Scent Communication in White-footed Mice

An Honors Thesis (HONRS 499)

by

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PURPOSE OF THESIS

Scent communication is an important component of behavior in mammals. Scent markings have been hypothesized to communicate a variety of messages, among them reproductive condition, ownership of territory and dominance status. This project examines the response to scent markings in the white-footed mouse, *Peromyscus leucopus*. In particular, this experiment tests whether or not dominance information is communicated by scent markings.
INTRODUCTION

Mammals tend to be very social creatures. As social creatures, they have evolved advanced modes of communication, such as a highly developed visual system, or finely tuned audio perception. Among rodents, the largest order of mammals (Whitaker, 1980), olfactory (scent) communication is perhaps the most important. Most rodents are nocturnal: visual communication isn't effective in the darkness of the night, and audio communication attracts the attention of predators. So, the rodents rely on olfaction to gather information about their environment.

Olfactory communication may be accomplished by marking. Scent marking is the deposition of chemical signals by one animal into the environment or, in some cases, onto another animal (Ralls, 1971). These chemical signals are found in urine, feces or glandular secretions, depending on the species in question (Ralls, 1971). Thus, the deposition of scent marks, and their subsequent detection by another animal, forms the basis of olfactory communication.

Scientists often debate the function of scent marking; several possibilities have been suggested. For example, scent marks may serve to:

1.) communicate ownership of territory;
2.) advertise sexual condition;
3.) label an animal's home range and assist in orientation and familiarity;
4.) indicate individual identity, such as age, species, and dominance status;
5.) alarm others of danger (Johnson, 1973).

Data supporting all of these possibilities has been collected (Thiessen and Rice, 1971).

Published evidence has confirmed the use of scent marking as territorial marking—that is, the marking of a home range that an individual will defend against rivals. However, research also suggests that mammals mark when dominant to others, not just when they possess a territory (Ralls, 1971). In one study, researchers found that male rats can discriminate between dominant and submissive males based on odors (scent markings) alone (Krames, Carr and Bergman, 1969). An individual cannot patrol its home range all the time. So, in effect, the scent markings
not only inform other individuals that an area is taken, but also the relative dominance status of the territory owner. In this way, potentially aggressive interactions may be avoided. This project explores this hypothesis; using white-footed mice, we have examined the response to scent markings and tested if scent communicates dominance information.

The white-footed mouse, *Peromyscus leucopus*, also known as the "wood mouse," is a common inhabitant of forests and fence rows in the Eastern United States. This mouse is active year-round, although they may hibernate in extremely cold weather. The white-footed mouse is omnivorous and makes its home in logs, underground burrows or similar concealed locations (Whitaker, 1980).

**METHODS**

The mice used in this experiment were obtained from Ball State University's Cooper Woods by live trapping. The traps were set in the late afternoon and retrieved the following morning. In all, 332 mice were collected in four seasons of sampling (from August of 1990 to August of 1991).

The mice were brought back to the laboratory (Cooper Science building) for investigation. Each specimen was categorized according to sex, reproductive condition and weight. (Mice that weighed more than 19 grams were considered adults, and mice that weighed less than 19 grams were considered juveniles.) Each mouse was also ear tagged for future reference in case of recapture.

The mice were then assigned to pairs. The two mice in a pair were assumed to be strangers to each other because their respective points of capture were greater than 150 meters apart. (For the purpose of clarity, one mouse of each pair will be designated the test mouse, and the other mouse will be the opponent.)
This is a general outline of the experimental procedure:

1.) Test the response of the test mouse to the unfamiliar scent of the opponent mouse;

2.) Measure "dominance status" by pairing the test mouse with the opponent and recording their behavior;

3.) Retest the response of the test mouse to the familiar scent of the opponent mouse—familiar because the two mice have interacted during the behavioral pairing (previous step).

A "T-shaped" maze was used to examine the test mouse's response to its opponent's scent (see diagram, Appendix A). At one end of the "T," a clean, unscented trap was set. At the opposite end, a scented trap was set. This scented trap contained the scent of the opponent mouse (the scent was deposited while the opponent was captured in the trap). At the bottom end of the "T," the test mouse was introduced into the maze. A fan was used to cap this end of the T-maze and draw air from the traps toward the test mouse. On the basis of olfactory cues, the mouse would choose either its opponent's scented trap or the clean, unscented trap.

The next step in the procedure was to introduce the two mice and determine which mouse was dominant and which was subordinate. This was done by observing the mice in an aquarium for 5 minutes; the following behaviors were scored each time they occurred:

**Approach** ............... movement to within 5 cm of an opponent
**Attack** ..................... biting or attempting to bite
**Avoid** ...................... movement away from an opponent which resulted from the presence of the opponent
**Chase** ...................... rapid pursuit of an opponent
**Close** ...................... remaining closer than 5 cm for more than 5 seconds
**Flight** ...................... rapid movement away from an opponent
**Following** ............... following an opponent as it walks
**Groom Self** ............. grooming behavior using the tongue and forelimbs
**Groom Opponent** .......... grooming or sniffing opponent

Following this five minute period of familiarization with its opponent, the test mouse was once again tested in the "T" maze. In this way, conclusions may be drawn by comparing the mouse's initial and final response to its opponent's scent. If dominance information is
communicated through scent marking, then the initial and the final response should be the same: the test mouse should recognize its opponent's status even before familiarization in the aquarium. If dominance information is communicated in another way, then the responses may differ: the test mouse might "learn" the scent of its opponent during familiarization.

RESULTS

In the initial introduction, 278 out of 332 mice (84%) chose the scented trap over the unscented trap. After familiarization with the opponent, only 232 mice (70%), chose the scented trap. These data have been organized in the following cross tabulation:

<table>
<thead>
<tr>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scented</td>
<td>Scented</td>
</tr>
<tr>
<td></td>
<td>Unscented</td>
</tr>
<tr>
<td>Unscented</td>
<td>Scented</td>
</tr>
<tr>
<td></td>
<td>Unscented</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi-square (McNemar test) = 18.08, \( p < .0001 \)

McNemar's test was used to determine whether or not the changes within the population sample were significant. If familiarization with an opponent had no influence on a mouse's response to the opponent's scent, then the number of animals that preferred scent before familiarization but not after should be approximately equal to the number that preferred scent after familiarization but not before; that is, the number of changers should be equal. This was not the case. A more than expected number of mice switched to the unscented trap after familiarization with its opponent. The number of changers in this population sample is significant (Chi-square=18.8, \( p < .0001 \)) and cannot be attributed to chance alone.

In order to pursue any hypothesis regarding the communication of dominance status, other variables must be considered. There are a significant number of mice that changed their response in the "T" maze, but they may be responding to something other than the dominance status of their opponent. An animal's response to scent in the maze may be a function of sex,
age, or opponent's sex. In addition, the response may be different in the reproductive season than in the non-reproductive season. For example, male mice may respond to female mice differently in the reproductive season than in the non-reproductive season.

A Log-linear logit model was used to explore these relationships. This model is a multivariate technique that is used to determine whether or not several independent variables are associated with a dependent variable.

In this case, the response in the maze (preference for scented or unscented) was used as a dependent variable and sex, age, reproductive season and opponent's sex were independent variables. For instance, the response of male mice might depend upon the season or the sex of the opponent.

The results of this test suggest that the initial response to scent was not associated with sex, age, reproductive season, or opponent's sex. A model that included only response to scent adequately explained the data (Chi square = 9.42, p = .854). Likewise, the response to scent after familiarization was not associated with the above variables (Chi-square = 8.39, p = .907).

It has been determined that the response to scent is not a function of sex, age, season or the sex of the opponent. Furthermore, there is a significant difference between the initial response and the post familiarization response. Is this difference associated with the dominance status of the mice?
In order to test this hypothesis, the data from the behavioral observations were used to determine dominance status. Factor analysis was used to reduce the 9 behavioral variables to a single dominance index (factor). This program reduces data to find significant correlations between factors. Factor 3 was highly correlated with attack and chase, indicating that it is a dominance factor:

**FACTOR LOADINGS ON FACTOR 3**

*(VARIMAX ROTATION)*

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Factor Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approach</td>
<td>-.08661</td>
</tr>
<tr>
<td>Attack</td>
<td>.72907</td>
</tr>
<tr>
<td>Avoid</td>
<td>-.18270</td>
</tr>
<tr>
<td>Chase</td>
<td>.73893</td>
</tr>
<tr>
<td>Close</td>
<td>-.20681</td>
</tr>
<tr>
<td>Flight</td>
<td>-.12935</td>
</tr>
<tr>
<td>Following</td>
<td>.06160</td>
</tr>
<tr>
<td>Groom Self</td>
<td>-.14499</td>
</tr>
<tr>
<td>Groom/Sniff Op.</td>
<td>-.25716</td>
</tr>
</tbody>
</table>

Scores from this factor were used to obtain a dominance index for each mouse; mice that attacked and chased frequently had the highest scores on this factor. These dominance scores were used in the following tests.

The test mouse's initial response to scent may depend upon its dominance status. Dominance scores for animals that initially chose the scent may significantly differ from scores for animals that initially avoided the scent. Dominance may also be associated with reproductive season. A two-way Analysis of Variance (ANOVA) was used to explore these relationships. The ANOVA procedure compares the means of the two groups and determines whether the difference between those means is significant. The comparison is between the mean dominance index of those mice that initially chose the scented trap versus the mean dominance index of those mice that initially avoided the scented trap. These means were analyzed in relation to the reproductive season.
**ANOVA - Dominance Index**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAIN EFFECTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-familiarization response</td>
<td>1</td>
<td>.143</td>
<td>.142</td>
<td>.707</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>.471</td>
<td>.468</td>
<td>.495</td>
</tr>
<tr>
<td>2-WAY INTERACTION</td>
<td>1</td>
<td>.331</td>
<td>.329</td>
<td>.567</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>326</td>
<td>1.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These numbers suggest that there is no significant difference between the means of the groups compared: Animals that exhibited dominant behaviors did not respond differently to a strange odor than did subordinate animals. Because season and the response by season interaction were not significant, there is no seasonal effect.

The previous test concerns the initial response to scent (pre-familiarization). Because there was a greater tendency to avoid scent after familiarization, a two-way ANOVA was used to determine if the final response was associated with dominance status or reproductive season.

**ANOVA - Dominance Index**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAIN EFFECTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-familiarization response</td>
<td>1</td>
<td>.551</td>
<td>.548</td>
<td>.460</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>.477</td>
<td>.474</td>
<td>.491</td>
</tr>
<tr>
<td>2-WAY INTERACTION</td>
<td>1</td>
<td>.275</td>
<td>.274</td>
<td>.601</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>326</td>
<td>1.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Once again, neither the main effects nor the interaction (reproductive season and response) was significant.

The primary interest of this project is whether or not dominance information is communicated by scent marking alone. If mice are able to determine the dominance status of an unfamiliar animal based on odor cues, then the difference in dominance status (of the test
mouse and the opponent) should be associated with response to the scent of an unfamiliar animal. The above tests only take into account the dominance status of the test mouse. But dominance is essentially a relative quality—a mouse is only dominant in comparison with another mouse. This final test takes into account the dominance status of the test mouse and the opponent mouse: the dominance status of one is subtracted from the other to arrive at a difference index.

**DIFFERENCE INDEX =**

\[ \text{Opponent's Dominance Score} - \text{Test Mouse's Dominance Score} \]

This hypothesis was also tested using a two-way ANOVA of difference index with response and reproductive season.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAIN EFFECTS</td>
<td>2</td>
<td>.165</td>
<td>.115</td>
<td>.891</td>
</tr>
<tr>
<td>Post-familiarization response</td>
<td>1</td>
<td>.331</td>
<td>.230</td>
<td>.632</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>.002</td>
<td>.001</td>
<td>.974</td>
</tr>
<tr>
<td>2-WAY INTERACTION</td>
<td>1</td>
<td>1.150</td>
<td>.801</td>
<td>.372</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>326</td>
<td>1.437</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Neither effect nor interaction was significant. A mouse's response to scent was not associated with the relative dominance status of the opponent.

**DISCUSSION**

The response of white-footed mice to scent marking was not associated with age, sex, reproductive season, or the sex of the scent donor. A significant number of mice changed their response to scent after familiarization with the opponent; mice preferred the scented trap over the unscented trap, but animals familiar with the scent donor showed less preference for the scented trap. The response to scent, whether familiar or unfamiliar, was not associated with the dominance status of the test mouse or the opponent mouse. Furthermore, the response to
unfamiliar scent was not associated with the relative dominance status of the opponent.

Overall, these results suggest that the period of familiarization alters the response of some mice to scent markings. However, there is no evidence to suggest that scent markings alone communicate dominance information. These findings contradict the research of Krames, Carr and Bergman (1969), who found that male rats can predict the dominance status of other males based on scent markings alone.

From an evolutionary point of view, one would not expect scent markings alone to communicate dominance status. If scent markings function to secure a territory by communicating an owner's dominance status, then "cheating" would be expected to evolve in subordinates and non-territory holders. Through natural selection, cheaters would be able to produce a scent that resembles that of a dominant animal and thus claim ownership of a territory without truly being dominant.

If dominance information was communicated by scent marking, then subordinates would avoid the territories of more dominant individuals. But Gosling (1990) has suggested that territorial marking probably does not function to prevent intrusion because animals without territories are frequently seen on marked territories. The scent marks probably serve to teach intruders the scent of the owner for future recognition. As our experiment has suggested, it may be that mice are able to "learn" the dominance status of other mice through the process of familiarization; perhaps they are able to associate an opponent's scent with the opponent's dominance status during a behavioral interaction. Whatever the case, the research presented here has eliminated some possibilities and created some new ones.

ACKNOWLEDGMENTS

I would like to thank Dr. Michael Gregory for all of his guidance in the completion of this project. Also, my sincere thanks to Mike Short for his help in the printing of this document.
Appendix A

A diagram of the T-maze used to conduct scent communication experiments.

"UNSCENTED" TRAP

"SCENTED" TRAP

INTRODUCE MOUSE

CAP

FAN
LITERATURE CITED


