

Does soil type drive social organization in southern hairy-nosed wombats?

FAITH M. WALKER, ANDREA C. TAYLOR and PAUL SUNNUCKS

Australian Centre for Biodiversity Analysis, Conservation and Management, School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia

Abstract

Spatiotemporal distributions of key resources are hypothesized to underpin sociobiological patterns. Burrow availability and quality is of paramount importance to fossorial animals. The southern hairy-nosed wombat (*Lasiorhinus latifrons*) burrows in both hard and friable soils. Theoretical and empirical studies suggest that the harder substrate should promote closer geographical clustering of burrows than in softer soils. Clustered burrows are expected to be associated with larger group sizes. If sociality is driven by constraint rather than advantage, patterns of spatial and temporal distribution of animals within and among groups may show indications of avoidance or even antagonism, and 'making the best of a bad job' via positive kin associations to offset the disadvantages of high-density living. To test these ideas, we compared warren relatedness and social structure of *L. latifrons* on friable soils (Nullarbor Plain) and hard calcrete (Brookfield Conservation Park, BCP). Individuals were sampled by noninvasive collection of hairs for genotyping to identify individuals and to estimate their space-use and associative behaviour with respect to relatedness. Burrows in calcrete were indeed more clumped, and warren and group size larger. Differences in spatiotemporal organization and relatedness structure between sites were in the expected direction: (i) Nullarbor males associated and shared warrens less than at BCP; and (ii) Nullarbor spatial relatedness patterning data were not consistent with proposed female breeding dispersal, in contrast to those at BCP. Under Nullarbor (low density) conditions, cooperation or tolerance between males may be less advantageous, and accessing or digging burrows should be less of a constraint for juvenile females.

Keywords: fossorial, marsupial, microsatellite, resource structure, sociobiology

Received 5 May 2006; revision received 26 July 2006; accepted 21 August 2006

Introduction

Studies attributing social evolution to environmental constraints are rare (Lott 1991; Foster & Endler 1999). Species exhibiting social plasticity are most informative regarding the evolution of social systems. Within such species, ecological variables can alter strategies employed by interacting conspecifics (Maher 2004; Schradin & Pillay 2005). Genetic relatedness is an important driver of levels of social interactions, and advances in molecular tools in the last decade or so allow kin theory to be integrated with assessment of resource and habitat structures (Travis *et al.*

1995; Faulkes *et al.* 1997; Cornuet *et al.* 1999; Stow & Sunnucks 2004; Randall *et al.* 2005; Waits & Paetkau 2005).

Soil type is a particularly influential ecological variable for fossorial (burrowing) animals because substrate can constrain burrow distribution and promote clustering by influencing the relative energetic costs of digging new burrows and expanding existing ones (Cowan 1987; Lovegrove 1989; Lacey & Sherman 1997; Ebensperger & Cofré 2001). For instance, in a classic example of sociality by constraint, soil type was found to influence rabbit (*Oryctolagus cuniculus*) social structure and population dynamics (Cowan & Garson 1985). In stable soils, burrows were clustered into complex warrens, were a limited resource for females, and were associated with substantial variation in male reproductive success. In contrast, rabbits on sandy soils (in which burrows are easy to dig) tended to live as

Correspondence: Faith M. Walker, 2700 Woodlands Village Blvd., Ste. 300-407, USA. Fax: (928) 773-9201; E-mail: faith.walker@sci.monash.edu.au

over-dispersed pairs in simple burrows and displayed much lower variance in male reproductive success. A similar situation was found in pika (*Ochotona* spp.), where nonburrowing, low-density species occupying talus slopes were asocial, whereas burrowing, high-density ones tended to be social, and exhibited several types of mating systems (Brandt 1989; Smith *et al.* 1990; Smith & Wang 1991). The relationship between environmental constraint and burrow distribution can take a number of forms, depending on the nature of the constraint.

Southern hairy-nosed wombats (*Lasiorhinus latifrons*) dig interconnected burrow systems (warrens) in several different soil types, including shallow calcareous loams, brown calcareous earths, solinized brown soils, brown sands, and red siliceous sands (Aitken 1971, 1973; soil classification system of the Atlas of Australian Resources, Stephens 1963). Warrens can be reliably identified on the basis of surface aggregations of burrow entrances; the distance among clustered entrances is much less than the distance between warrens (Steele & Temple-Smith 1998; Shimmin *et al.* 2002). As with some small mammalian species, soil properties can impact features such as burrow complexity, length and depth (Laundré & Reynolds 1993; Steele & Temple-Smith 1998). The least supportive substrate is sandy soil, which characterizes northern hairy-nosed wombat (*Lasiorhinus krefftii*) habitat, and on which burrows are commonly constructed near trees, for reinforcement (Gordon *et al.* 1985). Much of southern hairy-nosed wombat habitat contains calcrete (also called caliche or hardpan) — an accumulation of sand and gravel that is cemented by calcium carbonate into a hard mass. Calcrete is the most supportive soil for hairy-nosed wombat burrows. Often occurring in semi-arid areas, this subsurface material ranges from a very friable conglomeration to a massive, rock-like layer. Variations in the calcrete layer were found to influence warren distribution in the Murraylands, where Brookfield Conservation Park (BCP) is located (St. John 1998). Wombats appear unable to construct warrens where an unbroken layer lies close to the surface, but on the other hand, they also tend to be absent from or rarely use areas where calcrete is well below the surface and the soil above is not well developed. The optimal situation appears to be where deep soil is accessible through natural breaks in sheet-like calcrete. Under these conditions, the layer forms a ceiling of such durability that burrows are used for generations.

We wished to determine whether natural variation in wombat habitat structure (soil type and consequent burrow distributions) generated intraspecific variation in spatiotemporal social organization, with the potential to impact population processes and behaviours. Noninvasive genetic sampling of this species at BCP revealed male philopatry, spatiotemporal association between male kin, and female dispersal, putatively including females after

breeding (Walker, Taylor, and Sunnucks, unpublished data). Given evidence of low levels of interaction between individuals (Gaughwin 1981; Walker, Taylor, and Sunnucks, unpublished data), we postulated that southern hairy-nosed wombats have sociality only by constraint in habitat structure such as found at BCP. If wombat sociality is largely driven by constraint rather than advantage in clumped burrows and high-density living, under more permissive conditions for burrow construction we expect to see: (i) greater dispersion of individuals, (ii) less spatio-temporal association among closely related males, (iii) more of a response in female than male spatial genetic structure, and (iv) no evidence that is consistent with female breeding dispersal. Specific predictions under the hypotheses that soil drives burrow distribution and burrow distribution drives sociobiology are fully outlined in Table 1. To test these predictions, we sampled a large, continuous population in less calcareous soil and compared it to a temporally matched subset of BCP data. DNA extracts from noninvasively-collected hair samples were screened for 15 microsatellite loci. Thus this study presents a rare test of the effects of soil substrate on sociogenetic structure in a mammal, and the first for a wombat species — the largest burrowing mammals.

Methods

Study site

While intraregional variation in soil type and warren structure would provide an ideal comparison because it would enable exclusion of the confounding effects of climate and regional history, there is only minimal variation in soil type in the wombat-inhabited areas in the Murraylands, which may not be sufficiently extreme to lead to variation in sociobiology (Walker 2004). For this reason, an appropriate study site was identified in another South Australian region (Fig. 1), one in which departures from Murraylands warren structure have been attributed to soil type differences (Steele & Temple-Smith 1998). The selected site occurs in a medium warren density area inside Nullarbor National Park (GPS coordinates — WG584 54 604162 E 6518270N) and occupies the habitat type that contains the Nullarbor's largest number of wombats (Aitken 1973).

Soil characteristics and calcrete depth assessment

The Nullarbor Plain was a sea floor from the Lower Cretaceous until the limestone-rich plateau was uplifted in the Late Miocene (Lowry & Jennings 1981). This remote area is one of the largest karst landforms in the world, containing extensive networks of caves and other karst features. Little soil development has occurred owing to the

Table 1 Hypotheses and predictions of the effects of soil on southern hairy-nosed wombat burrow distribution and sociobiology

Expectations for Nullarbor (cf. BCP)	Analyses	Outcomes	Interpretations
Under hypothesis that soil drives burrows:			
Burrows more dispersed	Contingency χ^2	$P = 0.001$	Not constrained to expand existing warrens, as at BCP
Warrens smaller			
a. Number of burrows	Permutation	a. $P = 0.005$	Not constrained to expand existing warrens, as at BCP
b. Area		b. $P = 0.07$	
Warrens more dispersed	χ^2 goodness-of-fit	$P = 0.001$	Consistent with calcareous sheet driving warren distribution at BCP
Under hypothesis that burrow distribution drives sociobiology by constraint:			
Wombat distribution			
Number of wombats per warren smaller	Permutation	$P = 0.006$	Not constrained to live in groups, as at BCP*
Wombat density lower	NA	Supported	Not constrained to live in groups, as at BCP*
Wombat relationships			
Male			
a. Fine-scale spatial genetic structure not a consequence of <i>warren-sharing</i> between relatives	a. Spatial autocorrelation b. Permutation	a. Supported b. Supported	a. Male relatives not constrained to live in groups (hence reduced tolerance or cooperation) b. Appears to be no advantage to male relatives associating
b. Positive association between male relatives reduced			
Female			
a. Female dispersal over fine scale not as strong as at BCP	a. Spatial autocorrelation b. Spatial autocorrelation	a. Greater spatial structure within 100 m b. Supported	Burrow availability not as constrained at Nullarbor, so less local female dispersal
b. Greater difference than males to BCP spatiogenetic patterns, since female response to burrow distribution should exceed that of males			

*Result may also be driven by other factors.

homogenous parent material and to aridity (McKenzie & Robinson 1987).

The landscape is characterized by low parallel ridges bearing silt with nodular calcrete and limestone 30–40 cm below the surface (Beard 1975). These ridges are separated by depressions bearing relatively heavy soils (fine silky clay, with limestone-derived soils > 90 cm below the surface (McKenzie & Robinson 1987) in which wombats tend to construct warrens. Soils collected from burrow entrances at the study site were classified as red (2.5 YR 5\6) or light red (2.5 YR 6\6 and 2.5 YR 7\8) (Munsell soil colour chart).

The Murraylands, wherein BCP is located, lies between the Truro Hills and the Murray River. Towards the river, where most wombats reside, loam and clays overlay subsurface, sheet-like calcrete (Potter *et al.* 1973). Thus, the BCP site is likely more favourable for long-term burrow stability: the calcrete provides stability to support well-developed burrow systems, where it is penetrable.

To assess whether large-scale geological differences between study regions translate into calcrete depth differences where wombats are located, a steel rod was pounded up to 50 cm into the ground in the centre, 5 m north, and 30 m north of warrens at both sites. If sheet calcrete or calcareous rubble was encountered before 50 cm, another measurement was taken 1 m west from the first. The difference between Nullarbor and BCP in mean depth to calcrete was tested, for central, 5 m and 30 m samples, by permutation (RESAMPLING STATS 5.0.2; www.resample.com).

Hair sampling

Wombats occupying warrens within a 1.8 × 0.3 km area at the Nullarbor site, a similar sampling area to that at BCP (1.8 × 0.5 km), were sampled in October (spring) 2000 using a hair-trapping methodology described previously (Walker *et al.* 2006). A total of 249 burrows (i.e. every burrow of nondilapidated warrens) were taped for 6 days,

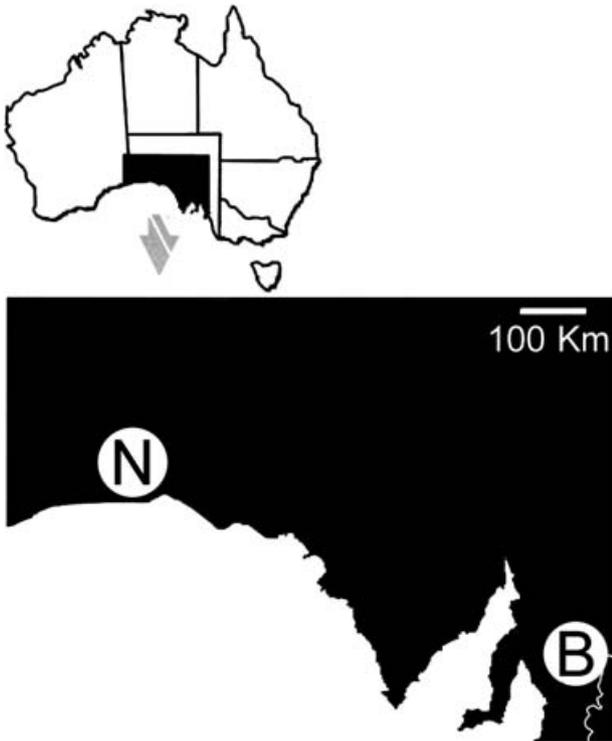


Fig. 1 Southern hairy-nosed wombats from South Australia's Nullarbor (N) and Brookfield Conservation Park (B) study sites were noninvasively sampled in October 2000.

providing 1524 opportunities for hair donation, yielding 151 'hit' tapes. Of these, 92% contained follicles suitable for DNA extraction. In comparison, at BCP in October 2000, 197 burrows were taped over the same number of days, procuring 229 hair-tapes, 93% of which yielded successful DNA extracts.

Burrow and warren density, warren size

Density of burrows was assessed by counting all burrows in each 100×100 m cell across sampling areas, and then sorting cells into categories based on burrow number as follows: none (0), low (1–5), medium (6–10), high (11–15), and very high (≥ 15). Contingency χ^2 tests were employed to determine if differences existed between Nullarbor and BCP sites. Additionally, warren density was examined to determine whether or not the warrens themselves were more clustered than expected. This was achieved by dividing the study sites into 400×400 m cells. Warrens were then counted and tested for departure from the expected (even) distribution (χ^2 goodness-of-fit test) at each site.

Measurement of maximum distances between burrows within warrens at both study sites was performed using a measuring wheel, to enable area calculations. Area and number of burrow entrances were compared using log-transformed data.

Analyses

To identify individuals, DNA extracts with 100% allelic sharing over five (BCP) and nine (Nullarbor) polymorphic microsatellite loci and a sexing marker (Walker *et al.* 2006) were considered to belong to the same wombat. Single DNA extracts from each wombat were then amplified for a total of 15 loci (Walker *et al.* 2006). Hair samples could be attributed to individual wombats with a high degree of certainty; less than one in a million randomly selected pairs could be expected to share a multilocus genotype ($P_{ID} = 4 \times 10^{-7}$; Waits *et al.* 2001). As described previously (Walker *et al.* 2006), rigorous procedures were followed to ensure that unique genotypes were not falsely declared. All unique genotypes differed by at least two alleles (at different loci), which was confirmed by re-amplification. Statistical analyses of genetic data proceeded as follows:

Sampling efficiency. To assess the effectiveness of the sampling regime in censusing wombats, a capture–mark–recapture (CMR) model was used to estimate population size (program CAPTURE; Rexstad & Burnham 1991). We used a model ($M_{h-jackknife}$) that accommodates heterogeneity in capture probability (Burnham & Overton 1978), and assumed a closed population because sampling took place over a short time period, and burrow-use data revealed high warren fidelity.

Space-use. Geographical coordinates were taken at each warren, enabling space-use examination via the program RANGES v (Kenward & Hodder 1996). This application calculates area, rangespan, and interfix distances for individuals. Fixes were determined by successive detection of matching DNA profiles in hair traps. We defined 'warren-use area' as the area circumscribed by warrens used (requiring detection in three or more warrens), 'rangespan' as the maximum distance between detections, and 'interfix distance' as the shortest path between successive detections (used as a measure of the minimum distance a wombat moved during the sampling period). Minimum convex polygons were selected for warren-use area estimation because they perform better than other methods when the number of detections is small (Harris *et al.* 1990).

Spatial genetic structure. Spatial autocorrelation methods utilizing permutation procedures were applied to examine spatial genetic structure (program GENALEX version 5.1; Smouse & Peakall 1999; Peakall & Smouse 2001; Peakall *et al.* 2003). The arithmetic mean of the X and Y GPS coordinates of detections was used to assign each individual a single point in space. Genotypic data were used to calculate genetic distances between wombat pairs of each sex class, and genotype pairs were shuffled 1000 times

among distance bins in order to determine statistical significance. The spatial distance classes used in general compare among rather than within warren distances.

Sex-biased dispersal. A sex-bias in dispersal was assessed via assignment tests (program GENECLASS, Cornuet *et al.* 1999), a procedure that uses population allele frequencies to determine the probability of each individual's genotype originating in the sampled population. We employed the Bayesian approach of Rannala & Mountain (1997), with the 'leave-one-out' option. A population of 10 000 genotypes was simulated using observed allele frequencies, to which individual likelihoods were compared. A sex-bias in assignment *P*-values was tested by randomization (10 000 permutations via RESAMPLING STATS 5.0.2).

Parentage determination. The most probable mother and/or father of each wombat were identified using the program CERVUS 2.0 (Marshall *et al.* 1998). The mean probability of excluding a randomly selected unrelated individual from parentage was > 0.999. Parameters included 50% of candidate parents sampled, 95% of loci typed, and an error rate of 0.3% (quantified for the present protocol in Sloane *et al.* 2000). Parentage was assigned when LOD > 3.0, delta > 95%, and a maximum of one allele mismatched (by no more than 2 base pairs).

Pairwise genetic relatedness. Multi-locus genotypes were used to estimate relatedness between wombat pairs (program KINSHIP 1.2, Goodnight *et al.* 1998). Relatedness of wombats that shared burrows and warrens on the same nights and on different nights was tested by permutation (RESAMPLING STATS 5.0.2).

Association between warren members. Wombat pairs were considered to be associated if they were detected to share the same warren during the same night. Strength of association was reflected by the simple ratio index, with values ranging from 0 (wombat pairs never detected together) to 1 (wombat pairs always detected together (Cairns & Schwager 1987), and significance was tested by permutation (program SOCPROG, Whitehead 1999).

In this study, where the statistical test used is not explicitly specified, permutation testing was performed with RESAMPLING STATS 5.0.2. Where appropriate, values are presented as mean \pm SD.

Results

Calcrete depth

The mean depth to calcrete was significantly greater at the Nullarbor ($n = 44$) than at the BCP ($n = 21$) site, at both 5 and 30 m from the warrens ($P = 0.0001$ for both; at 5 m:

BCP mean 20.4 ± 12.2 cm, Nullarbor 41.5 ± 11.3 ; at 30 m: BCP mean 15.2 ± 10.2 , Nullarbor 31.2 ± 13.6 cm; note: Nullarbor values are likely underestimates because the depth was only measured to 50 cm). No significant difference in the depth to calcrete was detected at the centre of warrens, likely because digging activity at both sites has resulted in accumulation of a pile of soft soil around burrow entrances ($P = 0.51$). Further evidence that the Nullarbor site lacked a hard, sheet-like layer close to the surface was obtained from a soil profile observable in a cutting, which revealed calcareous rubble but no sheet calcrete for at least 2 m.

Burrow and warren distribution and size

The spatial distribution of burrows over the Nullarbor and BCP sampling areas differed significantly ($P < 0.001$; Fig. 2). At BCP, there were more areas with no burrows and more with very high density. This difference would be expected if soil type represented a constraint to burrowing at BCP but not at Nullarbor, and if suitable digging sites were heterogeneously distributed. Warrens were also significantly clustered at BCP (goodness-of-fit test, $\chi^2 = 33.7$, d.f. = 9, $P = 0.001$), but not at the Nullarbor site ($\chi^2 = 6.86$, d.f. = 9, $0.05 < P < 0.1$).

Nullarbor warrens contained significantly fewer burrows than at BCP. This was the case when either the mean number of burrows (Nullarbor: 5.7; BCP: 14.0) or the mean number of only active burrows (Nullarbor: 2.8; BCP: 5.4) were compared ($P = 0.005$ and 0.01 , respectively). Active warrens at Nullarbor occupied smaller areas than those at BCP (Nullarbor: mean 94.6 ± 92.6 m²; BCP: mean 182.0 ± 230.4 m²; $P = 0.05$).

Demographics and space use

Twenty-seven wombats (10 males, 17 females) were detected in the Nullarbor sampling area over the 6-day

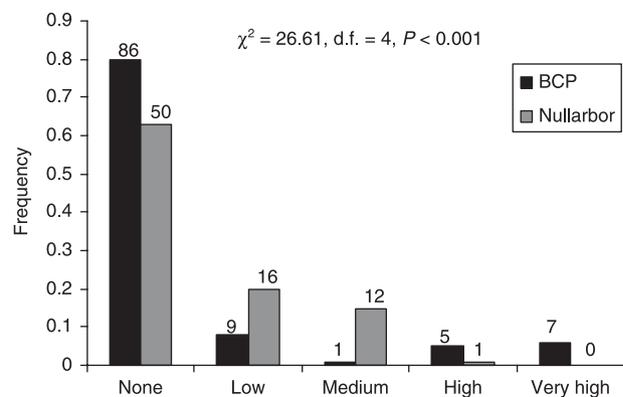


Fig. 2 Southern hairy-nosed wombat burrow density at Nullarbor and BCP in spring 2000: prevalence of areas with varying burrow number. Sample sizes are above each bar.

sampling period in spring 2000, whereas at BCP during this time a total of 48 wombats (18 male, 30 female) were detected. The number of new Nullarbor individuals detected declined to zero by the sixth sampling day, suggesting that most if not all wombats using the study area were detected. This interpretation was supported by a CMR population size estimate of 28 (s.e. 1.6) wombats. High detection of individuals also occurred at BCP: observed numbers did not differ significantly from asymptotic detection-model estimates (Walker *et al.* 2006). Nullarbor population density was estimated at 49.2 wombats/km², approximately half that at BCP. Of 702 tests for parentage at Nullarbor, nine wombat pairs were accepted as parent-offspring (1 MM, 3 FF, 5 MF), and one probable mother-father-offspring triad was identified. At BCP 18 wombat pairs were accepted as parent-offspring (6 MM, 3 FF, 9 MF), and 3 triads were detected. At both sites, within these pairs and trios, members occupied the same or neighbouring warrens.

As at BCP, there was no significant sex difference in the mean number of warrens used by Nullarbor wombats [Nullarbor mean (median): male 2.4 ± 1.6 (2), female 2.5 ± 2.1 (2), $P = 0.52$; BCP mean (median): male 1.47 ± 0.62 (1), female 1.54 ± 0.68 (1), $P = 0.54$], although Nullarbor wombats used more warrens than those at BCP (males $P = 0.01$; females $P = 0.008$). However, Nullarbor warrens were used by fewer than half as many wombats as at BCP (significant difference in the cumulative number of wombats detected in active warrens over 6 days – Nullarbor

mean 2.31, median 2; BCP mean 4.07, median 4; $P = 0.006$). While these results may be due in part to fewer opportunities for detection at the Nullarbor site (fewer burrows, thus hair-tapes, per warren), the lower incidence of multiple individuals detected on tapes (2%, compared with 10% at BCP for similar search effort) suggests that this was not the case. It is more likely that the difference in number of animals using warrens is simply a result of lower wombat density at the Nullarbor site. At Nullarbor, cumulative distance between detections of the same wombat ('interfix distance'), area encompassed by warrens utilized ('warren-use area'), and maximum distance between used warrens ('rangespan') did not differ between the sexes (log-transformed data; $P = 0.46, 0.30, \text{ and } 0.58$, respectively), and were similar to those at BCP (Walker *et al.* 2006).

Spatial genetic structure and sex-biased dispersal

At Nullarbor, both male and female wombat pairs exhibited significantly positive spatial genetic structure at low distances (0–100 m) – i.e. pairs living closer together were closer relatives – and significantly (male) or nearly significantly (female) negative spatial genetic structure at distances $> \sim 1700$ m (Fig. 3). The sexes did not differ significantly in this pattern (as signified by overlapping bootstrap error bars about the autocorrelation coefficient, Peakall *et al.* 2003). High genetic similarity among Nullarbor male pairs separated by less than 100 m was driven by four male pairs. Members of each pair occupied

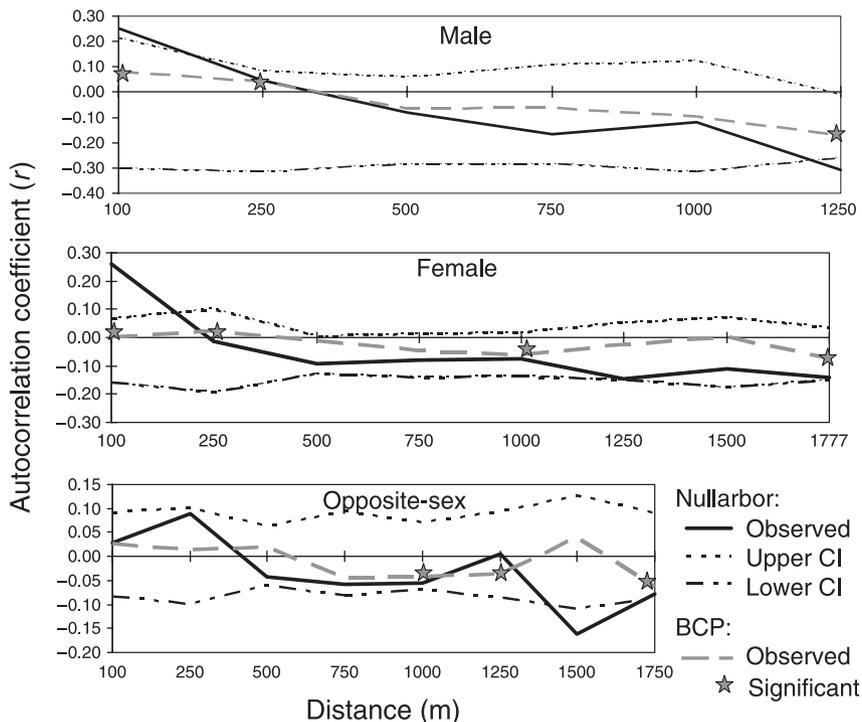


Fig. 3 Spatial genetic structure for each wombat pairwise sex-class at Nullarbor and BCP study sites in spring 2000. For Nullarbor, the observed relationship is significant where it lies outside the dotted lines denoting the 97.5% and 2.5% confidence intervals for each distance class. For BCP, significance under the same criteria is indicated by stars.

neighbouring warrens; three of the four pairs were feasibly brothers (one allele shared at each locus, $R = 0.50\text{--}0.57$). To determine whether the result among proximate Nullarbor females was caused by young-at-heel, the three mother-daughter pairs were removed from the analysis, with the effect that the autocorrelation coefficient declined but was still significantly greater than expected from random. This indicates that among females, relatives other than parent-offspring contribute to the greater-than-expected concentration of high relatedness at short distances. In contrast to the other sex-classes, opposite-sex wombat pairs did not exhibit positive spatial genetic structure at short distances (i.e. wombats tended to not live very near close relatives of the opposite sex). They did, however, at longer ones (1500 m).

Male pairs at BCP and Nullarbor exhibited a similar geographical pattern of genetic relatedness (Fig. 3), apart from significantly positive spatial genetic structure extending further (to 250 m) at BCP. Nullarbor female wombats, however, followed the male pattern (near neighbours significantly highly related, distant pairs less related), unlike at BCP, where spatially distant (100–250 m) female pairs were more closely related than local ones. This pattern among BCP females was also found in a larger-scale study (Walker, Taylor, and Sunnucks, unpublished data). The BCP result of significantly positive genetic structure among proximate opposite-sex pairs, caused by parent-offspring, was not found at Nullarbor.

Male Nullarbor wombats tended to be more likely to have been born locally than were females, as indicated by marginally nonsignificantly higher assignment P -values ($P = 0.06$). This result was also found at BCP ($P = 0.005$). Further, a lower percentage of Nullarbor males (10%) than females (18%) were not assigned to the sampling site area. This suggests that male wombats may immigrate less frequently into the study area than do females. Both of these results indicate that, like at BCP (Walker, Taylor, and Sunnucks, unpublished data), dispersal in the Nullarbor population is female-biased. Additional evidence for female-biased dispersal and comparative male philopatry was seen in pairwise relatedness within the sexes. Male wombats within the study sites were significantly more related to one another than were females (Nullarbor $P = 0.05$; BCP $P < 0.0001$), as would be expected with female-biased dispersal.

Relatedness of putatively interacting wombat pairs

At Nullarbor, each sex exhibited little burrow- or warren-sharing. Few male wombat pairs were detected to share warrens within or among nights ($n = 2$ for each), and this translated into no instances of significant association. For female pairs, same-night warren sharers ($n = 5$, mean $R = 0.15 \pm 0.31$) were similarly related to different-night

sharers ($n = 11$, mean $R = 0.26 \pm 0.27$) ($P = 0.29$), burrow sharers were similarly related to pairs that shared warrens but not burrows ($n = 11$ for both, mean $R = 0.17$ and 0.36 , respectively, $P = 0.14$), and the 5 pairs that associated positively (i.e. association value > 0) showed no apparent relationship between relatedness and association ($P > 0.2$). Two female pairs associated significantly; neither pair was mother-daughter, although one pair was highly related. These results are in contrast to those at BCP, where male pairs commonly shared warrens (only two males did not share a warren with other males) and associated according to their level of relatedness (significantly associating pairs were significantly more related than nearby potential associates; $P = 0.05$), and females associated with less-related females ($P > 0.0001$).

Interestingly, several marginally nonsignificant results in situations that might potentially reflect amorous interactions for opposite-sex Nullarbor wombat pairs were in the direction expected under outbreeding. First, heterosexual pairs that shared warrens on the same night were less related than wombats of this sex-class that shared warrens on different nights ($n = 11$ and 18 , mean $R = 0.02 \pm 0.23$ and 0.13 ± 0.25 , respectively, $P = 0.08$). This result became significant ($P = 0.03$) when the analysis allowed repeated occurrences of particular pairs. Second, burrow sharers were less related than pairs that shared warrens but not burrows ($n = 14$ for both, mean $R = 0.04$ and 0.10 , respectively, $P = 0.10$). Third, significantly associating pairs were less related than were pairs that shared warrens but did not associate significantly ($n = 5$, mean $R = -0.04$ and 0.11 , respectively, $P = 0.06$). Fourth, for pairs that associated at all, mean pairwise association was higher for less-related heterosexual pairs. While formally nonsignificant at the 5% level, this suite of four consistent results is sufficiently interesting to report — if sharing a burrow reflects a higher likelihood of a pair being mates.

Discussion

The soil and warren structure differences between the Nullarbor and BCP sites are consistent with the hypothesis that spatial patchiness and limitation of suitable digging sites causes an inflation of warren and group size (here, at BCP), with concomitant impacts on social organization and relatedness structure (Table 1). The fact that there were no shifts in warren locations or new warrens formed in the BCP study area over an 11-year period (F. Walker, unpublished data) also points to a digging constraint in less friable calcrete.

The difference in spatiotemporal association and relatedness structure between wombats at the Nullarbor and BCP sites is consistent with the hypothesis that burrow distribution drives sociobiology by constraint (Table 1), much as proposed for European wild rabbits (Cowan &

Garson 1985). The *a priori* expectations are supported that relaxation of the constraint — in this case deeper and more friable calcrete at Nullarbor — would reduce the need for aggregation with nonrelatives, and render less necessary any kin relationships whose primary driver is amelioration of the inevitable negative impacts of group living, such as competition for resources, and spread of disease. Unlike those at BCP (Walker, Taylor, and Sunnucks, unpublished data), male Nullarbor wombats did not share burrows, rarely shared warrens, and did not associate with their close male relatives. Sociality between male southern hairy-nosed wombats may thus be driven by the constraint of digging sites being in short supply.

Female wombats also showed different patterns of kin-based spatiotemporal association at Nullarbor than at BCP. At BCP, females associated with less-related females in their immediate neighbourhoods, and were more related to individuals at around the spatial scale of adjacent warrens. Along with other data, the pattern at BCP is consistent with the hypothesis of female breeding dispersal, first proposed by Johnson & Crossman (1991) with the suggestion that adult female *Lasiorhinus krefftii* bequeath their burrows to offspring who would find them much more taxing to construct. While the present results are not conclusive, it is intriguing that similar patterns were not present at Nullarbor, where substrate type and relative spatial homogeneity of burrow entrances indicate that they are easier to construct. Under these conditions, female breeding dispersal and burrow bequeathal would be less necessary, and the data are not consistent with it, unlike at BCP. The present results could also be due to adult females dispersing farther than at BCP, or to a smaller sample size precluding its detection. Further research should determine if either of these alternatives is occurring.

Soil-driven, disparate distributions of burrows occur in other mammalian species and have been found to influence social and mating systems (rabbits: Cowan & Garson 1985; pikas: Brandt 1989; Smith *et al.* 1990). Although there have been few studies of the effects of soil, social flexibility in the face of other types of resource variations can be instructive. For instance, in Gunnison's prairie dog (*Cynomys gunnisoni*), a highly patchy food resource distribution and/or high population density led to increased group size, group defence of resources by females, and polygyny (Travis & Slobodchikoff 1993; Travis *et al.* 1995). Conversely, uniformly distributed resources at moderate to low population density precluded group formation due to competition, and favoured monogamy. The small warren and group sizes at the Nullarbor site relative to those at BCP may translate into a variation in mating system and/or strategies of the sexes. For instance, one can envisage a similar situation to that observed in prairie dogs, except that males rather than females cooperate in resource (warren) defence.

It is unclear whether the disparity in wombat density between the two sites was also caused by soil type. This made it difficult to distinguish between soil or population density differences as causal factors in some instances. Thus, these results should be regarded as a first test of the hypotheses proposed here; the observed differences in ecology and associated demography provide a framework for generating testable hypotheses for future research. Additional populations in continuous habitat containing friable calcrete should be sampled, although it may prove challenging to locate ones that have been relatively undisturbed, and there appears to be no higher density populations in Nullarbor Plain National Park than the one investigated here (Walker 2004). It may be worthwhile to resample the Nullarbor site in the future so as to monitor increases or declines in population size and track previously genotyped individuals through time.

While sheet-like calcrete appears to be an environmental constraint promoting aggregation in southern hairy-nosed wombats, aggregation may be promoted by additional factors, as is usually the case (Caro & Bateson 1986; Lott 1991). It is also possible that warren complexity is a response to a harsh environment, providing a variety of microclimates for residents (Boggs *et al.* 1984; Shimmin *et al.* 2002), and that physiological advantages associated with complex warrens can be maintained even with some increase in group size. Alternatively, these advantages may encourage group-living if warren complexity increases with group size in a nonlinear fashion. Certainly, warrens consist of multiple burrow entrances even where calcrete is friable or not present (this species: Walker 2004; *L. krefftii*: Johnson 1991), and southern hairy-nosed wombats prefer warrens with more burrow entrances (Walker *et al.* 2006), both suggesting that there is an inherent advantage to multiple entrances, that it is energetically less expensive to add on to existing warrens than to dig fresh burrows, and/or that there is some advantage to living with more wombats (assuming that entrance and wombat number are positively correlated). The role of predator pressure in the evolution of group living in this species is unclear; currently, dingoes (*Canis familiaris dingo*) and humans are the only predators.

The present study examined how natural variations in resource structure were associated with wombat spatiotemporal association and relatedness structure. Despite substrate and population density differences between BCP and Nullarbor, several features are in common and thus may be considered properties of southern hairy-nosed wombat natural history in natural habitat: female-biased dispersal, female demographic bias, absence of a sex difference in warren and space-use, warren-sharing by parent-offspring and previously mated pairs, and plausibly inbreeding avoidance (Walker *et al.* 2006; Walker, Taylor, and Sunnucks, unpublished data).

Acknowledgements

Funding for this project was provided by The National Geographic Society and the Mark Mitchell Fund, while Monash University awarded a Logan Research Fellowship to A.C.T., and Monash Graduate, International Postgraduate, and Doctoral Completion Scholarships to F.M.W. We thank the Department of Environment and Heritage for permission to hair sample wombats, the National Parks and Wildlife Service SA at Ceduna for facilitating work on the Nullarbor, B. Hansen for assistance with fieldwork, and two anonymous reviewers for suggested improvements to the manuscript.

References

- Aitken PF (1971) The distribution of the hairy-nosed wombat [*Lasiorhinus latifrons* (Owen)] Part I: Yorke Peninsula, Eyre Peninsula, the Gawler Ranges and Lake Harris. *South Australian Naturalist*, **45**, 93–103.
- Aitken PF (1973) Report on hairy-nosed wombats in South Australia from Ceduna to the west of the State. Unpublished report prepared for the Department of the Post Master General, Adelaide.
- Beard JS (1975) *The Vegetation of the Nullarbor Area. Vegetation Survey of Western Australia, 1 : 1 000 000 Vegetation Series, Explanatory Notes to Sheet 4*. Nullarbor University of Western Australia Press, Perth.
- Boggs DF, Kilgore DL, Birchard GF (1984) Respiratory physiology of burrowing mammals and birds. *Comparative Biochemistry and Physiology*, **77A**, 1–7.
- Brandt CA (1989) Mate choice and reproductive success of pikas. *Animal Behaviour*, **37**, 118–132.
- Burnham KP, Overton WS (1978) Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika*, **65**, 625–633.
- Cairns SJ, Schwager SJ (1987) A comparison of association indices. *Animal Behaviour*, **35**, 1454–1469.
- Caro TM, Bateson P (1986) Organization and ontogeny of alternative tactics. *Animal Behaviour*, **34**, 1483–1499.
- Cornuet JM, Piry S, Luitart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
- Cowan DP (1987) Group living in the European rabbit (*Oryctolagus cuniculus*): mutual benefit or resource localization? *Journal of Animal Ecology*, **56**, 779–795.
- Cowan DP, Garson PJ (1985) Variations in the social structure of rabbit populations: causes and demographic consequences. In: *Behavioural Ecology: Ecological Consequences of Adaptive Behaviour. The 25th Symposium of the British Ecological Society* (eds Smith RM, Sibly RH), pp. 537–555. Blackwell Scientific Publications, Oxford, Reading.
- Ebensperger LA, Cofré H (2001) On the evolution of group-living in the new world cursorial hystricognath rodents. *Behavioral Ecology*, **12**, 227–236.
- Faulkes CG, Bennett NC, Bruford MW *et al.* (1997) Ecological constraints drive social evolution in the African mole-rats. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 1619–1627.
- Foster SA, Endler JA (1999) Geographic variation in behavior: perspectives on evolutionary mechanisms. Oxford University Press, New York.
- Gaughwin MD (1981) *Socio-ecology of the hairy-nosed wombat (Lasiorhinus latifrons) in the Blanche Town region of South Australia*. PhD Dissertation, University of Adelaide, Adelaide, South Australia.
- Goodnight KF, Queller DC, Poznansky T (1998) *KINSHIP 1.2. Goodnight Software*. Rice University, Houston, Texas.
- Gordon G, Riney T, Toop J, Lawrie BC, Godwin MD (1985) Observations on the Queensland hairy-nosed wombat *Lasiorhinus krefftii* (Owen). *Biological Conservation*, **33**, 165–195.
- Harris S, Cresswell WJ, Forde PG, Trehwella WJ, Woollard T, Wray S (1990) Home-range analysis using radio-tracking data – a review of problems and techniques particularly as applied to the study of mammals. *Mammal Review*, **20**, 97–123.
- Johnson CN (1991) Utilization of habitat by the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Journal of Zoology, London*, **225**, 495–507.
- Johnson CN, Crossman DG (1991) Dispersal and social organization of the northern hairy-nosed wombat, *Lasiorhinus krefftii*. *Journal of Zoology*, **225**, 605–613.
- Kenward RE, Hodden KH (1996) *RANGES v: An analysis system for biological data*. Institute of Terrestrial Ecology, Furzebrook Research Station, Wareham, Dorset, UK.
- Lacey EA, Sherman PW (1997) Cooperative breeding in naked mole-rats: implications for vertebrate and invertebrate sociality. In: *Cooperative Breeding in Mammals* (eds Solomon NG, French JA), pp. 267–301. Cambridge University Press, Cambridge, U.K.
- Laundré JW, Reynolds TD (1993) Effects of soil structure on burrow characteristics of five small mammal species. *Great Basin Naturalist*, **53**, 358–366.
- Lott D (1991) *Intraspecific Variation in the Social Systems of Wild Vertebrates*. Cambridge University Press, Cambridge, U.K.
- Lovegrove BG (1989) The cost of burrowing by the social mole rats (Bathyergidae) *Cryptomys damarensis* and *Heterocephalus glaber*: the role of soil moisture. *Physiological Zoology*, **62**, 449–469.
- Lowry DC, Jennings JN (1981) The Nullarbor karst Australia. In: *Benchmark Papers in Geology: 59: Karst Geomorphology* (ed. Sweeting MM), pp. 329–349. Hutchinson Ross, Penn.
- Maher CR (2004) Intrasexual territoriality in woodchucks (*Marmota monax*). *Journal of Mammalogy*, **85**, 1087–1094.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- McKenzie NL, Robinson AC (1987) A biological survey of the Nullarbor Region South and Western Australia in 1984, p. 284. South Australia Department of Environment and Planning, Western Australian Department of Conservation and Land Management, National Parks and Wildlife Service.
- Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.
- Peakall R, Smouse PE (2001) *GENALEX version 5: genetic analysis in Excel. Population genetic software for teaching and research*. Australian National University, Canberra, Australia.
- Potter JS, Weatherby KG, Chittleborough DJ (1973) *A Description of the Land in County Albert, County Alfred and Part of County Eyre, South Australia*. Department of Agriculture, South Australia.
- Randall JA, Rogovin K, Parker PG, Eimes JA (2005) Flexible social structure of a desert rodent, *Rhombomys opimus*: philopatry, kinship, and ecological constraints. *Behavioral Ecology*, **16**, 961–973.

- Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences, USA*, **94**, 9197–9221.
- Rexstad E, Burnham KP (1991) User's guide for interactive program CAPTURE. Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University, Fort Collins, Colorado.
- Schradin C, Pillay N (2005) Intraspecific variation in the spatial and social organization of the African striped mouse. *Journal of Mammalogy*, **86**, 99–107.
- Shimmin GA, Skinner JD, Baudinette RV (2002) The burrow architecture and environment of the southern hairy-nosed wombat *Lasiorchinus latifrons*. *Journal of Zoology*, **258**, 469–477.
- Sloane MA, Sunnucks P, Alpers DL, Beheregaray LB, Taylor AC (2000) Highly reliable genetic identification of individual northern hairy-nosed wombats from remotely collected hairs: a feasible censusing method. *Molecular Ecology*, **9**, 1233–1240.
- Smith AT, Formozov AN, Hoffmann RS, Zheng C, Erbajeva MA (1990) The pikas. In: *Rabbits, Hares, and Pikas: Status, Survey, and Conservation Action Plan* (eds Chapman JA, Flux JEC). IUCN/SSC Lagomorph Specialist Group, Gland, Switzerland.
- Smith AT, Wang XG (1991) Social relationships of adult black-lipped Pika (*Ocotona curzoniae*). *Journal of Mammalogy*, **72**, 231–247.
- Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.
- St. John BJ (1998) Management of southern hairy-nosed wombats *Lasiorchinus latifrons* in South Australia. In: *Wombats* (eds Wells RT, Pridmore PA), pp. 228–242. Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia.
- Steele VR, Temple-Smith PD (1998) Physical structure of warrens of a small colony of southern hairy-nosed wombats *Lasiorchinus latifrons*. In: *Wombats* (eds Wells RT, Pridmore PA), pp. 113–124. Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia.
- Stephens CG (1963) Soils. In: *Atlas of Australian Resources* (ed. Department of National Development). Canberra.
- Stow AJ, Sunnucks P (2004) Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology*, **13**, 443–447.
- Travis SE, Slobodchikoff CN (1993) Effects of food resource distribution on the social system of Gunnison's prairie dog (*Cynomys gunnisoni*). *Canadian Journal of Zoology*, **71**, 1186–1192.
- Travis SE, Slobodchikoff CN, Keim P (1995) Ecological and demographic effects on intraspecific variation in the social system of prairie dogs. *Ecology*, **76**, 1794–1803.
- 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.
- Waits LP, Paetkau D (2005) New non-invasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, **69**, 1419–1433.
- Walker FM (2004) *Sociobiology inferred from relatedness structure via remotely-collected DNA in southern hairy-nosed wombats* (*Lasiorchinus latifrons*). PhD Dissertation, Monash University, Clayton, Victoria, Australia.
- Walker FM, Sunnucks P, Taylor AC (2006) Genotyping of 'captured' hairs reveals burrow-use and ranging behavior of southern hairy-nosed wombats. *Journal of Mammalogy*, **87**, 690–699.
- Whitehead H (1999) socPROG 1.2 (for MATLAB 5.1): programs for analysing social structure. Dalhousie University, Halifax, Nova Scotia.

This is one of a quartet of primary data papers resulting from Faith Walker's PhD research, which aimed to understand the southern hairy-nosed wombat's sociobiology under normal conditions and under perturbation by employing large-scale noninvasive sampling of hairs to secure genotypes. Andrea Taylor is a Senior Research Fellow who has for many years focused on applying molecular genetic analysis to a variety of questions in marsupial biology and conservation. Paul Sunnucks is a Senior Lecturer in Zoology, whose collaborative research programs concentrate on the application of molecular population biology to structure and function of natural and human-influenced animal populations.
