

**Stranger Danger: the influence of scent cues on the foraging
behavior of *Peromyscus maniculatus gracilis***

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Laura M. Matthews

Mentor: Dr. Michael J. Cramer

Abstract

The “dear enemy” effect is a phenomenon observed among many species in which individuals show significantly less aggression toward familiar individuals with neighboring territories than to strangers. However, this phenomenon can vary among species and territory type, as it is generally observed more frequently in nesting or breeding territories than in foraging areas. Most related studies on mice have focused on the role of this phenomenon in sexual competition; this study seeks to investigate the effect by comparing how neighbor and stranger scent cues affect the foraging decisions of *Peromyscus maniculatus gracilis*. Response to scent cues of familiar and unfamiliar mice were not significantly different, possibly due to weakness in the scents used; further research should explore this facet of foraging behavior.

Introduction

Many animals—particularly vertebrates—establish and defend a territory for feeding, breeding, resting, or other survival purposes. This territory can be marked by sound, such as through birdsong, or scent, which in mammals often comes from skin, fur, scent glands, feces, and urine (Apfelbach et. al 2005). Territorial animals may respond to intruders with aggressive displays or attacks; however, the degree of aggression can depend on the type of territory being defended, as well as the status of the intruder as a neighbor or stranger (Temeles 1994). This phenomenon is known as the “dear enemy” effect, in which animals show less aggression toward neighboring animals when defending breeding territories, though this effect may be reduced or absent in feeding territories (Temeles 1994).

The “dear enemy” effect allows animals to reduce energy expenditure and health risks by avoiding unnecessary fights, as known neighbors with their own territories are considered less likely to pose a threat. First observed in birds by Fisher (1954), studies have shown the phenomenon to appear in a variety of other species, including deer mice, cichlids, and salamanders (Whitaker & Hamilton 1998, Leiser & Itzkowitz 1999, Jaeger 1981). However, the

presence of the “dear enemy” effect varies among species and appears to depend on the type of territory being defended, as nest territories tend to be more aggressively guarded than foraging areas (Temeles, 1994).

Mice live in high-density populations where territoriality plays an important role in group structure. The territories of *Peromyscus maniculatus* vary from 240 to up to 3000 m² and are characterized by a single dominant male territory owner, with home ranges of females overlapping the territories of neighboring males (Hurst 2005, Whitaker & Hamilton 1998). The social structures of *P. maniculatus* depend on neighbor recognition and aggression toward intruders; the mice typically defend a core territory area and show less aggression on the periphery of their home range, which often overlaps with the territory of other individuals (Wolff, Freeberg, & Dueser 1983, Whitaker & Hamilton 1998). Although direct interactions can demonstrate levels of aggression between individuals, territories are primarily marked by scent cues, especially from urine—which, particularly through major urinary proteins (MUPs) and major histocompatibility complexes (MHCs), can be used to identify individuals by a unique chemical signature (Cavaggioni et. al 2008, Yamazaki et al. 1976). These scent marks serve an important role in intrasexual competition, as dominant males enforce their boundaries with frequently renewed scent marks and attack males that place competing scent marks, while females select for the more competitive males that manage to keep competitor scent marks out of their territory (Hurst & Beynon 2004). However, although the “dear enemy” effect has been investigated thoroughly in the context of sexual competition, its influence on foraging behavior requires further research.

To identify the presence or absence of the dear enemy effect on *P. maniculatus gracilis* foraging, a recent study by McNally (2014) exposed subject mice to the presence of both a

neighbor mouse and a stranger. The study found that foraging preference varied between study sites; mice from one grid preferred neighbors, while another grid showed a preference for strangers, and a third seemed to exhibit no preference. However, the study did not find a significant overall effect for the species.

This study will explore the role of scent cues on *P. maniculatus* foraging behavior by placing scent cues—obtained from neighbor mice captured on the same grid or strangers from a different grid—near food sources and observing subject behavior. The null hypothesis is that the scent cues and will have no effect on foraging decisions, while the alternative hypothesis is that the mice will have a more negative foraging response to the stranger scent than to the neighbor or control, as the mice might wish to avoid the greater potential for aggression from strangers.

Methods

Trapping

Members of the deer mouse species *Peromyscus maniculatus gracilis*, which are abundant to the area, were trapped on the UNDERC property in the Upper Peninsula of Michigan. Sherman live traps were baited with sunflower seeds and millet and set spaced 15 meters apart in five, 60x60 meter grids. Each trapped mouse was tagged with metal ear tags, and their location, condition, sex, weight, size, and age were recorded. Juveniles and lactating females were released immediately to not keep mothers from their offspring for ethical reasons. Furthermore, pregnant females were not used in order to avoid the Bruce Effect, a phenomenon in which pregnant mothers abort their offspring when exposed to the scent of stranger males (Bruce, 1959). Adults usable for the experiment were housed in the laboratory for 4-6 days and then released to the area of capture after testing.

Handling

Subjects were housed in cages in the mammal room of the wet lab. Cages with mice from the same grid were kept next to each other, while those from different grids were separated by a short distance to prevent familiarization; additionally, the pane of the fume hood in which some cages were kept was lowered to inhibit the mixing of scents in the air.

A set of tubes was used for scent cue collection and transportation between the trial aquariums and cages in order to minimize stress and scent from human handling. A PVC pipe about 30 cm long with a funnel at the end was used to catch the subjects, which were then released into the middle of the trial aquarium or prompted into a centrifuge tube for scent collection. A cotton applicator was then stuck through the top of the tube to swab the flank of the mouse for several seconds; a hole at the end of the centrifuge tube also allowed for some cheek swabbing, though mice often backed out of range or simply bit the swab.

Experimental Set-up

During each trial, the subject mouse was placed in one of two aquaria divided into three equal sections (Figure 1). Each aquarium floor was covered in a layer of sand, and the sections on the sides each contained a small tin weigh boat filled with 5g of sunflower seeds, as well as a cotton applicator. During each trial, one applicator contained no scent while the other had swabbed a stranger mouse or a mouse from the same grid—presumed to be a “neighbor”—as the subject. The scent treatment for each side section and aquarium was randomized using a random number generator. The middle section served as a buffer between the two treatment areas. The aquarium was covered in paper on three sides to reduce outside visual cues, while the fourth was

open and used to observe the mouse's behavior by infrared video. Trials took 30 minutes and were conducted in a dark room between 11:00 pm and 2:00 am, when the mice would be most active. One trial was performed with each subject mouse, and two trials were conducted per night. The stranger treatment was tested first, and then the subject of that trial was used to collect the scent for the subsequent neighbor trial in order to reduce handling stress. The mass of seeds and shells left in and around each tin was then weighed after the trials; trial videos were used to determine the origin of any seeds scattered into the middle section, as well as to observe subject behavior.

Statistics

The data from this experiment was recorded as mass of seeds eaten from each tin. The differences in seeds eaten between the scent treatments and control were analyzed with a paired t-test for each treatment type (stranger and neighbor) to see if either scent had an effect; the paired t-test was used to control for variation among individuals, as each subject ate from both a control and a treatment tin. Furthermore, the difference in seeds eaten between the two tins used in each trial was calculated by subtracting the treatment mass eaten from the control mass eaten, and this data was analyzed with a two-sample t-test to compare the responses to the scent treatments. Normality was confirmed by plotting residuals and running a Shapiro-Wilk test. All statistical tests were run in RStudio.

Results

A total of twenty trials were run, with ten for each scent treatment. Amounts of seeds consumed was similar regardless of scent treatment (Figure 2). From the data collected, the

mean mass of seeds consumed from the control tins appeared greater than the mean of those consumed from the tins with the neighbor scent cue (mean \pm SE; control 0.490 ± 0.065 g; neighbor 0.389 ± 0.049 ; $t_9 = 1.2797$, $p = 0.23$); the mean mass of seeds eaten from the tin with the stranger scent appeared greater than the mean mass eaten from the control tins (mean \pm SE; control 0.342 ± 0.068 g; stranger 0.379 ± 0.048 ; $t_9 = -0.45396$, $p = 0.66$). However, any observed patterns were not statistically significant.

The differences in the responses were then compared by subtracting the amount eaten from each scent treatment tin from the corresponding controls (Figure 3). These differences were not statistically significant (mean \pm SE; neighbor 0.102 ± 0.079 ; stranger -0.036 ± 0.089 ; $t_{18} = 1.1593$; $p = 0.26$).

Discussion

Ultimately, the results of this study did not reveal any significant effects from scent cues on the foraging decisions of *Peromyscus maniculatus*. Though the subjects did eat more from the control tins than those with neighbor scent, and—contrary to the hypothesis—more from the tin with the stranger scent cues than from those corresponding controls, these differences were not statistically significant ($p > 0.05$). This may suggest that such scent cues are simply more important for defending established home territories than making foraging choices; the home ranges of *P. maniculatus* do tend to overlap, and thus it would be reasonable for mice to be used to encountering others while foraging (Whitaker & Hamilton 1998). However, no conclusions can be drawn for certain.

All trials were recorded by video, allowing for viewing of subject behavior during the trials. Most subjects spent a significant portion of their trials attempting to escape by jumping or

digging. Little time appeared to be spent investigating the swabs with the scent cues; unfortunately, this may indicate that the scent samples were not very strong. However, mice have a keen sense of smell particularly due to their specialized vomeronasal organ, which allows for recognition of individuals through urinary proteins (Keverne 1999, Hurst & Beynon 2004). A potential issue with this study could be that the swabbing did not always pick up the proteins necessary for recognition; as the mice tended to move around during scent collection, swabbing often focused on the more easily accessible flank than the groin. Any future repetitions of this experiment would likely benefit from more consistently targeting for a urine sample; alternatively, the litter from the cages the mice are kept in may provide a stronger scent source.

It would also be interesting to explore how a subject's own scent cues might affect their foraging behavior; the aquaria in the labs are, of course, not the home territory of the mice tested, but the presence of their scent could potentially simulate markers of their own territory, which could likely influence their foraging decisions. In any case, further research into the role of scent cues could provide insight for the social interactions among *P. maniculatus*, as well as other mammals. For example, video recordings of feeding trays placed in their natural habitats could be used to observe how the mice might interact with each other while foraging. Such social interactions are an integral part of understanding population dynamics in a given habitat or region. And as with most of the natural world, much remains to be explored.

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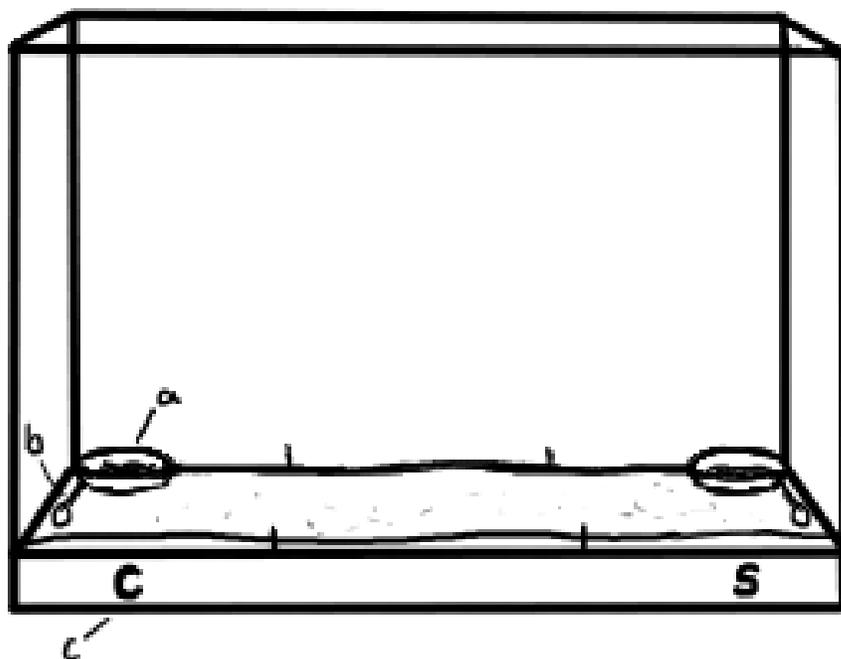
Figures

Figure 1. *Trial aquarium.* Each aquarium was divided into three sections; each side section contained a tin of seeds (a), a cotton applicator (b), and labels for each treatment (c), while the middle section was left bare. .

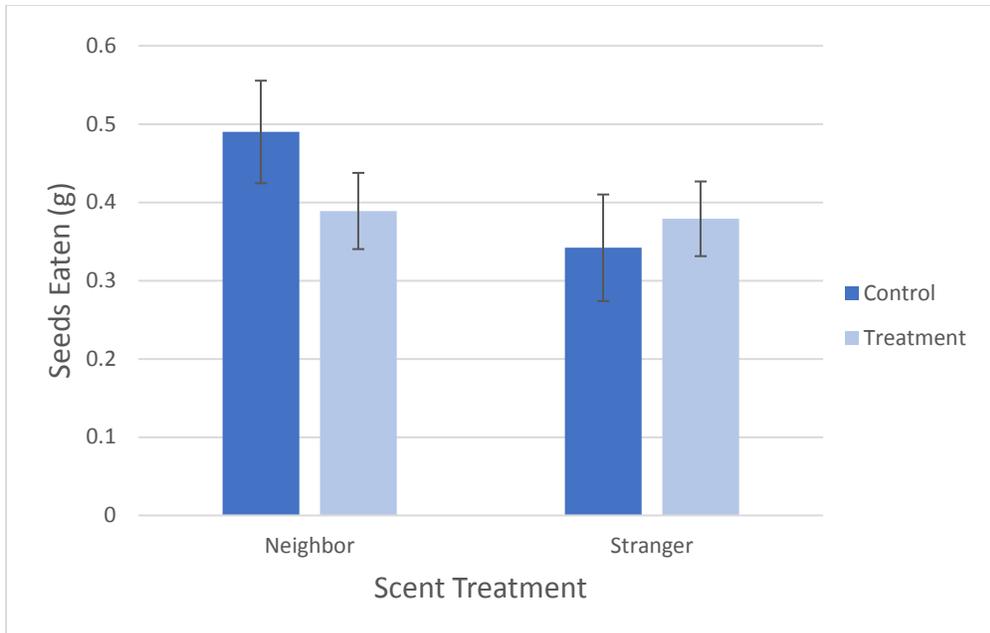


Figure 2. Average mass of seeds eaten from each treatment ($g \pm SE$). A paired t-test was run for the trials in each treatment group; neither neighbor nor stranger treatment appeared to have a significant effect (neighbor: $t_9 = 1.2797$, $p = 0.23$; stranger: $t_9 = -0.45396$, $p = 0.66$)

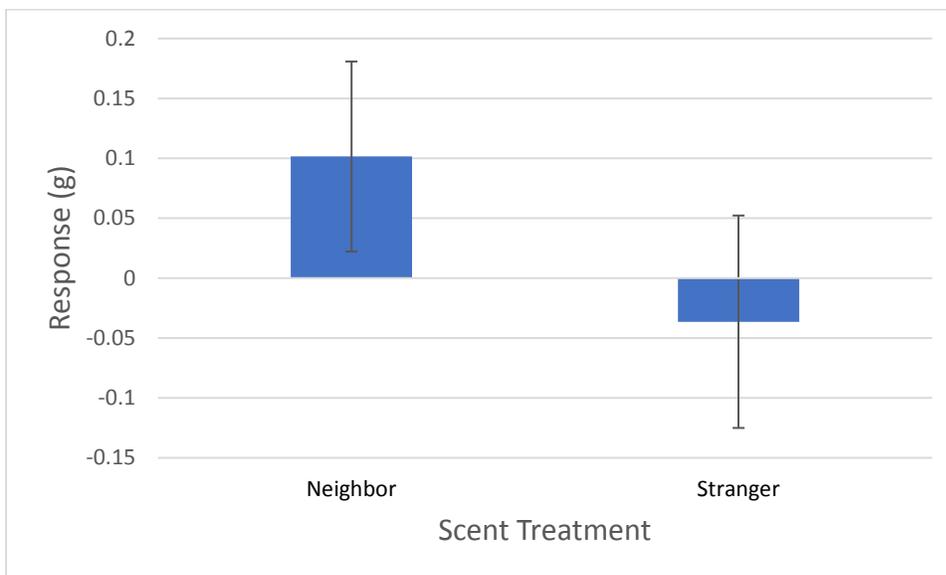


Figure 3. Foraging response to scent cues ($g \pm SE$). The response to scent cues was measured as the difference in the mass (g) of seeds eaten between treatment tins and the controls, using a two-sample t-test to compare the responses; differences were not statistically significant ($t_{18} = 1.1593$, $p = 0.26$).