Research Paper

Opportunity for female mate choice improves reproductive outcomes in the conservation breeding program of the eastern barred bandicoot (*Perameles gunnii*)

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ABSTRACT

Captive breeding is fundamental to recovery programs for many endangered species. Most programs seek to maximise retention of genetic variation and minimise inbreeding risk by assigning individuals to pre-determined pairings, but the reproductive outcomes of such pairings are often poor. There is evidence that pairing individuals in captivity that are instead genetically and behaviourally compatible can result in improved reproductive success, and that the resulting offspring perform better when released into the wild. Evidence is also mounting that such compatible outcomes can be achieved by allowing female mate choice. We investigated whether the reproductive success of captive endangered eastern barred bandicoots (*Perameles gunnii*) improved when females were permitted to choose their mate. Over a 21 day period, we allowed females to simultaneously evaluate two males that were unrelated to the female via interaction through perforated clear acrylic barriers, which permitted visual, auditory and olfactory, but not physical, contact. We determined female preferences by measuring how much time they spent interacting with each male, and whether they showed behavioural signs of receptivity. We then paired females with either their preferred or non-preferred male, and recorded the time to conception and number of pouch young produced. Based on the interaction choice trials, females paired with preferred males were significantly more likely to produce young (p = 0.03). These preferred pairings also resulted in earlier conception of young than non-preferred pairings (p = 0.008). These findings could improve productivity of the eastern barred bandicoot breeding program and, more generally, support the incorporation of mate choice into conservation breeding programs for other species wherever practical.

1. Introduction

Sexual selection theory predicts that females should be the more selective sex in choosing mates due to differential investment in gamete production and parental care (Trivers, 1972). For males, reproductive success tends to increase with the number of mates acquired (Bateman, 1948), but females are often limited in their reproductive opportunities, so should invest more selectively in reproduction (Andersson, 1994; Kokko et al., 2003). Where females can choose between numerous males, they will commonly increase their fitness, and that of their offspring, by selecting genetically compatible mates, or mates with 'good genes' that offspring may inherit (Ahtiainen et al., 2004; Tregenza and Wedell, 2000).

Mate choice may help maintain genetic diversity at both the individual and population levels. 'Heterozygosity theory' (Brown, 1997, 1999; Kempenaers, 2007) predicts directional female preference for males with greater allelic variety, and avoidance of inbreeding by preference for non-related males (Brown, 1997; Trivers, 1972; Weatherhead et al., 1999). Heterozygosity, mate preference, and subsequent reproductive success are united in studies across diverse taxa (Hoffman et al., 2007; Ilmonen et al., 2009; Marshall et al., 2003).

While genetic diversity is an important factor, *combinations* of genes, arising from the interactions of parental genes and linked to physiological and behavioural attributes, may be more beneficial than heterozygosity alone (Neff and Pritcher, 2005; Puurtinen et al., 2009). The prediction is that females will choose mates that produce offspring with optimum genetic variety and gene combinations (Colegrave et al., 2002; Neff and Pitcher, 2005).

Evaluation of potential mates generally involves multiple cues, with foundations in morphology, physiology and behaviour (Jennions and

Petrie, 1997). In mammalian reproduction, olfaction is likely the prime sensory modality (Roberts and Gosling, 2004), and the reliability of olfactory cues as indicators of male quality has been explored in some detail (Charpentier et al., 2008; Johansson and Jones, 2007). Females reportedly use scent to assess kinship (Parrott et al., 2007), social dominance (Drickamer et al., 2000), and genetic heterozygosity at key gene loci (Hoffman et al., 2007; Ilmonen et al., 2009), including at the Major Histocompatibility Complex which is active in immunity (Huchard et al., 2010, 2013).

Mate choice is thus an important element of reproduction in many taxa (Davies et al., 2012; Kappeler, 2010). However, mate selection in the wild typically contrasts with captive breeding, where individuals are assigned a mate, usually with the aim of minimising relatedness of paired individuals to retain maximum genetic diversity in the captive population (Ballou and Lacy, 1995; Earnhardt et al., 2001). This is an important objective, but provides no guarantee of genetic and/or behavioural compatibility between individuals, potentially constraining the productivity of captive breeding programs (Asa et al., 2011; Møller and Legendre, 2001; Wolf et al., 2000).

Captive-bred animals can also lack some physiological and behavioural attributes of their wild conspecifics, attributed to either inbreeding (Hedrick and Kalinowski, 2000; Robert, 2009) or adaptation to captivity (Araki et al., 2007; Frankham, 2008; Lynch and O'Hely, 2001). Both have negative implications for the fitness of released animals, as highlighted in some extensive reviews of reintroduction/relocation survival rates (Fischer and Lindenmayer, 2000; Jule et al., 2008). In the feathertail glider (*Acrobates pygmaeus*), for example, the poor expression of torpor and thermal performance seen in captive-bred animals may seriously impact their survival in the wild (Geiser and Ferguson, 2001).

Mate choice may enhance genetic and behavioural compatibility between mates, and thus improve both the reproductive success within captive breeding programs (Asa et al., 2011; Grahn et al., 1998), and fitness of released offspring, with such outcomes reported in insects (Anderson et al., 2007), birds (Bluhm and Gowaty, 2004, Ihle et al., 2015), and mammals (Drickamer et al., 2000). In one of only very few mate choice studies in marsupial species, female agile antechinus (*Antechinus agilis*) chose to mate with males least related to themselves based on olfactory cues, thus avoiding possibly deleterious effects of inbreeding (Parrott et al., 2007). Pairing of behaviourally compatible animals in captivity has led to better reproductive performance (Spoon et al., 2006), reduced aggression between mates (Gold and Maple 1994; Powell, 2010; Powell and Gartner, 2011), and, in species that provide bi-parental care, greater success in raising young when compared to random pairings (Ihle et al., 2015).

In this study, we investigated the potential benefits of mate choice within conservation breeding of the critically endangered mainland eastern barred bandicoot, *Perameles gunnii* (EBB). Once abundant on the basalt plains of south-western Victoria and south-eastern South Australia, this species is now extinct in the wild on mainland Australia (Department of Environment Land Water Planning, 2013). There are an estimated 1200 individuals, including reintroduced and captive populations, descended from only 19–23 founders taken into captivity from the last known wild population in the early 1990s (Coetsee, 2016). Reintroduced populations have since lost significant genetic diversity of 25–35% (Weeks et al., 2013). Twenty-seven years of captive breeding has produced nearly 900 offspring (Coetsee, 2016), but breeding records indicate that only about half of the assigned pairings produced offspring within three months of pairing (n = 180 pairs; Hartnett, 2015). This compares unfavourably to the mean percentage of 88% of free-ranging females found to be breeding each year over a four-year period (n = 294 females; Coetsee – unpubl. data, Woodlands Historic Park).

We conducted an experimental study in which female bandicoots were given the opportunity to interact with two different males over an extended period of time. We (i) assessed female preference for

particular males, and (ii) determined if measures of reproductive success were greater for females paired with preferred versus non-preferred males. These findings should determine whether the incorporation of mate choice could benefit the breeding program of this species, but also contribute to understanding the value of mate choice in conservation-based captive breeding more generally.

2. Methods

2.1. Study animals and maintenance

This study received approval from the Zoos Victoria Animal Ethics Committee (ZV14006), in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Captive eastern barred bandicoots were studied at Werribee Open Range Zoo (WORZ), Victoria, Australia. The breeding facility at this site can house up to 36 bandicoots, and contributes to the captive insurance and release program for this species.

We performed 22 mate choice trials over two years (2014–2015), with a total of 18 females (four being used twice in trials) and 20 males (ten being used in 2–4 trials) observed across the two years. The use of some animals in more than one trial was necessitated by the limited availability of animals, and was based on whether animals had already been designated for pairing in a previous trial. Two females used in preference trials were not paired but were released to the wild as part of a reintroduction program. Trials ran from early September to late October in 2014, and from early June to mid-October in 2015, falling within the peak breeding season (winter-spring) for the eastern barred bandicoot (Winnard, 2010). Although breeding in this species can be temperature-dependent, it has been observed year-round where conditions are favourable (Winnard, 2010). Like all Peramelid species, female EBBs are polyoestrous, and may produce up to five litters per year (Heinsohn, 1966), suggesting receptivity throughout the year.

In 2014, all study animals were sourced from the existing captive population. In 2015, ten new animals (five of each sex) were sourced from a wild population at the Mt. Rothwell Biodiversity Interpretation Centre. All animals were housed individually in purpose-built bandicoot enclosures at WORZ (7.2 m long × 2 m wide × 2.15 m high), until animals were paired, when they were given access to two enclosures simultaneously (see below). Each enclosure was furnished with a tanbark substrate (approximately 20 cm in depth), additional cover of native grasses and predominately eucalypt branches and logs, sheet metal and wire mesh walls, and shade cloth overlying the ceiling. Where males were moved between enclosures, all residual male scent was removed by thoroughly washing the walls and permanent foliage, and replacing the tanbark substrate, logs and branches. Standard animal-husbandry procedures were conducted daily by zoo staff, including provision of nesting material, fresh food and water, and remained consistent over the study period.

The males used in the trials ranged in age from 6.5 to 42 months (mean 26.3 months), and females from 7 to 48 months (mean 20.3 months), being within the appropriate reproductive age range for this species. Males reach sexual maturity at 5 months and females at 3 months, and females of up to four years old have produced young in captivity (Hartnett, 2015). The weight range was 565–1028 g (mean 769 g) for males and 525–821 g (mean 649 g) for females, with these ranges typical of reproducing males and females in both captive and free-ranging populations. (Hartnett, 2015; Coetsee – unpubl. data, Woodlands Historic Park, 2013–2017). In terms of origin, 67% of females and 30% of males were captive-born.

2.2. Experimental design

We assessed female preference for particular males by conducting mate choice trials based on encounter assays in which each female had voluntary, non-contact engagement with two potentially suitable
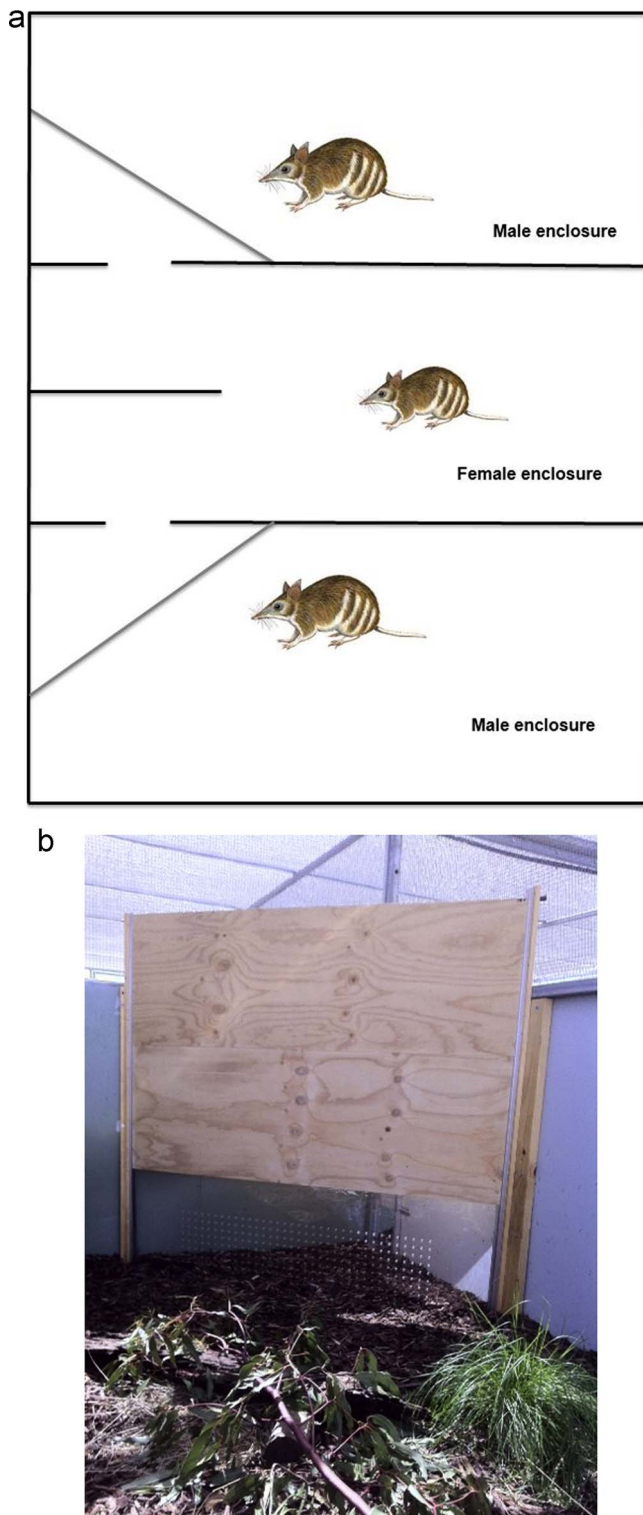
Fig. 1. 1a. Diagram of experimental mate preference set-up showing relative positioning of male and female eastern barred bandicoots and encounter screens. 1b. Image showing the position of the encounter screen within a male eastern barred bandicoot enclosure, and access doorway from the female enclosure in the back corner of the shared wall.

mating partners. This allowed them to make olfactory, visual and auditory assessments of each male. The female was placed in an individual pen with one male in each of the adjoining pens (Fig. 1a). The two males in each trial had been assessed as genetically suitable mating partners for the female, i.e. having low average kinship (coancestry) within the EBB meta-population (Ballou and Lacy, 1995; Parrott, 2015),

and low relatedness to the female subject, as determined by genetic microsatellite analyses, individual pedigree analysis (captive-born animals), and/or the pairing of animals from different source populations (wild-caught animals). For each trial, the three animals were housed in their enclosure for a minimum of three nights, allowing for familiarization with the area and providing an opportunity for males to scent-mark. Solid walls and doors prevented animals from directly interacting during this time. After this period, a new barrier (hereafter called the 'encounter screen') was added to the triangular corner area at the rear of both male enclosures. The encounter screen was 1500 mm wide × 1500 mm high, consisting of a lower portion of clear acrylic sheet, measuring 500 × 1500 × 6 mm, with the remaining height to 1500 mm composed of 6-mm thick plywood (Fig. 1b). The acrylic portion of each screen had 315 holes (9 rows × 35 holes, of 8 mm diameter) to facilitate scent transfer. Small doorways (250 × 200 mm) between female and male enclosures gave females access to this corner area of the male pens when opened.

Encounter assays were recorded using closed-circuit digital cameras with inbuilt infra-red lighting (VD-IR30VFS, Capture CCTV Ltd., Wiltshire, UK) suspended above the screened area in each male pen and at one end of the female pen, and connected to a digital video recorder (DR16HL Digimaster, CBC Americas Corp., Cary, NC, USA). These cameras could detect all female activity in the encounter areas and also male activity within 1 m of the encounter screen.

Trials commenced by removing the slides blocking the small doorways, giving the female voluntary access to the enclosed area within the pens of each of her two male neighbours. Trials were video-recorded between dusk and dawn (18:00–06:00 h) each night. Most trials (n = 18) ran for 21 nights, though the trials of four females in 2014 ran for a shorter period of three to five nights due to timing obligations within the larger breeding program. The length of the EBB oestrus cycle, based on studies of closely-related Peramelid species, is estimated to be 21 days (Lyne, 1964), but this has not been determined exactly, and little is known of the period of receptivity. The 21 day length of preference trials was determined to adequately overlap with the oestrus cycle and possible ovulation, when receptivity may increase.

At the conclusion of each trial, the screens were removed but the small doorway was left open on one side to facilitate pairing between the female and one of the two males. Pairings were determined either randomly in advance of trials, or in some cases, based on whether a male was required for a subsequent preference trial. Keepers captured females weekly after pairing to monitor the animals and check pouches for young. Any female with pouch young was separated from the male partner by blocking the access doorway between their enclosures. Pairs that had not produced young were kept together for a maximum of three months.

2.3. Assessment of behaviour and female preferences

A total of nearly 4700 h of video footage was recorded. For each trial, we collected behavioural data from a representative subset of this footage throughout each night and across the entire trial period. A primary observer (C. Hartnett) reviewed the bulk of this footage and checked the first night of data entered by two assistant viewers to ensure inter-observer consistency and reliability. The sub-sampling protocol involved watching the first 20 min of each hour following a female's emergence from her nest, until her return to the nest to sleep, totalling approximately 240 min per night. If the female did not visit either male in the first 20 min of an hour, we reviewed the next 20 min, or the third 20 min if no males were visited in either the first or second 20 min of each hour.

We recorded the number and duration of interactions that occurred between the female and each of the two males at the encounter screen. Other behaviours such as exploring, foraging and grooming were recorded as single events, as they tended to be performed in rapid succession so were difficult to time accurately. A description of the most

Table 1
Mean frequency of the most common behaviours performed by female eastern barred bandicoots during visits to the encounter areas in preference trials. Frequencies for each female were derived from observation of an average of 714 visits per female (SD = 659, range 125–2695, n = 22 females).

Behaviour	Mean (%) ± SD	Range (%)
Sniffing screen	64.1 ± 13.4	32–83
Observing: staring through screen into the male enclosure	51.9 ± 22.5	12–90
Exploring: sniffing walls/ground of general area	54.9 ± 13.7	21–74
Interacting: female and male simultaneously sniffing at screen	16.9 ± 15.8	2–59
Digging/foraging in substrate	9.7 ± 12.8	1–59
Nosing ground/digging in substrate at front of screen	10.1 ± 8.6	2–29
Grooming	2.2 ± 2.4	0–8

common female behaviours scored during video review, and average frequencies of these behaviours, is presented in Table 1.

We assessed female interest in each male as the relative amount of time that she spent interacting with each of the males in the encounter areas. This was extrapolated from the period of time the female was observed, and expressed as an amount of time for each male for each observation night. The male that had the higher average amount of time per observation night was classified as the preferred male. The strength of preference was determined by the degree of bias (e.g. 60:40%) and also by conducting paired *t*-tests (see below).

2.4. Measurement of breeding success

We measured breeding success using three variables (i) production of pouch young within three months, (ii) time from date of pairing to conception of young, estimated by size of pouch young when first detected, and (iii) litter size when first detected in the pouch.

2.5. Statistical analysis

Using the amount of time per observation night that females spent interacting with each male, paired-sample *t*-tests (paired for each night) were used to test if the average amount of time spent interacting with one male compared with the other was significantly different. These tests were repeated for each trial female. Female preference for each observation night of their trial was then expressed as the proportion of interaction time spent with the male that was preferred, on average, over all observation nights of that trial. A general pattern of change in the 'strength' of preference for a preferred male over the course of the trial was also investigated using a logistic regression mixed model with female as a random term, the proportion of time each night interacting with the preferred male as the binomial response variable, and the trial night (up to 21 in most cases – see above) as the potential explanatory variable.

The potential influence of male age and weight on female preference was assessed across females by using a paired *t*-test to determine if there was a significant mean difference in (i) the age and (ii) the weight of the preferred male compared with the non-preferred male. This allowed us to identify any pattern of systematic female preference (based on interaction time) for a particular age or body mass profile.

Pairing success (production of young) of females paired with preferred versus non-preferred males was analysed using the Pearson Chi-squared test, while one-way, between-subjects, analysis of variance (ANOVA) was used to compare time from pairing to conception and litter size for these females.

All statistical analysis was performed in the program Systat 13 (Systat Software Inc., San Jose, California, USA) except for the mixed model which was performed in MLWin 2.35 (University of Bristol, Bristol, UK). Standard deviation is reported with the mean at all times

within the text.

3. Results

3.1. Behavioural observations

The mean frequencies of the most common behaviours exhibited by female EBBs during visits to the encounter areas are given in Table 1. Females investigated the encounter screen (and very likely the scent of the male behind the screen), by sniffing in an animated and focused manner. They typically sniffed a large area of the screen, aided by raising themselves up on their hind legs to a 'full-stretch' position.

Interactions with males varied from animated nose-to-nose sniffing, with rapid back and forth movements in front of the screen, to brief periods where both moved up to 1 m away and explored the substrate or groomed with backs turned before returning once again to the screen (Table 1). The female usually left the encounter area if the time of male inattention exceeded approximately 15 s. Males typically ran around their pen after a short interaction at the screen, retreating to a distance of several metres and circling back to run past the screen, while the female stood still and observed. Females would often retreat from the male on his second approach, as this was generally made with less hesitation and greater pace than the initial approach. Males also commonly retreated to approximately 0.5 m from the screen, and either sniffed the substrate or groomed themselves with their back turned or body turned side-on to the female, until she left the encounter area. Occasionally females would retreat to a similar distance and also sit grooming, though grooming behaviour by females while in the encounter area was rare (an average of 2.2% of visits; Table 1).

3.2. Assessment of female preference

Female-male interactions occurred on 16.9% of female visits to the encounter area (Table 1). The average bias towards the preferred male (the male with which the female interacted for more time) was greater than 60% for 18 of the 22 females (Fig. 2). In 12 of these cases, the difference in amount of time interacting with these males was also statistically significant (Fig. 2; paired sample *t*-test: $p < 0.05$ for all).

Preferred males did not differ from non-preferred males in weight (mean = 775.9 g ± 108.9 vs 767.4 g ± 121.1, respectively; Paired *t*-test; $t_{21} = 0.36$, $p = 0.72$) or age (mean = 2.3 years ± 0.8 vs 2.1 years ± 0.9, respectively; Paired *t*-test; $t_{21} = 0.70$, $p = 0.49$).

The origin (captive vs wild) of the two males that a female could choose from differed in only seven preference trials, so statistical analysis was not possible. Captive origin males were preferred in five of these seven cases. Six of the seven females were of captive origin and in all but one case the female preferred a male of similar origin.

There was no consistent pattern of change in strength of preference over the 21-day period for which most females were observed ($X^2 = 0.65$, df = 1, $p = 0.42$; n = 22 females, 196 observation nights).

3.3. Reproductive outcomes

Females paired with preferred males were more likely to produce pouch young than those paired with non-preferred males (82%, n = 11 vs 33%, n = 9, respectively; $X^2 = 4.85$, df = 1, $p = 0.03$). Among females that produced pouch young (n = 12), those paired with preferred males produced young earlier than those paired with non-preferred males (ANOVA; mean = 12.8 d ± 8.9 vs 49.3 d ± 33, respectively; $F_{1,10} = 10.68$; $p = 0.008$). These females did not produce significantly larger litters when paired with preferred males than with non-preferred males, although the difference was in the predicted direction (ANOVA; mean = 1.4 ± 0.5 vs 1, respectively; $F_{1,10} = 2.0$, $p = 0.19$).
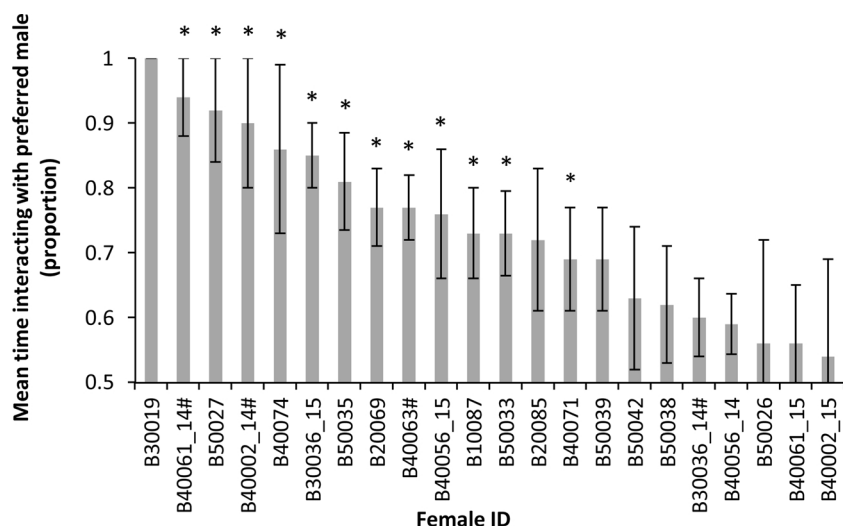
Fig. 2. Mean proportion of time (± SE) individual female eastern barred bandicoots spent interacting with preferred males (male with the greater average duration of interaction per night). An asterisk (*) indicates a significant difference between time spent interacting with the preferred and non-preferred male (p < 0.05). Females that were used twice across the two years 2014–2015 have the year (as _14 or _15) after their ID. Females that were analysed for a shorter period of time (3–5 nights) are denoted by a hash (#) after their ID.

4. Discussion

Female EBBs that were paired with a preferred male were more likely to produce offspring, and within a shorter period of time, than those paired with a non-preferred male. These findings have important implications for threatened species conservation. There is now considerable evidence for fitness benefits derived from genetic and behavioural compatibility between individual mates (Fedorka and Mousseau, 2002; Johnsen et al., 2000; Mays and Hill, 2004; Neff and Pritcher, 2005; Penn et al., 2002; Puurtinen et al., 2009; Wedekind and Füri, 1997; Wedekind et al., 2001). However, ours is one of only a handful of studies within conservation-based captive breeding (the golden hamster, *Mesocricetus auratus;* (Tang-Martinez et al., 1993), the Eurasian harvest mouse, *Micromys minutus*; Roberts and Gosling, 2004, the cheetah, *Acinonyx jubatus*; Mossotti, 2009, and the giant panda, *Ailuropoda melanoleuca*; Martin-Wintle et al., 2015) to demonstrate that providing females with the opportunity to choose their mates can improve reproductive success.

During preference trials in this study, individuals of both sexes were observed rubbing the chin and throat area across the encounter screen and along the ground directly in front of the screen, and residue from male urine was frequently seen on screen surfaces. Like most marsupials, the EBB has a well-developed ability to detect scent due to the large area of olfactory epithelium in the forebrain and a prominent vomeronasal region (Gemmell and Nelson, 1988; Johnson, 1977; Schneider et al., 2009). Many peramelid marsupials, including the EBB, have a subauricular scent gland, and the odour of the gland product becomes more pungent during the breeding season (Stoddart, 1980). The gland is present in both sexes, and the scent is thought to have the effect of subduing aggression in sexual encounters (Stoddart, 1980). Chemical communication is generally important for solitary species such as the EBB, which tend to associate only for mating (Heinsohn, 1966; Russell, 1985), as scent has the advantage of conveying persistent information about an animal's location (Gorman and Trowbridge, 2013; Salamon, 1996).

There is, however, little knowledge about the modes of sexual attraction in the EBB, and marking behaviour using gland product has never been empirically evaluated (Coulson, 1989). Whilst most likely prioritising olfaction, female EBBs also commonly observed males through the encounter screen, sometimes with ears turned toward the screen and nose raised to detect scent, suggesting the combined use of olfactory, visual and auditory cues in assessing males. Other behaviours that may indicate female mate preference in marsupials, such as grooming or licking the genitals or pouch (Parrott et al., 2007), were only rarely observed in female bandicoots within the encounter area

during this study.

In the absence of obvious sexual behaviours that would indicate attraction, we used the relative amount of time spent interacting with males as an indicator of a female's mate preference. When given access to two males, the trials revealed that most females spent significantly more time interacting with one male over the other. These findings suggest that females can discriminate between males, and female mate choice, likely based on genetic and behavioural attributes, seems clear in this research.

Although the mainland EBB population is descended from a small number of founders (Coetsee, 2016), there has been no decrease in fecundity or longevity across the life of the 27 year EBB breeding program (Hartnett, 2015), and the rate of reproduction in the wild has remained high and comparable with other Peramelid species (A. Coestee – pers com; Vernes and Pope, 2009). This suggests that there are few genetic issues in the mainland EBB at this point. However, in light of a founder bottleneck, and the subsequent 25–35% loss of genetic diversity in reintroduced populations, mate choice for increased heterozygosity and avoidance of inbreeding, as reported in other mate choice studies, could have important implications for this species' conservation breeding into the future. Parrott et al. (2015) found that female antechinus discriminated between mates based on very slight differences in genetic relatedness, and a similar sensitivity in preference may operate within the EBB mating system.

The magnitude of bias in time spent interacting with the two males varied from well above 2:1 in some females to a negligible and non-significant bias in others. Given that in each mate choice trial both males were considered genetically suitable (at least in terms of having low relatedness to the female), it may seem surprising that the preference displayed by many females was so pronounced. However, the two males in each pair could have differed genetically and behaviourally in many ways that affected their attractiveness and/or compatibility with the female. For example, individuals with higher variation at gene loci of the Major Histocompatibility Complex (MHC) show greater resistance to novel pathogens (Apanius et al., 1997; Penn et al., 2002; Von Schantz et al., 1996), and this variation is an important driver of mate choice in numerous vertebrate species, including fish (Consuegra and de Leaniz, 2008; Landry et al., 2001), mammals (Arcaro and Eklund, 1998; Eklund et al., 1991; Schwensow et al., 2008; Setchell and Huchard, 2010), birds (Bonneaud et al., 2006; Freeman-Gallant et al., 2003; Strandh et al., 2012), and reptiles (Olsson et al., 2003). Variation in proteins at the MHC is most likely communicated by odour (Schaefer et al., 2002; Tregenza and Weddel, 2000), aligning well with the probable importance of olfaction-based assessment of males by females in the EBB.

Our study also provided some further resolution on the social behaviour of the EBB. Being a cryptic, nocturnal species, social interactions in wild EBBs have been only infrequently observed. The mechanisms of mate selection and degree of male competition for mates remain largely unknown, and documentation of breeding behaviour comes primarily from captive-based studies involving manipulated encounters of short duration and with few animals (Clunie, 1987; Krake and Halley, 1993; Moloney, 1982; Murphy, 1993). Wild bandicoots of the Tasmanian sub-species have been seen chasing others from foraging areas (Heinsohn, 1966), however observations of males directly competing for breeding opportunities have not been recorded.

Our observations revealed that females given voluntary access to males within a captive environment will repeatedly initiate contact. This was a novel approach to studying breeding behaviour in captive EBBs, as previous studies (Clunie, 1987; Moloney, 1982) manipulated inter-sexual encounters in a way that did not permit females to escape from a pursuing male. With the focus on female choice in this study, it was essential to prevent forced copulations. The presence of the screen meant that females could assess males at close proximity, but leave the encounter area at any time. In our study, female bandicoots readily ventured into the encounter area, and explored it primarily by sniffing, consistent with scent being an important sensory channel in this species' evaluation of its environment. Female sniffing was likely to be motivated by 'curiosity-exploration' or 'sociability' (Gosling and John, 1999), because foraging behaviours such as nosing or digging were rarely performed within the encounter area, and food was provided elsewhere in the female's own enclosure. Bandicoots are essentially solitary in the wild, avoiding interactions with conspecifics outside of territory defence, and mate-seeking during peak breeding periods (Heinsohn, 1966; Russell, 1985). Thus, our observations of females repeatedly visiting an area known to be inhabited by a male, and initiating contact with that male, are likely to be reliable signs of sexual interest. The interaction bias of greater than 60% shown by 82% of females suggests that females may be receiving important information about male attributes during these close encounters.

The experimental design facilitated female assessment of male quality via scent-based, auditory, and visual cues, and therefore, a male's behavioural signs of interest in the female possibly played an important role in female preference. The behaviour of males more generally may also have provided females with important cues for assessing mate compatibility, particularly given the high heritability and offspring fitness consequences of certain behavioural traits (Dingemanse et al., 2004; Watters and Meehan, 2007). Pre-pairing assessment of individual behavioural characteristics, and testing whether preferred males in mate choice trials consistently exhibit behavioural traits linked to better survival outcomes in the wild, or assessing if pairings based on particular combinations of behavioural traits are reflected in reproductive success, would be an interesting avenue for future research in the EBB captive breeding program.

Females that were assigned their preferred male showed superior outcomes in two of the three measures of reproductive performance that we investigated: production of young (pairing success), and time from pairing to conception, compared to females paired with their non-preferred male. There is no practical method for determining the exact timing of ovulation for this species, particularly due to its propensity to suffer capture stress, and therefore animals are commonly paired without the timing of ovulation as a consideration. This would likely have introduced some variation between pairs for the time to conception, with females being at different stages of the cycle when paired. However, females were allocated randomly to be paired with their preferred or non-preferred male, so this could not have influenced our findings that females paired with a preferred male were more likely to conceive, and conceived sooner after pairing than those assigned a non-preferred male, giving us some confidence that mate choice played a role in these outcomes.

The study results also suggest that, at the very least, female mate

choice can facilitate behavioural compatibility, with some exciting implications for improving the performance of captive breeding programs. Assessing any potential fitness benefits of providing mate choice (arising from genetic compatibility) would require monitoring of offspring, particularly after release into the wild. This was not within the scope of this study, but in light of recent improvements to tracking and monitoring techniques for this species (Coetsee et al., 2016), could be conducted in the future.

4.1. Management implications

Refining management procedures is essential in order to achieve the best possible outcomes for threatened species breeding programs (Chargé et al., 2014). Improving the success of pairings is fundamentally important for most captive programs, as it allows more complete genetic representation of founders and therefore retention of genetic diversity over generations. A higher likelihood of pairs reproducing at a younger age, and doing so in a shorter period of time, is also critical when the program includes a reintroduction component. One of the priorities of the EBB breeding program is to supplement wild populations and produce young at an appropriate time for planned reintroductions. Our study revealed female preference for males that was linked to improved reproductive outcomes, with females paired with preferred males having an increased likelihood of producing pouch young, and a shorter period from pairing to conception. These findings suggest that providing mates with an opportunity to interact, and assessing female preference for particular males prior to assigning mating pairs, could help maximise the number (and possibly quality) of animals for subsequent release to the wild.

5. Conclusion

Our findings add to a growing body of empirical and theoretical studies that provide evidence for a clear link between mate choice and fitness benefits (Alatalo et al., 1998; Drickamer et al., 2000; Hoffman et al., 2007; Huchard et al., 2010, 2013; Ilmonen et al., 2009; Parrott et al., 2015; Puurtinen et al., 2009; Schwensow et al., 2008; Tregenza and Wedell, 2000). The findings of this study suggest that providing females in captive breeding programs with the opportunity to choose their mate may help to improve productivity. A fundamental requirement of conservation breeding programs is that healthy young can be produced to maintain the population, and in many cases, supply animals for release to the wild (Asa et al., 2011). To this end, implementing animal management methods that improve the quantity, and possibly the quality, of offspring produced are of clear benefit to these programs. Consequently, mate choice is likely to be of benefit to the breeding programs of many species.

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