

MICROSATELLITE DNA FINGERPRINTING CONFIRMS DIZYGOTIC TWINNING AND PATERNITY IN THE ALLIED ROCK-WALLABY, *PETROGALE ASSIMILIS* (MARSUPIALIA: MACROPODIDAE).

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WITH the exception of *Hypsiprymnodon moschatus*, which gives birth to two or more young (Johnson 1983), macropods are monovular, producing one young per oestrus cycle. In macropods, the incidence of twins (two offspring born to the same mother on the same day, van Oorschoot and Cooper 1988) is very low. In the genus *Macropus*, 0.1 to 0.7% of births recorded are twins (Inns 1980; Norbury 1986). It is difficult to compare twinning rates between studies (see van Oorschoot and Cooper 1988), as rates obtained from longitudinal studies (e.g., Poole 1975; van Oorschoot and Cooper 1988; this study) are confounded by unequal numbers of repeated observations on individual mothers and can not be compared directly with cross-sectional studies in which each female is examined only once, such as those of Norbury (1986) and Frith and Sharman (1964).

Since 1986, 306 pouch young births have been recorded from 69 females in a long-term study of *Petrogale assimilis* at Black Rock (19°05' S, 144°27' E) on Lyndhurst Station, 250km west of Townsville. Three sets of twins were recorded, representing 0.98% of births.

The genetic identity of five of the six twin pouch young was characterised using microsatellite markers designed specifically for *P. assimilis* (Spencer, Odorico, Jones, Marsh and Miller 1995). These markers are ideal for paternity and kinship analysis as they are highly polymorphic (> 80%) at each locus, allowing individuals to be indisputably identified. A blood sample was obtained from one pouch young (ID#1; Table 1) from the first set of twins observed.

No sample was obtained from the second young (ID#2) as it died before it could be sampled. Of the remaining two sets of twins, DNA was obtained from small (~20mg) tissue samples. Microsatellite-PCR analysis showed that, for five highly polymorphic loci, the twin pouch young were dizygotic (i.e. having different genotypes) in both of the cases tested (Table 1). In all pouch young, one allele was inherited from the mother and the other from the father.

At birth, the sex ratio of *P. assimilis* does not differ significantly from parity (Yates corrected $\chi^2 = 0.61$; $n = 306$; $df = 1$; $p = 0.4348$). If the young were dizygotic, the sex ratio of twins should not be expected to differ from this 1:1 ratio. At least five of the six young were males (Table 1) and assuming the link between sex ratio and monozygosity as discussed by van Oorschoot and Cooper (1988), the young may have been monozygotic, but this was not the case. There is no comparative genetic evidence from macropods of zygosity of twinning.

We have recorded 15 occasions on which female RW001 has given birth. Twins were produced on two occasions. Both sets were fathered by her long term pair bonded mate (RW026). RW001 produced seven single young between producing the twins. The third set of twins (from the mother RW "1E") were also fathered by her pair-bonded partner (RW "U").

This study reveals several interesting factors. Rock wallabies (*Petrogale*) have low twinning rates similar to those in other macropods (Norbury 1986). The two sets of twins tested were both dizygotic and the female was therefore dioovular (producing two

	Twin I.D. #	Genotype at each of five microsatellite loci ^a					Estimated DOB ^b	Sex	Min age at death (days)
		pPas297	pPas595	pPas593	pPas597	pPas385			
Pair 1	Mother (RW001)	DD	HI	GJ	JQ	FH			
	Twin 1	1	-	-	-	-	16 Jun 1989	M	233
	Twin 2	2	DI	IG	GK	QQ		?	8
	Father (RW026)		HI	GI	CK	CQ			
Pair 2	Mother (RW001)		DD	HI	GJ	JQ			
	Twin 1	3	DH	HI	JC	JC	9 Aug 1992	M	109
	Twin 2	4	DH	HI	JC	JQ		M	109
	Father (RW026)		HI	GI	CK	CQ			
Pair 3	Mother (RW"1E")		IJ	JM	FF	MP			
	Twin 1	5	JN	JG	FE	MA	1 Dec 1993	?M ^c	12
	Twin 2	6	JN	JG	FE	MC		M	53
	Father (RW"U")		NN	GJ	EJ	AC			

Table 1. Summary information from the twins recorded from the colony of *P. assimilis* at 'Black Rock'. The loci that prove dizygosity (pPas597 and pPas385) are underlined in bold. For each locus and each twin, the maternally inherited allele is indicated on the left and the paternal on the right. ^a see Spencer et al (1995). ^b estimated from head length at first capture (Spencer, unpubl. data). ^c sex determination tentative due to small developmental size.

fertilised eggs) at the time of oestrus. In the three sets of twins examined, the young were fathered by the long term consort of the female. It is premature to confer any genetic control of twinning as discussed by van Oorschot and Cooper (1988), but one of the females from Black Rock has produced two sets of twins and in both cases, the young were fathered by the same male.

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