Ethology **108,** 815—823 (2002)

ISSN 0179–1613

Kin Discrimination in a Macropod Marsupial

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Abstract

Differential treatment of kin is ubiquitous in social animals. Parents often behave preferentially towards their dependent offspring. Species in several taxa also bias behaviour towards non-descendent kin. This latter phenomenon has not been demonstrated in marsupials, which are reportedly less social than eutherian mammals. We report the first evidence of non-parental kin-biased behaviour in a macropodid marsupial. Experimental pairing of individuals based on kinship reliably altered the rate of aggression between individuals in pairs of female tammar wallabies (*Macropus eugenii*). This effect is probably attributable to relatedness rather than to familiarity. Marsupial sociality may be substantially more complex than is currently recognized.

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Introduction

Animals in a variety of vertebrate and invertebrate taxa differentiate kin from non-kin (Holmes and Sherman 1983; Halpin 1991). Such kin discrimination is an important mechanism allowing individuals to maximize their inclusive fitness (Hamilton 1964). Kinship influences reproductive decisions, thus preventing inbreeding and facilitating optimal outbreeding (Shields 1993; Pusey and Wolf 1996). Kinship may also influence decisions about time or energy allocation. In some species, helping behaviour varies as a function of relatedness (Emlen 1991). Similarly, relatedness has been reported to influence the rate of aggressive and other social behaviour (Holmes 1986; Pfennig 1999). Given the potential functional importance of kin discrimination, it is surprising that researchers

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working on the social behaviour of marsupials over the last three decades have apparently ignored the question of whether or not these animals can discriminate kin from non-kin.

The degree of sociality in macropodids (kangaroos, wallabies and rat kangaroos) ranges from solitary living to relatively simple open-membership groups, in which males associate with oestrous females, while mothers and their older daughters may associate facultatively (Jarman 1991; Jarman and Kruuk 1996). Some of these social systems clearly afford opportunities for animals to benefit from kin discrimination. For instance, when alarmed by predators macropods produce foot-thumps that warn conspecifics (Blumstein et al. 2000). Individuals that selectively foot-thumped more when surrounded by kin might increase their fitness compared with those that did not. If kin aggregate under natural conditions, kin discrimination mechanisms would also be useful to avoid mating with close relatives. To our knowledge, there have been no reports of non-parental kin discrimination in macropods (Johnson 1989). Perhaps this is because marsupials are generally presumed to be behaviourally less complex than eutherian mammals (Flannery 1994), and thus have not been as closely scrutinized for this phenomenon. Kin discrimination might also be undetected because investigators have focused principally on the mother–offspring relationship (e.g. Russell 1984; Jarman 1991; Dawson 1995), rather than looking for mechanisms that might explain other types of interactions.

We conducted an experimental study of kin discrimination in female tammar wallabies (*Macropus eugenii*), a 4–10-kg moderately social macropod marsupial (Kinloch 1973; Inns 1980; Smith and Hinds 1995). In nature, tammars spend their days resting in dense vegetative cover and forage by night in loose aggregations that may contain > 20 individuals (Inns 1980; D. T. Blumstein, unpubl. data). Adults have well-developed dominance relationships (Blumstein et al. 1999). Previous results reveal that tammars modify their behaviour as a function of the number of nearby conspecifics, both in captivity and in the field. Wallabies increase the time allocated to foraging, and decrease that allocated to vigilance, as a function of group size (Blumstein et al. 1999; Blumstein and Daniel in press). Such group size effects are inferred to reduce the risk of predation by historically important predators (e.g. the now-extinct thylacine, *Thylacinus cynocephalus*), or more recent predators (dingoes, *Canis lupus dingo*; cats, *Felis catus*; foxes, *Vulpes vulpes*; and wedge-tailed eagles, *Aquila audax*). More detailed knowledge about the behaviour of this nocturnal species in the field is presently lacking. However, detailed information about social predispositions can be obtained in captivity; such work can then generate testable hypotheses for future field studies. Our goal in the present study was to understand how kinship influences time allocation. We manipulated kinship and quantified variation in both social behaviour (affiliative and aggressive interactions) and socially influenced behaviour (foraging and vigilance).

Methods

Subjects

Kinship was manipulated by housing a wild-caught adult female tammar wallaby (i.e. the focal subject) with either a close female relative or with an unrelated female. We used a within-subjects design in which each animal received both of these treatments in a random order. The behaviour of the focal subject was observed for 10 h over 2 d in each condition. The 12 focal wallabies participating in this experiment were captured on Kangaroo Island, South Australia, 3.5 yr before the experiment began and formed part of the Macquarie University Fauna Park breeding colony. At the time of this experiment, they were $\geq$ 4 yr old. Four additional females were used to create 'pair'; three of these were wild caught and one was the captive-born adult daughter of a focal subject.

Estimating Kinship

The genealogy of our subjects was not known, so we estimated kinship using eight highly variable microsatellite loci (details in Blumstein et al. 1999; see also Queller and Goodnight 1989). We defined pairs with a coefficient of relatedness $r = 0.25$ ($\bar{x} \pm SE = 0.365 \pm 0.018$) as related, and those pairs with $r = 0$ ($\bar{x} \pm SE = -0.160 \pm 0.057$) as unrelated. The 95% confidence intervals of the two kinship treatments did not overlap. A negative kinship coefficient implies that a pair of subjects are less closely related to each other than would be expected by chance, given the frequency of alleles for each locus in the reference population (Queller and Goodnight 1989). We used allele frequencies of animals on Kangaroo Island (Taylor and Cooper 1999; K. Zenger, unpubl. data) as the reference population.

Experimental Protocol

Wallabies were normally housed in large semi-natural outdoor breeding yards, each of which held 6–10 adult females and a single adult male. Two weeks before the experiment began, we moved the 16 typed subjects into three 'holding yards' each containing four to six adult females. To control for recent familiarity, animals that were to be subsequently paired were not housed together during this period, or while the experiment was being run.

Once each week, for 12 consecutive weeks, we moved two pairs of animals from the holding yards and placed them into two adjacent, but visually isolated, 9 × 11 m 'test yards'. The wire fencing of these yards was covered with a 2-m high band of 90% shade cloth and the partition between them was hung with a 2-m high strip of opaque black plastic 'weedmat'. Animals being tested were thus visually isolated from all conspecifics other than their companion. Subjects were observed from a centrally located external hide that permitted the simultaneous viewing of both experimental yards by a single observer. In addition to natural vegetation in both the holding and test yards, subjects were given ad libitum access to kangaroo pellets (Gordon stock feed) and water. Concrete tubes (1.0 m long, 0.6 m diameter) were provided for shelter. The location and type of food, water and shelter was fixed at the beginning of the experiment and was identical in both yards and for all treatments.

Each of the two focal subjects was paired with a randomly selected companion from their respective pools of high-kin or low-kin typed wallabies. No subject was tested with the same individual twice, but animals were 're-used' as non-focal animals an average (± SE) of 1.4 (± 0.26) times. Our 10-h observation period was selected so as to encompass periods of peak activity: sunrise + 2.5 h; sunset − 2.5 h (Blumstein et al. 1999). Following the kinship manipulation and observations, animals were returned to their holding yards.

We recorded the time each focal subject allocated to antipredator vigilance, foraging, affiliative social behaviour and aggression (ethogram in Blumstein et al. 1999). By focusing on pairs of females, aggression was limited to simple displacements; these were occasionally preceded by a jab with a forepaw. Time allocation for the common behaviours (vigilance and foraging) was quantified by noting the activity of the focal subject every 5 min during the 10 h of observation. The rate of the remaining rare behaviours was estimated by noting each occurrence.

Our experimental design controlled for several factors that might potentially influence behavioural time allocation (e.g. Elgar 1989; Blumstein et al. 1999). All focal animals were wild-caught on Kangaroo Island, South Australia, and had been captive for 3.5 yr. We studied only interactions between adult females and assigned animals to pairs randomly with respect to reproductive status and body mass. The distribution of food, water, and shelter was standardized. Experimental yard was randomly assigned, as was treatment order. Most importantly, we controlled for recent familiarity by housing separately animals that were to be subsequently paired. We were unable to control for one potential confounding variable – long-term familiarity, and two potential obscuring variables – reproductive status and the mass difference between each subject and their companion in the high- and low-kinship treatments. We were, however, able to test statistically for possible effects attributable to these factors.

We assumed that animals were unfamiliar with each other when initially captured, but are unable to test this assumption. Once in captivity, wallabies were housed with each other for different amounts of time prior to our study, and this long-term familiarity might influence time allocation. Using the Fauna Park records, we calculated the number of days that each focal subject was housed with each of its two experimental companions and correlated the average number of days with time allocated to behaviour that was significantly influenced by kinship.

Ten of the 12 focal subjects carried joeys in their pouches. Joey age could be an obscuring variable that affected social behaviour. We correlated average joey age with time allocation estimates for those behaviours that were significantly influenced by kinship. We found a tendency for joey age to influence the rate of aggressive behaviour and fitted a repeated-measures ANCOVA model to remove this effect.

We anticipated that the body mass differential between the focal subject and its companion could vary in such a way as to obscure an effect of our kinship manipulation. We tested for this in two ways. First, we compared the body mass of each subject, relative to its companion, in the high- and low-kinship treatment. Second, we correlated the difference in body mass with the difference in aggressive behaviour (the only variable for which kinship explained significant variation between paired individuals).

We used either non-parametric tests or transformed variables to meet the distributional assumptions of parametric tests. We report the effect size (d) to better understand the relative importance of kinship on each dependent variable (Cohen 1988).

Results

Kinship specifically affected the rate of aggressive behaviour (Fig. 1). Despite marked individual variation, in 10/12 pairs, wallabies engaged in more aggressive interactions with their companion in the low-kinship treatment than in the high-kinship treatment (Wilcoxon test, $p = 0.045$, $d = 0.59$). Within the high-kinship treatment, there was a tendency for a negative relationship between kinship and the number of aggressive acts per hour ($r_S = -0.54$, $p = 0.07$). This difference in the rate of aggressive behaviour was not accompanied by changes in the type of interaction; the majority of encounters were simple displacements, occasionally

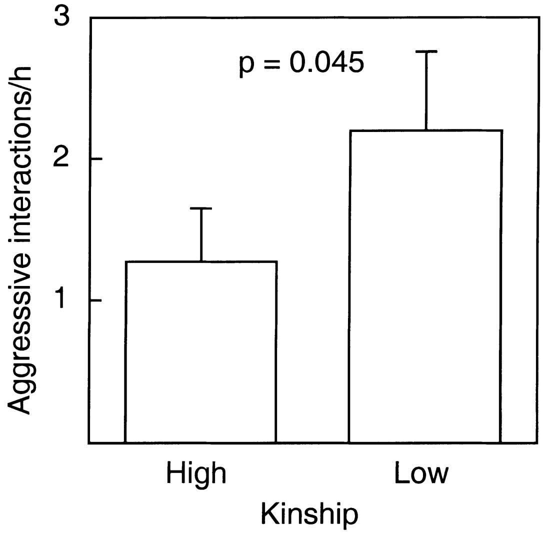

Fig. 1: $\bar{x}$ (+SE) rate of aggressive behaviour for 12 adult female tammar wallabies while paired with a close relative and with an unrelated individual

preceded by a single jab with a forepaw. No animals were injured during this experiment.

The effect of experimental kinship manipulation was not attributable to familiarity or to differences in body mass. There was no significant relationship between the number of days for which wallabies were housed together prior to the experiment and aggression ($r_s = -0.48$, $n = 12$, $p = 0.12$). Similarly, body mass differences between the focal subject and its high- and low-kin companion did not differ significantly between the two treatments ($\bar{x}_{high} = 212 \pm 191$ g SE, $\bar{x}_{low} = -67 \pm 248$ g SE, Wilcoxon test, $p = 0.27$). Moreover, there was no relationship between body mass differences and differences in the rate of aggressive displacements in the high- and low-kinship treatments ($r_s = 0.25$, $p = 0.43$).

There was a non-significant tendency for joey age to influence the rate of aggressive behaviour ($r_s = -0.59$, $n = 10$ females with joeys, $p = 0.08$). The effect of kinship was more pronounced once this obscuring effect of reproductive state was removed by adding the log of joey age as a covariate in a repeated-measures ANCOVA (kinship $F_{1,8} = 20.26$, $p = 0.005$; joey age $F_{1,8} = 5.55$, $p = 0.08$).

Kinship did not influence the time ($\bar{x} \pm SE$) that wallabies allocated to affiliative behaviour ($\bar{x}_{high} = 0.35 \pm 0.14$ events/h, $\bar{x}_{low} = 0.25 \pm 0.05$ events/h, $p = 0.959$, $d = 0.27$), or to the socially influenced behaviours of foraging ($\bar{x}_{high} = 0.19 \pm 0.02$ proportion time, $\bar{x}_{low} = 0.20 \pm 0.02$ proportion time, Wilcoxon test, $p = 0.39$, $d = 0.22$), and vigilance $\bar{x}_{high} = 0.65 \pm 0.03$ proportion time, $\bar{x}_{low} = 0.62 \pm 0.03$ proportion time, $p = 0.388$, $d = 0.32$).

Discussion

Female tammar wallabies bias their most common social behaviour, aggression, selectively toward non-kin. The discrimination mechanism responsible for this phenomenon is not likely to be based on familiarity, nor does it occur only among siblings or between parents and offspring. We believe that this is the first demonstration of non-parental kin-biased behaviour in marsupials.

Our understanding of many aspects of tammar social structure in nature would benefit from additional study. However, captive studies such as ours can be used to help focus field investigations. Depending upon the costs and benefits of aggregation, kin discrimination may facilitate either associating with kin or avoiding them (Giraldeau and Caraco 2000). Our results suggest that females are likely to aggregate with kin and imply that they gain some fitness benefit from doing so. We were able to create groups of relatives that did not consist solely of parents and offspring from wild-caught animals, which implies that kin may be aggregated in nature. Wallabies might receive indirect and direct fitness benefits by warning kin of nearby predators with foot-thumps. Similarly, because young kangaroos and wallabies are associated with their mothers for an extended period of time (Dawson 1995), adult females may associate with kin to reduce aggression on their more vulnerable young. Female–female kin discrimination may also reflect a more general cognitive 'ability' found in tammars of both sexes. Such discrimination is potentially useful in mate choice decisions as it would help individuals avoid mating with close relatives. It would be valuable to determine whether kinship influences mating decision in tammars.

To our knowledge, ours is also the first study to explore whether kinship influences the time allocated to vigilance and foraging in any species. Many birds and mammals (Bednekoff and Lima 1998), including tammars (Blumstein et al. 1999), decrease the time allocated to antipredator vigilance and forage more as group size increases. Several other factors influence this trade-off (Elgar 1989; Blumstein et al. 1999), but it appears that kinship may not, at least directly. However, if kin discrimination leads to clumping of relatives, only relatives would benefit from aggregating. It is thus possible that kinship has an indirect influence on the trade-off between vigilance and foraging.

The cues tammars used to assess kinship remain unidentified, but discrimination apparently does not rely on familiarity, as has so often been reported in mammals (Holmes and Sherman 1983). Perhaps this is because familiarity is not reliably correlated with relatedness. Tammars suckle only one large young at a time, and this joey interacts mostly with its mother. Thus, siblings do not grow up together and a familiarity mechanism is not likely to have evolved to mediate kin discrimination.

Recognition may indeed depend on phenotype matching using the well-developed olfactory sense of marsupials (Salamon 1996). Olfactory cues are clearly important in macropod sexual behaviour: males exhibit flehmen-like motor patterns while sniffing females and their urine (Salamon 1996). Similarly, when two female tammars first meet, they often sniff each other or the air, suggesting that olfactory information is also important in same-sex social interactions. Major histocompatibility (MHC) odour-types are important mechanisms of individual recognition in other mammals (Boyse et al. 1991). Future work could capitalize on these findings and explore the role of MHC genes in tammars (Slade et al. 1994) to better understand the mechanisms of macropodid kin discrimination.

Our findings suggest that marsupial sociality may be substantially more complex than is currently recognized. Specifically, there is the potential for marsupials, like other mammals, to enhance fitness by selectively biasing their behaviour toward non-descendent kin.

Acknowledgements

We thank Ron Claassens, Anne Mouland, and Scott Peel for wallaby wrangling; Des Cooper, John Rodger, and The Marsupial CRC for access to animals and facilities; Des, Andrea Taylor and Kyall Zenger for discussions about molecular studies of relatedness; Kyall for laboratory work; Alan Taylor and Janice Daniel for advice on statistical analyses, Jim Hare, Jean-Guy Godin and two anonymous referees for constructive comments. We are grateful for the support from the Australian Research Council (via an ARC-APD to D.T.B.), the Australian Cooperative Research Centre Programme, Macquarie University (MURG programme), and the Departments of Psychology and Biological Sciences.

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Received: January 24, 2002

Initial acceptance: March 28, 2002

Final acceptance: May 2, 2002 (J.-G. Godin)