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## High Levels of Genetic Variability in an Isolated Colony of Rock-wallabies (*Petrogale assimilis*): Evidence from Three Classes of Molecular Markers

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### Abstract

Estimates of genetic variation for a small ( $N_e = 39$ ) colony of allied rock-wallabies (*Petrogale assimilis*) were calculated with three different categories of molecular marker. Average heterozygosity was estimated at 3.8% for allozymes, 47.3% for multilocus 'DNA fingerprints' and 85.5% for microsatellite markers. Overall these values indicate that this small isolated colony of rock-wallabies maintains a high level of genetic variation despite its relative isolation and the apparently low levels of migration between colonies. It is likely that mechanisms exist (such as kin avoidance, multiple mating systems, high and variable selective pressure in extreme and fluctuating environmental conditions) that promote the maintenance of high levels of genetic variation in isolated colonies of *P. assimilis*. These mechanisms are discussed in the context of the results obtained from the molecular markers.

### Introduction

Rock-wallabies are highly specialised members of the marsupial family Macropodidae (kangaroos and their relatives) with a distribution throughout Australia and some offshore islands (Eldridge and Close 1993). Unique amongst the macropodids, rock-wallabies have evolved a lifestyle that sees them restricted to rocky outcrops and adjacent feeding areas. This contrasts markedly with their nearest relatives, the kangaroos and other wallabies, which are found mostly in woodlands, grasslands, or semi-arid rangelands (Strahan 1983).

There are 15 species of rock-wallabies (*Petrogale*) and the group exhibits exceptional chromosome diversity with 20 distinct chromosomal races (Eldridge and Close 1993). Animals are usually found within localised colonies, ranging in size from a single pair to several hundred individuals (Briscoe *et al.* 1982). Although colonies may be as close as a few hundred metres apart, in some regions there may be no suitable rocky habitat for many kilometres and, even in optimal habitats, colonies are patchy (Briscoe *et al.* 1982). As a result of their fragmented habitat and generally small colony size, individual colonies of rock-wallabies would generally be expected to show low levels of genetic variation.

The allied rock-wallaby (*Petrogale assimilis*) is found in north-eastern Australia (Eldridge and Close 1993). 'Black Rock' is a small isolated rocky outcrop (800 m long and 400 m wide; Horsup 1994) and the nearest major colony ( $N > 20$ ) is eight kilometres to the west. Radio-tracking studies indicate the greatest distance that individual rock-wallabies have been observed to move from the escarpment is 750 m and this occurred during the 'dry season' as wallabies searched further from the rock to feed (Horsup 1994). These data suggest indirectly that genetic exchange between colonies of *P. assimilis* is likely to be very limited. Six generations of wallabies have resided in the colony over the study period and information is available for much of their life histories. This colony provides an ideal opportunity to assess in some detail the level of within-colony genetic variability found in rock-wallabies.

Allozyme electrophoresis has been a standard technique for detecting genetic variability within and between individuals for much of the past 30 years. As such, there is now a wealth of comparative information on genetic variability in protein-encoding loci for most animal groups (Nevo *et al.* 1984). Other techniques now available enable the direct assessment of genetic diversity at the DNA level. Molecular VNTR (variable number of tandem repeat) markers such as minisatellites (Burke 1989; Scribner *et al.* 1994; Wetton *et al.* 1995) and more recently microsatellites (Paetkau and Strobeck 1994; Kellogg *et al.* 1995) have been used to study mating systems and population-level diversity. These markers reveal much greater levels of genetic variability than allozyme electrophoresis (Tautz *et al.* 1986; Tautz 1989), and are now considered superior as intra- and inter-population markers (Burke and Bruford 1987; Bruford and Wayne 1993; Scribner *et al.* 1994).

Surprisingly few studies have measured within-population variability with allozyme, minisatellite and microsatellite markers (however, see Scribner *et al.* 1994; Bancroft *et al.* 1995). Such comparative results should prove useful and important, allowing the costs and benefits of each technique to be evaluated and giving a more detailed picture of the variation resident in the gene pool under study. In this paper, we assess genetic variability in the 'Black Rock' colony of *P. assimilis* by a combination of allozyme electrophoresis, multilocus 'DNA fingerprints', and microsatellite methods. Somewhat unexpectedly, the members of the colony show evidence of moderate to high levels of genetic variability with all three markers. Mechanisms that may contribute to the maintenance of this genetic variation are discussed.

## Materials and Methods

### *Study Site and Field Methods*

The study colony occupies 'Black Rock' (19°01'S, 144°27'E), a small rock outcrop on 'Lyndhurst' Station in the wet-dry tropics of north-western Queensland, Australia. Wallabies were caught in wire cage traps (50 × 35 × 35 cm) with a standard peanut-butter bait. Individuals were marked for identification by ear tags/tattoos and/or implanted passive integrated transponder (PIT) tags. Blood samples (1–5 mL) were collected from the lateral caudal vein of each wallaby on initial capture. Blood samples for allozyme analyses were centrifuged in the field and the separate fractions (red blood cell and plasma) stored in liquid nitrogen. For DNA extraction, whole blood in EDTA was immediately frozen and stored at –20°C. DNA was extracted from the lymphocyte fraction according to protocols described in Spencer *et al.* (1995).

### *Detection of Allozyme Variation*

Allozyme electrophoresis was conducted on cellulose acetate gels ('Cellologel', Chemetron) according to protocols and procedures described in Richardson *et al.* (1986). Erythrocytes were lysed in an equal volume of homogenising solution (deionised water containing 0.1 mg NADP and 1 mL of 2-mercaptoethanol per mL) and screened for a large number of enzymes or non-enzymatic proteins. Of these displayed zymograms, 27 were of sufficient activity and resolution to enable individual allozyme profiles to be determined (Table 1). The nomenclature used for referring to loci and allozymes follows Adams *et al.* (1987). The data were analysed by the BIOSYS program (Swofford and Selander 1981) to give estimates of average heterozygosity from both direct counts and Nei's unbiased estimate of heterozygosity (Nei 1978).

### *Multilocus DNA Fingerprints*

Multilocus 'DNA fingerprints' were generated according to methods described by Odorico *et al.* (1992). Aliquots of up to 10 mg of genomic DNA were digested with *Hinf*I and applied to 35 cm, 0.8% agarose gels in TBE buffer (0.13 M Tris, 75 mM boric acid, 2.5 mM EDTA, pH 8.0) and run at 1.5 V cm<sup>-1</sup> for approximately 48–72 h. After electrophoresis, gels were denatured, neutralised and transferred to nylon membranes (Hybond NFp; Amersham). The B10 probe (Odorico *et al.* 1992) was oligolabelled (with <sup>32</sup>P-dATP) and added to the membranes and hybridised at 47°C in 6× SSPE; 7% SDS; 30% formamide; 0.5% skim milk powder.

Highly polymorphic banding patterns were obtained from three separate autoradiographs encompassing 33 individuals. This sample included all sexually mature animals in the colony plus any independent young in which either one or both parents were never sampled. The inclusion of the latter ensured representation of their genotypes in analyses, even though the parents were not caught. Individuals were not included if they

**Table 1. List of enzymes and non-enzymatic proteins used in the allozyme study on the 'Black Rock' colony**

Protein	Abbreviation of locus	Enzyme Commission No.
acid phosphatase	<i>Acp</i>	3.1.3.2
adenosine deaminase	<i>Ada</i>	3.5.4.4
adenylate kinase	<i>Ak</i>	2.7.4.3
albumen	<i>Alb</i>	–
carbonate dehydratase	<i>Ca</i>	4.2.1.1
diaphorase	<i>Dia</i>	1.6.99
enolase	<i>Enol</i>	4.2.1.11
esterase	<i>Est-1, Est-2, Est-3</i>	3.1.1
fumarate hydratase	<i>Fum</i>	4.2.1.2
glyceraldehyde-3-phosphate dehydrogenase	<i>Gapd</i>	1.2.1.12
lactoylglutathione lyase	<i>Glo</i>	4.4.1.5
aspartate aminotransferase	<i>Got</i>	2.6.1.1
glucose-6-phosphate dehydrogenase	<i>G6pd</i>	1.1.1.49
glucose-6-phosphate isomerase	<i>Gpi</i>	5.3.1.9
haemoglobin	<i>Hb</i>	–
isocitrate dehydrogenase	<i>Idh</i>	1.1.1.42
L-lactate dehydrogenase	<i>Ldh-1, Ldh-2</i>	1.1.1.27
malate dehydrogenase	<i>Mdh</i>	1.1.1.37
mannose-6-phosphate isomerase	<i>Mpi</i>	5.3.1.8
nucleoside-diphosphate kinase	<i>Ndpk</i>	2.7.4.6
purine-nucleoside phosphorylase	<i>Np</i>	2.4.2.1
dipeptidase	<i>Pep-A</i>	3.4.13
tripeptide aminopeptidase	<i>Pep-B</i>	3.4.11
proline dipeptidase	<i>Pep-D</i>	3.4.13
phosphoglycerate mutase	<i>Pgam</i>	5.4.2.1
phosphogluconate dehydrogenase	<i>6Pgd</i>	1.1.1.44
phosphoglycerate kinase	<i>Pgk</i>	2.7.2.3
phosphoglucomutase	<i>Pgm</i>	5.4.2.2
triose-phosphate isomerase	<i>Tpi</i>	5.3.1.1

had not become sexual mature and if their parents were identifiable. Genetic variability was estimated directly from the phenotypic multilocus (DNA fingerprinting) patterns obtained. Parameters calculated include heterozygosity ( $H$  and  $H_{\max}$ ), and the estimated number of loci ( $L$ ) as described in Stephens *et al.* (1992). Estimates of  $H$  and  $L$  have been shown to be upwardly biased and estimates of  $H_{bc}$  and  $L_{bc}$  were calculated with a bias-corrected factor (*sensu* Jin and Chakraborty 1993). As the number of bands within each gel is a function of the number of loci, only within-gel comparisons were made, although average levels of heterozygosity were estimated over the three fingerprint membranes.

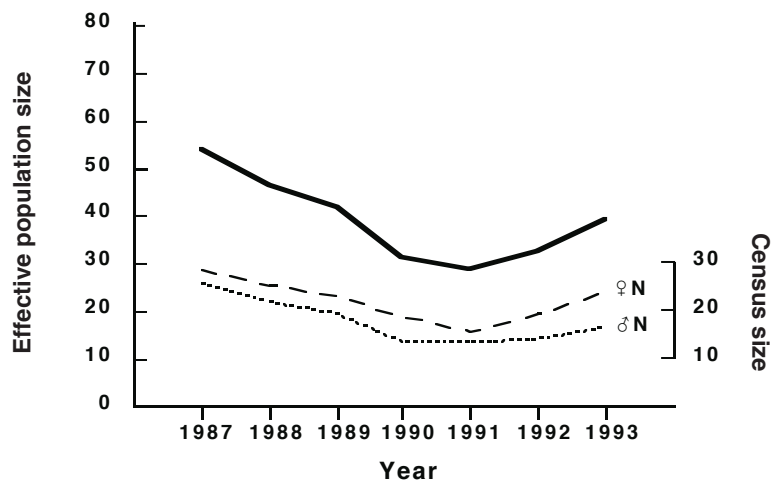
#### PCR Amplification of Microsatellite Loci

Polymerase chain reaction (PCR) amplified microsatellites were produced by protocols detailed in Spencer *et al.* (1995) for 128 unrelated individuals. Statistical problems are typically encountered with microsatellite loci as many of the genotypes are rare or never detected. As such, it is often not possible to directly test the data for conformation to Hardy–Weinberg expectations because of the presence of small expected values in  $\chi^2$  analyses (Hartl and Clarke 1989). Pooling rare difficulty but may result in a loss of information. Therefore, we avoided pooling rare alleles by running Monte-Carlo simulations of the data (Roff and Bentzen 1989; Zaykin and Pudovkin 1993).

## Results

### *Calculation of the Demographic Effective Population Size*

The effective population size (demographic) was estimated from the temporal changes in demographic population size of the 'Black Rock' colony with pooled census data (Crow and Kimura 1970) in conjunction with Manly-Parr recapture estimates (Manly and Parr 1968). The estimates of effective population size were not corrected for variance in reproductive success between sexes, a procedure that would reduce the estimate of effective size even further (see Crow and Kimura 1970, p. 70; Frankham 1995). The demographic effective population size ranged from 28.1 to 53.5 animals over the period 1987–93 (mean = 38.6; Fig. 1).



**Fig. 1.** Temporal changes in the demographic effective population size ( $N_e$ ; thick line) and the census size of males ( $\text{♂}N$ ) and females ( $\text{♀}N$ ) over the study period.

### *Allozyme-based Estimates of Heterozygosity*

The 25 enzymes and two non-enzymatic proteins characterised in this study (see Table 1) were encoded by a total of 32 putative loci. Of these, seven loci were polymorphic, displaying either two allozymes (*Gpi*, *Mpi*, *Pep-D*, *6Pgd* and *Pgm*) or three allozymes (*Est-1* and *Fum*) per locus. A summary of the allozyme frequencies found at each polymorphic locus is presented in Table 2. The average ( $\pm$  s.e.) heterozygosity per protein-encoding locus for the 'Black Rock' colony was estimated to be  $3.8 \pm 1.6\%$ , with Nei's unbiased estimate of heterozygosity (Nei 1978). The seven polymorphic loci displayed an average of  $2.29 \pm 0.18$  alleles per locus. The average heterozygosity at these seven variable loci was  $17.2 \pm 4.7\%$ .

### *Multilocus Probe-based Estimates of Heterozygosity*

Analysis of the B10 multilocus probe identified 36–51 bands greater than three kilobases in size over the three gels (Table 3). Measures of heterozygosity (*sensu* Stephens *et al.* 1992) ranged from 59.1 to 65.8% and were consistently higher than the 43.4 to 52.2% range encountered when corrected for bias as advocated by Jin and Chakorborty (1993). The mean heterozygosity averaged over three membranes from 33 unrelated individuals was estimated to be 47.3% ( $H_{\max} = 71.4$ ), an order of magnitude higher than the levels observed with the allozyme loci.

**Table 2. Levels of genetic variation detected by allozyme electrophoresis in eight populations of *Petrogale***  
 Qld, Queensland; NSW, New South Wales; WA, Western Australia. Species names as per Eldridge and Close (1993). All the results were from wild populations, except *P. penicillata* from Jenolan Caves in NSW which was a captive colony in a large enclosure

Taxon	Locality	Sample size	No. of loci examined	No of loci polymorphic	Locus	Allele frequencies	Estimated heterozygosity	Reference
<i>P. assimilis</i>	Black Rock, Qld	35	32	7	<i>Est-1</i>	0.01, 0.87, 0.12	0.038 ± 0.016	This study
					<i>Fum</i>	0.01, 0.97, 0.02		
					<i>Gpi</i>	0.97, 0.03		
					<i>Mpi</i>	0.04, 0.96		
					<i>Pep-D</i>	0.81, 0.19		
					<i>6Pgd</i>	0.06, 0.94		
<i>P. assimilis</i>	Mt Stuart, Qld	10	22	1	<i>6Pgd</i>	0.10, 0.50, 0.40	0.021	Briscoe, unpublished
					<i>P. herberti</i>	Cania Gorge, Qld	8	35
<i>Ldh-A</i>	0.75, 0.25							
<i>Pgm-C</i>	0.75, 0.25							
<i>P. herberti</i>	Mt Ball, Qld	7	44	4	<i>Adh</i>	0.88, 0.12	0.021	Briscoe, unpublished
					<i>Ldh-B</i>	0.75, 0.25		
					<i>Pgm-C</i>	0.88, 0.12		
					<i>Mpi</i>	0.67, 0.33		
<i>P. lateralis purpureicollis</i>	Dajarra, Qld	10	30	3	<i>Gpi</i>	0.80, 0.20	0.033	Briscoe, unpublished
					<i>Gdh</i>	0.11, 0.89		
					<i>Fum</i>	0.40, 0.60		
<i>P. lateralis lateralis</i> <sup>A</sup>	Nageen Hill, WA	10	22	1	<i>Tf</i>	0.69, 0.19, 0.12	0.011	Briscoe, unpublished
<i>P. penicillata</i>	Jenolan Caves, NSW	17	25	1	<i>Gpi</i>	0.76, 0.15, 0.09	0.016	Ingleby and Briscoe, unpublished
<i>P. sharmani</i>	Mt Claro, Qld	16	43	5	<i>Acp-C</i>	0.97, 0.03	0.030	Briscoe, unpublished
					<i>Pgm-C</i>	0.97, 0.03		
					<i>Idh-A</i>	0.80, 0.20		
					<i>Ldh-A</i>	0.577, 0.423		
					<i>6Pgd</i>	0.63, 0.25, 0.09		

<sup>A</sup> Population has been suppressed by fox predation for many years (Kinnear *et al.* 1988).

**Table 3. Estimates of average and maximum heterozygosities ( $H$ ), number of loci ( $L$ ) and variance of heterozygosity ( $V_s$ ) with the 'B10' multilocus probe**

\*\* , sampling variance of heterozygosity (*sensu* Stephens *et al.* 1992);  $H_{bc}$ , biased-corrected estimates (Jin and Chakraborty 1993)

Gel	Scorable bands	No. of individuals per gel	$L$	$H$	$H_{max}$	$V_s(H)^{**}$	$L_{bc}$	$H_{bc}$
1	36	11	8.744	0.591	0.714	0.026	9.50	0.463
2	57	8	10.568	0.620	0.766	0.019	11.253	0.522
3	36	14	5.772	0.658	0.714	0.037	6.675	0.434
Average heterozygosity				0.623		0.473		

#### Microsatellite-based heterozygosity

The five microsatellite loci were highly polymorphic with 9–19 alleles per locus [mean  $\pm$  s.e. =  $11.60 \pm 1.72$ ] compared with seven of the 32 protein-encoding loci tested. On the basis of the five polymorphic loci, heterozygosity ( $\pm$  s.e.) averaged  $85.5 \pm 1.5\%$  (Table 4), higher than estimates based on multilocus 'DNA fingerprint' patterns (Table 5) and five times more variable than the allozyme data (polymorphic loci only). The Monte-Carlo simulation of allele

**Table 4. Estimated allele frequencies for each microsatellite locus characterised in the 'Black Rock' rock-wallaby colony**

The number of individuals analysed for each locus is given in parentheses. Zero frequencies in any locus indicate that alleles were found in other rock-wallaby colonies, but not in the 'Black Rock' colony

Allelic designation	Locus				
	pPas385 (127)	pPas593 (108)	pPas297 (128)	pPas595 (123)	pPas597 (127)
A	0.000	0.023	0.000	0.122	0.146
B	0.020	0.000	0.000	0.037	0.020
C	0.118	0.065	0.152	0.000	0.205
D	0.307	0.005	0.133	0.000	0.000
E	0.189	0.241	0.027	0.004	0.051
F	0.063	0.060	0.016	0.000	0.004
G	0.059	0.157	0.234	0.167	0.020
H	0.059	0.019	0.160	0.053	0.071
I	0.185	0.042	0.023	0.065	0.004
J	–	0.144	0.000	0.134	0.083
K	–	0.245	0.000	0.183	0.031
L	–	0.000	0.000	0.016	0.028
M	–	–	0.000	0.024	0.094
N	–	–	0.141	0.089	0.047
O	–	–	0.031	0.106	0.051
P	–	–	–	–	0.008
Q	–	–	–	–	0.110
R	–	–	–	–	0.016
S	–	–	–	–	0.012
Heterozygosity	0.814	0.826	0.850	0.877	0.897
Mean heterozygosity	0.855 $\pm$ 0.015				

**Table 5. Comparisons of measures of genetic variability within the 'Black Rock' colony of *P. assimilis*, estimated for each of the three nuclear genetic markers**Values are given as mean ( $\pm$  s.e.). *H* is Nei's unbiased estimate of heterozygosity (Nei 1978)

Variable	Allozyme	Multilocus B10 probe <sup>A</sup>	Microsatellites
Number of loci	7	6.8–11.3	5
Heterozygosity <i>H</i>	0.172 ( $\pm$ 0.047)	0.473	0.855 ( $\pm$ 0.015)
<i>H</i> (range)	0.056–0.353	0.356–0.767	0.814–0.897
<i>H</i> <sub>max</sub> (range)	–	0.714–0.766 <sup>B</sup>	–
No. of alleles per locus	2.29 ( $\pm$ 0.18)	–	11.60 ( $\pm$ 1.72)

<sup>A</sup>Unbiased estimate from Jin and Chakraborty (1993) and <sup>B</sup>Stephens *et al.* (1992).

frequencies in the 'Black Rock' colony revealed that one of the five microsatellite loci, p*Pas*297, showed a heterozygote excess, deviating significantly from expected Hardy–Weinberg genotype frequencies ( $\chi^2 = 165.645$ , d.f. = 120,  $P = 0.004$ ). This effect may be due to pooling generations, as it was not observed when individuals were grouped into different age cohorts.

## Discussion

### *Comparison of Levels of Heterozygosity with the Three Classes of Molecular Marker*

#### *Allozyme variation*

Contrary to *a priori* expectations, the level of allozyme variation in the 'Black Rock' population is 'normal'. This is despite the small (demographic) effective population size of the colony and the presumed low rate of immigration from surrounding colonies. Compared with the average heterozygosity of 183 other mammalian species or subspecies surveyed by Nevo *et al.* (1984), the average heterozygosity of this isolated colony of rock-wallabies lies near the mean ( $\pm$  s.e.) for mammals in general (mean heterozygosity =  $4.14 \pm 0.25\%$ ; Nevo *et al.* 1984).

Sherwin and Murray (1990) reviewed the heterozygosity estimates found in a range of allozyme studies on marsupials, and concluded that marsupials had levels of within-population variability similar to those of eutherian mammals. The mean average heterozygosity presented for Australian marsupials is 3.3% (value calculated by excluding the result for the one non-Australian example, *Didelphis*), whilst an earlier study of 14 species of macropodids found an average heterozygosity of 4.6% (Cooper *et al.* 1979). Levels of heterozygosity at protein-encoding loci in other *Petrogale* species (see Table 2) are all lower than for this study. However, we would be surprised if heterozygosities from *P. assimilis* were significantly different from other species of *Petrogale*, such as *P. purpureicollis* ( $H = 3.3\%$ ) or even the closely related *P. sharmani* ( $H = 3.0\%$ ), especially given the differences in sample size.

'Black Rock' wallabies have significantly higher levels of heterozygosity ( $H = 3.8\%$ ) than the Jenolan Caves population of *P. penicillata* ( $H = 1.6\%$ ) and Nangeen Hill population of *P. lateralis* ( $H = 1.1\%$ ) populations (Table 2). However, this is not surprising given their recent history. The population of *P. lateralis* at Nangeen Hill has been suppressed by fox predation for many years (see Kinnear *et al.* 1988) while the captive Jenolan Caves population of *P. penicillata* was established from 10 founders in 1966 (R. L. Close, unpublished data). These data suggest that small population size and long-term isolation in rock-wallabies does result in a detectable loss of variation.

#### *Multilocus DNA fingerprint variation*

Multilocus band patterns display levels of heterozygosity that are an order of magnitude greater than those detected by allozyme analysis. However, such VNTR methods are subject to bias as measurement errors within and between gels may exceed the repeat unit length, making definitive assignment of alleles difficult (Scribner *et al.* 1994). Additionally, despite the strict

demonstration of Mendelian inheritance, multiple band patterns cannot be attributed to specific loci (Lynch 1991). Consequently, allele frequency distributions are not attainable and require selection of procedures to identify population level information (Lynch 1991; Stephens *et al.* 1992). These problems make the testing of classic genetic models difficult with minisatellites as discussed by Budowle *et al.* (1991) and Weir (1992a, 1992b). However, analytical interpretations are increasingly becoming clearer (Weir 1992a; Charkarborty *et al.* 1993; Shriver *et al.* 1993), allowing minisatellite alleles a future role in the understanding of short-term population processes, such as population structure, relatedness and dispersal (Webb *et al.* 1995).

#### *Microsatellite variation*

We are able to make some comparisons within *P. assimilis*. Interestingly, similar average heterozygosities (i.e.  $H > 82\%$ ) were observed in other less-isolated colonies of *P. assimilis* with the same primer sets (Spencer *et al.* 1995), suggesting that there has been no significant detectable loss of variation in the 'Black Rock' population. Whether other species of rock-wallabies carry similar levels of within-colony genetic variability remains to be determined, with work already underway to investigate this question. There are, however, a few studies in which to compare microsatellite variation in other marsupials. Pope *et al.* (1996) found expected levels of heterozygosity in the yellow-footed rock wallaby (*P. xanthopus*), of 67–72% for four (different) loci. Additionally, Taylor *et al.* (1994) found (expected) levels of heterozygosity of 27% in an inbred population of the northern hairy-nosed wombat (*Lasiorhinus krefftii*), compared with 66% in a closely related species, the southern hairy-nosed wombat (*L. latifrons*).

Microsatellites should be extremely informative for patterns across closely related species. Different microsatellite loci are likely to be subject to quite different selective mechanisms (Moore *et al.* 1991) as they do not seem to be conserved over large taxonomic distances (although see FitzSimmons *et al.* 1995). The comparison of microsatellite variation at phylogenetic distances further than subfamily level in mammals may not be particularly useful, as loci are unlikely to be conserved in distantly related species (Moore *et al.* 1991).

#### *Maintainance of High Levels of Heterozygosity in the 'Black Rock' Colony*

Three scenarios for the observed levels of heterozygosity in this colony are possible. First, and most obvious, is that this colony may not be as genetically isolated over time as the radio-tracking and other studies might suggest. Genetic variability in the colony may be augmented at regular intervals via migration from other colonies. Second, these levels may not be adaptive, but may reflect either some base level peculiar to this species or to rock-wallabies in general, or a level intrinsic to this set of loci. A third possible explanation is that these levels may be adaptive, that is, heterozygote individuals have higher levels of fitness than homozygotes (e.g. reproductive success; see Vrijenhoek and Leberg 1991), with such levels being maintained by any of several mechanisms. It should be noted from the outset that none of these three scenarios is mutually exclusive.

It would be possible to explain the high levels of variability resident within the 'Black Rock' colony if nearby colonies possessed distinct gene pools and acted as sources of occasional gene flow. Whilst immigration into 'Black Rock' colony may be greater than we have assumed, behavioural evidence suggests indirectly that genetic exchange between *P. assimilis* colonies is likely to be very limited, as Horsup (1994) found that individuals moved only short distances (< 750 m) from the colony to feed. The size of home ranges during the 'wet-season' was significantly reduced, as animals fed closer to the rock (Horsup 1994). The nearest major rock-wallaby colony is 8 km to the west of the colony at 'Black Rock'. Most significantly, a colony of rock-wallabies on nearby 'Horse' mountain, 40 km to the south of 'Black Rock', hosts a different louse species (Barker and Close 1990), strongly inferring that physical contact and therefore genetic exchange between different colonies is limited. Future characterisation of colonies near to 'Black Rock' with the five microsatellite loci will help assess the degree of

similarity between their gene pools and hence provide more insight into the possibility that gene flow could be a contributing factor to observed levels of genetic variability found in this study.

As discussed by Caughley (1994), it is difficult to explain relative fitness in terms of differences in species-level heterozygosity. It may be that the pattern of generally high levels of heterozygosity observed in the colony are due to processes (for example, the mating system and inbreeding avoidance) intrinsic in this genus. The levels of allozymic heterozygosity presented in Table 2 suggest that the other species within the genus *Petrogale* display similar levels of genetic variation at allozyme loci. Few data are available to determine whether the levels of variability encountered within the 'Black Rock' colony are characteristic of rock-wallabies in general. Levels of genetic variability at the five microsatellite loci for other colonies of *P. assimilis* are presently the subject of further investigation.

The maintenance of relatively high levels of heterozygosity may be explained if the colony experiences continuous cycles of near extinction as a result of harsh acyclic environmental extremes (see below) followed by immigration from other colonies possessing either extraordinarily high levels of heterozygosity or significant genetic dissimilarities. This would seem reasonable for a newly founded population, for example, as found in Soay sheep where the population had only recently been introduced to an island (Bancroft *et al.* 1995). However, it seems an unsatisfactory explanation for *P. assimilis*. Other naturally occurring colonies of *P. assimilis*, which have presumably persisted for considerable evolutionary rather than ecological time (Briscoe *et al.* 1982), also show high levels of genetic variation (Spencer *et al.* 1995).

A range of mechanisms may maintain high levels of genetic variance in allied rock-wallabies. In particular, multiple mating systems and kinship avoidance have been observed in the colony (Spencer, unpublished data). Moreover, rock-wallabies such as *P. assimilis* undergo acyclic environmental extremes in the wet-dry tropics which result in fluctuations in population size. Much of Australia and 'Black Rock' in particular have been under drought conditions throughout the 1980s. This has seriously affected recruitment into the 'Black Rock' colony. Delaney and Marsh (1995) suggest that the age structure of the colony has been highly skewed towards old animals during the early to mid-1980s. Such a finding is consistent with low capture rate of non-adult rock-wallabies and low survival of pouch young and is quite different from the usual pattern in a species with a long lifespan. For example, a study of culling in grey kangaroos revealed that almost 60% of those shot consisted of young animals (Quin 1989). The low recruitment, the presence of a very old age cohort during the early part of the study and the higher than average rainfall during the 1970s support the view that localised colonies of *P. assimilis* fluctuate acyclically, concomitant with periods of high rainfall (Delaney and Marsh 1995). Such fluctuations may contribute to the maintenance of high levels of genetic variability in *P. assimilis* via selection through non-random mortality of particular genotypes during population crashes (Ellner and Hairston 1994), when selection would presumably be intense (Takahata 1981). Fluctuating population size may be very important to the genetic integrity of the colony. Frankham (1995) found that the most important influence on estimates of  $N_e$  were fluctuations in population size, and suggests that the effective population size, with demographic estimates, is likely to be an order of magnitude larger than those estimated with genetic data (*sensu* Frankham 1995).

The presence of an excess of heterozygotes at one of the microsatellite loci may reflect the existence of overdominance for some of the heterozygotes at this locus. Maruyama and Nei (1981) showed that a combination of overdominant selection (heterozygote advantage) and mutation was eminently effective at increasing the mean heterozygosity and variance in heterozygosity. They found that, for loci displaying overdominance, only a very low mutation rate is required to explain high levels of polymorphism in a finite population, even for loci with a large number of alleles. The high mutation rates typical of microsatellite loci (exceeding  $10^{-2}$  per locus per generation for dinucleotides; Bruford and Wayne 1993) are clearly sufficient to enable high levels of heterozygosity to persist even without overdominance.

Prior to the use of minisatellites and microsatellites in population genetic studies, allozymes were the marker of choice. Their modes of mutation are well understood and they remain a cost-effective and rapid method for generating data. However, the extraordinary levels of heterozygosity and their high level of mutation (Dallas 1992) make VNTR loci ideal for the examination of changes in the size, structure and dispersal at the population level (Scribner *et al.* 1994). The ease of using microsatellite markers for population studies also make them a powerful tool for the population and conservation geneticist to investigate population-level variation where other markers may lack sensitivity to these changes. This is especially true in species with little allozyme polymorphism, for example, many of the social insects (Hughes and Queller 1993). Microsatellites also have the potential to provide significant insights into the evolutionary process. However, the modes of mutation (see Valdes *et al.* 1993; Goldstein *et al.* 1995) are still under investigation.

Unlike protein loci, microsatellite loci may not be particularly informative when different loci have been sampled from distantly related species (Moore *et al.* 1991). The primers developed for *P. assimilis*, however, have revealed polymorphic loci across a range of diverse macropod species (Spencer *et al.* 1995), making them potentially useful for many endangered species of macropods from Australia. In addition, these primers show no linkage and are inherited in a Mendelian fashion, making them ideal for population studies.

It is likely that mechanisms exist that promote the maintenance of genetic variation in isolated colonies of *P. assimilis*. Behavioural mechanisms, such as kin avoidance, multiple mating systems, and high and variable selective pressure in extreme and fluctuating environmental conditions, presumably act to maintain variation. These are thought to have consequently led to the maintenance of high levels of genetic variation observed in the *Petrogale* genus.

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#### References

- Adams, M., Baverstock, P. R., Watts, C. H. S., and Reardon, T. (1987). Electrophoretic resolution of species boundaries in Australian Microchiroptera. I. *Eptesicus* (Chiroptera:Vespertilionidae). *Australian Journal of Biological Sciences* **40**, 143–162.
- Bancroft, D. R., Pemberton, J. M., and King, P. (1995). Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. *Heredity* **74**, 326–336.
- Barker, S. C., and Close, R. L. (1990). Zoogeography and host associations of the *Heterodoxus octoseriatus* group and *H. ampullatus* (Phthiraptera:Boopiiidae) from rock-wallabies (Marsupialia:*Petrogale*). *International Journal for Parasitology* **20**, 1081–1087.
- Briscoe, D. A., Calaby, J. H., Close, R. L., Maynes, G. M., Murtagh, C. E., and Sharman, G. B. (1982). Isolation, introgression and genetic variation in rock-wallabies. In 'Species at Risk: Research in Australia'. (Eds R. H. Groves and W. D. L. Ride.) pp. 73–87. (Australian Academy of Science: Canberra.)
- Bruford, M. W., and Wayne, R. K. (1993). Microsatellites and their application to population genetic studies. *Current Opinions in Genetics and Development* **3**, 939–943.
- Budowle, B., Giusti, A. M., Wayne, J. S., Baechtel, F. S., Fournay, R. M., Adams, D. E., Presley, L. A., Deadman, H. A., and Monson, K. L. (1991). Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons. *American Journal of Human Genetics* **48**, 841–855.
- Burke, T. (1989). DNA fingerprinting and other methods for the study of mating success. *Trends in Ecology and Evolution* **4**, 139–144.

- Burke, T., and Bruford, M. W. (1987). DNA fingerprinting in birds. *Nature* **327**, 149–152.
- Caughley, G. (1994). Directions in conservation biology. *Journal of Animal Ecology* **63**, 215–244.
- Chakraborty, R., Srinivasan, M. R., and DeAndrade, M. (1993). Intraclass and interclass correlations of allele sizes within and between loci in DNA typing data. *Genetics* **133**, 411–419.
- Cooper, D. W., Johnson, P. G., Sharman, G. B., and VandeBerg, J. L. (1979). A comparison of genetic variability at X-linked and autosomal loci in kangaroos, man, and *Drosophila*. *Genetics Research* **33**, 243–252.
- Crow, J. F., and Kimura, M. (1970). 'An Introduction to Population Genetics Theory.' (Harper and Row: New York.)
- Dallas, J. F. (1992). Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. *Mammalian Genome* **5**, 32–38.
- Delaney, R., and Marsh, H. (1995). Estimating the age of wild rock-wallabies by dental radiography: a basis for quantifying the age structure of a discrete colony of *Petrogale assimilis*. *Wildlife Research* **22**, 547–559.
- Eldridge, M. D. B., and Close, R. L. (1993). Radiation of chromosomal shuffles. *Current Opinion in Genetics and Development* **3**, 915–922.
- Ellner, S., and Hairston, N. G. (1994). Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *American Naturalist* **143**, 403–417.
- FitzSimmons, N. N., Moritz, C., and Moore, S. S. (1995). Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution* **12**, 432–440.
- Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genetical Research* **66**, 95–107.
- Goldstein, D. B., Linares, A. R., Cavalli-Sforza, C. C., and Feldman, M. W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.
- Hartl, D. L., and Clarke, A. G. (1989). 'Principles of Population Genetics.' 2nd Edn. (Sinauer Associates Inc.: USA.)
- Horsup, A. (1994). Home range of the allied rock-wallaby, *Petrogale assimilis*. *Wildlife Research* **21**, 65–84.
- Hughes, C. R., and Queller, D. C. (1993). Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology* **2**, 131–137.
- Jin, L., and Chakraborty, R. (1993). A bias-corrected estimate of heterozygosity for single-probe multilocus DNA fingerprints. *Molecular Biology and Evolution* **10**, 1112–1114.
- Kellogg, K. A., Market, J. A., Stauffer, J. R., and Kocher, T. D. (1995). Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proceedings of the Royal Society, London Series B* **260**, 79–84.
- Kinnear, J. E., Onus, M. L., and Bromilow, R. N. (1988). Fox control and rock-wallaby population dynamics. *Australian Wildlife Research* **15**, 435–450.
- Lynch, M. (1991). Analysis of population genetic structure by DNA fingerprinting. In 'DNA Fingerprinting: Approaches and Applications'. (Eds T. Burke, G. Dolf, R. Wolff and A. J. Jeffreys.) pp. 113–126. (Birkhauser: Basel.)
- Manly, B. F. J., and Parr, M. J. (1968). A new method of estimating population size, survivorship and birth rate from capture–recapture data. *Translations of the Society of British Entomologists* **18**, 81–89.
- Maruyama, T., and Nei, M. (1981). Genetic variability maintained by mutation and overdominant selection in finite populations. *Genetics* **98**, 441–459.
- Moore, S. S., Sargeant, L. L., King, T. J., Mattick, J. S., Georges, M., and Hetzel, J. S. (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* **10**, 654–660.
- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.
- Nevo, E., Beiles, A., and Ben-Shlomo, R. (1984). The evolutionary significance of genetic diversity: ecological, demographic and life-history correlates. In 'Evolutionary Dynamics of Genetic Diversity'. (Ed. G. S. Mani.) pp. 213–213. (Springer-Verlag: Berlin.)
- Odorico, D. M., Spencer, P. B. S., and Miller, D. J. (1992). Improved DNA fingerprints for macropods generated using a (CA)<sub>n</sub>-containing satellite fragment derived from the allied rock-wallaby, *Petrogale assimilis*. *Wildlife Research* **19**, 397–404.
- Paetkau, D., and Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* **3**, 489–495.

- Pope, L. C., and Moritz, C., and Sharp, A. (1996). Population structure of the yellow-footed rock-wallaby, *Petrogale xanthopus* (Gray, 1854) inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* **5**, 629–640.
- Quin, D. G. (1989). Age structures reproduction and mortality of the eastern grey kangaroo (*Macropus giganteus* Shaw) from Yan Yean, Victoria. In 'Kangaroos, Wallabies and Rat-kangaroos'. (Eds G. Grigg, P. Jarman and I. D. Hume.) pp. 787–794. (Surrey Beatty and Sons: New South Wales.)
- Richardson, B. J., Baverstock, P. R., and Adams, M. (1986). 'Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies.' (Academic Press: Sydney.)
- Roff, D. A., and Bentzen, P. (1989). The statistical analysis of mitochondrial DNA polymorphism:  $\chi$  the problem of small samples. *Molecular Biology and Evolution* **6**, 539–545.
- Scribner, K. T., Arntzen, J. W., and Burke, T. (1994). Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite and multilocus minisatellite data. *Molecular Biology and Evolution* **11**, 737–748.
- Sherwin, W. B., and Murray, N. D. (1990). Population and conservation genetics of marsupials. *Australian Journal of Zoology* **37**, 161–180.
- Shriver, M. D., Jin, L., Chakraborty, R., and Boerwinkle, E. (1993). VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. *Genetics* **134**, 983–993.
- Spencer, P. B. S., Odorico, D. M., Jones, S. J., Marsh, H. D., and Miller, D. J. (1995). Highly variable microsatellites in isolated colonies of the rock wallaby (*Petrogale assimilis*). *Molecular Ecology* **5**, 523–525.
- Stephens, J. C., Gilbert, D. A., Yuhki, N., and O'Brien, S. J. (1992). Estimation of heterozygosity for single-probe multilocus DNA fingerprints. *Molecular Biology and Evolution* **9**, 729–743.
- Strahan, R. (1983). 'The Complete Book of Australian Mammals.' (Angus and Robertson: Sydney.)
- Swofford, D. L., and Selander, R. B. (1981). BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 281–283.
- Takahata, N. (1981). Genetic variability and rate of gene substitution in a finite population under mutation and fluctuating selection. *Genetics* **98**, 427–440.
- Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**, 6463–6471.
- Tautz, D., Trick, M., and Dover, G. A. (1986). Cryptic simplicity in DNA is a major source of genetic variation. *Nature* **322**, 652–656.
- Taylor, A. C., Sherwin, W. B., and Wayne, R. K. (1994). Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorchinus krefftii*. *Molecular Ecology* **3**, 277–290.
- Valdes, A. M., Slatkin, M., and Freimer, N. B. (1993). Allele frequencies at microsatellite loci: the stepwise mutation model revisited. *Genetics* **133**, 737–749.
- Vrijenhoek, R. C., and Leberg, P. L. (1991). Let's not throw the baby out with the bathwater: a comment on management for MHC diversity in captive populations. *Conservation Biology* **5**, 252–254.
- Webb, N. J., Ibrahim, K. M., Bell, D. J., and Hewitt, G. M. (1995). Natal dispersal and genetic structure in a population of the European wild rabbit (*Oryctolagus cuniculus*). *Molecular Ecology* **4**, 239–247.
- Weir, B. S. (1992a). Independence of VNTR alleles defined as fixed bins. *Genetics* **130**, 873–887.
- Weir, B. S. (1992b). Independence of VNTR alleles defined as floating bins. *American Journal of Human Genetics* **51**, 992–997.
- Wetton, J. H., Burke, T., Parkin, D. T., and Cairns, E. (1995). Single-locus DNA fingerprinting reveals that male reproductive success increases with age through extra-pair paternity in the house sparrow (*Passer domesticus*). *Proceedings of the Royal Society, London, Series B* **260**, 91–98.
- Zaykin, D. V., and Pudovkin, A. I. (1993). Two programs to estimate significance of  $\chi$  pseudo-probability tests. *Journal of Heredity* **84**, 152.