

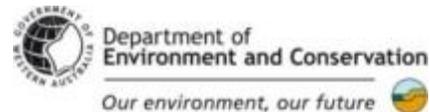
**CONSERVATION CONUNDRUM: THE
POPULATION DYNAMICS ASSOCIATED WITH
RECENT DECLINE OF WOYLIES (*BETTONGIA
PENICILLATA*) IN AUSTRALIA.**



Prepared by

**Carlo Pacioni
Peter Spencer**

Adrian Wayne



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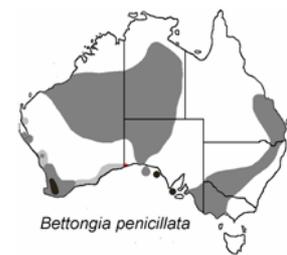
PACIONI C., P.B.S. SPENCER & A. WAYNE (2009). CONSERVATION CONUNDRUM:
THE POPULATION DYNAMICS ASSOCIATED WITH RECENT DECLINE OF
WOYLIES (*BETTONGIA PENICILLATA*) IN AUSTRALIA. UNPUBLISHED REPORT TO
THE SOUTH COAST NATURAL RESOURCE MANAGEMENT INC.

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. The woylie is now listed as 'Critically Endangered' in the IUCN Redlist



Conservative measures of the population decline (Wayne 2006) show that the woylie population at Dryandra has declined by >93% 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 >95% since 2002. Similarly, woylies at Batalling (a 1982 reintroduction site east of Collie) have declined by 97% since 2002. 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. The declines continue in the Upper Warren region, now affecting the last

remaining moderate-density wild woylie population in WA. 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 (StPI), Wedge (WEDI) and Venus Bay Island (VBIA), which remain stable and a fenced mainland peninsula (Venus Bay Peninsula) that has concurrently undergone around a 90% decline (P. Copley and J. Van Weenen, pers. comm.). The fenced Scotia sanctuary (AWC) in New South Wales also supports a moderate woylie population, where moderate declines have also been observed (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; DEC 2008). 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 South Coast Natural Resource Management Board. 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.

For these reasons this study focuses on wild populations and aimed to: 1) establish the overall genetic variability and differences, within and among populations. 2) Identify

genetically distinct populations and possible sub structuring. 3) Establish population relationships both historically and contemporarily. In order to achieve these objectives we used two different genetic markers: mitochondrial DNA (mtDNA) and microsatellite loci (MS).

RESULTS & DISCUSSION

Using the Bayesian assignment approach implemented in STRUCTURE (Pritchard et al. 2000), we investigated the genetic structure of the indigenous populations and identified four genetically distinct groups: Tutanning Nature Reserve (TUT), Dryandra Forest (DRY), Kingston (the western part of Upper Warren, KING) and Perup (the eastern part of Upper Warren, PER). Final parameters of genetic variability (average number of alleles, effective number of alleles, average number of private alleles and expected heterozygosity) for these populations are reported in **Table 1**, and in **Table 2** are showed the indexes for the translocated populations. While DRY, KING and PER showed a relatively high genetic variability, indicating that these populations can ensure a medium-long term genetic viability, lower values were detected in TUT.

Table 1. Mean Allelic Patterns Across Indigenous Populations

Na: No. of Different Alleles. **Ne:** No. of Effective Alleles. **No.**

Private Alleles: No. of Alleles Unique to a Single Population. **He:**

Expected Heterozygosity. All values are given as a mean \pm 1

Standard error.

	DRY	TUT	PER	KING
Na	8.92 (\pm 0.92)	5.5 (\pm 0.61)	15 (\pm 1.81)	12 (\pm 1.33)
Ne	5.76 (\pm 0.66)	3.23 (\pm 0.31)	7.55 (\pm 0.88)	5.91 (\pm 0.62)
No. Private Alleles	0.92 (\pm 0.31)	0.67 (\pm 0.41)	2.42 (\pm 0.89)	1.17 (\pm 0.42)
He	0.8 (\pm 0.03)	0.64 (\pm 0.05)	0.83 (\pm 0.03)	0.79 (\pm 0.04)

Amongst the translocated populations, StPI, VBIA and WEDI have little genetic variability making dubious their genetic viability for long term conservation. Sampson (1971) forecast that without restoration of the habitat in TUT, this population could be compared to an island population. Unfortunately, almost forty years later, we found very similar results between TUT and the two bigger SA's islands. These considerations put some uncertainty on their short-medium term genetic viability unless active management is put in place at these locations. As expected, VBIA present little genetic variability due to its small area and consequently small population size. On the other

hand, BAT and KAR have high genetic variability. Analysis of allele frequencies showed that KAR is a good representation of its source population DRY as opposite to BAT that changed its genetic make up and became a completely genetically different population from PER (from where the founder animals were sourced).

Table 2. Mean Allelic Patterns Across Translocated Populations**Na:** No. of Different Alleles. **Ne:** No. of Effective Alleles. **No.****Private Alleles:** No. of Alleles Unique to a Single Population. **He:**Expected Heterozygosity. All values are given as a mean \pm 1

Standard error.

	BAT_2007	KAR_2004	StPI	VBIA	WEDI
Na	7 (\pm 0.79)	7.5 (\pm 0.82)	4.92 (\pm 0.54)	2.67 (\pm 0.28)	4.17 (\pm 0.3)
Ne	4.01 (\pm 0.41)	4.87 (\pm 0.66)	3.02 (\pm 0.27)	2.1 (\pm 0.3)	2.83 (\pm 0.22)
No. Private Alleles	0.17 (\pm 0.11)	0 (\pm 0)	0.08 (\pm 0.08)	0 (\pm 0)	0 (\pm 0)
He	0.72 (\pm 0.03)	0.74 (\pm 0.04)	0.63 (\pm 0.04)	0.42 (\pm 0.07)	0.6 (\pm 0.06)

Based on the results of the assignment tests, we were able to identify migrants (i.e. recent migrants) or animals whose ancestors were of different population from where they were sampled (i.e. offspring of migrants) and provided an understanding of the population relationship. As summarized by **Table 3** there is a current, relatively intense, gene flow between KING and PER while there is some indication of connection between KING and DRY.

Table 3. Migrants detected in the indigenous populations.

%Miss: proportion of genotyped missing. **P As:** probability of assignment. **P Anc:** probability of having ancestors from the population identified as origin.

ID	Sex	(%Miss)	INTO	FROM	GENERATION	P As	P Anc
07-366_UW_C	M	0	KING	PER	F2	0.069	0.929
07-370_UW_C	M	0	KING	DRY	F2	0.01	0.988
07-381_UW_C	F	0	KING	PER	F2	0.546	0.434
07-341_UW_B	F	0	PER	KING	F1	0.04	0.951
07-389_UW_C	M	0	PER	KING	F0	0	1
07-585_UW_M	M	0	PER	KING	F0	0	1

Analysis of D-Loop of the mtDNA showed that the haplotype distribution (mtDNA) is well mixed between the two KING and PER populations (**Fig 1**). We calculated the evolutionary distance between sequences using the Maximum Composite Likelihood method (Tamura et al. 2004) implemented in the software Mega version 4 (Tamura et al. 2007) with complete deletion of sites with gaps or missing data, leaving 353 sites for

analysis. The rate variation among sites was modeled with a gamma distribution. Interestingly, Dryandra's haplotypes are closely related to KING and PER while haplotypes isolated from Tutanning have a high evolutionary distance from all the others. These results support the relatively high level of gene flow between KING and PER and suggest that historically the three populations (DRY, PER and KING) were probably more connected than what is evident nowadays from MS. At this stage, we are not able to provide a robust explanation of why Tutanning has been genetically isolated for such long time. Possibly, the small population size at which the population has been for the last few decades increased the effect of the genetic drift.

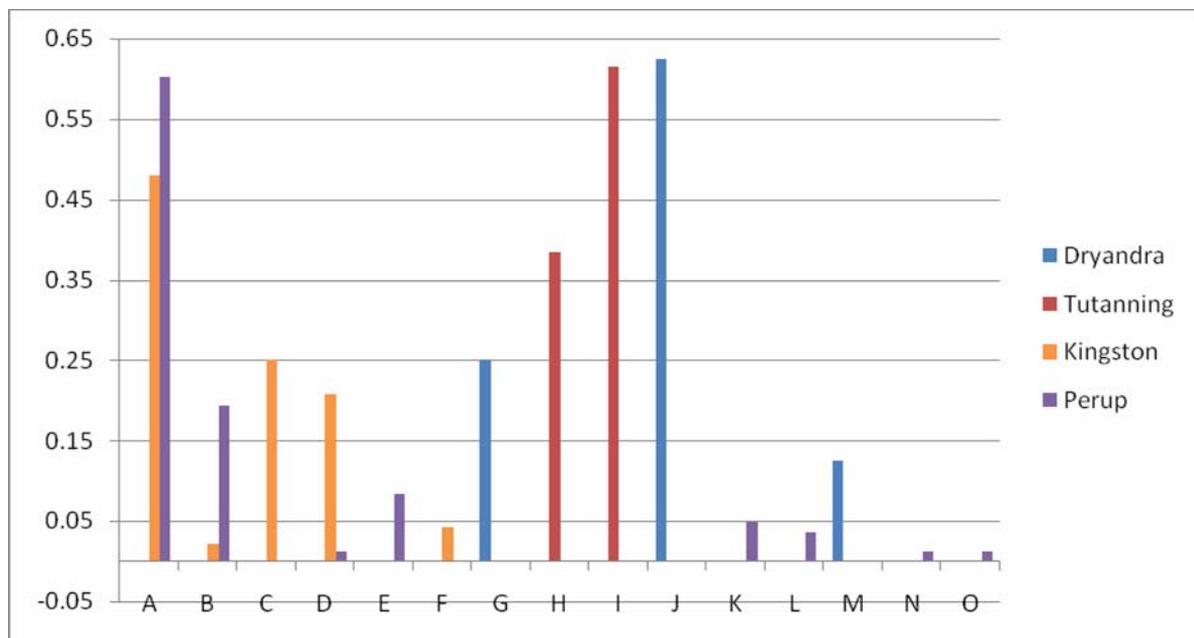


Figure 1. Haplotypes distribution within indigenous populations. On the x-axis different haplotypes. On the y-axis proportion within population.

Table 4. Pairwise evolutionary distance between haplotypes.

UW: haplotypes found in both PER and KING.

Hapl_A	Hapl_A	Hapl_B	Hapl_C	Hapl_D	Hapl_E	Hapl_F	Hapl_G	Hapl_H	Hapl_I	Hapl_J	Hapl_K	Hapl_L	Hapl_M	Hapl_N	
Hapl_A	UW	UW	UW	KING	UW	PERUP	KING	DRY	TUT	TUT	DRY	PERUP	PERUP	DRY	PERUP
Hapl_B	UW	0.03538													
Hapl_C	KING	0.00962	0.04												
Hapl_D	UW	0.02187	0.04712	0.01977											
Hapl_E	PERUP	0.01564	0.04471	0.01361	0.00962										
Hapl_F	KING	0.02865	0.00962	0.02865	0.04001	0.03768									
Hapl_G	DRY	0.0116	0.04233	0.01361	0.01361	0.00379	0.03538								
Hapl_H	TUT	0.01769	0.04001	0.01976	0.02398	0.01769	0.03313	0.02185							
Hapl_I	TUT	0.01976	0.04236	0.02186	0.02612	0.01976	0.0354	0.02397	0.00188						
Hapl_J	DRY	0.00765	0.03768	0.00571	0.02187	0.01564	0.03087	0.01564	0.02186	0.02398					
Hapl_K	PERUP	0.03539	0.00765	0.04001	0.03769	0.04	0.00962	0.04235	0.0354	0.0377	0.03769				
Hapl_L	PERUP	0.01769	0.04711	0.02398	0.01976	0.00962	0.04	0.01361	0.02397	0.02612	0.02186	0.04235			
Hapl_M	DRY	0.03539	0.00961	0.04001	0.03769	0.04	0.0116	0.04235	0.0354	0.0377	0.03769	0.00188	0.04235		
Hapl_N	PERUP	0.04442	0.02628	0.0468	0.04442	0.04205	0.02413	0.04678	0.04207	0.04443	0.04442	0.022	0.0444	0.022	
Hapl_O	PERUP	0.00188	0.03768	0.00765	0.01977	0.01361	0.03087	0.00962	0.01564	0.01769	0.00962	0.03769	0.01976	0.03769	0.0468

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 managers 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, we were allowed us to work with reasonable statistical confidence.

Overall, the findings of this study suggested that

1. Molecular ecology can make a valuable contribution to the planning and management of the woylie.
2. All indigenous populations have to be considered equally important because each of them retain unique genetic material.
3. The genetic viability of KING, PER and DRY is high enough to sustain medium-long term genetic conservation. Unfortunately, the same cannot be said about TUT and active management actions may be contemplated in order to improve the heterozygosity of this population.
4. The primary focus of management activities should be directed to support the rapid recovery of the woylie populations in order to guarantee large population sizes, which in turn would ensure long term conservation of the genetic pool.
5. The translocated population at Karakamia can be considered a genetic subset of Dryandra and, as such can be considered an insurance population.

6. The same cannot be said about Batalling. There is enough genetic divergence to suggest that the Batalling translocation has resulted in the subsequent population being unique from its source population (Perup).
7. SA's island populations are showing low level of heterozygosity and we anticipated that they might be at risk of being affected by inbreeding depression.

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