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Species differences in urine scent-marking and counter-marking in *Peromyscus*

Elizabeth A. Becker[a,b,*], Frank R. Castelli[a], Christine N. Yohn[b,1], Lindsey Spencer[a], Catherine A. Marler[a,c]

[a] Department of Psychology, University of Wisconsin-Madison, 1202 W Johnson St., Madison, WI 53706, United States
[b] Department of Psychology, Saint Joseph's University 5600 City Ave., Philadelphia, PA 19131, United States
[c] Department of Zoology, University of Wisconsin-Madison, 250 N Mills St., Madison, WI 53706, United States

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ABSTRACT

Species comparisons indicate that scent-marking may differ as a function of mating system and co-housing with the opposite sex ("pairing"). We previously demonstrated that pairing may decrease male solicitation to unfamiliar females in the monogamous *Peromyscus californicus* but not in the non-monogamous *P. leucopus*. Whether urine scent-marking of females changes following pairing and whether scent-marking of paired males varies in response to scent-marks of their cagemate versus those of an unfamiliar female has not been examined. Therefore, we tested *P. californicus* and *P. leucopus* for within and between species differences in urine scent-marking of: 1) paired and non-paired females in an unscented arena, and 2) paired males in response to their female cagemate's or an unfamiliar female's scent-marks (counter-marking). Consistent with previous findings, *P. californicus* of both sexes deposited more urine scent-marks and covered greater surface area than *P. leucopus*. In both species, female scent-marking did not differ according to pairing status and male counter-marking did not differ in response to the scent-marks of their female cagemate versus an unfamiliar female. More females of both species and more *P. leucopus, but not P. californicus,* males scent-marked more around the perimeter than centrally. Potential explanations for these findings are discussed.

1. Introduction

Pair-bonds, which are characteristic of socially monogamous species, influence behavior. This study examines the effect of pair-bonding with an opposite-sex individual on urine scent-marking, an important communicative behavior, by comparing the monogamous California mouse (*Peromyscus californicus*) with the non-monogamous white-footed mouse (*Peromyscus leucopus*). In *Peromyscus*, co-housing with an opposite-sex individual ordinarily leads to copulation and, in the case of *P. californicus*, but not *P. leucopus,* the formation of a pair-bond. Therefore, we specifically examined whether urine scent-marking behavior of females varies depending on pairing status (defined as co-housing with an opposite sex conspecific) and whether urine scent-marking behavior of paired males in response to the scent-marks of females (counter-marking) varies depending on whether the scent-marks are from their female cagemate versus an unfamiliar female.

Social monogamy, a complex behavioral phenomenon defined by the existence of a pair-bond in which one male and one female maintain a high degree of close proximity and concurrent engagement in the same activities (Wickler and Seibt, 1983), is rare among mammals (Kleiman, 1977). In a theoretical framework, Kleiman (1981) argued that pair-bonds would be stronger among species adopting obligate rather than facultative monogamy, and thus, behaviors between mates would be more affiliative, more synchronous, less aggressive, and levels of territoriality more similar. A strong pair-bond is important, particularly in socially obligate monogamous species, because it influences behaviors that maximize reproductive success, such as bi-parental care, which in many contexts is critical for offspring survival (Cantoni and Brown, 1997; Gubernick and Nordby, 1993; Gubernick and Teferi, 2000; Wynne-Edwards, 1987; Wynne-Edwards and Lisk, 1989). Pair-bonding may also influence communication for the purposes of mate advertisement, mate-guarding, territoriality, and pair-bond maintenance (Becker et al., 2012; Benedict, 2010; Blaich et al., 1996; Gubernick, 1990; Inman, 1986; Mays et al., 2006; Servedio et al., 2013; Woodward et al., 2000).

Among rodents and other mammals, urine scent-marking is a

* Corresponding author at: Department of Psychology, Saint Joseph's University 5600 City Ave., Philadelphia, PA 19131, United States.
E-mail address: ebecker@sju.edu (E.A. Becker).
[1] Present Address: Department of Psychology, Rutgers University, 152 Frelinghuysen Rd., Piscataway, NJ 08854, United States.

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common form of communication (Brown, 1979; Brown and Macdonald, 1985; Eisenberg and Kleiman, 1972; Roberts, 2007; Thiessen and Rice, 1976). The chemical quality of urine can communicate many pieces of information, including species membership, individual identity, sex, reproductive status, age, and dominance (Brown, 1979). The pattern of deposition of urine scent-marks may also convey information. For example, the frequency of scent-marks may communicate mating interest (Hurst, 1990a; Rich and Hurst, 1998, 1999; Wolff and Powell, 1984; but see Mech et al., 2003; Thomas, 2002) or dominance (Desjardins et al., 1973; Fuxjager et al., 2015; Gosling and Roberts, 2001; Hurst, 1990b, 1990c, 1990a; Roberts, 2007), while scent-marking along the perimeter of territories may communicate ownership (Gosling and Roberts, 2001; Wynne-Edwards et al., 1992). The placement of scent-marks near those previously deposited by others (counter-marking), or the subset of which that are at least partially directly on top of those previously deposited by others (over-marking), may communicate dominance to rivals or attractiveness to a potential mate (Ferkin, 1999; Hurst, 1990c, 1990a, 1990b; Hurst and Rich, 1999; Johnston, 2003; Kohli and Ferkin, 1999; Rich and Hurst, 1999, 1998; Woodward et al., 2000; but see Thomas and Wolff, 2002; Wolff et al., 2002). Additionally, urine itself may cause physiological changes in conspecifics including the blocking of pregnancy and the changing of the timing of puberty (Brown, 1985; Koyama, 2004), and patterns of scent-marking may influence which conspecifics encounter the urine and are consequently affected.

To identify behaviors and their functions associated with pair-bonding, it is useful to compare behaviors and functions both within and between species. Scent-marking in the socially monogamous prairie vole (*Microtus ochrogaster*) has been compared to the non-monogamous meadow vole (*M. pennsylvanicus*), but how pair-bonding affects scent-marking has received little attention overall (Ferkin et al., 2001, 2004b; Woodward et al., 2000). Mice of the genus *Peromyscus* provide excellent opportunities for such comparative studies because the mating systems of *Peromyscus* species vary widely. For instance, white-footed mice (*Peromyscus leucopus*) are sexually promiscuous (Xia and Millar, 1991), show weak to no association with mates (Ribble, 2003; Wolff, 1989; Wolff and Cicirello, 1991; Xia and Millar, 1988), do not cohabit with a mate during the breeding season (Nicholson, 1941), and males show minimal paternal care (Bester-Meredith et al., 1999; Bester-Meredith and Marler, 2003; Marler et al., 2003; Xia and Millar, 1988). In addition, *P. leucopus* show overall low levels of male–male aggression (Bester-Meredith et al., 1999; Dewsbury, 1983), although varying levels of territoriality have been documented (Ribble, 2003). The California mouse (*P. californicus*), in stark contrast, forms strong, lifelong pair-bonds (Gubernick and Nordby, 1993; Ribble, 1991), cohabits with their mate (Eisenberg, 1963, 1962) shows high levels of bi-parental care (Bester-Meredith et al., 1999; Gubernick and Alberts, 1987; Marler et al., 2003), and is mostly sexually and genetically monogamous (Gubernick and Nordby, 1993; Ribble, 1991). Additionally, *P. californicus* maintain pair-exclusive territories with their mate (Ribble and Salvioni, 1990), and show high levels of aggression towards non-mates of either sex (male–male aggression: Bester-Meredith et al., 1999; Rieger and Marler, in press; female–female aggression: Davis and Marler, 2003; Rieger and Marler, in press; opposite-sex aggression: Gubernick and Nordby, 1993;).

In a previous study from our laboratory, urine scent-marking differed between these two *Peromyscus* species and, for *P. californicus*, depended on whether an individual was co-housed with the opposite sex (defined here as "paired"; Becker et al., 2012). *P. leucopus* males scent-marked less frequently overall than *P. californicus* males, and co-housing with a female did not affect scent-marking in an arena that was either unscented, contained scent-marks from unfamiliar males, or contained unfamiliar stimulus females. In contrast, male *P. californicus,* co-housed with a female, and thus likely pair-bonded, increased urine scent-marking in an unscented arena and decreased scent-marking in an arena containing unfamiliar stimulus females.

The goal of the current study was to expand on the previous comparison of *Peromyscus* species and the effect of co-housing with a member of the opposite sex (which leads to pair-bonding in *P. californicus*) on urine scent-marking behavior. *P. californicus* and *P. leucopus* were the subjects of two experiments. In *Experiment 1*, females that were either paired (co-housed with a male) or not paired (co-housed with other females) were observed scent-marking in a clean unscented arena. Since pairing status influenced the amount of scent-marking in *P. californicus* males in the previous study, and males and females are both monogamous and territorial, we predicted similar differences in females. If the function of urine scent-marking in *Peromyscus* is for communicating territorial ownership, then we predicted that *P. californicus* females, but not *P. leucopus* females would deposit a higher proportion of their scent-marks in the perimeter versus the center as a form of display behavior in the absence of a social stimulus. In *Experiment 2*, males that were paired (co-housed with a female) were either placed alone in an arena containing scent-marks of their cagemate or scent-marks of an unfamiliar female to observe counter-marking and over-marking behavior. Because of the many possible functions of counter-marking and over-marking, we took an exploratory approach as to whether these behaviors by males would differ in response to scent-marks from their familiar cagemate versus an unfamiliar female. Therefore, we did not have specific predictions for within-species differences or a species-by-stimulus-female interaction. We did, however, predict that an overall higher amount of scent-marking in both sexes of *P. californicus* than *P. leucopus* based on our previous study.

2. Methods

2.1. Animals

Mice used in this study, aged 6–12 mo, were randomly selected from our breeding colony at the University of Wisconsin-Madison. *P. californicus* and *P. leucopus* were housed in separate colony rooms. All animals were housed under a 14L:10D reverse light cycle in opaque polypropylene cages (L x W x H: 48.3 × 26.7 × 15.6 cm) with aspen wood shavings (Nepco, Northeastern Products Corp., Warensburg, NY, USA), one-half of a cotton Nestlet (Ancare Corp., Bellmore, NY, USA) for enrichment, and access to food (LabDiet 5001, PMI Nutrition International, LLC, Brentwood, MO, USA) and water *ad libitum*. Animal rooms were set to 22.2 °C and 60%RH.

Twenty-five male-female pairs of *P. californicus* and 28 of *P. leucopus* were formed 7 d prior to testing since co-housing for this amount of time is sufficient for behavioral changes indicating pair-bond formation in *P. californicus*. After 7 d of co-habitation with the opposite-sex, individuals sleep in the same nest (Becker, Castelli, Rieger, Zhao, and Marler, unpublished observations) and will groom each other (Rieger and Zhao, unpublished observations). Furthermore, in a forthcoming study from our laboratory (Pultorak et al., 2014), female subjects were introduced to a cage containing a male and behavioral interactions were measured upon first encounter and then again 7 d after cohabitation. Compared to day 0, females approached males sooner and the dyad showed lower amounts of agonistic behavior including a lower emission of aggressive bark vocalizations (Rieger and Marler, in press) on day 7 (Pultorak et al., 2014). Additionally, in a second forthcoming study from our laboratory (Zhao and Marler, unpublished data), *P. californicus* males that had been co-housed with a female for 7 d spent less time investigating a novel female in estrus presented across a wire mesh barrier than did males that had been co-housed with other males. This decreased interest in a potential mate further suggests pair-bond formation within the 7 d period.

Pregnancy can alter a female's behavior, including communication with her partner (*P. californicus*: Pultorak et al., 2017) and scent-marking behavior (Coquelin, 1992; Johnston, 1979; Kleiman and Mack, 1980; Wallace et al., 1973). Furthermore, the odor of a pregnant female might have a different stimulus value than a non-pregnant female and