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

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ABSTRACT

Thirty-nine endangered brush-tailed rock-wallabies (*Petrogale penicillata*) were reintroduced to Grampians National Park, western Victoria, between 2008 and 2012. Subsequent high mortality, low breeding, and no recruitment were linked to fox predation and physical disturbance during monitoring. From 2014 to 2017, the colony was left undisturbed and monitored only by remote camera. Five adult animals were identified across this period (1 ♂ and 3 ♀s – all tagged; and one untagged female), and an average of 0.7 pouch young were birthed per tagged female per year. In 2019, camera-monitoring and non-invasive genetic monitoring (faecal) were used to identify colony members, genetic diversity, and breeding. Camera monitoring in 2019 identified the same five individuals, whereas genetic monitoring using 12 microsatellites identified eight individuals (two male and six female genotypes). Genetic diversity within the colony was moderate (expected heterozygosity (He) = 0.655, observed heterozygosity (Ho) = 0.854). Leaving the colony undisturbed after 2013 correlated with improved adult survival, increased breeding, and successful recruitment of young to the population. Recommendations for the Grampians colony include continuation of regular camera- and scat monitoring to improve our understanding of the reintroduction biology of *P. penicillata* and other marsupials in open, unfenced landscapes.

Keywords: camera trap, conservation genetics, genetic monitoring, macropod, marsupial, reintroduction biology, *Petrogale*, threatened species.

Introduction

Reintroduction is a common conservation tool to help threatened species and prevent wildlife extinctions. However, the success rates of reintroductions are highly variable. The factors that influence success or failure are numerous and vary with species and release site (Bubac *et al.* 2019; Berger-Tal *et al.* 2020). Within Australia, the main factor identified in the failure of many reintroductions is predation by exotic carnivores (e.g. Moseby *et al.* 2011; Sheean *et al.* 2012; Hardman *et al.* 2016; Short 2016; Watkins *et al.* 2018; Robinson *et al.* 2020).

While post-release monitoring of reintroduction attempts is now frequently conducted, it is often conducted poorly, and difficulties with monitoring are commonly reported (Bubac *et al.* 2019; Berger-Tal *et al.* 2020; Gant *et al.* 2020). Survival, abundance, population growth, breeding, and recruitment are all parameters that determine reintroduction success and can be assessed only through post-release monitoring (Richards and Short 2003; White *et al.* 2003; Parlato and Armstrong 2013; Johnson *et al.* 2018). Although genetic diversity is highly valued when selecting groups of animals for release (e.g. Lapbenjakul *et al.* 2017), without post-release monitoring, it cannot be known whether the genetics of the reintroduced population remain viable for long-term success (Zeng *et al.* 2007; Scott *et al.* 2020). Post-release monitoring data are vital to guide management decisions such as interventions to ensure population viability, understanding why a reintroduction succeeded or failed, to assess effectiveness of predator control, and to optimise future reintroduction attempts (Richards and Short 2003).

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Monitoring reintroduced animals through observation, trapping, or mark–recapture can be difficult in steep or hard-to-access terrain, or when animals are cryptic or shy, and can have occupational safety risks to researchers and animal welfare risks for the study species (Piggott *et al.* 2006*a*; Berger-Tal *et al.* 2020). Trap-shy animals can lead to the detection of only a subset of a population (Hoyle *et al.* 1995; Cutler and Swann 1999; Wegge *et al.* 2004).

Alternatively, non-invasive wildlife monitoring requires no contact with animals and can often be preferable owing to its efficacy in labour, cost, and animal and staff welfare (De Bondi *et al.* 2010). Camera traps can be used to obtain information on population abundance, behaviour, habitat occupancy, and population structure (Silveira *et al.* 2003; Claridge *et al.* 2010). Non-invasive genetic monitoring, including the use of faecal samples, can provide information on population abundance, population structure, sex, diet, habitat occupancy, and population genetic diversity (Piggott *et al.* 2006*a*; Lachish *et al.* 2011; Tende *et al.* 2014; Moßbrucker *et al.* 2015; Morales-Contreras *et al.* 2016; Camp *et al.* 2020; Bannister *et al.* 2020).

The brush-tailed rock-wallaby (BTRW; *Petrogale penicillata*) is one of many currently declining, threatened mammal species in Australia (Woinarski *et al.* 2014). The small to medium-sized, cryptic macropod species historically occurred in abundance throughout much of the Great Dividing Range and has a strong dependence on complex rocky habitat (Wakefield 1961; Kaufmann 1974). In the Grampians, Victoria, the species became locally extinct in 1999 and, in response, a translocation strategy was devised to reintroduce captive-bred animals into the Moora Moora Creek area within the Grampians National Park (Delaney *et al.* 2005; Bramwell *et al.* 2008; Taggart *et al.* 2008, 2015).

Reintroduction of 39 animals began in November 2008 and concluded in November 2012, with only seven animals surviving by December 2013 (Taggart *et al.* 2015). Predation by exotic predators was recognised as a key cause of mortality, despite the presence of an intensive fox control program around the site (Robley *et al.* 2014; Taggart *et al.* 2015). The frequency of colony disruption from supplementations, physical monitoring (e.g. cage-trapping and handling) and release group size were hypothesised to result in poor levels of social cohesion during the establishment phase, leading to increased predation vulnerability (Taggart *et al.* 2015).

Few pouch young were identified within the colony between 2008 and 2013, no juveniles or subadults were observed or trapped, and no animals were recruited into the adult population (M. Stevens, Parks Victoria, pers. comm.). From 2014 onward, the colony was left undisturbed by physical monitoring and supplementation and was monitored only by remote camera.

Non-invasive faecal genetics was pioneered in this species by Piggott and colleagues to test its potential for monitoring elusive or endangered species, especially in rough terrain (e.g. Browning *et al.* 2001; Piggott and Taylor 2003; Eldridge *et al.* 2004; Hazlitt *et al.* 2004, 2006*a*, 2006*b*, 2014; Piggott 2004; Piggott *et al.* 2006*a*, 2006*b*, 2018).

The aim of this study was to examine the efficacy of remote cameras and faecal genotyping as non-invasive post-release monitoring methods to better understand the population structure, current distribution, recruitment, genetic diversity, and parentage of the remaining reintroduced colony. This enabled us to assess the difference in the population growth following a period of 6 years with no animal supplementations or other human interference, which can provide useful insights to help optimise reintroduction of BTRW and similar species.

Materials and methods

Study site

This study was located at Moora Moora Creek, within the Grampians National Park in western Victoria, Australia (Fig. 1). The reintroduced BTRW colony is located centrally within the national park, approximately 10 km southwest of the township of Halls Gap.

Camera-monitoring data

Three motion triggered remote cameras (BuckEye Orion XIR; BuckEye Cam, Australia) were installed at the Moora Moora Creek release site by Parks Victoria staff in 2014. Images recorded by these cameras were used to determine successful matings and breeding of the BTRW in the colony from 2014 to mid-2017 and to monitor the ongoing survival of tagged animals. Cameras were not operational between mid-2017 and December 2018.

Previously published BTRW growth data were used to calculate the approximate dates of birth of the pouch young recorded by remote cameras in the colony. Calculations were based on estimates of gestation length (~30 days), first pouch exit (~180 days), permanent pouch exit (190–210 days) and weaning (220–240 days; Taggart *et al.* 2005).

In January 2019, six new motion-triggered remote cameras (BuckEye X80; BuckEye Cam Australia) were installed at Moora Moora Creek. The new cameras were set up ~250 m apart, along a 1.2 km length of the Moora Moora Creek escarpment above Moora Moora Creek where the BTRWs were released, and which now forms the main colony site. Cameras uploaded photos, *via* a cellular base station, directly to an office computer on weekdays.

Recorded sightings of BTRWs on the cameras from the 19 January 2019 to the 3 August 2019 were obtained from Parks Victoria staff. The identity of the BTRW based on clear visualisation of an identifying ear tag or definite absence of an ear tag was provided with the date and camera location of the sighting.

Camera locations and BTRW ear-tag identity were used to estimate the escarpment length occupied by each individual

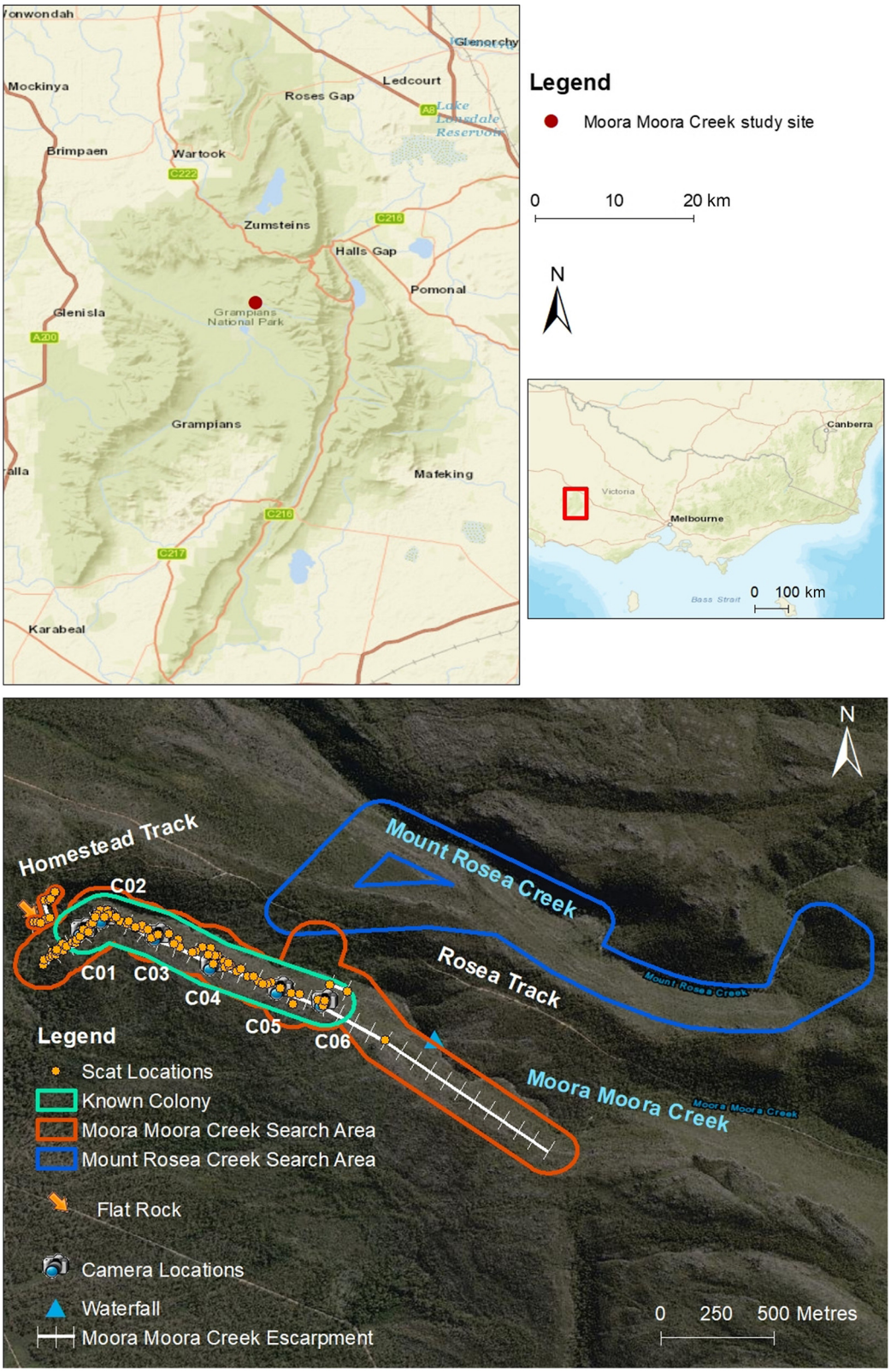

Fig. 1. Location of the study site Moora Moora Creek within the Grampians National Park (top). Location of features of study site and locations of scats collected from the reintroduced population of brush-tailed rock-wallabies (*Petrogale penicillata*) present at the site, including locations searched for scats (bottom). Also included are the locations of six remote cameras at the site.

BTRW (referred to as their 'escarpment range'). The escarpment range of each animal is defined as the number of remote cameras on which an individual was captured, signifying how much of the escarpment each individual was using. Home-range studies of rock-wallaby species have typically found that core ranges were best estimated using 50–75% of radio tracking/GPS/spotlight locations (Horsup 1994; Laws and Goldizen 2003; Telfer and Griffiths 2006; Sharp 2009; Hayward *et al.* 2011). For this study, a cumulative frequency across multiple cameras where >60% of sightings were recorded were considered an animal's 'core escarpment range'.

For visualisation purposes, a buffer zone of 100 m was created around the camera location where each animal was recorded to represent an area in which an animal was thought to occur using ArcGIS ArcMap 10.8.1 (Esri, USA). Additionally, the escarpment range of an individual was considered identical to its core escarpment range if the proportion of images of an individual recorded at each camera was equal, because it was assumed that the animal spent similar amounts of time around each camera location along the escarpment.

Genetic sample collection

BTRWs are highly dependent on their rocky habitat, and so their scats are easy to locate if present. Rock-wallaby scats can be readily distinguished from other macropod scats in the region (*Macropus fuliginosus*, *M. giganteus*, *M. rufogriseus* and *Wallabia bicolor*; Aussavy *et al.* 2011) by their size, shape (typically cylindrical with a single pointed end), and texture (mix of small and larger plant fragments; Jarman and Capararo 1996). Fresh scats (<2 weeks old) can be identified by their glossy, black colour, and strong odour (Piggott 2004; David Taggart and Lauren Werner, pers. comm.).

In this study, fresh BTRW scats were collected opportunistically from across the length of the rocky escarpment area in and around the known colony site (the Moora Moora Creek Escarpment) in September 2018 and February to April 2019. Only one scat, selected at random, was taken from locations where multiple scats were present in close proximity to one another.

The scat search and collection began at the western end of the escarpment, approximately 0.3 km south-west of Camera C01 and concluded 1.13 km past the eastern end of the main colony, including the entire colony (approximately 1.2 km in length; Fig. 1). A 0.08 km length of escarpment on the north-western side of the creek under a landmark known as 'flat rock', directly opposite the western end of the main colony, was also searched (Fig. 1). Additionally, an adjacent 2.3 km of escarpment on both the northern and southern sides of Mount Rosea Creek, located north of the known Moora Moora Creek BTRW colony site, was searched in autumn 2019 (Fig. 1). The area searched represented the closest escarpments to the main colony, with potentially suitable habitat that dispersing BTRWs might have moved to.

Scats were collected in individual zip-lock bags to avoid DNA contamination among scats. Scat locations were recorded using a hand-held GPS unit. The scat samples were then stored in a portable cooler while travelling and on site. Scat samples were kept frozen at −20°C in the laboratory before DNA extraction (e.g. Walker *et al.* 2009; Kolodzieg *et al.* 2013; Sabino-Marques *et al.* 2018). For comparison of genetic diversity, DNA extracted from stored ear biopsies of 10 BTRWs (progeny of three males and four females) of known sex were included in the study from the animal facility at the Waite Campus of University of Adelaide, Adelaide, Australia. The translocated animals and the captive Waite Campus animals were founded from the same set of individuals. These founder individuals came from two distinct evolutionarily significant units (ESU), the southern and the central ESU (Browning *et al.* 2001; Paplinska *et al.* 2011; David Taggart, pers. comm.).

Genomic DNA extraction

Faecal DNA was extracted from scats by using the Bioline Isolate II Genomic DNA Kit protocol (Bioline, Australia), with modifications that included using sterile cotton swabs soaked in 200 µL of nanopure water (Sigma-Aldrich, Australia) to remove epithelial cells from all surfaces of the scats (e.g. Akomo-Okoue *et al.* 2015; Ramón-Laca *et al.* 2015; Dimsoski 2017; Bourgeois *et al.* 2019) and using an additional centrifugation step to remove excess debris before binding onto the silica membrane.

Genomic DNA from the ear biopsies was extracted using the Bioline Isolate II Genomic DNA Kit (Bioline, Australia), using approximately 20 mg of each ear biopsy and extending the proteinase digestion at 56°C to 24 h.

Total DNA extracted from faecal and ear biopsy samples were quantified and assessed for quality by using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., USA) and stored at −20°C until analysis.

Genotyping

A panel of 12 nuclear microsatellite markers, seven di-nucleotide (PI22 (Luikart *et al.* 1997), BT_1, BT_3, BT_6, BT_11, BT_17, BT_25; Accession numbers: OK070787, OK070788, OK070789, OK070790, OK070791, OK070792) and five tri-nucleotide (SK8, SK62, SK65; accession numbers: OK031000, OK031001, OK031002; ET32, ET35; unpubl. data, Adam Croxford, pers. comm., Accession numbers: XM_036734328.1, XM_020966173.1; mean He = 0.7) were used to genotype the BTRW faecal DNA (Supplementary Table S1). A subset of six microsatellite markers (PI22, SK8, SK62, SK65, ET32, ET35) (mean He = 0.74) were used to genotype captive ear-tissue DNA due to budget constraints. Two *Y*-chromosome linked markers (SRY; Watson *et al.* 1998,

SRY; Katsura *et al.* 2018) were checked for amplification in all samples (tissue and faecal DNA) for sex determination. Flanking primers of unpublished microsatellite markers were designed using Primer3 software (Koressaar and Remm 2007; Untergasser *et al.* 2012; Koressaar *et al.* 2018) with a length of between 80 and 300 bp. Markers were selected on the basis of reliability of amplification ease of scoring (low stuttering), and polymorphism. The markers were modified with truncated Illumina linkers to allow the amplicons to be converted into Illumina libraries (sequences: forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG; reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG). PCRs of the ear-biopsy DNA were conducted in 10 μL volumes containing 1× Biomix (Bioline, Australia), 0.2 nM of each forward and reverse primers, and 10 ng of DNA. The same protocol was used for faecal DNA analysis following a 1 in 10 dilution of the DNA.

Polymerase chain reactions were conducted using a BioRad T100 Thermal Cycler (Bio-Rad, USA), using the following PCR protocol: an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 15 s, 55°C for 30 s, 72°C for 45 s and a final extension at 72°C for 5 min. The annealing temperature for the *Y*-linked SRY marker (Watson *et al.* 1998) was set to 64°C instead of 55°C and the cycle was repeated 40 times for both *Y*-linked markers. The sexing PCR was performed with ET32 and ET33 to ensure that there were no false negatives. PCR products were visualised after electrophoresis on a 1% agarose gel stained with SybrSafe (Invitrogen, USA).

Polymerase chain reaction products for each individual (including both ear-tissue samples and scat samples) were pooled and purified using Ampure XP beads (Agencourt Bioscience Corporation, USA) at a ratio of 1:1. The two-step Illumina indexing approach described by Wilkinson *et al.* (2017) was used to develop sequencing libraries for each sample and all samples were pair-end sequenced using a 300-cycle MiSeq version 2 kit (Illumina, USA) at Australian Genome Research Facility (AGRF), Melbourne.

The MiSeq sequences were demultiplexed using the MiSeq Reporter software (Illumina) and paired reads were then merged using the Geneious Prime 2019.2 software (Biomatters, New Zealand). Genotypes were determined from the amplicon length of the microsatellite. To test the fidelity of the genotyping, three replicate amplifications from 10 different samples (two ear-tissue and eight scat DNA samples) were performed and run on the MiSeq, including the single scat genotyped as F6.

Individual identification

Hardy–Weinberg equilibrium and linkage disequilibrium tests were conducted across all loci for all samples, by using the Markov-chain algorithm of Raymond and Rousset (1995) in Genepop version 4.7.5 (Rousset 2008). The presence of null alleles and allele dropout were assessed using the MICRO-CHECKER 2.2.3 program (Van Oosterhout *et al.* 2004). Owing to budget constraints, samples with non-amplifying loci could not be re-analysed in this study, and were excluded.

The probability of identity (P_{ID}), meaning the probability of two individuals having the same genotype in a randomly mating population, was calculated among full siblings ($P_{(ID)sib}$) for the Moora Moora Creek individuals (12 loci; 1.0×10^{-4}) by using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). GenAlEx 6.5 was also used to match genotypes, with zero mismatches allowed. Samples with 100% allelic sharing at all 12 loci were considered to be the same individual because of the low P_{ID} values and exclusion of genotypes with non-amplifying loci.

Basic genetic-diversity parameters, including mean number of alleles per locus (Na), observed (Ho) and expected heterozygosity (He), and an inbreeding parameter (F_{is}) were obtained using GenAlEx 6.5 (Peakall and Smouse 2006, 2012), by using the unique scat genotypes and the 10 tissue samples. Because only six loci were amplified in tissue DNA, genetic diversity parameters were calculated using the six-locus subset for both the Waite (tissue) and Moora Moora Creek (scat) samples and all 12 loci for Moora Moora Creek (scat) samples only.

Parentage analysis

Parentage of scat genotypes (conducted with 12 loci) was assigned using the program Cervus 3.0.7 (Kalinowski *et al.* 2007). Because the identity of all BTRW genotypes was unknown compared with release records, all genotypes were considered as both offspring and candidate parents with sexes known. Parentage was determined using parent-pair analysis where the simulation was conducted with the following parameters: 10 000 offspring, three candidate mothers with a proportion of 0.9 sampled, two candidate fathers with a proportion of 0.9 sampled, a proportion of 0.9 loci typed and 0.1 loci mistyped, and a minimum typed number of loci of 12. The parent-pair analysis was then conducted with a strict confidence level of 95% and a relaxed confidence level of 80%. Of all parentage analyses, there was one locus pair mismatch between individuals F6 and F3. However, because of the low number of individuals and lack of known relationships, the power to detect error was low. GenAlEx 6.5 was used to calculate the probability of exclusion with no parents known.

Results

Camera-monitoring along the escarpment (2019)

Brush-tailed rock-wallaby was the only macropod species recorded by the remote cameras in 2019. Images of BTRWs in the colony showed that the animals were in good body

condition with clean glossy pelts and no obvious signs of disease.

Camera data suggested that at least five BTRWs persisted at the Moora Moora Creek site in early 2019, including four tagged founder animals (1 ♂ and 3 ♀) and an untagged animal. The identity, age, and sex of the known Moora Moora Creek BTRWs are listed in Table 1. During 2019, 76 images of an untagged BTRW were recorded (Table 2). All sightings of the untagged animal suggested that it was a young adult female, including a visible pouch. Tagged male 167 was twice recorded mating an untagged animal at Camera C03 in April and May of 2019. The total escarpment and core escarpment ranges of known BTRWs overlapped (Fig. 2*a*–*e*; Table 2).

Camera records of breeding events (2014–2017, 2019)

Breeding events in the colony deduced from camera images of females with pouch young were recorded between 2014 and 2017 (Supplementary Table S2). All tagged adult females (Ki1, Ki2, and TFS76) were detected with pouch young, indicating that all tagged adult females and the tagged male in colony were fertile and breeding. Camera data from 2014 to 2017 and in 2019 indicated that the majority of mating events in the colony occurred in late summer and autumn (69%), with pouch young emergence occurring in late spring and early summer (Supplementary Table S2). Less frequent mating events were also detected on camera in late spring (15%) and winter (15%).

Remote camera images indicated that nine pouch young were born between 2014 and mid-2017. A further five attempted matings were recorded between January and August 2019, including four of unknown outcome and one failed attempt. All three tagged females (Ki1, Ki2 and TFS76) were observed mating with male 167 in 2019. An untagged female was also observed with a pouch young (Supplementary Table S2). No other images of pouch young or young at foot were recorded in 2019.

From the camera observations made between 2014 and 2017, the three tagged adult females produced, on average, 0.7 pouch young per female per year (Supplementary Table S3). No female weaned a young every year between 2014 and 2017. Three pouch young were detected only as a pouch 'bulge' late within the observation period and so their status remains unknown. Of six pouch young detected on camera with heads out of pouch, five (83%) reached at least the young-at-foot stage. Two (33%) young were later observed as subadults, three young (50%) were not observed past the young-at-foot stage and the sixth young had just reached pouch emergence when the cameras were removed in mid-2017 (Supplementary Table S2).

Table 1. Identification, sex, and age of the individuals in the reintroduced brush-tailed rock-wallaby (*Petrogale penicillata*) colony at Moora Moora Creek, Grampians National Park, Australia.

BTRW ID	Sex	D.O.B.
Ki1 – tagged	Female	1 November 2012
Ki2 – tagged	Female	1 February 2014
TFS76 – tagged	Female	1 June 2011
167 – tagged	Male	7 June 2011
Untagged	Unknown	Unknown

Individuals listed had been observed on remote camera in 2019 and were known to be alive as of October 2019.

Table 2. Number and proportion of images of brush-tailed rock-wallabies (*Petrogale penicillata*) in the Moora Moora Creek colony recorded by six remote cameras positioned along the escarpment.

BTRW ID	C01	C02	C03	C04	C05	C06	Total
Ki1	27	17	6	20	–	–	70
Proportion	0.39	0.24	0.09	0.29	–	–	1
Ki2	13	14	33	11	–	–	71
Proportion	0.18	0.20	0.47	0.15	–	–	1
TFS76	2	3	2	39[A]	24[A]	24[A]	94
Proportion	0.02	0.03	0.02	0.42	0.26	0.26	1
167	26	10	12	27	32	13	120
Proportion	0.22	0.08	0.1	0.23	0.27	0.11	1
Untagged	53[A]	5	4	3	10	1	76
Proportion	0.70	0.07	0.05	0.04	0.13	0.01	1

Images taken between 19 January and 3 August 2019. C01–C06 are camera IDs.
[A]Core escarpment range of animal.

Scat DNA genotypes identified

In total, 218 BTRW scats were collected from the colony site (44 collected in September 2018 and 174 collected across February and April 2019). No scats were found more than 250 m away from the known colony area (Fig. 1). No BTRW scats were found in the adjacent Mount Rosea Creek search area, despite the presence of potentially suitable habitat (Fig. 1). In addition to BTRW scats, swamp wallaby (*Wallabia bicolor*) scats were frequently found at the base and top of the rocky escarpments in and around the known colony site at Moora Moora Creek and in the Mount Rosea Creek area. All Waite BTRWs tested were of known sex. Genotyping confirmed their sex, and thus that the technique was working. DNA extracted from 4 of the 218 scats collected at Moora Moora Creek failed to amplify and it was deemed that the scats were too old to produce viable DNA. Genotypes including loci that did not amplify were excluded from further analysis. This reduced the sample size from 218 to 169. The P_{ID} for the Moora Moora Creek BTRWs is within the recommended values of 1×10^{-2} to 1×10^{-4} for accurate population estimates and individual identification (Mills *et al.* 2000; Waits *et al.* 2001). The Hardy–Weinberg

Fig. 2. Satellite image of the brush-tailed rock-wallaby (*Petrogale penicillata*) reintroduction site at Moora Moora Creek, in the Grampians National Park, Australia. Figure shows core and entire escarpment ranges of (*a*) female Ki1, (*b*) female Ki2, (*c*) female TFS76, (*d*) male 167 and (e) untagged animal. The range estimates were taken from images captured by six remote cameras set up at the site along the escarpment. C01–C06 indicate the positions of the six remote cameras.

equilibrium test showed no significant deviation from equilibrium after Bonferroni correction and no linkage disequilibrium was present for the loci used in the study. The analysis in MICRO-CHECKER revealed no evidence of null alleles being present and no allelic dropout occurring across all the microsatellite loci. The results from the replicated samples showed 100% genotyping match and all three replicates were scored with no failure of PCR.

This supports the high-fidelity of the genotyping system employed in this study.

Analysis of faecal DNA from the Moora Moora Creek colony identified eight unique BTRW genotypes (Supplementary Table S4). Of the genotypes identified, two were male and six were female. This increased the total number of BTRWs known to be alive at Moora Moora Creek from five to eight and established a sex ratio in the colony of 1:3 male to female (Supplementary Table S5). The female genotypes were designated F1 (34 scats), F2 (54 scats), F3 (37 scats), F4 (13 scats), F5 (18 scats) and F6 (1 scat). The male genotypes were designated M1 (48 scats) and M2 (9 scats) (Supplementary Table S5).

The approximate range of each individual BTRW along the Moora Moora Creek Escarpment was mapped on the basis of the GPS locations of the scats collected and genotyped (Fig. 3*a*–*h*). Although habitat sharing occurred among all individuals, genotyped BTRW females F4, F5 and F6 and male M2 had far more localised escarpment ranges than did females F1, F2, F3 and male M1.

Genetic diversity

An assessment of the genetic diversity of the BTRWs at the Moora Moora Creek colony showed that the average number of alleles per locus (Na) was similar in both the Waite (6 loci; 3.3 ± 0.7) and the Moora Moora Creek (6 loci; 3.3 ± 0.3, 12 loci; 3.8 ± 0.4) BTRW colonies (Supplementary Table S6). The same was true for both expected heterozygosity (He) (Waite, 6 loci; 0.598 ± 0.052; Moora Moora Creek, 6 loci; 0.620 ± 0.051, 12 loci; 0.655 ± 0.029) and observed heterozygosity (Ho) (Waite, 6 loci; 0.865 ± 0.095; Moora Moora Creek, 6 loci; 0.813 ± 0.070, 12 loci; 0.854 ± 0.053). Negative F_{is} values (Waite, 6 loci; −0.476 ± 0.196; Moora Moora Creek, 6 loci; −0.315 ± 0.052, 12 loci; −0.310 ± 0.067) were calculated for both groups.

Parentage analysis

The probability of exclusion with no parents known was calculated as 1.000. The male M1 was assigned as the most likely sire of two BTRWs (F4 and M2) with at least 95% confidence and of another two BTRWs (F5 and F6) with less than 80% confidence (Table 3). The female F2 was assigned as the most likely mother of F4, F5, and M2 (at least 80% confidence) (Table 3). Female F3 was the most likely mother of female F6 (95% confidence; Table 3), while female F1 was designated as neither mother nor offspring.

Discussion

The current study used images gathered from non-invasive camera monitoring and genetic monitoring *via* scats to assess the status of the reintroduced Moora Moora Creek BTRW colony, and the impact of leaving the colony undisturbed (no supplementation or trapping) for 6 years.

Breeding and recruitment

Camera monitoring showed that all tagged females were breeding. The Moora Moora Creek pouch young appeared to have a higher rate of survival to pouch emergence at 83% than the 73% recorded previously for this species by Wynd *et al.* (2006). During the 6-year (2008–2013) BTRW colony-establishment phase at Moora Moora Creek, while physical animal/colony interference was occurring, a total of seven pouch young was recorded from 6 of 23 released females, two young were recorded out of pouch and no recruitment into the adult population was observed (Taggart *et al.* 2015). An increase in the number of pouch young produced per female (0.7/year), in pouch emergence (83%), and in survival of young to subadulthood (n = 2) was thus confirmed during 2014–2017 *via* camera-monitoring.

In this study, BTRW births in the Moora Moora Creek colony were found to peak in late summer and autumn, with pouch emergence occurring in spring (Taggart *et al.* 2005; Wynd *et al.* 2006). This contrasts with earlier reports from Wynd *et al.* (2006) that found that female BTRWs bred in synchrony in autumn. Tight breeding synchrony, such as that described by Wynd *et al.* (2006), was observed in female BTRWs at Moora Moora Creek only during 2015, with all young emerging from the pouch within a month of each other.

Previous research suggests that one birth per year is common for BTRWs (Taggart *et al.* 2005; Wynd *et al.* 2006); however, the Moora Moora Creek females birthed on average 0.7 pouch young per year. Although the body condition of all individuals recorded on camera at Moora Moora Creek appeared very good, the tagged females were of older ages (between 5 and 8 years old in 2019), which might account for the lower breeding rate as age is known to decrease breeding success (Cassinello and Aldos 1996; Tettamanti *et al.* 2015). Another explanation may be poor mate choice, with potentially only one dominant adult male available to females at this site.

As the Moora Moora Creek BTRW colony is only small, further monitoring of breeding, which is planned for the colony, will provide more robust estimates of reproductive rates, survival of young and recruitment to the adult population.

Genetic monitoring and diversity

The microsatellite markers used in the current study are different from those used in previous genetic studies on BTRWs, and, so, direct comparisons of genetic diversity cannot be made between the Moora Moora Creek colony and previously published works. Both the Moora Moora

Fig. 3. Locations of brush-tailed rock-wallaby (*Petrogale penicillata*) scats of genotyped (*a*) female F1, (*b*) female F2, (*c*) female F3, (*d*) female F4, (e) female F5, (*f*) female F6, (*g*) male M1 and (*h*) male M2. Animals are part of the reintroduced colony in Grampians National Park, Australia. C01–C06 indicate the positions of the six remote cameras monitoring the site.

Table 3. Parentage of untagged wild-born brush-tailed rock-wallabies in the Moora Moora Creek colony at the Grampians National Park, Australia.

BTRW ID	Sire	Sire confidence (%)	Dam	Dam confidence (%)	Trio confidence (%)
F4	M1	≥95	F2	≥80	≥95
F5	M1	<80	F2	≥80	≥95
F6	M1	<80	F3	≥80	≥80
M2	M1	≥95	F2	≥80	≥80

Parentage determined from 12 microsatellite markers amplified in faecal DNA.

Creek and the Waite individuals had a higher Ho than He and negative F_{is} values are typically representitive of an excess of heterozygotes. As the founder animals of the Moora Moora Creek colony and the Waite individuals come from both the southern and the central ESU (David Taggart, pers. comm.), this genetic mixing may account for a heterozygosity excess. However, another likely explanation is that the small sample size and related individuals of both groups is skewing the diversity statistics (e.g. Hale *et al.* 2012). The mixed source of the colonies (central and southern ESU) may also explain deviations from Hardy–Weinberg equilibrium because of the Wahlund effect.

The genetic diversity of the Moora Moora Creek BTRW colony is highly comparable to the genetic diversity of the captive Waite individuals, regardless of whether it is calculated at 6 or 12 loci. As the colony is very small and includes only two or possibly three generations, continued breeding without intervention will lead to reduced genetic diversity because of the small number of successful founders, which included a single male and four females. This can have serious consequences, including the reduced ability of a population to adapt to change, loss of fitness through inbreeding depression (Willoughby *et al.* 2019) and the potential to reduce long-term population growth rates, which are crucial to establishing a reintroduced population (Bozzuto *et al.* 2019).

Only one male was assigned as a father (M1), and, so, is likely to be the dominant male in the colony. This sire and the dams were predominantly assigned with a high confidence (80–95%); however, M1 was assigned as the father of F5 and F6 with a lower confidence (<80%). This low confidence may be due to the suitability of the microsatellite markers used, because the assessment of markers was limited by the small sample size, as well as because M2 is the full or half sibling of all offspring and so shares many alleles with these individuals. Because M1 shares half its alleles with both F5 and F6, and none of the founders was a full sibling, it is highly likely that M1 is also the father of F5.

Three of the four surviving offspring were assigned to a single dam, female (F2). This bias may be random, may be due to scat sampling effort, with other offspring yet to be discovered, or may indicate that F2 is acting as a dominant breeding female within the colony. Observations of dominant female BTRWs supressing the breeding and/or limiting the successful reproduction of subordinate females has previously been reported (Jarman 1991). This uneven genetic founder representation could increase the rate of inbreeding and loss of genetic fitness within this colony and, ultimately, reduce its population viability if left unchecked (Jamieson 2011).

Genetic monitoring and camera-monitoring

As previously reported in BTRWs and other macropod species, both monitoring techniques identified significant habitat sharing among colony members (Jarman 1991; Laws and Goldizen 2003; Hazlitt *et al.* 2006*b*; Molyneux *et al.* 2011).

Monitoring through faecal genetics and remote cameras produced different population estimates in this study. Camera-monitoring showed that four of the original tagged animals (1 male and 3 females) still survived and one untagged BTRW was present. Faecal genetic monitoring identified eight genotypes within the colony. The results of previous comparisons between these techniques in wildlife species have varied from being highly comparable (Rodgers *et al.* 2014) to detecting different subsets of the same population (Bluff *et al.* 2011; Velli *et al.* 2015).

From the parentage analysis, it is likely that M1, F1, F2, and F3 are the tagged animals. However, the corresponding genotype of the untagged animals identified on camera cannot be determined. As a result of the difficulty in distinguishing untagged BTRWs on camera, we could not be certain how many untagged BTRWs were actually recorded. It is possible, therefore, that the two techniques are detecting different subsets of the colony here as well. These results support previous studies suggesting that non-invasive genetic monitoring and camera-monitoring should be used in combination to obtain the most accurate monitoring results (Bluff *et al.* 2011; Velli *et al.* 2015). Such systems can also support each other, because scat searches of sites such as Moora Moora Creek could provide guidance on where to place additional cameras for further monitoring, and both techniques offer unique benefits, such as behavioural observations or genetic analyses.

Both monitoring techniques also had limitations. The information gathered from remote cameras was determined by the number and placement of cameras across the colony.

A large limitation of non-invasive faecal genotyping was that the genotypes identified is impacted by the location of the scat searches and the impact of terrain on access across the site.

In this study, less than half the number of scats was found of animals identified as offspring of that of older, parent animals, and all scats of offspring were highly localised within the colony area compared with those of older, parent animals. In particular, only nine scats, over a small area, were identified as belonging to male M2, and DNA from one scat was genotyped as belonging to female F6. This may indicate that older BTRWs at Moora Moora Creek are defending their established home ranges, as has been observed in the allied rock-wallaby (Bleistein *et al.* 1994; Horsup 1994), thus restricting the location and movement of subordinate animals and potentially forcing them into much smaller areas, or areas less accessible for monitoring. As such, it is possible that BTRWs additional to those currently detected may be present at Moora Moora Creek (Bluff *et al.* 2011), although, because considerable lengths of escarpment within, and adjacent to, the known colony were searched for scats, it is likely that the majority of individuals in the colony has been detected. Considering the rugged terrain used by BTRWs at the study site, the use of non-invasive genetic methods for population estimates appears appropriate and advantageous.

Insights into the reintroduction success of BTRWs at Moora Moora Creek

Taggart *et al.* (2015) hypothesised that either regular supplementation of small numbers of naïve captive-bred BTRWs into the establishing Moora Moora Creek colony, or a single larger release of these animals, disrupted colony social cohesion and increased the animal visibility and vulnerability to predation, negatively affecting colony establishment. This hypothesis correlates with the results and observations obtained in this, and other, studies (Moehrenschlager and MacDonald 2003; Shier and Swaisgood 2012). In this study, leaving the BTRW colony undisturbed between 2014 and 2019 through non-invasive monitoring correlated with a decline in BTRW predation, increased breeding, and the recruitment of at least four animals into the adult population. It would appear now that the social structure within the colony has settled without continual disturbances, and along with successful fox baiting and the elimination of resident predators, has promoted breeding and recruitment. This encouraging result justifies continual monitoring of this colony because there is still much to be learnt to inform and improve future reintroduction efforts and recovery of this species.

Despite the benefits of non-invasive monitoring observed in this study, management interventions, such as supplementation, are to be expected if the population is established only on a small number of animals. Non-invasive monitoring can provide information on when such intervention is required, with minimal disturbance to the target population while ensuring that issues, such as inbreeding, do not reach a stage where they severely compromise the population.

Management intervention in the Moora Moora Creek colony took place post-study to address the need for increased genetic diversity. Male 167 was replaced with two new unrelated males. This one-off event of adding new genetics into the colony was deemed worth the risk, despite the colony interference. This lower-level physical interference stands in contrast to that which occurred between 2008 and 2013, when the colony was being regularly interfered with associated trapping for monitoring purposes and the frequent addition of new animals across the colony establishment period. However, the following 8 years (2014–2021), which include the current study, have seen minimal interference owing to camera monitoring and scat genotyping, allowing the animals within the colony to settle and establish.

The results of this study should be considered when planning future reintroductions of highly territorial and social species such as the BTRW (Hazlitt *et al.* 2004), where limited information is available on breeding rates, survival, recruitment, site carrying capacity, and animal home range in open, unfenced, wild populations.

Supplementary material

Supplementary material is available online.

References

Akomo-Okoue EF, Inoue E, Atteke C, Nakashima Y, Hongo S, Inoue-Murayama M, Yamagiwa J (2015) Noninvasive genetic analysis for assessing the abundance of duiker species among habitats in the tropical forest of Moukalaba, Gabon. *Mammal Research* **60**, 375–384. doi:10.1007/s13364-015-0233-1

Aussavy M, Bernardin E, Corrigan A, Hufschmid J, Beveridge I (2011) Helminth parasite communities in four species of sympatric macropodids in western Victoria. *Australian Mammalogy* **33**, 13–20. doi:10.1071/AM10020

Bannister H, Croxford A, Brandle R, Paton DC, Moseby K (2020) Time to adjust: changes in the diet of a reintroduced marsupial after release. *Oryx*, **55**, 755–764. doi:10.1017/S0030605319000991

Berger-Tal O, Blumstein DT, Swaisgood RR (2020) Conservation translocations: a review of common difficulties and promising directions. *Animal Conservation* **23**, 121–131.

Bleistein S, Horsup A, Gansloßer U (1994) Border conflicts among territorial rock wallabies (*Petrogale assimilis*). *Aggressive Behaviour* **20**, 325–333. doi:10.1002/1098-2337(1994)20:4#325::AID-AB2480200406#3.0.CO;2-V

Bluff LA, Clausen L, Hill A, Bramwell MD (2011) A decade of monitoring the remnant victorian population of the brush-tailed rock-wallaby (*Petrogale penicillata*). *Australian Mammalogy* **33**, 195–201. doi:10.1071/AM10037

Bourgeois S, Kaden J, Senn H, Bunnefeld N, Jeffery KJ, Akomo-Okoue EF, Ogden R, McEwing R (2019) Improving cost-efficiency of faecal genotyping: new tools for elephant species. *PLoS ONE* **14**, e0210811. doi:10.1371/journal.pone.0210811

Bozzuto C, Biebach I, Muff S, Ives AR, Keller LF (2019) Inbreeding reduces long-term growth of Alpine ibex populations. *Nature Ecology & Evolution* **3**, 1359–1364. doi:10.1038/s41559-019-0968-1

Bramwell M, Andrews L, Brook L, Corrigan T, Stevens M, Schultz D, Taggart D (2008) Reintroduction plan for the release of brush-tailed rock-wallabies (*Petrogale penicillata*) into Moora Moora Creek, Grampians National Park, Victoria. Department of Sustainability and Environment, Bairnsdale, Vic., Australia.

Browning TL, Taggart DA, Rummery C, Close RL, Eldridge MDB (2001) Multifaceted genetic analysis of the "critically endangered" brush-tailed rock-wallaby *Petrogale penicillata* in Victoria, Australia: implications for management. *Conservation Genetics* **2**, 145–156. doi:10.1023/A:1011850201944

Bubac CM, Johnson AC, Fox JA, Cullingham CI (2019) Conservation translocations and post-release monitoring: identifying trends in failures, biases, and challenges from around the world. *Biological Conservation* **238**, 108239. doi:10.1016/j.biocon.2019.108239

Camp A, Croxford AE, Ford CS, Baumann U, Clements PR, Hiendleder S, Woolford L, Netzel G, Boardman WSJ, Fletcher MT, Wilkinson MJ (2020) Dual-locus DNA metabarcoding reveals southern hairy-nosed wombats (*Lasiorhinus latifrons* Owen) have a summer diet dominated by toxic invasive plants. *PLoS ONE* **15**, e0229390. doi:10.1371/journal.pone.0229390

Cassinello J, Aldos CL (1996) Female reproductive success in *Ammontragus lervia* (Bovidae, Artiodactyla). Study of its components and effects of heriarchy and inbreeding. *Journal of Zoology* **239**, 141–153. doi:10.1111/j.1469-7998.1996.tb05442.x

Claridge AW, Paull DJ, Barry SC (2010) Detection of medium-sized ground-dwelling mammals using infrared digital cameras: an alternative way forward? *Australian Mammalogy* **32**, 165–171. doi:10.1071/AM09039

Cutler TL, Swann DE (1999) Using remote photography in wildlife ecology: a review. *Wildlife Society Bulletin* **27**, 571–581.

De Bondi N, White JG, Stevens M, Cooke R (2010) A comparison of the effectiveness of camera trapping and live trapping for sampling terrestrial small-mammal communities. *Wildlife Research* **37**, 456–465. doi:10.1071/WR10046

Delaney R, Temple-Smith P, McLean N (2005) Strategy for the translocation of the brush-tailed rock-wallaby in Victoria. Report for the Victorian Brush-tailed Rock-wallaby Recovery Team, Department of Sustainability and Environment and Parks Victoria, Vic., Australia.

Dimsoski P (2017) Genotyping horse epithelial cells from fecal matter by isolation of polymerase chain reaction products. *Croatian Medical Journal* **58**, 239–249. doi:10.3325/cmj.2017.58.239

Eldridge MDB, Rummery C, Bray C, Zenger KR, Browning T, Close RL (2004) Genetic analysis of a population crash in brush-tailed rock-tailed rock-wallabies (*Petrogale penicillata*) from Jenolan Caves, South-Eastern Australia. *Wildlife Research* **31**, 229–240. doi:10.1071/WR03030

Gant JR, Mair L, McGowan PJK (2020) Fragmented evidence for the contribution of ex situ management to species conservation indicates the need for better reporting. *Oryx* **55**, 573–580. doi:10.1017/S0030605319000784

Hale ML, Burg TM, Steeves TE (2012) Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS ONE* **7**, e45170. doi:10.1371/journal.pone.0045170

Hardman B, Moro D, Calver M (2016) Direct evidence implicates feral cat predation as the primary cause of failure of a mammal reintroduction programme. *Ecological Management & Restoration* **17**, 152–158. doi:10.1111/emr.12210

Hayward MW, Bellchambers K, Herman K, Bentley J, Legge S (2011) Spatial behaviour of yellow-footed rock-wallabies, *Petrogale xanthopus*, changes in response to active conservation management. *Australian Journal of Zoology* **59**, 1–8. doi:10.1071/ZO11007

Hazlitt SL, Eldridge MDB, Goldizen AW (2004) Fine-scale spatial genetic correlation analyses reveal strong female philopatry within a brush-tailed rock-wallaby colony in southeast Queensland. *Molecular Ecology* **13**, 3621–3632. doi:10.1111/j.1365-294X.2004.02342.x

Hazlitt SL, Goldizen AW, Eldridge MDB (2006*a*) Significant patterns of population genetic structure and limited gene flow in a threatened macropodid marsupial despite continuous habitat in Southeast Queensland, Australia. *Conservation Genetics* **7**, 675–689. doi:10.1007/s10592-005-9101-x

Hazlitt SL, Goldizen AW, Nicholls JA, Eldridge MDE (2014) Three divergent lineages within an Australian Marsupial (*Petrogale penicillata*) suggest multiple major refugia for mesic taxa in Southeast Australia. *Ecology and Evolution* **4**, 1102–1116. doi:10.1002/ece3.1009

Hazlitt SL, Sigg DP, Eldridge MDB, Goldizen AW (2006*b*) Restricted mating dispersal and strong breeding group structure in a mid-sized marsupial mammal (*Petrogale penicillata*). *Molecular Ecology* **15**, 2997–3007. doi:10.1111/j.1365-294X.2006.02985.x

Horsup A (1994) Home range of the allied rock-wallaby, *Petrogale assimilis*. *Wildlife Research* **21**, 65–83. doi:10.1071/WR9940065

Hoyle SD, Horsup AB, Johnson CN, Crossman DG, McCallum H (1995) Live-trapping of the northern hairy-nosed wombat (*Lasiorhinus krefftii*): population-size estimates and effects on individuals. *Wildlife Research* **22**, 741–755. doi:10.1071/WR9950741

Jamieson IG (2011) Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conservation Biology* **25**, 115–123. doi:10.1111/j.1523-1739.2010.01574.x

Jarman PJ (1991) Social behaviour and organization in the macropodoidea. *Advances in the Study of Behaviour* **20**, 1–50. doi:10.1016/S0065-3454(08)60318-6

Jarman PJ, Capararo SM (1996) Use of rock-wallaby faecal pellets for detecting and monitoring populations and examining habitat use. *Australian Mammalogy* **19**, 257–264. doi:10.1071/AM97257.

Johnson TF, Brown TJ, Richardson DS, Dugdale HL (2018) The importance of post–translocation monitoring of habitat use and population growth: insights from a Seychelles warbler (*Acrocephalus sechellensis*) translocation. *Journal of Ornithology* **159**, 439–446. doi:10.1007/s10336-017-1518-8

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**, 1099–1106. doi:10.1111/j.1365-294X.2007.03089.x

Katsura Y, Kondo HX, Ryan J, Harley V, Satta Y (2018) The evolutionary process of mammalian sex determination genes focusing on marsupial SRYs. *BMC Evolutionary Biology* **18**, 3. doi:10.1186/s12862-018-1119-z

Kaufmann JH (1974) Habitat use and social organization of nine sympatric species of macropodid marsupials. *Journal of Mammalogy* **55**, 66–80. doi:10.2307/1379257

Kolodzieg K, Nikolov I, Schulz HK, Theissinger K, Schulz R (2013) Evaluation of fecal storage and DNA extraction methods in wild boar (*Sus scrofa*). *Biochemical Genetics* **51**, 406–412. doi:10.1007/s10528-013-9573-1

Koressaar T, Lepamets M, Kaplinski L, Raime K, Andreson R, Remm M (2018) Primer3_masker: integrating masking of template sequence with primer design software. *Bioinformatics* **34**, 1937–1938. doi:10.1093/bioinformatics/bty036

Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3. *Bioinformatics* **23**, 1289–1291. doi:10.1093/bioinformatics/btm091

Lachish S, Passmore A, Jones M (2011) A new PCR assay for reliable molecular sexing of endangered tasmanian devils (*Sarcophilus harrisii*) from non-invasive genetic samples. *Conservation Genetics Resources* **3**, 279–281. doi:10.1007/s12686-010-9341-z

Lapbenjakul S, Thapana W, Twilprawat P, Muangmai N, Kanchanaketu T, Temsiripong Y, Unajak S, Peyachoknagul S, Srikulnath K (2017) High genetic diversity and demographic history of captive Siamese and saltwater crocodiles suggest the first step toward the establishment of a breeding and reintroduction program in Thailand. *PLoS ONE* **12**, e0184526. doi:10.1371/journal.pone.0184526

Laws RJ, Goldizen AW (2003) Nocturnal home ranges and social interactions of the brushtailed rock-wallaby Petrogale penicillata at Hurdle Creek, Queensland. *Australian Mammalogy* **25**, 169–176. doi:10.1071/AM03169

Luikart G, Painter J, Crozier RH, Westerman M, Sherwin WB (1997) Characterization of microsatellite loci in the endangered long-footed potoroo *Potorous longipes*. *Molecular Ecology* **6**, 497–498. doi:10.1046/j.1365-294X.1997.00208.x

Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications* **10**, 283–294. doi:10.1890/1051-0761(2000)010[0283:EAAUND]2.0.CO;2

Moehrenschlager A, MacDonald DW (2003) Movement and survival parameters of translocated and resident swift foxes *Vulpes velox*. *Animal Conservation* **6**, 199–206. doi:10.1017/S1367943003251

Molyneux J, Taggart DA, Corrigan A, Frey S (2011) Home-range studies in a reintroduced brush-tailed rock-wallaby (*Petrogale penicillata*) population in the Grampians National Park, Victoria. *Australian Mammalogy* **33**, 128–134. doi:10.1071/AM10039

Morales-Contreras J, Escalante P, Martínez-Ruíz M, Matías-Ferrer N (2016) Black hawk-eagle (*Spizaetus tyrannus*) idenitfied by DNA from a feather recovered in the rain-forest region of Veracruz. *The Southwestern Naturalist* **61**, 137–142. doi:10.1894/0038-4909-61.2.137

Moseby KE, Read JL, Paton DC, Copley P, Hill BM, Crisp HA (2011) Predation determines the outcome of 10 reintroduction attempts in arid South Australia. *Biological Conservation* **144**, 2863–2872. doi:10.1016/j.biocon.2011.08.003

Moßbrucker AM, Apriyana I, Fickel J, Imron MA, Pudyatmoko S, Sumardi SH (2015) Non-invasive genotyping of sumatran elephants: implications for conservation. *Tropical Conservation Science* **8**, 745–759. doi:10.1177/194008291500800312

Paplinska JZ, Taggart DA, Corrigan T, Eldridge MDB, Austin JJ (2011) Using DNA from museum specimens to preserve the integrity of evolutionarily significant unit boundaries in threatened species. *Biological Conservation* **144**, 290–297. doi:10.1016/j.biocon.2010.09.005

Parlato EH, Armstrong DP (2013) Predicting post-release establishment using data from multiple reintroductions. *Biological Conservation* **160**, 97–104. doi:10.1016/j.biocon.2013.01.013

Peakall R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research: an update. *Molecular Ecology Notes* **6**, 288–295. doi:10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research: an update. *Bioinformatics* **28**, 2537–2539. doi:10.1093/bioinformatics/bts460

Piggott MP (2004) Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. *Wildlife Research* **31**, 485–493. doi:10.1071/WR03096

Piggott MP, Banks SC, Stone N, Banffy C, Taylor AC (2006*a*) Estimating population size of endangered brush-tailed rock-wallaby (*Petrogale penicillata*) colonies using faecal DNA. *Molecular Ecology* **15**, 81–91. doi:10.1111/j.1365-294X.2005.02783.x.

Piggott MP, Banks SC, Taylor AC (2006*b*) Population structure of brush-tailed rock-wallaby (*Petrogale penicillata*) colonies inferred from analysis of faecal DNA. *Molecular Ecology* **15**, 93–105. doi:10.1111/j.1365-294X.2005.02784.x

Piggott MP, Hansen B, Soderquist T, Eldridge MDB, Taylor AC (2018) Population monitoring of small and declining brush-tailed rock-wallaby (*Petrogale penicillata*) colonies at the extreme of their range using faecal DNA sampling. *Australian Mammalogy* **40**, 58–66. doi:10.1071/AM16056

Piggott MP, Taylor AC (2003) Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. *Wildlife Research* **30**, 1–13. doi:10.1071/WR02077

Ramón-Laca A, Soriano L, Gleeson D, Godoy JA (2015) A simple and effective method for obtaining mammal DNA from faeces. *Wildlife Biology* **21**, 195–203. doi:10.2981/wlb.00096

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, 1280–1283. doi:10.1111/j.1558-5646.1995.tb04456.x

Richards JD, Short J (2003) Reintroduction and establishment of the western barred bandicoot *Perameles bougainville* (Marsupialia: Peramelidae) at Shark Bay, Western Australia. *Biological Conservation* **109**, 181–195. doi:10.1016/S0006-3207(02)00140-4

Robinson NM, Dexter N, Brewster R, Maple D, MacGregor C, Rose C, Hall J, Lindenmayer DB (2020) Be nimble with threat mitigation: lessons learned from the reintroduction of an endangered species. *Restoration Ecology* **28**, 29–38. doi:10.1111/rec.13028

Robley A, Ramsey D, Holmes B (2014) Grampians arc fox monitoring research partnership project 2013–2014. Arthur Rylah Institute for Environmental Research, unpublished client report for Parks Victoria, Department of Environment and Primary Industries, Melbourne, Vic., Australia.

Rodgers TW, Giacalone J, Heske EJ, Janecka JE, Phillips CA, Schooley RL (2014) Comparison of noninvasive genetics and camera trapping for estimating population density of ocelots (*Leopardus pardalis*) on Barro Colorado Island, Panama. *Tropical Conservation Science* **7**, 690–705. doi:10.1177/194008291400700408

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103–106. doi:10.1111/j.1471-8286.2007.01931.x

Sabino-Marques H, Ferreira CM, Paupério J, Costa P, Barbosa S, Encarnação C, Alpizar-Jara R, Alves PC, Searle JB, Mira A, Beja P, Pita R (2018) Combining genetic non-invasive sampling with spatially explicit capture–recapture models for density estimation of a patchily distributed small mammal. *European Journal of Wildlife Research* **64**, 44. doi:10.1007/s10344-018-1206-x

Scott PA, Allison LJ, Field KJ, Averill-Murray RC, Shaffer HB (2020) Individual heterozygosity predicts translocation success in threatened desert tortoises. *Science* **370**, 1086–1089. doi:10.1126/science.abb0421

Sharp A (2009) Home range dynamics of the yellow-footed rock-wallaby (*Petrogale xanthopus celeris*) in central-western Queensland. *Austral Ecology* **34**, 55–68. doi:10.1111/j.1442-9993.2008.01882.x

Sheean VA, Manning AD, Lindenmayer DB (2012) An assessment of scientific approaches towards species relocations in Australia. *Austral Ecology* **37**, 204–215. doi:10.1111/j.1442-9993.2011.02264.x

Shier DM, Swaisgood RR (2012) Fitness costs of neighbourhood disruption in translocations of a solitary mammal. *Conservation Biology* **26**, 116–123. doi:10.1111/j.1523-1739.2011.01748.x

Short J (2016) Predation by feral cats key to the failure of a long-term reintroduction of the western barred bandicoot (*Perameles bougainville*). *Wildlife Research* **43**, 38–50. doi:10.1071/WR15070

Silveira L, Jácomo ATA, Diniz-Filho JAF (2003) Camera trap, line transect census and track surveys: a comparative evaluation. *Biological Conservation* **114**, 351–355. doi:10.1016/S0006-3207(03)00063-6

Taggart DA, Schultz D, White C, Whitehead P, Underwood G, Phillips K (2005) Cross-fostering, growth and reproductive studies in the brush-tailed rock-wallaby, *Petrogale penicillata* (Marsupialia: Macropodidae): efforts to accelerate breeding in a threatened marsupial species. *Australian Journal of Zoology* **53**, 313–323. doi:10.1071/ZO05002

Taggart DA, Schultz DJ, Corrigan TJ, Schultz TJ, Stevens M, Panther D, White CR (2015) Reintroduction methods and a review of mortality in the brush-tailed rock-wallaby, Grampians National Park, Australia. *Australian Journal of Zoology* **63**, 383–397. doi:10.1071/ZO15029

Taggart D, Clausen L, Stevens M, Bramwell M, Corrigan A (2008) Brush-tailed rock-wallaby reintroduction site assessment guide for the Grampians National Park, Victoria. Report for the Victorian Brush-tailed Rock Wallaby Recovery Team, Victorian Department of Sustainability and Environment, Melbounrne, Vic., Australia.

Telfer WR, Griffiths AD (2006) Dry-season use of space, habitats and shelters by the short-eared rock-wallaby (*Petrogale brachyotis*) in the monsoon tropics. *Wildlife Research* **33**, 207–214. doi:10.1071/WR05032

Tende T, Hansson B, Ottosson U, Åkesson M, Bensch S (2014) Individual identification and genetic variation of lions (*Panthera leo*) from two protected areas in Nigeria. *PLoS ONE* **9**, e84288. doi:10.1371/journal.pone.0084288

Tettamanti F, Grignolio S, Filli F, Apollonio M, Bize P (2015) Senescence in breeding success of female Alpine chamois (Rupicapra rupicapra): the role of female quality and age. *Oecologia* **178**, 187–195. doi:10.1007/s00442-014-3197-y

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3: new capabilities and interfaces. *Nucleic Acids Research* **40**, e115. doi:10.1093/nar/gks596

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538. doi:10.1111/j.1471-8286.2004.00684.x

Velli E, Bologna MA, Silvia C, Ragni B, Randi E (2015) Non-invasive monitoring of the european wildcat (*Felis silvestris silvestris* Schreber, 1777): comparative analysis of three different monitoring techniques and evaluation of their integration. *European Journal of Wildlife Research* **61**, 657–668. doi:10.1007/s10344-015-0936-2

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology* **10**, 249–256. doi:10.1046/j.1365-294X.2001.01185.x

Wakefield N (1961) Victoria's rock-wallabies. *Victorian Naturalist* **77**, 322–332.

Walker FM, Horsup A, Taylor AC (2009) Leader of the pack: faecal pellet deposition order impacts PCR amplification in wombats. *Molecular Ecology Resources* **9**, 720–724. doi:10.1111/j.1755-0998.2009.02582.x

Watkins GE, Willers N, Raudino H, Kinloch J, van Dongen R (2018) Success criteria not met, but valuable information gained: monitoring a reintroduction of the tammar wallaby. *Wildlife Research* **45**, 421–435. doi:10.1071/WR17102

Watson CM, Margan SH, Johnston PG (1998) Sex-chromosome elimination in the bandicoot *Isoodon macrourus* using Y-linked markers. *Cytogenetics and Cell Genetics* **81**, 54–59. doi:10.1159/000015008

Wegge P, Pokheral CP, Jnawali SR (2004) Effects of trapping effort and trap shyness on estimates of tiger abundance from camera trap studies. *Animal Conservation* **7**, 251–256. doi:10.1017/S1367943004001441

White PCL, McClean CJ, Woodroffe GL (2003) Factors affecting the success of an otter (*Lutra lutra*) reinforcement programme, as identified by post-translocation monitoring. *Biological Conservation* **112**, 363–371. doi:10.1016/S0006-3207(02)00333-6

Wilkinson MJ, Szabo C, Ford CS, Yarom Y, Croxford AE, Camp A, Gooding P (2017) Replacing Sanger with next generation sequencing to improve coverage and quality of reference DNA barcodes for plants. *Scientific Reports* **7**, 46040. doi:10.1038/srep46040

Willoughby JR, Waser PM, Brüniche-Olsen A, Christie MR (2019) Inbreeding load and inbreeding depression estimated from lifetime reproductive success in a small, dispersal-limited population. *Heredity* **123**, 192–201. doi:10.1038/s41437-019-0197-z

Woinarski JCZ, Burbidge AA, Harrison PL (2014) Taxon profiles. In 'The Action Plan for Australian Mammals 2012'. pp. 51–857. (CSIRO Publishing: Melbourne, Vic., Australia)

Wynd NM, Sigg DP, Pople AR, Hazlitt SL, Goldizen AW (2006) Factors affecting female reproductive success and the survival of pouch young in the threatened brush-tailed rock-wallaby, *Petrogale penicillata*. *Australian Journal of Zoology* **54**, 61–70. doi:10.1071/ZO05064

Zeng Y, Jiang Z, Li C (2007) Genetic variability in relocated Père David's deer (*Elaphurus davidianus*) populations: iImplications to reintroduction program. *Conservation Genetics* **8**, 1051–1059. doi:10.1007/s10592-006-9256-0

Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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