COVER SHEET

TITLE: Female California mouse scent marking behavior and its implications for the pair bond

AUTHOR'S NAME: Marie Kuneau

MAJOR: Genetics

DEPARTMENT: Psychology / Zoology

MENTOR: Catherine Marler

DEPARTMENT: Psychology / Zoology

MENTOR(2):

DEPARTMENT(2):

YEAR: 2013

ABSTRACT

Female California scent marking behavior and it's implications for the pair bond

Scent marking has been shown to be an active form of communication for male mice in claiming territory, advertising for females, and mate guarding. Yet little is known about how females use scent marking behaviorally. California mouse is a traditionally monogamous species. Pair bonding in male Caliornia mice has shown to reduce advertisement through scent marking to novel females. This study aims to further explore the nature of the pair bond in the female California mouse through interactions with her mate relative to a naïve male. Females were exposed to a pretest, a control condition, a substrate previously marked by a naïve male and one marked by their mate. Size, volume, distribution and overmarkings were assessed in a preliminary analysis. Early data shows that females may preferentially overmark their mates to a naïve male. This could further explain for the scent marking strategies employed by female mice.

Marie Kimaro, Genetics
Author Name/Major

Marie Kimaro
Author Signature

05/10/13
Date

Catherine Marler, Psychology
Mentor Name/Department

Catherine Marler
Mentor Signature

Marie Kumerow

Senior Thesis

Title: Female California mouse scent marking behavior and its implications for the pair bond

Summary

Scent marking has been shown to be an active form of communication for male mice in claiming territory, advertising for females, and mate guarding. Yet little is known about how females use scent marking behaviorally. California mouse is a traditionally monogamous species. Pair bonding in male Caliornia mice has shown to reduce advertisement through scent marking to novel females. This study aims to further explore the nature of the pair bond in the female California mouse through interactions with her mate relative to a naïve male. Females were exposed to a pretest, a control condition, a substrate previously marked by a naïve male and one marked by their mate. Size, volume, distribution and overmarkings were assessed in a preliminary analysis. Early data shows that females may preferentially overmark their mates to a naïve male. This could further explain for the scent marking strategies employed by female mice.

Introduction

Urinary scent marking is used by many mammals to relay information about their health and reproductive status, or territorialism and social dominance. Relative to vocalizations and other auditory cues, scent markings last a longer duration, but come with an inherent lag between the signal emission and reception (reviewed by Arawaka et al. 2008). Additionally, lingering chemo-signaling can also be responsible for increased predation (reviewed by Arawaka et al. 2008).

In mice, many traditional male behaviors have been associated with scent marking, including opposite sex attraction using sex-specific volatiles in the urine, mate guarding by covering up a female partner's estrus status, and territoriality by overmarking and countermarking (reviewed by Ferkin, 1999). Female mice have been shown to distinguish partner viability for mate selection using markers, opting for the more dominant, top-marking male (Johnston et al. 1997). Components called major urinary proteins

(MUPs) and the major histocompatibility complex (MHC) in the urine have been shown to allow for individual recognition based on chemical signatures (Hurst et al. 2001; reviewed by Arawaka et al. 2008). These distinguishable components of urinary scent marking could be responsible for both mate selection and recognition.

Female scent marking has been shown to vary throughout the reproductive cycle, but little is known about the behavioral nature of urinary marks. One study which tracked female mouse scent marking output throughout the reproductive cycle showed a decline of markings at parturition, but no changes related to sexual receptivity (Coquelin, 1992). This is dissimilar to many other species, in which show a positive association between sexual receptivity and volume of urine (Ferkin et al. 2004). Studies of the female scent marking behavioral response to male scent marking have been limited, but have suggested a communicatory nature of the female urine mark (Maruniak et al. 1975; Powell and Wolff, 1982; Wolff and Powell, 1984; Hurst, 1990).

The California mouse, *Peromyscus caliornicus*, is monogamous and biparental species thought to exhibit traditional pair bonding (Gubernick and Nordby, 1993). In a study by Becker et al (2012), paired male California mice were shown to reduce the level of sexual advertisement to receptive females compared to a polygamous *Peromyscus* species or to virgin California males. Becker et al.'s (2012) study used scent marking in the presence of a male or female stimulus to elicit response. In this study, we will be characterizing the pair-bonded female scent marking response of California mouse to a control condition, an unrelated naïve male, and the focal female's mate. Volume, size of marks, placement and overmarks will be analyzed to better determine the nature of female response. I hypothesize that 1) female urinary responses will not be random, but can be characterized into perceivable communicatory patterns, 2) paired females will respond at greater length to their pair-bonded mate than a naïve male.

Methods and Analysis

Animal Care

We used 50 paired female *Peromyscus californicus* mice, ranging from six to thirty six months, that have been reared in our laboratory colonies at the University of Wisconsin, Madison. Animals are

maintained in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Animal treatment and research protocols have been approved by the University of Wisconsin, Madison College of Letters and Sciences Institutional Animal Care and Use Committee (IACUC); L0021-0-03-10. Throughout the testing session, focal females were housed with their mates and litters in cages (48.3 cm×26.7 cm×15.6 cm); and naïve males were housed with same-sex conspecifics. Food and water will be available *ad libitum*. During testing, individuals were placed in a clean glass aquarium (20x11x12 in) lined with filter paper (Fisher Brand; Qualitative P8; flow rate: fast). Animals will be kept to a 14L:10D hour light cycle. Testing began immediately after initiation of the dark phase in the according to the following protocol.

Timeline for Female Testing

Paired female P. Californicus mice were deemed eligible to be inducted into the study upon having a new birth as either a primiparous or multiparous female. Each focal female was tested in four different conditions. The "Pre-test" session was scheduled between one and four days postpartum, which consisted of the female being exposed to the testing chamber and scent marking paper apparatus for thirty minutes. Following this, the female was tested on the fifth, tenth and fifteenth day after this primary session under three alternative conditions: control, exposed to naïve male, or exposed to her mate. The control condition mimicked the Pre-test in which the female is placed on a clean filter paper for a half hour. The naïve male condition consisted of thirty minutes in which an unrelated naïve male is marking on the filter paper, followed by the focal female marking the same paper for thirty additional minutes. The mate condition consisted of the paired female's mate marking for thirty minutes, followed by the focal female marking the same paper for an additional thirty minutes. The order of the latter three conditions will be varied for each focal female to account for any order effects.

Labeling markings on the filter papers

The filter paper was removed from the scent marking chamber after each animal to be labeled. Labeling consisted of tracing the perimeter of each mark under ultraviolet light. To differentiate female

markings from those in which a naïve male or her mate is used, different colors were used for first and second animal marking each filter paper (Figure 1).

Analyzing scent marking data

To analyze the tracings, a grid was used to differentiate marks by size and location. The relative size of any mark was discriminated based on the number of squares a mark contacts. Large marks and pooling of urine were grouped to identify changes in pooling behavior. Similarly, marks of one or two box dimensions were used to identify changes in spraying behavior. The total volume was measured as a sum of all the box units counted while identifying them by size. Marks were counted in relation to a 5cm perimeter around the score sheet, indentifying those that fell outside, on the line or inside relative to the boundary. For papers that were m arked by both a male and female individual, overmarks were identified as any location in which tracings from both individuals touch or overlap. Overmarks were also counted in relation to their location relative to the perimeter (Figure 1).

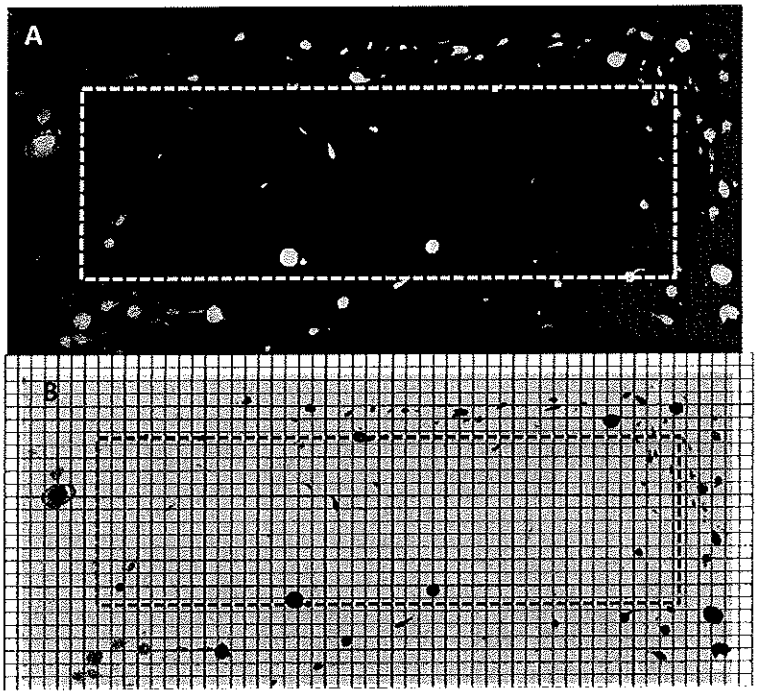


Figure 1. A. Visualization of scent marking during labeling under ultraviolet light. Urine marks are traced in colored pencil to be quantified later. B. Scent marking scoring. Scent marking observations were made using a grid and drawing a 5cm perimeter boundary. Number, volume, size and overmarks will all be identified.

Preliminary Statistical Analysis

Preliminary data was collected for 16 animals in each of our groups (out of 50 animals total). Early data was analyzed using a series of paired t tests to measure volume of urine, size of marks, number of marks, placement of marks relative to the periphery, and female overmarks between conditions. A p value of .1 was used as an upper indicator of being a trait of interest for the final results. Future data analysis will include ANOVA tests and the use of urine volume as a co-variate.

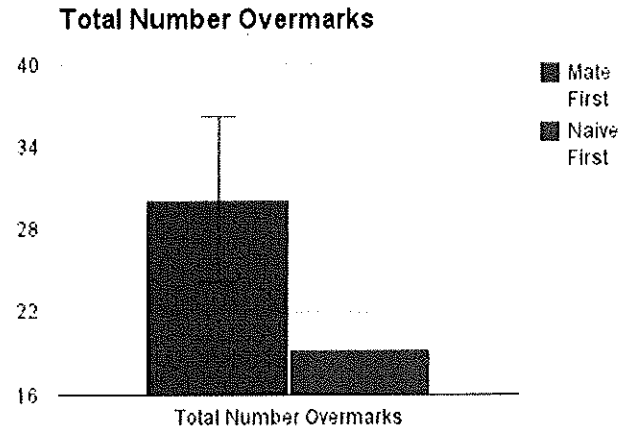
Preliminary Results

No statistically significant results were found in comparing volume, totally marks, spraying or pooling between conditions ($p>0.1$).

Overmarking Analysis

Early analysis provides a number of potential trends to watch as final data is collected and analyzed. The most notable is the total number of overmarks being increased in the mate response condition compared to the naïve male response condition ($p=0.06$). This could have potential implications as to message inscribed in a female mouse overmark. Additionally, a strong trend was detected in the overmarks made inside the perimeter boundary, again preferring the mate condition over the naïve male ($p=0.07$).

A less notable trend was detected in the number of overmarks around the perimeter favoring the mate condition. This could simply be a byproduct of having more overmarks overall ($p=0.1$).



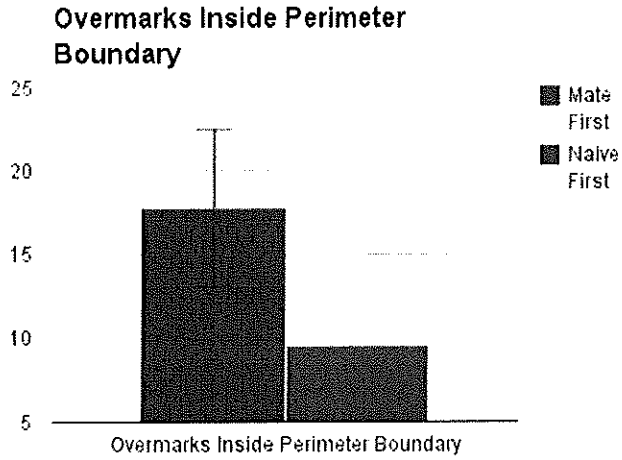


Figure 2. Preliminary data trends in increased overmarking in response to mate over naïve condition. A. Paired females showed increased over-marking in response to their mate. Graph depicts mate first conditions with an average of 30.2±6.29 SE, compared to a naïve male, average 19.25±4.28 SE ($p=.06$). B. Paired females also showed increased levels of overmarking inside the perimeter boundary in response to their mates. Graph depicts mate first conditions with an average of 17.8±4.7 SE, compared to a naïve male, average 9.68±2.4 SE ($p=.07$).

Perimeter Analysis

Another less distinct trend was increased female markings in response to the naïve male around the perimeter relative to the female only condition, but not to the mate condition ($p=0.1$). This may suggest advertisement or territoriality.

Discussion

Female California mice are monogamous and bipaternal, and therefore likely to maintain their pair bond. Gubernick and Norby (1993) showed that females at day 0 postpartum preferred to associate with their mate than an unfamiliar naïve male, and that females preferred to mate with their partner when give the choice of a an alternative naïve male. Additionally, pair-bonded male California mice have been shown to mark less in response to exposure to a receptive female relative to virgin California mice or a related polygamous species (Becker et al. 2012). However, the nature of female scent marking and pair bonding behavior is not well elaborated. Because of the monogamous and pair-bonded nature of this species, female California mouse was predicted to show preference to her pair bonded mate's markings relative to a naïve male's markings. Female behavioral response was measured quantitatively through urine volume, number of markings, distribution, number of overmarks and their distribution.

Preliminary results revealed a trend towards increased female response preferential to her mate relative to a naïve male condition through increased total overmarks. An overmark is a behavioral tool that can be used for mate guarding by covering attractants in your mate's scent, reestablishment of territorial boundaries, passive attack of a conspecific, or potentially as an advertisement. Males are known to have chemical attractants in their urine, providing support that overmarking behavior could be evidence of female mate guarding. In a second theory, females may be recognizing their pair-bonded partner and responding monogamously to their familiar. This is further supported by a second trend identified: females overmarked more medially, within the boundary of the perimeter to her mate more frequently than to a naïve male. This could be communicating increased familiarity and comfort in the "home territory," relative to marking selectively along the perimeter as is noticed in territorial amd aggressive encounters.

A third trend identified increased overmarking response by females to her mate along the perimeter relative to a naïve male. Being a less significant trend, this one may be a by-product of more total overmarking by females in response to their mates.

The final trend identified by preliminary results was increased levels of female marking on the perimeter in response to the naïve male condition relative to the mate first condition. An increased number of marking may represent advertisement of her receptivity. Scent marking has been shown to contain clues of gender, reproductive status, social dominance and overall health unique to an individual marker (Drickamer, 2001; reviewed by Arakawa et al. 2008). Urine also contains MHC, volatile odorants and non-volatile MUPs which are used for mate attraction (reviewed by Arawaka et al. 2008; Hurst et al. 2001). A receptive female might use markers to identify a potential mate, showing that females use scent marking as a behavioral tool to select a mate preference in the absence of her mate. However, an increased level of markings specifically around the perimeter may also be indicative of territorial or protective behavior. One could conjecture that a female with new pups might be protective of her mate's territory. Overmarking that was refreshed in small patches a number of times could be parallel to male territorial behaviors.

This preliminary analysis provides concrete evidence that female scent marking is communicative in nature and shows distinct patterns. Further extrapolation will be provided with analysis of the other 34 data points (a mouse tested under all four conditions). Based on this early data, ANOVA and covariates with male markings and overall urine volume will be used in regards to number of marks, total volume, pooling and spraying. Because of the wide range of marking patterns and volume of urine used in female scent marking, these tools will help create a more thorough analysis beyond a two group paired t test.

Future studies in female scent marking behavior could look at how social cues and chemical changes affect a females' preference to maintain a pair bond. Male dominance and territoriality has been shown to affect female preference in mate selection in rodents, in which the female prefers the top-marker (Johnston et al. 2007). Pair bonded females exposed to a condition in which her mate has been overmarked by a "dominant" male could futher extrapolate on the nature of the pair bond in California mouse (Rich and Hurst 1999). Lastly, this paper is assuming neurochemical status of females in a pair bond. Prior studies have shown that both oxytocin null mice and oxytocin antagonists disrupts social

memory in mice (Ferguson et al. 2000), so altering the neurochemical status of female mice may alter scent marking behavior and maintenance of the pair bond.

Sources

Arawaka, H, Blancahrd, DC, Arawaka, K, Dunlap, C, and Blanchard RJ. (2008) Scent marking behavior as an odorant communication in mice. Neuroscience and Biobehavioral Reviews 32(7): 1236-1248.

Coquelin, A. (1992) Urine-marking by female mice throughout their reproductive cycle. Hormones and Behavior 26 (2): 255-271.

Drickamer, L. 2001. Urine marking and social dominance in male house mice (Mus musculus domesticus). Behavioral Processes 52 (1-2): 113-120.

Ferguson, JN, Young, LJ, Hearn, EF, Matzuk, MM, Insel, TR, and Winslow, JT. (2000). Social amnesia in mice lacking the oxytocin gene. Nature Genetics 25: 284-288.

Ferkin, MH. (1999) Scent over-marking and adjacent-marking as competitive tactics used during chemical communication in voles. Advances in chemical signals in vertebrates: 239-246.

Ferkin, MH, Lee, DN, and Leonard, ST. (2004). The reproductive state of female voles affects their scent marking behavior and the responses of male conspecifics. Ethology 110 (4): 257-272.

Gubernick, DJ and Nordby, JC. (1993) Mechanisms of sexual fidelity in the monogamous California mouse, *Peromyscus californicus*. Behav Ecol Sociobiol 32:211-219.

Hurst, J. (1990). Urine marking in population of wild house mice Mus domesticus Rutty. III. Communication between the sexes. Animal Behavior 40(2): 233-243.

Hurst, JL, Payne, CE, Nevison, CM, Amr, DM, Humphries, RE, Robertson, DH, Cavaggioni, A, and Beynon, RJ. (2001) Individual recognition in mice mediated by major urinary proteins. Nature 414: 631-634.

Johnston, R, Sorokin, ES and Ferkin, MH. (1997). Sent counter-marking by male meadow voles: females prefer the top-scent male. Ethology 103(6): 443-453.

Maruniak, JA, Owen, K, Bronson, FH and Desjardins, C. 1975. Urinary marking in female house mice: Effects of ovarian steroids, sex experience and type of stimulus. Behavioral Biology 13(2):211-217.

Powell, AJ and Wolff, PR. (1982) Sex differences in mouse urination patterns. Animal Behavior 30 (4):1207-1211.

Rich, TJ, and Hurst, JL. (1999). The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. Animal Behavior 58 (5): 1027-1037.

Wolff, PR, and Powell, AJ. (1984) Urine patterns in mice: An analysis of male/female counter-marking. Animal Behavior 32 (4): 1185-1191.