

PRIMER NOTE

## Highly variable microsatellites in isolated colonies of the rock-wallaby (*Petrogale assimilis*)

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Habitat fragmentation is a major concern of conservation biologists, since reduced gene flow between isolated sub-populations may further decrease the effective population size of a species. Rock wallaby (genus *Petrogale*) colonies provide a naturally occurring system to study the genetic consequences of habitat fragmentation. Colonies of less than 10 to more than 50 adult rock wallabies are restricted to isolated rock outcrops, and are thus expected to exhibit the genetic and demographic consequences of small population size. In this paper, we describe the characterization of a series of microsatellite loci from the allied rock-wallaby, *Petrogale assimilis*, and their use to estimate genetic variation. Despite the small population sizes, a high degree of heterozygosity was observed at all the loci investigated.

Single-locus microsatellite typing exploits the natural variability of simple repetitive sequences, and has been extensively employed in the medical sciences and in animal husbandry. Despite the potential applications of this technology in conservation genetics, few marsupial microsatellite loci have been characterized to date. In a wide range of eutherian mammals, microsatellites with the general form (TG)<sub>n</sub> are by far the most common class in the genome (see, for example, Moore *et al.* 1991). Our preliminary studies (Odorico *et al.* 1992) imply that this is also true for marsupials. We therefore characterized a series of (TG)<sub>n</sub> microsatellite loci from *P. assimilis*, with the aim of developing microsatellite PCR systems for kinship and population studies.

A partial genomic library was constructed for *P. assimilis* and screened by standard techniques (Weber & May 1989; Tautz 1989; reviewed in Queller *et al.* 1993). In the initial round of screening, seven clones containing (TG)<sub>n</sub> microsatellites with  $n > 20$  were identified; several additional clones contained imperfect or lower numbers of (TG)<sub>n</sub> repeats. A further single clone contained (CT)<sub>20</sub> and another a complex locus containing two tetranucleotide

repeats (Table 1). Oligonucleotide primers were designed to enable PCR amplification at several of these loci (Table 1); in one case the repeat region was too close to the end of the clone to permit this, and in a second case the microsatellite was monomorphic. For two other (TG)<sub>n</sub> loci we do not yet have allelic data.

PCR reactions were performed in 10-mM Tris containing 50-mM KCl, 1.5-mM MgCl<sub>2</sub>, 1 unit of *Taq* polymerase (Promega) and 50–100 ng of template DNA in a total volume of 25 µL. For each locus, the dNTP (0.2–0.8 mM) and primer (1–8 nM) concentrations were optimized, and in each case one of the primers was end-labelled using [<sup>32</sup>P]-ATP. Varying the primer and dNTP concentrations were found to have major effects on the amount of 'band-stuttering' (Tautz 1989; Luty *et al.* 1990) observed, and hence the interpretability of results. After an initial denaturation period of 5 min at 95 °C, 30 cycles of PCR were performed, each cycle consisting of 1 min denaturation at 93 °C, 30 s at the annealing temperature (Table 1) and 1 min extension at 72 °C. A final extension period of 2 min (at 72 °C) was employed. PCR products were resolved on urea-polyacrylamide (6%) sequencing gels, and size estimates for fragments obtained by comparison with control (M13mp8 DNA) sequencing reactions.

Tables 1 and 2 summarize allelic data for five loci in four colonies of *P. assimilis*. We found no evidence for sex-linkage or linkage between any of the loci. In most cases, alleles were shared across all of the populations. However, two 'private' alleles of the Pa595 locus were detected, which were unique to either the 'Black Rock'/'Little Black Rock' or Mt. Stuart/Magnetic Island populations.

'Black Rock' and 'Little Black Rock' are two panmictic colonies about 2 km apart and 265 km WNW of the other two colonies. Mt. Stuart and Magnetic Island are 19 km apart, but for the last 6000 years this distance has included a 4 km wide sea barrier. For the 'Black Rock' and 'Little Black Rock' colonies, the samples obtained are known to reflect most or all of the animals captured since 1986, respectively. The sizes of the other colonies are not accurately known. However, we consider that the samples represent ≈ 50% of the animals at Mt Stuart and a small proportion (< 5%) of the Magnetic Island population, which comprises several colonies.

**Keywords:** conservation genetics, macropods, marsupials, microsatellites

Received 30 January 1995; revision accepted 26 April 1995

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**Table 1** Characteristics of five polymorphic loci in *P. assimilis*

Locus	Core repeat sequence	<i>n</i>	No. of alleles	<i>F</i>	Primer sequences (5' → 3')	Primer melting temp* (°C)	Annealing temp (°C)
Pa385	(TG) <sub>16</sub>	168	9	0.271	GCTCTACCAGGCTGATTGGGA TGAGTATCTCTTTTGCTGCTTGAA	66	60
Pa593	(TG) <sub>23</sub> CG(TG) <sub>5</sub>	167	12	0.204	TAGGGCACGTCATAAGGATGCAG GCTTCCAGTTTCTGACTTTTCATG	70	55
Pa297	(TG) <sub>21</sub>	169	15	0.219	CCACCTTTGAGGGTATGGCTTT GATCTTGCTGTGTTTACTGTTTATTG	68	60
Pa595	(GATA) <sub>19</sub> (GACA) <sub>2</sub> GGTA(GATA) <sub>21</sub>	161	15	0.205	CTGAAGATGCACCCAGATA AATTCATCACTGGACTCTA	56	49
Pa597	(CT) <sub>20</sub>	167	19	0.147	ACATACTATGCAACATTGGCTT CTAGTAGAAAGGAAAAGAATTCAGA	66	55

*n* = total number of animals scored; *F* = frequency of most common allele.

\*Melting temperature of lower melting primer.

The DNA sequences from which the primers were derived have been deposited with NCBI, and have the following accession numbers: (Pa385) U30632; (Pa593) U30633; (Pa297)U30634; (Pa595) U30635; (Pa597) U30636.

Given the small and isolated nature of these colonies, we were surprised at the high heterozygosity values (comparable with those predicted by the Hardy-Weinberg equilibrium) and low allele frequencies (Table 2). We had expected to observe evidence of inbreeding. In addition, we had anticipated that the effect of population size in *P. assimilis* would be affected by: (1) behavioural traits which can lead to nonrandom mating, such as male dominance hierarchies and long-term pair bonds (Barker 1990); and (2) significant maternal differences in the survivorship of pouch young. The effects of these traits on individual differences in reproductive success have not yet been quantified.

One of the goals of microsatellite research is to develop primers which are informative for a wide range of species. To test the general usefulness of the primers developed for *P. assimilis*, we used them in reactions with DNA from the following macropods as templates: *P. persephone* (*n* = 6), *Thylogale stigmatica* (*n* = 6) or *Dendrolagus lumholtzi* (*n* = 1).

Each primer pair appeared to be informative for all of these species, as multiple loci were detected in each case (Table 2). These preliminary results suggest that the primers described above may have applications in population genetics across a broad range of macropods.

### Acknowledgements

This work was supported by the 1993 Prestige Grant from James Cook University of North Queensland. We would like to thank Peter Johnson for access to animals, other than allied rock wallabies, held at the Queensland National Parks and Wildlife Service, Pallarenda, Townsville.

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Species/Colony	<i>n</i>	Heterozygosity (% ± standard error)		Mean number of alleles
		Observed (Direct count)	Expected (Hardy-Weinberg)†	
<i>Petrogale assimilis</i>				
Black Rock	153	82.2 ± 0.02	85.9 ± 0.01	12.0
Little Black Rock	15	85.7 ± 0.06	85.4 ± 0.03	8.6
Mt. Stuart	20	84.8 ± 0.05	88.6 ± 0.02	6.6
Magnetic Island	6	83.3 ± 0.09	74.1 ± 0.04	4.2
<i>Petrogale persephone</i>	6	51.0 ± 0.21	46.6 ± 0.19	3.0
<i>Thylogale stigmatica</i>	6	52.0 ± 0.14	54.2 ± 0.14	3.0
<i>Dendrolagus lumholtzi</i>	1	-	-	2*

†Unbiased estimate (see Nei 1987).

\*Allelic polymorphism was not investigated for this species.

**Table 2** Genetic variance at five loci in four colonies of *P. assimilis* and in other macropods

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