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Genetic monitoring of southern hairy-nosed wombats over two decades reveals that individuals can live for at least 18 years in the same warrens

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Abstract. Survival and growth rates are important demographic parameters to understand for long-term management of populations. Eighteen years have elapsed since non-invasive genetic methods were used to identify southern hairy-nosed wombats (*Lasiorhinus latifrons*), and determine space use and relatedness at Brookfield Conservation Park, South Australia. Because the species is long-lived (>30 years) and genetic methods can identify all or most wombats that use an area, it is possible to determine whether population size or warren use have changed and if any individuals are still alive. To this end, in April 2017 we collected hair from wombats from the same warrens as the earlier study using sticky tape suspended across burrows. We subjected DNA from selected hairs to 10 microsatellite loci and a Y-linked sex marker, and identified 76 wombats. Five wombats were detected 16–18 years before, and four of them were found in warrens that they had used previously. The number of tapes hit, wombats detected, and warrens used were greater than in April 2001 and similar to September 2001. This study illustrates that non-invasive sampling methods can be used to track free-ranging individuals in continuous habitat across decades, despite rapidly evolving genetic technology that can strand older datasets.

Additional keywords: legacy data, molecular analysis.

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Introduction

Current rates of global environmental change are projected to influence the density and distribution of wildlife populations and to result in a rapid loss of biodiversity (Dirzo *et al.* 2014; Pimm *et al.* 2014; Newbold *et al.* 2016). The mechanisms and interactions of population size changes through time and density changes and movement in space can be elusive, and many approaches that are used to better understand these fundamental questions in ecology are cost prohibitive or invasive (Hebblewhite and Haydon 2010; Burgar *et al.* 2018). Non-invasive or minimally invasive genetic sampling, used alone or in concert with other methods, provides a powerful, efficient, and cost-effective approach to better understanding population processes (Walker *et al.* 2008*a*; Arandjelovic *et al.* 2010; Potter *et al.* 2012). Such methods commonly use remotely collected hair (Happe *et al.* 2020), faeces (Bradley *et al.* 2008), or feathers (Roy and Gregory 2019) as a DNA source.

Remote sampling and analytical methods for genetic monitoring were developed in the 1990s (Morin and Woodruff 1992; Taberlet *et al.* 1997; Sloane *et al.* 2000) and have been refined over the past 25 years (Carroll *et al.* 2018; Franklin *et al.* 2019). The early days of microsatellite DNA research used radioisotope incorporation during polymerase chain reaction (PCR) followed by autoradiography to visualise alleles, which was later replaced with capillary electrophoresis. More recently, high-throughput sequencing of microsatellite markers has shown great utility (De Barba *et al.* 2017). Each progression of technology leaves behind legacy datasets, as it is either not possible or not cost-effective to migrate 'old' data onto new platforms. Yet, for many studies using microsatellite DNA, incorporating these datasets into new genetic monitoring efforts will be extraordinarily valuable for insight into population changes over time.

One species with legacy microsatellite data is the southern hairy-nosed wombat (*Lasiorhinus latifrons*), a large (23–38 kg),

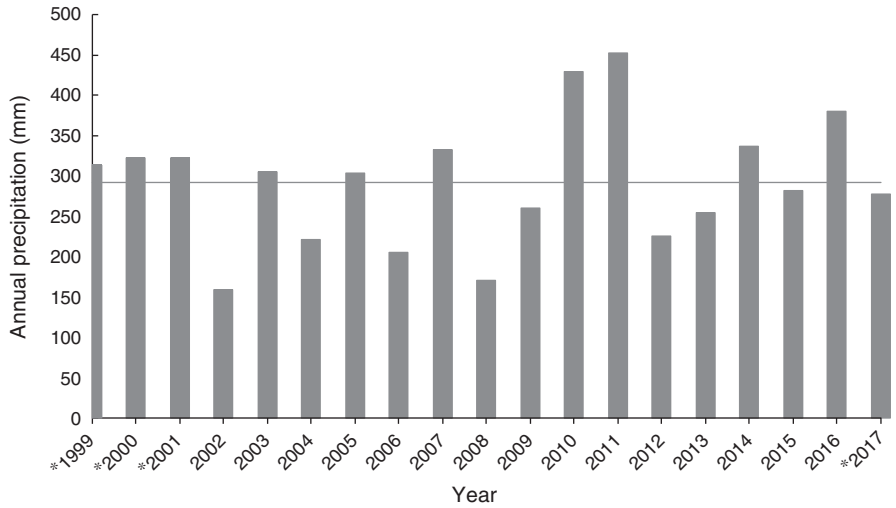

Fig. 1. Annual rainfall from 1999 to 2017. Data are from the Bureau of Meteorology's Blanchetown station, 14 km from the study site. Asterisks indicate years of genetic tagging, and the horizontal line represents mean rainfall over these years.

long-lived (>30 years) herbivorous marsupial distributed in semiarid regions in southern Australia (Aitken 1971; Taggart and Temple-Smith 2008; Swinbourne *et al.* 2017). This species, which inhabits interconnected burrow systems called warrens, is problematic to study because it is nocturnal, shy, and difficult to capture. In the late 1990s and early 2000s, we deployed one of the largest hair trapping studies of the time with the aim of genetically identifying and tracking wombats via microsatellite DNA (Walker 2004). We found female-biased dispersal, male philopatry, and male kinship-based associations (Walker *et al.* 2008*b*), conservative space use with wombats using multiple warrens in a small area (<7.83 ha) (Walker *et al.* 2006), soil type as a driver of sociobiology (Walker *et al.* 2007), and increased population density and altered kin relationships in fragmented habitat (Walker *et al.* 2008*a*).

Our main study site for this work was at Brookfield Conservation Park in the Murraylands of South Australia, which has a large population of southern hairy-nosed wombats in relatively continuous habitat (McGregor and Wells 1998; Swinbourne *et al.* 2017). Since the time of our genetic studies, the Murraylands has experienced episodes of drought and is vulnerable to a changing climate (Marshall *et al.* 2018), and sarcoptic mange has cyclically impacted wombats (Ruykys *et al.* 2009). In order to better understand population size, survival, and warren use over a long period, we resampled our study area 18 years after the initial research. Specifically, we wished to determine: (1) how numbers and sex ratio of wombats compared with the earlier study, and (2) whether any of the same wombats from 1999–2001 could be detected and, if so, whether they used the same or different warrens. Such a study required navigating the disjunction between the original and currently used sequencing methods, while keeping laboratory costs low. This was achieved by applying a combination of recently developed (White *et al.* 2014) and original loci (Taylor *et al.* 1994; Alpers 1998; Beheregaray *et al.* 2000) along with positive control DNA of known genotype that had been used in our earlier work.

Materials and methods

This study was approved by the University of Adelaide Animal Ethics Committee (project no. S-2016-163) and South Australia's Department of Environment, Water, and Natural Resources (permit no. Y26582-1). Sample export/import permits were acquired from the Australian Department of the Environment and Energy (permit no. PWS2018-AU-001967) and the US Fish and Wildlife Service (DEC control no. 2017141018). All genetic sampling and sequencing complied with relevant guidelines and regulations, and no wombats suffered injury or mortality as part of this study. Our non-invasive sample collection methods follow guidelines of the American Society of Mammalogists for animal care and use (Sikes 2016).

Study site and genetic sampling

Our field site was at Brookfield Conservation Park (−34.34618298, 139.48006), South Australia. This semiarid area has low, irregular rainfall (Fig. 1) and a large population of southern hairy-nosed wombats (McGregor and Wells 1998; Swinbourne *et al.* 2017). In April 2017, we collected hair samples from wombats in a 1.8 × 0.5 km area that was used in our hair-based genetic studies 16–18 years previously (Walker *et al.* 2006, 2007, 2008*b*). To do so, we suspended double-sided carpet tape (TESA Tape product 4970) between wooden garden stakes placed at either side of burrow entrances of 25 main study warrens. These were the same warrens, and were named the same, as in the previous studies, with the exception of warrens X and Alpha, which were abandoned, and Col, Suz, and PR, which were new and added to the study. GPS coordinates are provided in Supplementary Table S1. We taped all burrows at each warren except for those with no indication of recent use (spider webs or collapsed entrances). Tapes were suspended ~25 cm above the centre of burrow entrance floors. As wombats moved in and out of burrows during the night they left hairs behind on the tapes. Tapes were checked for hair each morning for five consecutive days, and were collected and replaced if hair was present.

Five nights of taping detected all or nearly all wombats in the sampling area in our previous studies (Walker *et al.* 2006). We also taped seven peripheral warrens to the main study area, as was done in the early 2000s, in order to increase the number of wombats detected (Walker *et al.* 2006), and collected tapes with hair on Days 1 and 4. The total number of burrows taped across all warrens was 350, as in our previous work.

We performed same-day DNA extractions at the field camp using 5% Chelex solution (Sloane *et al.* 2000; Walker *et al.* 2006). Two DNA extractions were performed per hair tape, each from an individual hair follicle. We selected hair follicles based on size, and used hair from opposite ends of the tape when possible in order to maximise the chances that burrow sharing would be detected. DNA extractions were stored in a refrigerator for 10 days, after which they were transported to a −80°C freezer. For this study, we analysed the first four of the five days' samples.

Microsatellite loci and PCR conditions

Our previous studies used radioisotope incorporation during PCR followed by autoradiography to visualise alleles, as it preceded capillary electrophoresis with fluorescently labelled primers that is now standard practice for microsatellite studies. Further, most of the early microsatellite loci developed for wombats (Taylor *et al.* 1994; Alpers 1998; Beheregaray *et al.* 2000) consist of dinucleotide repeats. This makes transferring to the newer technology problematic because they tend to suffer slippage during PCR, which appears as stutter peaks on chromatograms. Thus, in order to assign hairs to individual wombats and to recognise individuals from our previous studies, we used a combination of new tri- and tetranucleotide microsatellite loci developed by White *et al.* (2014) along with a subset of the original loci.

Loci for assigning hairs to wombats

Using the universal tail PCR labelling system of U'Ren *et al.* (2007), we tested the eight new primer sets of White *et al.* (2014), which were developed for northern hairy-nosed wombats (*Lasiorhinus krefftii*), the two older loci that they found to perform well with capillary electrophoresis (Ll68CA and Ll2), and the sexing marker we used in our earlier study (bSRY: Watson *et al.* 1998). We selected a panel of six loci that reliably amplified, were readily scored, and were polymorphic (6412, ELZRS, F3184, I85G2, DR470, Ll68CA). We PCR-amplified ELZRS, F3184, I85G2, DR470, 68CA, and bSRY as a multiplex or singleplexes (or in the case of bSRY, a duplex with DR470), and 6412 as a singleplex. The multiplex PCR contained 1X Mg-free PCR buffer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), 2 mM of $MgCl_2$, 0.2 mM dNTPs, 0.2 μM fluorescently labeled forward primer, 0.2 μM unlabeled reverse primer, 0.4 U/μL Platinum *Taq* DNA polymerase (Invitrogen), 0.02 μg/μL Ultrapure non-acetylated Bovine Serum Albumin (BSA), and 5 μL of DNA template (mean 1.27 ng/μL) in a 15 μL reaction. PCRs for singleplexed loci contained 1X Mg-free PCR buffer (Invitrogen), 2 mM of $MgCl_2$, 0.2 mM dNTPs, 0.05 μM unlabeled forward primer, 0.1 μM universal forward label (VIC, PET, 6-FAM, NED) (U'Ren *et al.* 2007), 0.1 μM unlabeled reverse primer, 0.1 U/μL Platinum *Taq* DNA polymerase (Invitrogen), 0.02 μg/μL BSA, and 5 μL of DNA template in a 15 μL reaction. PCRs for the sexing duplex of DR470 and bSRY contained the same quantities as the singleplexes aside from the primers and *Taq* polymerase: 0.1 μM of the smaller DR470 unlabeled forward primer, 0.2 μM universal forward label (PET) (U'Ren *et al.* 2007), 0.2 μM DR470 unlabeled reverse primer, 0.4 μM of the larger bSRY unlabeled forward primer, 0.8 μM universal forward label (6-FAM) (U'Ren *et al.* 2007), 0.8 μM bSRY unlabeled reverse primer, and 0.2 U/μL Platinum *Taq* DNA polymerase (Invitrogen). We performed thermal cycling on an Applied Biosystems SimpliAmp Thermal Cycler. Cycling conditions for all PCRs began with a denaturation step of 94°C for 5 min, followed by 45 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, then concluded with a final extension step of 72°C for 5 min.

After PCR amplification, product was diluted as follows before fragment analysis on an Applied Biosystems 3130 Genetic Analyzer: 1 : 50 for singleplexes, 1 : 100 for the duplex, and no dilution for the multiplex. We used GeneMarker software to score alleles, and program GeneAlEx 6.5 (Peakall and Smouse 2012) to match genotypes of wombats. DNA extractions with 100% allelic sharing across loci were treated as belonging to the same wombat. The power of these six loci for differentiating between individuals was high; the probability of two randomly selected wombats sharing a multilocus genotype was 4.6×10^{-8} (Waits *et al.* 2001).

Loci for identifying wombats from the early 2000s

To match wombats detected in 2017 with those from our 1999–2001 studies, we applied four loci (71CA, Lk23, Lla109, Ll2: Taylor *et al.* 1994; Alpers 1998; Beheregaray *et al.* 2000) to a representative sample from each wombat. These loci, along with Lla68CA above, were PCR amplified with positive control DNA of 10 Brookfield Conservation Park wombats from Taylor *et al.* (1994), as was done for our earlier studies (e.g. Walker *et al.* 2006). PCR reagents and conditions were as above for singleplexes. Any genotype with 100% allelic matching (at all five loci plus the sexing marker) with an individual from the 1999–2001 studies was considered to be the same individual. We repeated PCRs for all questionable matches using an additional representative sample from each wombat. The probability of identity for these five loci was 1.2×10^{-5}.

Quality control

We used a suite of quality control measures to reduce and detect genotyping errors (Bonin *et al.* 2004; Waits and Paetkau 2005). As ~3671 PCR reactions were performed for this study, 11 single-locus genotypes can be expected to be false based on the 0.3% error rate of Sloane *et al.* (2000), who were the first to survey a hairy-nosed wombat population with these methods. Genotyping both hair samples from each tape assisted with scoring accuracy, since in our previous work 90.5% of these samples were from the same wombat. Further, we distinguished scoring errors, null alleles, and PCR artifacts by repeating PCRs for samples that were poorly amplifying (*Taq* polymerase increased to 0.2 U/μL), had novel alleles, and for multilocus genotypes differing at three or fewer loci. Samples that failed to amplify at four or more loci or that had high homozygosity were removed from the dataset. To facilitate scoring of questionable

Fig. 2. Photograph from a remote camera showing a southern hairy-nosed wombat at Warren A in front of a burrow, which has double-sided carpet tape suspended between two garden stakes to catch hairs. A second wombat is coming into the frame on the lower left. A video of this event, showing the first wombat ducking under the tape, can be found here: https://doi.org/10.6084/m9.figshare.11774562.v1.

individuals, PCRs were repeated on the same PCR plate, placed on the Genetic Analyzer together, and scored side-by-side. Additionally, any putative individual wombats that were genetically similar and geographically more distant than wombats were detected to move in Walker *et al.* (2006) were also reamplified for all loci and compared side-by-side. As an additional conservative measure, after matching genotypes with wombats from the early 2000s, we included only unique genotypes that were detected at least twice, either on the same tape or different tapes.

Locus behaviour

We tested for deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium between pairs of loci with program GENEPOP 4.7.3 (Rousset 2008). We tested for heterozygote or homozygote deficits in addition to the non-directional Hardy–Weinberg test, and we used a sequential Bonferroni procedure (Rice 1989) to adjust for multiple tests where required. We used program MICRO-CHECKER (Van Oosterhout *et al.* 2004) to test for the possibility of scoring errors, allelic dropout, and null alleles.

Results

Rate of attrition and samples analysed

Over the five sampling nights and 350 taped burrows, wombats had 1750 opportunities to donate hair to this study, 460 of which were taken (e.g. Fig. 2). Most tapes (95%) that were 'hit' by wombats had hair follicles, which indicates that tapes were set at an appropriate height to pluck hairs and that tape condition did not deteriorate between hits due to environmental variables (e.g. dew, dust). Of the hit tapes, 373 were from the main study warrens, which is similar to the number of hits in September 2001 (n = 370) but greater than in April 2001 (n = 172)

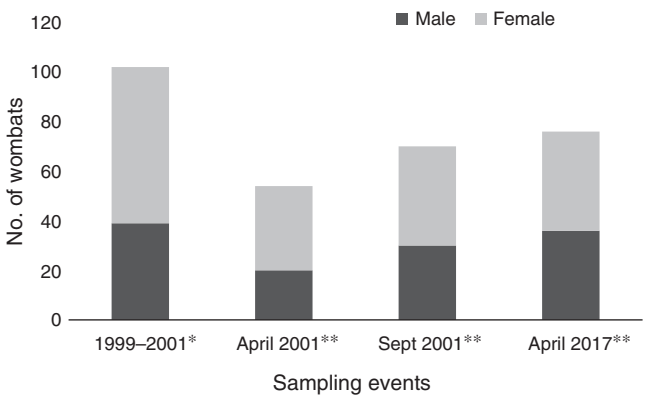

Fig. 3. Number and sex ratio of wombats genetically detected at our 1-km^2 study site at Brookfield Conservation Park during (*) 1999–2001 (five sampling periods of five nights each); (**) the first four nights of 2001 and 2017 sampling periods.

(Walker *et al.* 2006). We analysed 663 DNA extractions (Days 1–4) of the 810 total extractions from five days of sampling. In our five sampling periods between 1999 and 2001, an average of three new wombats were detected on the fifth day (Walker *et al.* 2006), which suggests that analysis of the first four days allows detection of most wombats that use the area.

Wombat detection success and locus behaviour

We identified 76 distinct multilocus genotypes (Supplementary Table S2): 63 in the main study warrens and 13 in peripheral warrens. More females than males were detected (40 females and 36 males) (Fig. 3), but this difference was not statistically significant (P = 0.37; exact binomial test). The number of new

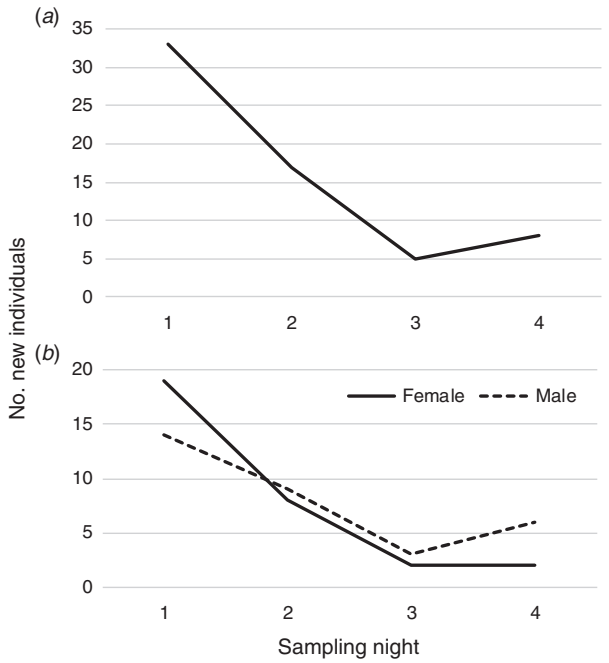

Fig. 4. Number of new wombats identified per night across the main study warrens at South Australia's Brookfield Conservation Park: (*a*) total, (*b*) by sex. As in Walker *et al.* (2006), new wombats declined to low numbers by Night 3.

wombats detected per night in the main study area declined to five and eight on Nights 3 and 4, respectively (Fig. 4). Individual wombats were detected up to 15 times ($M = 4.79$, s.d. = 2.92; Supplementary Table S2). There were 18 instances of within-night burrow sharing (two different wombats detected on the same hair tape), and 101 cases of both hair samples taken from a tape belonging to the same wombat. In 2001 we detected eight instances of within-night burrow sharing in April and 11 instances in September.

Mean expected heterozygosity for the 10 loci was 0.61, and mean number of alleles per locus was 5.4. All loci adhered to Hardy–Weinberg expectations for the probability test as well as tests for heterozygote or homozygote deficits. Likewise, no locus pairs were in linkage disequilibrium, and there was no evidence of null alleles for any locus.

Warren and burrow use

Warrens that were heavily used during this study were also heavily used in the early 2000s as well as when the authors first surveyed the study area in 1994 (Walker *et al.* 2006). In this study, two small warrens were new, and two small warrens had been abandoned. Wombats were detected to use 1–3 warrens (mean = 1.32, s.d. = 0.53). Up to 10 individuals used a single warren (mean = 4.11, s.d. = 2.63), and warrens with high numbers of individuals in 2000 and 2001 had similar numbers in this study (Table 1). In April 2017 our main study warrens were used by more wombats than in April 2001 and fewer wombats than in September 2001 (standardised warrens used and the number of sampling nights; Wilcoxon signed-ranks test: $Z = 26$, $P < 0.021$, $Z = -28.5$, $P < 0.047$, respectively). The number of wombats per active burrow, where an active burrow was defined as wombat hair on a tape, averaged 0.63 across all main study warrens (Table 1).

Wombats detected 16–18 years previously

Five wombats (6.6%) that were detected during five sampling periods in 1999–2001 were also detected during this study. Four of the five wombats were detected in a warren used by that individual 16–18 years previously. The exception was a female found in a warren 87 m from the cluster of warrens she was detected to use in 2001 (Table 2). There was a female sex bias in wombats detected over this timespan (four females, one male).

Discussion

This work is comparable to 347 captures of 76 individuals in a capture–mark–recapture study, and is the first study we are aware of to track the same individuals of a wild population occupying continuous habitat through nearly 20 years by using non-invasively collected samples. The five wombats that we detected again in this study were at least 16–18 years old, and likely older, since they were large enough to leave hair on a tape at the time of the original study. Species of this genus are known to be long-lived: two wild adult female southern hairy-nosed wombats in the Murraylands were recaptured 18 years after first capture (D. Taggart, pers. comm.), a captive female southern hairy-nosed wombat lived to be 33 years old (B. Cleaver, pers. comm.), and three wild northern hairy-nosed wombats (one male and two females) were determined to be at least 28 years old (Taylor 2012). Warren fidelity, which was documented previously (Gaughwin 1981; Walker *et al.* 2006), was particularly striking in that four of the five wombats were found in a warren they had used in 1999–2001. One of the long-lived wombats (female W30) was using the same burrow in the same warren as she used in 2001; in general, it was not possible to number the burrows to match the earlier study, but in some cases the burrows were recognisable.

The number of wombats detected and number of hit tapes was similar to those detected in September 2001, when the greatest number of wombats was detected in our earlier study. The numbers in April 2017, particularly given it was the non-breeding season, suggest that wombat population size in this area is similar to that in 2001, despite periods of drought in 2002 and 2008 (Fig. 1). There was higher than average rainfall in 2010 and 2011. Southern hairy-nosed wombat reproduction is linked to the plant growth index between July and September, which is correlated with rainfall (Gaughwin *et al.* 1998). Oogenesis and spermatogenesis are reduced or terminated when rainfall is low during these months (Gaughwin 1981). The sex ratio in this study was not significantly biased toward females, unlike in our previous work (Walker *et al.* 2006). Interestingly, there was a female sex bias in 'old' wombats: four of the five were female, and five of the six other long-lived hairy-nosed wombats (mentioned above) were also female. This may be related to greater mortality in male mammals, especially polygynous mammals (Clutton-Brock and Isvaran 2007).

We successfully migrated our early wombat data to what is now a traditional sequencing platform, and were able to do so cheaply (approximately US$14 500 for the 663 samples in this

Table 1. Number of wombats detected in the main study warrens in 2017 and 2001, and number of wombats per active burrow in 2017
Data were standardised to include only the first four nights of sampling. Number of males and females are in parentheses. Warrens that were not present before 2017 are indicated with 'n.a.'. April 2001 data followed three months of below-average rainfall; lower numbers of wombats (and lower space use) during this sampling period may be due to wombats being more sedentary after short-term drought (Walker *et al.* 2006)

Warren	No. of wombats			Taped and active burrows in 2017		
	Apr. 2017	Sep. 2001	Apr. 2001	No. of burrows taped[A]	No. of active burrows[B]	No. of wombats per active burrow
A	9 (6M, 3F)	9 (5M, 4F)	8 (3M, 5F)	23	16	0.56
B	1 (1M, 0F)	1 (0M, 1F)	0	9	1	1.00
G	10 (3M, 7F)	6 (3M, 3F)	3 (1M, 2F)	20	16	0.63
H	4 (0M, 4F)	5 (1M, 4F)	0	14	6	0.67
I	4 (3M, 1F)	7 (3M, 4F)	4 (1M, 3F)	28	13	0.31
J	2 (0M, 2F)	8 (5M, 3F)	1 (1M, 0F)	11	2	1.00
Q	2 (1M, 1F)	5 (2M, 2F)	2 (0M, 2F)	9	6	0.33
Gam	8 (5M, 3F)	8 (3M, 5F)	6 (3M, 3F)	27	14	0.57
Psi	4 (1M, 3F)	5 (2M, 3F)	5 (1M, 4F)	17	7	0.57
Z	7 (3M, 4F)	9 (1M, 8F)	4 (2M, 2F)	23	17	0.41
n	4 (2M, 2F)	8 (2M, 6F)	0	8	6	0.67
Eps	5 (4M, 1F)	3 (1M, 2F)	2 (1M, 1F)	10	8	0.63
Y	2 (1M, 1F)	4 (1M, 3F)	1 (0M, 1F)	8	4	0.50
NN	4 (2M, 2F)	6 (3M, 3F)	3 (0M, 3F)	10	6	0.67
XX	1 (1M, 0F)	4 (1M, 3F)	2 (0M, 2F)	7	1	1.00
YY	5 (4M, 1F)	6 (2M, 4F)	3 (1M, 2F)	4	4	1.00
Pi	1 (1M, 0F)	0	0	2	2	0.50
Rho	2 (1M, 1F)	0	0	18	7	0.29
Om	0	3 (0M, 3F)	1 (0M, 1F)	15	0	–
W	0	0	0	4	0	–
Phi	0	0	1 (0M, 1F)	1	0	–
F	0	0	0	6	0	–
Suz	2 (1M, 1F)	n.a.	n.a.	11	6	0.33
Col	3 (3M, 0F)	n.a.	n.a.	3	3	1.00
PR	0	n.a.	n.a.	9	0	–

[A]All burrows were taped except obviously unused ones (spider webs, collapse).
[B]Determined by wombat hair on tapes.

Table 2. Sex and warren use of five wombats that were detected 16–18 years after the initial study
The wombats (6.6% of wombats detected in 2017) used the same warrens or sets of warrens that they did 16–18 years previously. The one exception (W2) was found in a warren that is 87 m from the warrens used in the previous study

Wombat ID			Warren use		No. of detections		
2017	1999–2001	Sex	2017	1999–2001	2017	1999–2001	No. of sampling periods[A] (1999–2001)
W2	86	Female	J	G, I, H, VV-P	4	16	1
W9	79	Female	H, I	A, B, G, H, I, J, K, DD-P, EE-P	6	19	1
W30	87	Female	Y	Omega, Y	2	6	2
W74	38	Male	A	A, B, Q	1	20	5
W90	36	Female	Psi	Gamma, Psi, Z	1	19	4

[A]Number of sampling periods (of five) in which wombat was detected (Walker 2004).

study, or US$22 per sample). We did not move our wombat data into the genomics era by high-throughput sequencing of microsatellite loci (De Barba *et al.* 2017) or SNP analysis (Kraus *et al.* 2015) due to the time and costs involved, but this would be a valuable future direction for hairy-nosed wombat research. For future hair censuses that use capillary electrophoresis, it is now possible to apply only the subset of loci from White *et al.* (2014), since they are discriminatory to the individual level even for our 'old' wombats. In order to keep hair censuses inexpensive, we recommend using the universal tail PCR labelling system of U'Ren *et al.* (2007) (the length of which should be subtracted from allele sizes in order to match with earlier work) and running singleplexes instead of multiplexing using fluorescently labelled primers. This is because we found that multiplexing

had a high failure rate and required multiple PCR amplifications, and hence did not save time and was more costly overall. Our next step in analysis of these data is to examine parentage, pairwise relatedness, and relatedness of burrow sharers.

Conclusion

Genetic monitoring to estimate population demographics and track genetic diversity will likely become increasingly more important as wildlife populations decline due to anthropogenic changes. Tying in legacy microsatellite datasets has tremendous value for a long-term perspective of these parameters, as well as reidentifying individuals of long-lived species. Here, we show that it is possible to migrate between the earliest sequencing platform (involving radioisotopes) and a commonly used platform (capillary electrophoresis) in order to identify wombats that were at least 18 years old. The warren fidelity of these wombats over this time highlights the lack of vagility and hence vulnerability of this iconic species to a warming climate (Marshall *et al.* 2018). There is potential value in monitoring populations regularly to better estimate life-history parameters and to detect any effects of change in climate or disease. We recommend that studies using microsatellite markers bank DNA from each individual as well as high-quality DNA from a set of positive controls, so that high-throughput sequencing or other yet-to-be-developed methods can be used in the future.

Conflicts of interest

The authors declare no conflicts of interest.

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