

Supplementary Material

Non-invasive monitoring and reintroduction biology of the brush-tailed rock-wallaby (*Petrogale penicillata*) in the Grampians National Park, Australia

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Supplementary Material

Supplementary Material Table S1. Microsatellite regions amplified for individual identification, genetic diversity, sex identification, and parentage analysis of brush-tailed rock-wallabies (*Petrogale penicillata*). Primer sequences for Y-linked markers used are also included.

Microsatellite	Reference	Primer sequences	Repeat motif	Amplicon size (bp)
ET 32	Unpublished	GTCAGAACCGATTTCACCTC GGGGATCTTCTCGATCACTA	(CAG) ₁₈	194
ET 35	Unpublished	CCTCACAGACAAGTGCTCCT ATCAATCCCTGTTGGAGATG	(CAG) ₁₄	179
SK 8	Unpublished	GATTCTCCAGCAGCAAGTCC CTCCTTTGCTTGCTTTCCAG	(CAG) ₇	259
SK 62	Unpublished	CACGCTGCCATTTTATTTC TTGGTAGCCTCTTGGTCCAC	(CAG) ₉	300
SK 65	Unpublished	TGCCTCCAGCCTCCTTACTA GCTTGTCCAAGGAGATGAGG	(CAG) ₉	148
PI 22	Luikart et al. (1997)	GGTGCTCATTTTATTAGAGTTTG ATTCTTCCTTTCCAAACTCAAGG	(AC) ₁₅	124-156
BT_1	Unpublished	GAAGACCTAGGCCACAAGGA ACCTTTTATGTGCGCTGGAC	(CA) ₂₁	488
BT_3	Unpublished	GAGCAGGGGTCATTTGAACT CCCATTCGCTTTGTCTCATT	(AC) ₂₃	480

BT_6	Unpublished	ACATGAGGGGCAAACAGAG TCTAGCCACCTCCTCCTCAA	(AC) ₂₂	460
BT_11	Unpublished	ACCCAAATGAACTGCAAAGG TTCCCTCCCCTCTACCACT	(AC) ₂₀	429
BT_17	Unpublished	TCATTTCTCTGATGGCATGG ACTGGGGGACCTCAACTTTC	(AC) ₂₅	399
BT_25	Unpublished	GTCAAGCTTCCTGCCCTAGA AGGAGAGTGGGGAGGGAATA	(TC) ₂₀	480
SRY	Watson, Margan and Johnston (1998)	ATCATATGGTCACGGAGTCAGCGG GTGTTTAGCGCGCAAACGTTTGGC	-	181
SRY	Katsura et al. (2018)	TTGAGTCCGTGAAAAGTGGGTC TTGTGAATCTGCCACGCTTGTC	-	252

Supplementary Material Table S2. Breeding events and stages of development of pouch young born to three reintroduced brush-tailed rock-wallaby (*Petrogale penicillata*) females (Ki1, Ki2, TFS76) observed on remote cameras at the Moora Moora Creek release site, Grampians National Park, Australia.

Recorded by remote camera from 2014 to 2017. The predicted birth date indicated from date of observed developmental stages. Mating attempts observed by remote camera in 2019 were

also included. PY, pouch young; HOOP, head out of pouch; OOP, out of pouch; YAF, young at foot; SA, sub-adult.

Date	Ki1	Ki2	TFS76	Untagged
September/October 2013			(A) Approx. month of birth	
January 2014			Large PY (A)	
November 2014			YAF (A)	
March 2015		(C) Approx. D.O.B: 9/3/15		
April 2015	(B) Approx. D.O.B: 1/4/15			
May 2015			(D) Approx. D.O.B: 5/5/15	
September 2015	PY HOOP (B)	PY HOOP (C)		
November 2015		YAF (C) sighted. Fox sighted two hours later.	PY HOOP (D)	

		No further YAF sightings.	
December 2015	YAF (B) (E) Approx. D.O.B. 6/12/15		YAF (D)
May 2016	PY HOOP (E)		
June 2016	SA (B) YAF (E)		SA (D) exploring rocks
August 2016	(H) Approx. D.O.B.		
September 2016			(F) Approx. D.O.B. 8/9/16
December 2016			PY positive (F) Untagged animal PY positive (G) born Nov/Dec 16
February 2017	PY OOP (H)		
March 2017		(I) Approx. D.O.B. 9/3/17	
April 2017		PY positive (I)	

February 2019	Possible mating attempt with 167	Mating attempt with 167 (Failed)	
April 2019		Mating attempt with 167 (Unknown)	Mating attempt with 167 and untagged female
May 2019		Possible Pouch Bulge	Mating attempt with 167 and untagged female

Supplementary Material Table S3. Summary of camera observations on the number of young born to tagged brush-tailed rock-wallaby (*Petrogale penicillata*) females at the Moora

Moora Creek release site in the Grampians National Park, Australia, between 2014 and 2017.

Year	Ki1	Ki2	TFS76	Total
2014	0	0	1	1
2015	1	1	1	3
2016	1	0	1	2
2017	1	1	0	2

Total	3	2	3	8
Average	0.75	0.5	0.75	
PY/Year				
Average PY/Year/Female				0.667

Supplementary Material Table S4. Brush-tailed rock-wallaby genotypes detected at Moora Moora Creek through non-invasive faecal genetic monitoring genotyped at twelve loci. Newly designed (di-) BT_1, BT_3, BT_6, BT_11, BT_17, BT_25, newly designed (tri-) SK8, SK62, SK65, unpublished (tri-) ET32, ET35, (di-) Pi22 (Luikart et al. 1997).

Markers were scored according to size of microsatellite (base-pairs) or number of motif repeats dependent on easiest scoring method for each marker.

Supplementary Table S4. Number and sex of genotyped brush-tailed rock-wallabies (*Petrogale penicillata*) at Moora Moora Creek, Victoria, Australia in August 2019.

Animals were originally reintroduced to the site between 2008 and 2012 and had only been monitored by remote cameras and trapping prior to this study.

BTRW ID	Sex	Number of Scat Samples
F1	Female	34
F2	Female	54
F3	Female	37

F4	Female	13
F5	Female	18
F6	Female	1
M1	Male	48
M2	Male	9

Supplementary Material Table S5. Summary of number of individuals, number of alleles, and measurements of genetic diversity of the Moora Moora Creek colony of reintroduced brush-tailed rock-wallabies (*Petrogale penicillata*), and the captive BTRW colony held at University of Adelaide (Waite Campus).

Faecal DNA (Moora Moora Creek) was genotyped using twelve microsatellite markers.

Tissue DNA (Waite) was genotypes using a subset of six loci. Na, number of alleles; He, expected heterozygosity; Ho, observed heterozygosity; Fis, fixation index.

Locus	Moora Moora Creek				Waite			
	Na	Ho	He	Fis	Na	Ho	He	Fis
SK_8	4.0	0.875	0.727	-0.204	3.0	1.000	0.611	-0.636
SK_62	4.0	0.875	0.633	-0.383	2.0	1.000	0.500	-1.000
SK_65	3.0	0.750	0.633	-0.185	2.0	1.000	0.500	-1.000
PI22	2.0	0.500	0.375	-0.333	2.0	0.400	0.480	0.167
ET32	4.0	0.875	0.695	-0.258	6.0	0.900	0.770	-0.169
ET35	3.0	1.000	0.656	-0.524	5.0	0.889	0.728	-0.220
BT_1	4.0	1.000	0.734	-0.362				
BT_3	6.0	1.000	0.773	-0.293				

BT_6	3.0	1.000	0.656	-0.524				
BT_11	3.0	1.000	0.594	-0.684				
BT_17	6.0	0.875	0.719	-0.217				
BT_25	3.0	0.500	0.664	0.247				
Mean (six loci)	3.3 ±0.3	0.813 ±0.070	0.620 ±0.051	-0.315 ±0.052	3.3 ±0.7	0.865 ±0.095	0.598 ±0.052	-0.476 ±0.196
Mean (twelve loci)	3.8 ±0.4	0.854 ±0.053	0.655 ±0.029	-0.310 ±0.067				
