CHAPTER 6 CONSERVATION GENETICS

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Abstract

A crucial component of the Woylie Conservation and Research Project (WCRP) is a sound understanding of the genetics of this species.

Our aims were to characterise woylie genetics and use this information to focus on outcomes that will be directly relevant to conservation and recovery of the species and to incorporate these findings into the demographic management of declining populations.

6.1.1. Introduction

The role of genetics as an important factor influencing population dynamics has been analysed on both: theoretical and empirical grounds (Frankham, 1995). The recent development of the study of microsatellite loci for population genetics offers the opportunity to use non invasive sampling techniques and, at the same time, to have a good understanding of the genetic dynamics of wild populations (Goldstein and Schlèotterer, 1999; Sunnucks, 2000). The species appears to have undergone two recent (genetic) bottlenecks. One occurred following European settlement, and another as recently as four years ago. A possible outcome of this well-known genetic phenomenon would be a decreased capacity of the population to cope with changes as a result of the loss of variability and this may ultimately impact on the long-term survivorship of the population. As such, this issue should be considered an important aspect in the investigation of the recent woylie decline.

6.1.2. Methods

We used mtDNA and microsatellite loci to achieve the following aims:

- Characterise the historical genetic profile of the woylie species across its former range since the settlement of Australia by Europeans using ancient DNA approaches.
- Characterise the genetics of remaining contemporary extant woylie populations in Western Australia (i.e. Upper Warren, Dryandra and Tutanning), include quantifying to the levels of genetic variability, estimating the effective population size within and among extant remnant.
- 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).
- 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 and assessing the reproductive success of translocated animals; estimating the effective size of the founding population; and, quantifying the extent of loss of genetic diversity.
- 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.

- 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).
- Describe information on paternity, social structure, home range and individuals movements between adjacent populations.

Tissue samples have been collected from animals trapped at the Population Comparison Study (PCS) sites, and Western Australian populations (Dryandra, Tutanning and Batalling) as well as populations that were reintroduced to South Australia. In addition, tissue samples collected during (previous) translocations have been made available for this study by other researchers. Using this material, we optimised a range of microsatellite primers that were developed from studies of other macropods and potoroos. These primers were tested to verify their suitability for investigating the molecular ecology of the woylie.

6.1.3. Results

To date, more than 1200 tissue samples have been collected, collated, incorporated into a sample database. All samples are stored in the wildlife genetic collection at Murdoch University (Table 6.1.1).

Table 6.1.1. Sample collection field sites detailing the number (N) of tissue samples collected for use in this study.

Sampling locality	Number of samples (N)
Upper Warren	535
Karakamia Wildlife Sanctuary	256
Batalling State Forest	89
Dryandra Nature Reserve	60
Tutanning Nature Reserve	44
St Peter's Island, SA	119
Venus Bay Island A, SA	31
Venus Bay Peninsula Conservation Park, SA	50
Wedge Island, SA	80
Total	**1264**

DNA extractions were carried out on a subset of more than 250 of these samples. Twenty-eight primers that were originally developed for different Macropodidae species were tested to assess their suitability and polymorphism in woylies (Table 6.1.2). Nineteen of those produced an amplified PCR product when tested with woylie DNA. Of those, 13 produced reproducible and variable information. For a small test-sample (N=8), we recorded heterozygosity values of between 70 and 86% (mean heterozygosity = 79 ± 5.7%). All 13 loci were highly polymorphic, with between four and 12 alleles at any locus and the average of number of alleles per locus of 6.69 (S.D. = ± 2.05;) (Table 6.1.3).

Table 6.1.2. Information available related to the primers described from a range of Macropodidae species that were tested for polymorphism in samples from the woylie. Readability is how easily the DNA profiles could be interpreted and indicated as a score from 1 (very low) to 5 (very good).

Locus	Gene Bank	Size range	Readability	Reference
Bt64			4	(Pope *et al.*, 2000)
Bt76			5	(Pope *et al*.., 2000)
Bt80			5	(Pope *et al.*, 2000)
Pl2	Y09050	150-160	1	(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	4	(Luikart *et al.*, 1997)
Y175			4	(Zenger *et al.*, 2002)
Y170				(Pope *et al.*, 1996)
Y151			4	(Pope *et al.*, 1996)
Y76			1	(Pope *et al.*, 1996)
Pa597	U30636		3	(Spencer *et al.*, 1995)
Pa593	U30633		5	(Spencer *et al.*, 1995)
Pa297	U30634		2	(Spencer *et al.*, 1995)
Pa385	U30632		3	(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	4	(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)

Table 6.1.3. Locus characteristics: preliminary results.

Locus	*N*	H_O	H_E	N_A	N_E
Bt76	14	1.000	0.857	9	7.00
Bt80	16	0.875	0.820	7	5.57
T172	16	0.500	0.773	5	4.41
Pl2	16	1.000	0.797	6	4.92
Bt64	14	0.571	0.704	7	3.38
Pa297	10	0.400	0.800	6	5.00
Pa597	16	0.000	0.719	4	3.56
Pa595	14	0.286	0.816	6	5.44
Pl26	16	0.625	0.695	4	3.28
Y151	16	0.750	0.898	12	8.00
Y170	16	0.875	0.789	6	4.74
Y175	16	0.875	0.844	8	6.40
Pa593	16	0.875	0.773	7	4.41
Mean	**15.1**	**0.664 ± 0.291**	**0.791 ± 0.057**	**6.69 ± 2.05**	**4.84 ± 1.10**

H_0 Observed heterozygosity.; H_E Expected heterozygosity (Nei 1973). ; N_A Number of alleles.; N_E Number of expected alleles.

6.1.4. Discussion

We have made significant progress towards achieving the development of a set of polymorphic microsatellite markers for the study of the molecular ecology of the woylie. Once these informative primers have been further refined and optimised we will be able to generate a rapid amount of information to interpret any underlying patterns of genetic contributions in the recent demographic changes that appear to have occurred in populations of the species.

The reliable interpretation of the PCR-amplification products is extremely good for eight primers (Bt 64, Bt 76, Bt 80, Pl 26, Pa 593, T17-2, Y151, and Y175) while the remaining (Pl 2, Pa 385, Pa 597, Pa 297 and Y76) are yet to be fully optimised. The remaining 15 primers were either difficult to interpret, monomorphic or did not produce a PCR product.

Based on the allele frequencies, we found from our preliminary screening that the average Hardy-Weinberg expected heterozygosity (in the small number of woylies tested) to be very high (79%; see Table 6.1.3 for details; Nei, 1973). This level of heterozygosity is similar to the amount of variability found in other marsupial species (Pope *et al.*, 2000, Eldridge *et al.*, 2004). In addition, this result suggests that the probability of obtaining identical DNA profiles from two randomly chosen woylies would be more than one in 100 million. As a result, the DNA profiles are unique to a each individual and consequently great confidence can be placed in correctly identifying individuals and their relationship to other woylies.

6.1.5. Future directions

We are planning to optimise the polymorphic primers in October. Once an efficient set of conditions has been achieved, most of the remaining genetic data should be generated by the end of this year. It is anticipated that the genetic analysis should be concluded in early 2008.

6.1.6. Conclusion

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 samples from within each, this should allows to work with reasonable statistical confidence.

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6.1.8. References

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