and our intention is to also amplify microsatellites loci, if possible, from the same individuals. To date, one primer (Pa593) has been successfully used to amplify two of the extractions. Work is continuing on this phase of the project.

2. *Characterise the genetic profiles of remaining contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning). This will include quantifying to the levels of genetic variability and heterozygosity, estimating the effective population sizes within and among extant remnants.*

A substantial sampling effort was achieved with tissue samples collected from over 1260 animals (Table 1). These include samples from all the major sampling sites proposed, including the Western Australia populations (Dryandra, Tutanning and Batalling) as well as populations that were reintroduced to South Australia (St Peter, Venus Bay & Wedge Island) and one that was used as previous translocations site (Karakamia site). All samples are stored for availability for other laboratories in the wildlife genetic collection at Murdoch University (Table 1).

Table 1. Sample collection field sites detailing the number of tissue samples collected for use in this study.

Sampling locality	Additional information	No. of samples
Batalling State Forest	Translocated population from the Upper Warren	89
Upper Warren (UW)	Wild population. Upper Warren. It was sampled in 1998 (samples available from wildlife genetic collection at Murdoch University) and 2006.	535
Karakamia Sanctuary	Translocated population from Dryandra. Possibly a few animals from Upper Warren have been moved in the sanctuary after the establishment. This population is currently growing .	256
Dryandra Forest (Dry)	Wild population	60
Tutanning Nature Reserve (Tut)	Wild population	44
St Peter Island, SA	Translocated population. There is no clear record if founders of the breeding centre (Monarto) were from Tutanning or Dryandra or both (most likely both)	119
Wedge Island, SA	Translocated population. There is no clear record if founders of the breeding centre (Monarto) were from Tutanning or Dryandra or both (most likely both)	80
Venus Bay Island (VBI) & Venus Bay Conservation Park (VBCP), SA	Translocated population. There is non clear record if founders of the breeding centre (Monarto) were from Tutanning or Dryandra or both (most likely both)	31 & 50
Total		**1264**

We optimised for a very large number of microsatellite markers that had been developed from studies of other macropods and potoroos. These primers were tested to verify their suitability for investigating the population genetics of *B. penicillata*. Thirty two primers that were originally developed for different Macropodidae species were tested to assess their suitability and polymorphism in woylies (Table 2). Twenty one of those produced an amplified PCR product when tested with woylie DNA. Of those, 13 produced reproducible and variable information (Fig. 2).

Table 2 Information available related to the primers described from a range of Macropodidae species that were tested for polymorphism in samples from the woylie.

Locus	Gene Bank accession number	Size range (base pairs)	Reference
Bt64		156-202	(Pope et al., 2000)
Bt76		193-241	(Pope *et al.*, 2000)
Bt80		180-202	(Pope *et al.*, 2000)
Pl2	Y09050	150-160	(Luikart et al., 1997)
Pl3	Y09051	141-159	(Luikart *et al.*, 1997)
Pl13	Y09052	77-113	(Luikart *et al.*, 1997)
Pl18	Y09053	126-134	(Luikart *et al.*, 1997)
Pl22	Y09054	124-156	(Luikart *et al.*, 1997)
Pl26	Y09055	164-184	(Luikart *et al.*, 1997)
Y175		252-282	(Zenger et al., 2002)
Y170		124-156	(Pope et al., 1996)
Y151		200-300	(Pope *et al.*, 1996)
Y148		**-**	(Pope *et al.*, 1996)
Y112		**185-235**	(Zenger *et al.*, 2002)
Y105		**223-251**	(Zenger *et al.*, 2002)
Y76		-	(Pope *et al.*, 1996)
Pa55		**-**	(Spencer *et al.*, 1995)
Pa597	U30636	-	(Spencer et al., 1995)
Pa593	U30633	100-130	(Spencer *et al.*, 1995)
Pa297	U30634	80-110	(Spencer *et al.*, 1995)
Pa385	U30632	-	(Spencer *et al.*, 1995)
Pa595	U30635	-	(Spencer *et al.*, 1995)
B90		-	(Pope *et al.*, 2000)
B123		-	(Zenger *et al.*, 2002)
G31-1	AF322629	118-136	(Zenger and Cooper, 2001b)
Me15	AF025909	225-270	(Taylor and Cooper, 1998)
Me16	AF025910	240-280	(Taylor and Cooper, 1998)
Me17	AF025911	110-140	(Taylor and Cooper, 1998)
T17-2	AF326948	115-147	(Zenger and Cooper, 2001a)
T31-1	AF326953	115-137	(Zenger and Cooper, 2001a)
MeY01	DQ641481	340-344	(Macdonald et al., 2006)
MeY37	DQ641488	179-181	(Macdonald *et al.*, 2006)

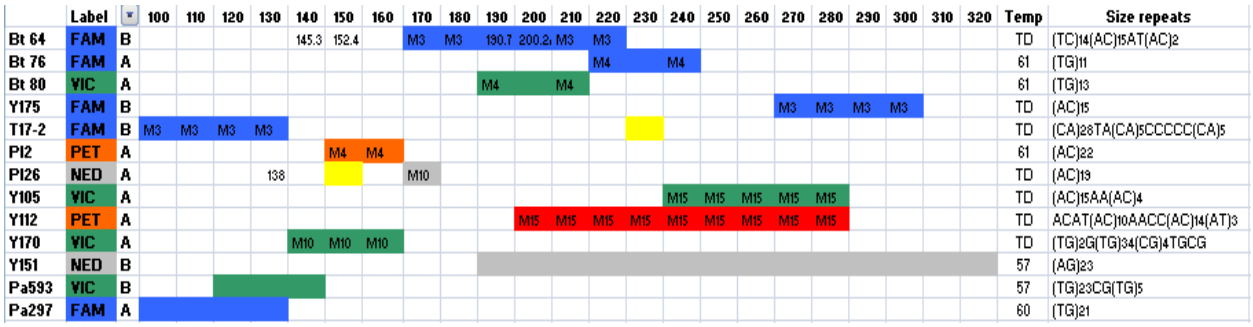


Figure 2. The multiplex approach used to amplify the 13 polymorphic woylie microsatellite markers. The details of these markers are given in Table 2. TD = touchdown PCR.

Here we report on the results of DNA extractions carried out on a subset of > 250 of these samples. We recorded heterozygosity values between 70 and 93% (mean heterozygosity of 75 ± 1.2%). All loci were highly polymorphic, with between four and 35 alleles at any locus and the average of number of alleles per locus of 18.0 (± 8.2 S.D; Table 3).

Table 3 Preliminary results obtained from 582 genic individuals (based on all available data) that was used to measure different values of genetic diversity, including observed (H_0) and expected (H_E)heterozygosity (Nei 1973), number of alleles.

3.

Locus	***H*$_O$**	***H*$_E$**	***N*$_A$**
Bt76	0.816	0.862	12
Bt80	0.830	0.866	9
T172	0.518	0.856	14
Pl2	1.000	0.797	6
Bt64	0.743	0.922	24
Pa297	0.400	0.800	6
Pa597	0.000	0.719	4
Pa595	0.286	0.816	6
Pl26	0.625	0.695	4
Y151	0.779	0.933	35
Y170	0.830	0.894	15
Y175	0.767	0.905	19
Pa593	0.767	0.898	16
Mean	**0.756 ± 0.012**	**0.892 ± 0.009**	**18.0 ± 8.21**

We made significant progress towards achieving the development of a set of polymorphic microsatellite markers for the study of the molecular ecology of the woylie (Table 4).

Based on the allele frequencies, we found that genetic variability from the contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning) from our preliminary screening had a very high average heterozygosity (80%; see Table 4). This level of heterozygosity is similar to the amount of variability described in other marsupial species (Pope et al., 2000, Eldridge et al., 2004).

Table 4. Measures of genetic variability from the contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning); mean observed (H_o) and mean expected heterozygosity (H_e) and mean observed (N_A) number of alleles, for the original wild woylie populations. Values given as mean ± S.D.

Population	**N**	**Heterozygosity**		**No. of alleles**
		Unbiased	**Observed**	
Upper Warren				
Warren	32	0.8231 ± 0.013	0.7685 ± 0.027	9.63 ± 1.19
Kingston	24	0.819 ± 0.033	0.842 ± 0.044	9.67 ± 1.53
Tutanning N.R.	32	0.767 ± 0.008	0.797 ± 0.036	6.75 ± 0.96
Dryandra Forest	28	0.856 ± 0.019	0.827 ± 0.028	10.38 ± 2.92

Somewhat surprisingly, we could detect only a single genetic bottleneck in the contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning). The Tutanning population had a significant bottleneck, but showed no evidence of any mode-shift in allele frequencies (Table 5). Interestingly that population had an effective population size 13-times larger than any of its contemporaries (Table 5). This result is somewhat surprising, and is being investigated further as we accumulate further data from this population. The data also showed that the South Australian populations are genetically close, with F_{ST} values below 0.1 (Table 6). It also shows that the Upper Warren populations (i.e. Upper Warren and Kingston) are genetically identical.

Table 5. Estimates of a genetic bottleneck and the effective population size from the contemporary indigenous woylie populations in Western Australia (i.e. Upper Warren, Dryandra, and Tutanning) for the microsatellite loci. n.s. = not significant ($P > 0.05$). Bottleneck: probability of a recent genetic bottleneck detected;

	Genetic bottleneck		Effective Population size	
Population	P	Mode-shift	Ne (LD)	range
Upper Warren				
Warren	0.562 (n.s.)	No	47	35.4 – 68.5
Kingston	0.812 (n.s.)	No	34	18.8 – 91.1
Tutanning N.R.	0.031 (sig)	No	268	44.6 - ∞
Dryandra Forest	0.062 (n.s.)	No	35	26.5 – 49.5

Table 6. Pairwise F_{ST} (below the diagonal) values between all the populations of woylies sampled and estimated from available polymorphic microsatellite loci.

	Population							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. Battaling								
2. Dryandra	0.113							
3. Karrakamia	0.141	0.042						
4. Kingston	0.129	0.112	0.114					
5. St. Peter Isl	0.218	0.200	0.241	0.218				
6. Tuttaning	0.132	0.155	0.136	0.165	0.273			
7. Venus Bay	0.213	0.179	0.207	0.201	0.065	0.272		
8. Warren	0.102	0.118	0.151	0.002	0.217	0.150	0.219	
9. Wedge Isl	0.179	0.158	0.194	0.206	0.051	0.251	0.115	0.225

4. *Investigate the genetic consequences of previous population declines and range contractions by comparing available historical material with contemporary material from remaining indigenous populations (see above).*

   We were unable to detect previous or long-term population bottlenecks in the historically healthy populations. Most of our effort has been on generating data for the extant populations, but we are continuing to accumulate the data necessary to complete this area of study.

5. *Model and quantify the genetic responses within translocated populations in Western Australia and South Australia based on the characteristics of the founding cohort (e.g. size, genetic diversity, etc), the characteristics of the translocation population establishment (e.g. rate of increase, population size, density, time since establishment, etc) and the effect of post-establishment animal supplementation (e.g. introduction of additional animals post-establishment for the purposes of increasing genetic diversity). Genetic responses include correlating & assessing the reproductive success of translocated animals; estimating the effective size of the founding population; and, quantifying the extent of loss of genetic diversity.*

Table 7. Measures of genetic variability from the translocated woylie populations from Western and South Australia, including the mean observed (H_o) and mean expected heterozygosity (H_e) and mean observed (N_A) number of alleles, for the original wild woylie populations. Values given as mean ± S.D.

Population	N	Unbiased Hz	Obs Hz	No Alleles
Batalling State Forest	56	0.841 ± 0.044	0.848 ± 0.024	7.00 ± 3.00
Karakamia Sanctuary	43	0.795 ± 0.057	0.781 ± 0.030	9.80 ± 2.17
St Peter Island, SA	30	0.648 ± 0.031	0.531 ± 0.041	5.60 ± 2.19
Venus Bay Island, SA	14	0.666 ± 0.063	0.595 ± 0.076	3.33 ± 0.58
Wedge Island, SA	32	0.687 ± 0.026	0.594 ± 0.050	4.67 ± 1.15

Table 8. Estimates of a genetic bottleneck and the effective population size from the translocated woylie populations from Western and South Australia for the microsatellite loci. n.s. = not significant ($P > 0.05$). Bottleneck: probability of a recent genetic bottleneck detected;

	Genetic bottleneck		Effective Population size	
Population	P	Mode-shift	Ne (LD)	range
Batalling State Forest	0.031	No	0.5	Not available
Karakamia Sanctuary	0.063	No	44	29.8 – 77.6
St Peter Island, SA	0.437	No	34	18.8 – 91.1
Venus Bay Isl, SA	0.062	Yes	∞	13.4 - ∞
Wedge Island, SA	0.062	No	∞	34.8 - ∞

Translocated populations sent to South Australia exhibit low levels of variability, with all populations containing only a ~60% of the variation contained in their parental stocks (Table 7). The Venus Bay populations, as expected, have signatures of genetic bottlenecks and should not be considered as "insurance population", although on an island and safe from predators.. The populations from Venus Bay, St Peter Island and Wedge Island appear to be genetic similar and the results of their F_{ST} suggest that they originate from closely related population (Table 6). Similarly, the Upper Warren and Kingston samples showed a similarly low F_{ST} (F_{ST} =0.002), suggesting that they could also be from a common origin.

Regrettably, due to the extreme low density and a bush fire, it has not been possible to collect a sufficient number of samples from Kalgarin National Park and Kalbarri National Park despite the effort. Further attempt are planned for October 2008 under the Western Shield monitoring activities. It would be particularly interesting to be able to study these populations given the fact that DNA samples of the founders are available at the wildlife genetic collection at Murdoch University.

6. *Based on the findings of the above objectives, predict the genetic implications of recent woylie declines, and the likely consequences for future species recovery and conservation. This may allow specific populations to be identified as high priority for conservation efforts.*

This study has successfully identified that the populations from the Upper Warren region are highly variable and do not appear to retain signatures of either long or short-term genetic bottlenecks. Of more concern are those original populations from Tutanning and Dryandra. They both are, or near to having a significant bottleneck, but display no mode-shift in allele frequencies. They both retain genetic diversity, suggesting that a genetic bottleneck has been a recent impact on the populations, but this is not evident from a long term diagnosis, as they contain healthy amounts of genetic variability, and as such should still be considered as candidate populations when individuals are being considered for reintroduction, or in the establishment of insurance populations.

7. *Investigate whether there are any relationships between woylie population declines (and associated demographic change such as survivorship, reproductive success, etc) and the genetic attributes of affected and unaffected populations (i.e. a complimentary component of the existing population comparison study).*

Progresses have been achieved in centralising all the various demographic data available. This includes information on sex, age, fertility and survivorship of woylies as well as capture rates in eleven forest blocks in the Upper Warren and in Karakamia Sanctuary. An outstanding effort has been made to standardize and validate the entries that have been recorded under protocols that have changed over the time. In fact, we have been able to collate almost thirty years of data. The centralized database

would enable a formal analysis of the possible relationships between genetic attributes and demographic trends of various locations in different point in time once the genetic data that we have been working on, will be finalised.

CONCLUSION & FUTURE DIRECTIONS

Issues of genetic drift, inbreeding and loss of genetic diversity are becoming more relevant as populations of many threatened species decline. This trend is exacerbated in small and isolated populations (e.g. island populations). In many cases, these populations are becoming the only source of reproductive potential for many endangered species. Where there are several isolated populations of a threatened species and limited funds, it is often difficult for conservation mangers to identify which populations should be targeted for conservation efforts, and to identify those populations which could be used for captive breeding or translocation. Ideally, populations that retain the greatest genetic variation should be favoured for conservation efforts, but these are not easily identified.

We believe that this investigation represents an interesting case study, which provides not only important information directly relevant to woylie management and conservation but also a model for other species that share similar management history and ecology. We are confident that the number of primers that we have been able to use for this genetic study is appropriate to answer our original aims. A large robust set of primers now exist to answer these questions. Coupled with samples obtained from such a large number of locations and a large number of individuals from within each, this should allow us to work with reasonable statistical confidence.

This was an ambitious project and we believe that we have achieved the original objectives. Due to the success we anticipate genotyping many more specimens than originally proposed, and this is continuing. Overall, the findings of this preliminary study suggest that molecular ecology can make a valuable contribution to the planning and management of the woylie as it has declined. A wider geographic distribution of samples is continuing, larger sample numbers and the scoring of more loci would have improved this study considerably

ACKNOWLEDGMENTS

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