Effect of Fluorescent Powder Marking of Females on Mate Choice by Male White-Footed Mice (*Peromyscus leucopus*)

By: MATINA C. KALCOUNIS-RÜPPELL, AMANDA PATRICK, JOHN S. MILLAR

Kalcounis-Rüppell, M. C., A. Patrick, and J. S. Millar. 2001. Effect of fluorescent powder marking of females on mate choice by male white-footed mice (*Peromyscus leucopus*). *American Midland Naturalist* 146: 429-433.

*****Note: Figures may be missing from this format of the document**
TABLE 1 CAN BE FOUND AT THE END OF THE ARTICLE

Abstract:

Although fluorescent powder marking is a well established and common marking technique used by small-mammal ecologists, few studies have commented on how it might affect behavior. Potential effects on behavior are particularly relevant in studies that use fluorescent powder marking as a method of quantifying mating behavior. We performed a laboratory experiment using white-footed mice (*Peromyscus leucopus*) to test the null hypothesis that the presence of fluorescent powder has no significant effect on mate choice. We tested this hypothesis by establishing choice between a pair of females by a male and determining if the choice changed when one of the females was powdered. The presence of fluorescent powder on females did not alter mate choice by males after a choice had been established. Our results demonstrate that using fluorescent powder on females should not alter male mating activity.

Article:

Introduction

Fluorescent powder marking involves dusting individuals with fluorescent powder and enables field researchers to indirectly trace movements and interactions of secretive, nocturnal small mammals (Lemen and Freeman, 1985; Kaufman, 1989). Fluorescent pigments commonly are used because they are inexpensive, have low toxicity and result in few pathological effects on powdered individuals (Stapp *et al.*, 1994; Ebensperger and Tamarin, 1997; Mullican, 1988). In addition to being used to follow trails left by powdered individuals, the transfer of powder between individuals has been used to infer social interactions (Kaufman, 1989) and parent-offspring relationships (Ribble, 1991; Millar *et al.*, 1992; Aars *et al.*, 1994; Duquette and Millar, 1995a, b). Use of fluorescent powder marking has increased in field studies of social systems and behavior of rodents (Ribble, 1991; Getz *et al.*, 1992; Ribble and Millar, 1997; Gubernick and Teferi, 2000; Kalcounis-Rüppell, 2000), in part, because the transfer of fluorescent powder provides an independent estimate of putative mates and offspring for studies that use molecular markers for determining kinship among individual rodents (*e.g.*, Ribble, 1991). For nocturnal, secretive, nonconspicuous rodents, the transfer of fluorescent powder is one of the only direct means of estimating of putative mates and offspring in field studies.

In addition to providing information about putative relationships, fluorescent powder marking can be used to determine the relative mating frequencies of individuals as a supplement to studies on genetic relatedness (Kalcounis-Rüppell, 2000). However, few studies have examined the effects of fluorescent powder marking on behavior (*for exception see* Ebensperger and Tamarin, 1997). Olfactory cues in rodents are needed for advertising physiological states (Singer, 1991; Coquelin, 1992; Kavaliers and Kinsella, 1995), intersexual and intrasexual recognition (Bowers and Smith, 1979) and kin recognition and familiarity (Barnard and Fitzsimons, 1989; Kumar and Dominic, 1993; Liebenauer and Slotnick, 1996). Although odorless to humans, fluorescent powder on female mice could interfere with olfactory cues thereby altering mate choice by males. Fluorescent powder could either interfere with female production of cues (*e.g.*, blocking pores or a scent gland) or male perception of cues (*e.g.*, masking pheromone odor). Regardless of the mechanism of olfactory interference, it is conceivable that fluorescent powder marking of females could alter mate choice by males. If fluorescent powder marking is to be used to investigate social systems and behavior of rodents, the effects of fluorescent powder marking on mate selection need to be quantified.

To evaluate the effect of fluorescent powder marking of females on mate selection by males, we conducted a laboratory experiment using white-footed mice (*Peromyscus leucopus*). We tested the null hypothesis that the presence of fluorescent powder has no significant effect on mate choice, by establishing choice between a pair of females by a male and determining if the choice changed when one of the females was powdered.

Methods

We conducted our experiment from September to December 1997, using wild caught, adult white-footed mice. Twenty-six males and 16 females were maintained in separate rooms on a 12-h light/dark photoperiod at an ambient temperature of 25 C. When not involved in experiments, mice were individually housed in plastic cages (28 by 17 by 12.5 cm) with hard chip bedding and *ad-lib* food (RMH 2000 rat/mouse/hamster chow) and water.

Mice were tested during the dark (active) portion of the photoperiod when they would be most active. All testing took place in a circular arena (142 cm in diameter) placed in an isolated dark room maintained at 25 C and illuminated with a 40 W red light. The arena was constructed from sheet metal and was lined with newspaper to completely cover the floor. For each trial a scrotal (distended testes) male was placed in the arena with two empty test cages placed opposite one another along the edge of the arena and permitted to acclimate for 3 min before the initiation of the experiment. Two perforate females were then chosen at random and placed separately in two clear empty plastic cages (28 by 17 by 12.5 cm). Following the 3 min acclimation period the empty cages were removed from the arena and replaced with the cages containing females. If the male was not already in the center of the arena, it was placed there and acclimated for another 30 s. The choice behavior of the male was observed from outside the room through a glass window and recorded with a tape recorder for seven minutes. The male was then removed and all newspaper was replaced. The procedure was repeated for each of the 26 males.

Mate choice was subjectively assessed as a combination of the duration of time the male spent in one half of the arena divided by the total time for which the male was observed (7 min), and the behavioral response of males to females. The following were considered as mate-choice

behaviors: sitting/walking on top of the female's cage, standing against the female's cage on hind legs, sniffing the female and physically contacting the female through the top of the cage. The following were not considered mate-choice behaviors: grooming, sitting near the cage without sniffing the female and pacing in the arena.

The experiment consisted of 16 trials. For each trial a random pair of perforate females was selected and tested against all 26 scrotal males. A new pair of randomly chosen females was used for each trial. Although some females were used more than once (up to 3 trials), each trial consisted of a different pair of females. After all 26 males had been exposed to a particular pair of unpowdered females, and a choice of female was established, one of the females of each pair was randomly chosen, removed from her cage and powdered. One female in each of the 16 pairs was assigned one of four commonly used fluorescent powder colors (magenta, deep green, orange-yellow or chartreuse; Radiant Color, Richmond, California). Thus, each color was assigned to four females. For powder application one teaspoon of fluorescent powder was placed in a clean plastic bag with the female. After the powder was distributed, the female was returned to her cage in the arena. The same 26 males were then individually reintroduced to the center of the arena and the same protocol followed, with the same pair of females, to document mate choice. Thus, in each trial males were exposed to a pair of unpowdered females and allowed to choose one female. They were subsequently re-exposed to the same pair of females with one female having been marked with fluorescent powder.

This protocol allowed us to test for any effect of powder on mate choice consistency by documenting change in the choices that males made pre- and postfluorescent powder marking. We tested the null hypothesis that males would choose unpowdered perforate females at random (0.5 probability of selecting each female in a pair) using chi-square goodness of fit tests (Zar, 1984). If the fluorescent powder marking treatment of females has no affect on mate choice, we expected the distribution of male-choice, postfluorescent, powder marking treatment to be the same as the prefluorescent powder marking treatment. For example, if in the prefluorescent powder marking treatment, 52% of the males in a given treatment choose female A and 48% of the males choose female B, we expected the same distribution in the postfluorescent powder marking treatment. We also tested for consistency of male choice pre- and postfluorescent powder marking treatment by determining if the distribution of nonconsistent males was random (the expected distribution being 0.5:0.5 because the pretreatment choice should not affect the posttreatment choice if there is no effect of fluorescent powder marking).

Results

All males showed choice of a particular female within the first 2 min of exposure to the two perforate females. Mate choice by males between two unpowdered females did not differ from random association in all sixteen trials nor did the pattern of mate choice change after one female was marked with fluorescent powder (chi-square 0.00–0.62, df = 1). Males consistently chose the same female pre- and post-treatment (Table 1). Of the 416 (26 males × 16 trials) postfluorescent, powder marking, treatment choices made, there were only 35 changes in choice. Of the 35 changes, 20 were to a powdered female and 15 were to an unpowdered female. All four colors were represented in the 15 changes from a powdered to an unpowdered female. The same individuals did not always change; 23 different males made the 35 changes in choice.

Discussion

It is known that transfer of fluorescent powder from females to males indicates copulation (Ebensperger and Tamarin, 1997), but the effects of this technique on mate selection previously have not been tested. Our purpose was to determine the effect of fluorescent powder on the mating behavior of white-footed mice. Our results demonstrate that the presence of fluorescent powder has no effect on mate choice in white-footed mice after a choice has been made.

Despite regular grooming, fluorescent powder remains on mice in both the laboratory and the field for several days (M.C. Kalcounis-Rüppell, pers. obs.) and has been recorded as remaining on free-ranging mice for 7–14 d (Kaufman, 1989). The presence of powder on females may interfere with olfactory cues, and olfactory cues in rodents are needed for advertising physiological states (Singer, 1991; Coquelin, 1992; Kavaliers and Kinsella, 1995) and recognition (Bowers and Smith, 1979; Barnard and Fitzsimons, 1989; Kumar and Dominic, 1993; Liebenauer and Slotnick, 1996). Presumably, olfactory cues are important in mate selection, and if a male's choice of a female can be attributed to physical or behavioral characteristics, our results demonstrate that fluorescent powder marking does not alter the male's perception of these characteristics. For example, any signals advertising the physiological state of an estrous female are relayed regardless of powdering. Clearly, the application of fluorescent powder will change the physical appearance and behavior of mice. For example, increased frequency of autogrooming was exhibited by female eastern chipmunks (*Tamias striatus*) after powdering (Hallman *et al.*, 1993). Despite physical and behavioral changes associated with fluorescent powder marking, we believe that the cues used by males to select a female are not changed when a female is dusted with fluorescent powder.

Mice do not distinguish among colors (Jacobs, 1993), so we were not specifically concerned with how each of the four colors affected male choice of female white-footed mice. Nevertheless, some pigment-specific constituent of the powder could have affected female choice by male mice. Our sample size was not sufficient to test for pigment-specific effects, but the low frequency of changing choice postfluorescent powder marking and the fact that all pigments were involved in the changes suggests that our overall result of no effect of fluorescent powder marking on mate selection is true for all four pigments.

Fluorescent powder marking can be used in the field to determine patterns of interaction between males and females. Some goals of field use of fluorescent powder marking are to establish putative genetic mates for subsequent laboratory analyses or to determine mate choice by individual males and females in a population. Our results suggest that the use of fluorescent powder marking on females will not alter male mating activity. Without question, our experiment differs from the natural situation. In the field, mate choice by males involves a number of complex cues from females and the environment, is rarely between two females simultaneously and is defined by more than proximity to, or sniffing of, females. Despite these discrepancies, we argue that our results are applicable to the field because we used wild-caught mice, and observed a clear lack of an effect of fluorescent powder marking in a simple situation with a limited number of cues and a single choice. Thus, in the field, putative mates and/or mating choice should not be affected by the use of fluorescent powder marking.

Acknowledgments

We thank Bob Young for access to Young's Tree Farm where the animals for this study were captured. M. Brimson provided assistance with animal care. This manuscript was improved by the comments of R.C. Bailey, T.M. Laverty, A.G. McAdam, D.P. Moreno, A.I. Schulte-Hostedde, R.J. Gegear and the Small Mammal Ecology Discussion Group at the University of Western Ontario. This study complied with guidelines of the Canadian Council on Animal Care.

Literature Cited

Aars, J. , H. P. Andreassen , and R. A. Ims . 1994. The use of fluorescent pigments to establish mother-offspring relationships in small mammals. Mammal 58:649–655.

Barnard, C. J. and J. Fitzsimons . 1989. Kin recognition and mate choice in mice: fitness consequences of mating with kin. Anim. Behav 38:35–40.

Bowers, M. A. and H. D. Smith . 1979. Differential habitat utilization by sexes of the deermouse,. Peromyscus maniculatus. Ecology 60:869–875.

Coquelin, A. 1992. Urine-marking by female mice throughout their reproductive cycle. Horm. Behav 26:255–271.

Duquette, L. S. and J. S. Millar . 1995a. The effect of supplemental food on life-history traits and demography of a tropical mouse *Peromyscus mexicanus*. J. Anim. Ecol 64:348–360.

Duquette, L. S. 1995b. Reproductive response of a tropical mouse, *Peromyscus mexicanus,* to changes in food availability. J. Mammal 76:596–602.

Ebensperger, L. A. and R. H. Tamarin . 1997. Use of fluorescent powder to infer mating activity of male rodents. J. Mammal 78:888–893.

Getz, L. L. , D. F. Gudermuth , and S. M. Benson . 1992. Pattern of nest co-occupancy of the prairie vole *Microtus ochrogaster* in different habitats. Am. Midl. Nat 128:197–202.

Gubernick, D. J. and T. Teferi . 2000. The adaptive significance of male parental care and the evolution of monogamy in a mammal. Proc. Roy. Soc. Lond B 267:147–150.

Hallman, T. M. , L. C. Adams , D. J. Mullins , and J. R. Tester . 1993. Duration of effectiveness of fluorescent pigment when tracking small mammals. Can. Field-Nat 107:370–372.

Jacobs, G. H. 1993. The distribution and nature of color among the mammals. Biol. Rev 68:413–471.

Kalcounis-Rüppell, M. C. 2000. Breeding systems, habitat overlap, and activity patterns of monogamous and promiscuous mating in *Peromyscus californicus* and *P. boylii*. Ph.D. dissertation, University of Western Ontario. 185 p.

Kaufman, G. A. 1989. Use of fluorescent pigments to study social interactions in a small nocturnal rodent, *Peromyscus maniculatus*. J. Mammal 70:171–174.

Kavaliers, M. and D. M. Kinsella . 1995. Male preference for the odors of female mice is reduced by the neurosteroid pregnenolone sulfate. Brain Res 682:222–226.

Kumar, A. and C. J. Dominic . 1993. Male-induced implantation failure (the Bruce effect) in mice: protective effect of familiar males on implantation. Physiol Behav 54:1169–1172.

Lemen, C. A. and P. W. Freeman . 1985. Tracking mammals with fluorescent pigments: a new technique. J. Mammal 66:134–136.

Liebenauer, L. L. and B. M. Slotnick . 1996. Social organization and aggression in a group of olfactory bulbectomized mice. Physiol. Behav 60:403–409.

Millar, J. S. , E. M. Derrickson , and S. T P. Sharpe . 1992. Effects of reproduction on maternal survival and subsequent reproduction in northern *Peromyscus maniculatus*. Can. J. Zool 70:1129–1134.

Mullican, T. R. 1988. Radio telemetry and fluorescent pigments: a comparison of techniques. J. Wildl. Manag 52:627–631.

Ribble, D. O. 1991. The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. Behav. Ecol. Sociobiol 29:161–166.

Ribble, D. O. and J. S. Millar . 1997. The mating system of northern populations of *Peromyscus maniculatus* as revealed by radio telemetry and DNA fingerprinting. Ecoscience 3:423–428.

Singer, A. G. 1991. A chemistry of mammalian pheromones. J. Steroid Biochem 39:627–632.

Stapp, P. , J. K. Young , S. Vandewoude , and B. Van Horne . 1994. An evaluation of the pathological effects of fluorescent powder on deer mice (*Peromyscus maniculatus*). J. Mammal 75:704–709.

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. New Jersey: Prentice Hall. 718 p.

Table 1. Mate choice by males between two females is consistent pre- and postfluorescent powder treatment. The number of males (of 26 total) in each replicate that were consistent (C) or nonconsistent (NC) is shown. Expected values are 13 and 13, because previous choice should not affect the result. For all replicates df = 1; (**P < 0.01; ***P < 0.001)

Trial	C	NC	Chi-square	P	Trial	C	NC	Chi-square	P
1	24	2	18.61	***	9	24	2	18.61	***
2	26	0	26	***	10	25	1	22.15	***
3	23	3	15.38	***	11	24	2	18.61	***
4	25	1	22.15	***	12	26	0	26	***
5	22	4	12.46	***	13	23	3	15.38	**
6	24	2	18.61	***	14	21	5	9.84	***
7	23	3	15.38	***	15	25	1	22.15	***
8	22	4	12.46	***	16	24	2	18.61	***