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Termite Trail-Following Behavior Elicited by Ballpoint Pen Ink

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Abstract

Termite trail-following behavior occurs because of pheromones produced in the sternal gland of the termites and secreted when the sternal gland touches a substance. 2-phenoxyethanol, a chemical found in ballpoint pen ink, is structurally similar to the trail-following pheromones and causes the termites to respond with trail-following behavior. This study investigated the difference in trail-following behavior between ballpoint pen ink, which contains 2-phenoxyethanol, and Sharpie ink, which does not contain 2-phenoxyethanol. The termites were placed on a square drawn with either ballpoint pen ink or Sharpie pen ink. Time spent on each of the squares was measured. We predicted that termites would spend more time with the ballpoint pen ink and less time with the Sharpie ink, due to the presence of 2-phenoxyethanol. Our class results show that there is a significant difference between time spent on the ballpoint pen ink condition and time spent on the Sharpie ink condition, which supports our prediction and is supported by the results of previous studies. While our class results are significant, our group results are not significantly different between the ballpoint pen ink condition and the Sharpie ink condition, due to a lower sample size which is easily skewed by outliers.

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It is well known that termites' sternal gland produces trail-following pheromones (1-3). The sternal gland discharges pheromones when it presses against a substance (1). There is a chemical in ballpoint pen ink that is similar in composition to the trail-following pheromones, called 2-phenoxyethanol (1,4,5). This compound elicits trail-following behavior in termites as do the pheromones.

Chen et al. (4) demonstrated that termites are attracted to pen ink because of the 2-phenoxyethanol present in it. The experiment involved isolating 2-phenoxyethanol from pen ink and using it to draw circles. The termites were then timed for how long they spent following the 2-phenoxyethanol circle (4). Costa-Leonardo et al. (1) explained that trail-following pheromones are produced when exploring new territories. Other termites will then follow this pheromone in order to find food sources. The 2-phenoxyethanol may produce behavior related to trail-following behavior because both the compound and trail-following pheromones are pure alcohols (1,4). 2-phenoxyethanol has also been studied when combined with nonrepellent insecticides. Since 2-phenoxyethanol is an attractant to termites, Fei et al. (5) investigated the effect that it would have on trail construction in combination with three different nonrepellent insecticides. The termites constructed more trails when 2-phenoxyethanol was present than in the absence of it. The 2-phenoxyethanol was also potent enough that termites continued using its trail for 13 weeks (5).

Gazal et al. (2) looked at behavioral and chemical stimuli and how these stimuli affected the trail-following behavior of termites. The trails that the termites constructed using their pheromones were in continuous use for 48 months because the pheromones were continuously layered on the trail, making the trail more potent. Gazal et al. (2) found that termites preferred to

follow their own trail rather than the behavioral trail made of extracted feces of dead termites, or the chemical trail, made of extract from sternal glands of dead termites. These results provide evidence that there is more at work in termite trails than singularly the trail-following pheromones (2). Tokoro et al. (3) investigated if there were specific trail-following pheromones that differed between termite species. They found that termites prefer to follow the trail-following pheromones of their species rather than those of other species of termites (3).

The previous studies form a basis for the current study, which investigates if termites will spend more time on a square drawn with ballpoint pen ink, which has 2-phenoxyethanol, or a square drawn with Sharpie ink, which does not have 2-phenoxyethanol. We predict that the termites will spend more time on the pen ink square because of the presence of 2-phenoxyethanol which will cause trail-following behavior similar to the behavior caused by termite trail-following pheromones.

Method

One hundred *Reticulitermes flavipes* (hereafter referred to as termites) were supplied by Dr. Theodoraki. Termites were housed in containers at room temperature with wood and moistened paper towels until testing. At test they were moved to small tubes along with wood and moistened paper towels. Each termite was used for one trial and then transferred to a "used" tube also with wood and moistened paper towels.

We used blue ballpoint BIC Roundstic pens and blue Sanford Sharpie Extra Fine Point Markers as the ink treatments for our experiment. In the middle of a large, standardized sheet of paper, we drew and filled in one 1cm x 1cm ballpoint pen ink square, left 5 cm of empty space, and drew and filled in one 1cm x 1cm Sharpie ink square. Termites were divided into two treatment conditions – ballpoint pen ink (n = 50) and Sharpie ink (n = 50). Each termite was

placed directly onto the square corresponding to its ink condition. Each termite was used once in one of the conditions. Ten termites utilized each square, 20 termites per paper. They were timed for how long they remained on the square and were removed from the paper after 60 seconds. Small paintbrushes were used to place, keep, and remove the termites from the paper.

We manipulated ink type and measured the time each termite spent on their ink condition (either ballpoint pen ink or Sharpie ink) in seconds out of a minute to test our hypothesis and see if our prediction that termites will follow the ballpoint pen ink more was correct, performing one replication using the 100 termites. We standardized the paper used, the size of the squares, distance between squares, and which square was on which side of the paper (ballpoint pen ink square on the left, Sharpie ink square on the right), and the time each termite was left on the paper (Table 1).

Table 1. Experimental design.

Key Observation	Termites followed ballpoint pen ink more than pencil or Sharpie ink.
Hypothesis	Termites will follow one ink more than the other ink.
Prediction	Termites will follow ballpoint pen ink more than Sharpie ink because of the presence of 2-phenoxyethanol.
Variables	
Independent	Ink Type (Ballpoint Pen or Sharpie)
Dependent	Time (seconds)
Standardized	Size of: paper, squares, distance between squares, and which square was on which side of the paper; color of ink; time limit
Components of Procedure	
Levels of Treatment	2
Replications	1
Sample Size	Class n = 50, Group n = 30
Control Treatment	None

Results

We designed our experiment from our original observations of termite behavior. We observed that termites followed pen ink more than they followed the Sharpie ink or pencil, but

we were unsure if that was due to the color of the inks or the ink itself, because our ballpoint pen was red while our Sharpie ink was blue. We then hypothesized that the termites will follow one ink more than the other when the ink color was standardized. Our prediction was that termites would follow the ballpoint pen ink more, due to the presence of 2-phenoxyethanol in the ballpoint pen ink as opposed to the absence of 2-phenoxyethanol in the Sharpie ink. We then ran our experiment and analyzed the data, which was combined into class data and split into group data.

An independent samples *t*-test was conducted using the class data to compare time spent on the squares in the ballpoint pen ink condition and in the Sharpie ink condition. There was a significant difference at a 95% confidence interval between the ballpoint pen ink condition (M = 9.49, SD = 10.65) and the Sharpie ink condition (M = 4.87, SD = 9.28); $t(98) = 2.31$, $p = .023$. These results support our prediction that termites prefer pen ink to Sharpie ink (Figure 1, Table 2).

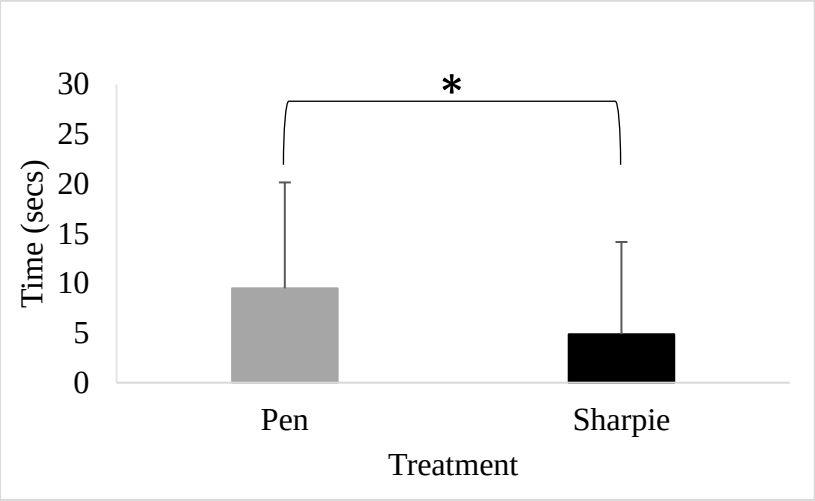

Figure 1. Mean time spent in each treatment condition with error bars showing the positive standard deviations using the class data. There is statistical significance between the treatment conditions.

Another independent samples *t*-test was conducted using the group data to compare time spent on the squares in the ballpoint pen ink condition and in the Sharpie ink condition. There was not a significant difference at a 95% confidence interval between the ballpoint pen ink condition (M = 7.39, SD = 10.48) and the Sharpie ink condition (M = 5.48, SD = 9.27); $t(58)$ = .75, p = .459. These results would suggest that termites did not have a preference between the inks (Figure 2, Table 3).

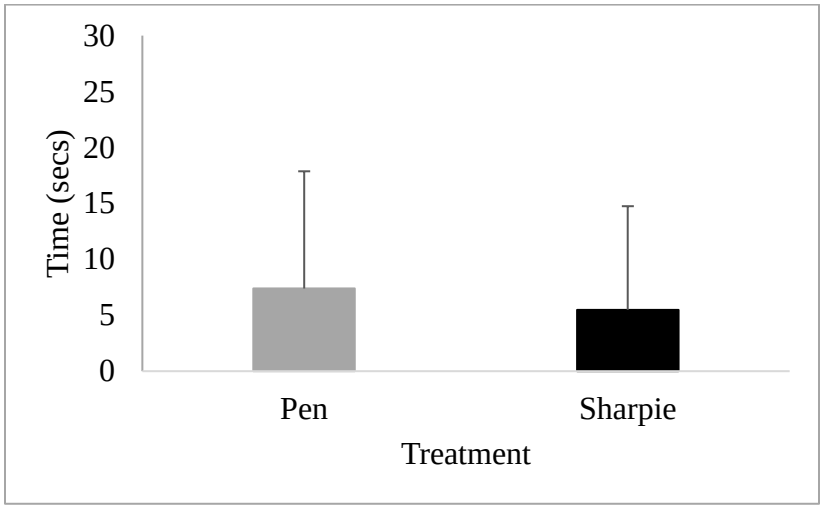

Figure 2. Mean time spent in each treatment condition with error bars showing the positive standard deviations using the group data. There is no statistical significance between the treatment conditions.

Table 2. Statistical class values.

t-calculated	t = 2.312
t-critical	t = 1.990
Degrees of Freedom	98
Confidence Interval	95%
Significance	p = .023
Sample Size	n = 50

Table 3. Statistical group values.

t-calculated	t = .75
t-critical	t = 2.009
Degrees of Freedom	58
Confidence Interval	95%
Significance	p = .459
Sample Size	n = 30

Discussion

Our class results supported our prediction that termites preferred the ballpoint pen ink condition to the Sharpie ink condition. This is most likely because of the presence of 2-phenoxyethanol which has a similar structure as the pure alcohol trail-following pheromone (1,4). This elicits trail-following behavior and, in our experiment, caused the termites to remain on the ballpoint pen ink condition significantly longer than they remained on the Sharpie ink condition in the class results. Contrarily, the group results do not support our prediction. This is most likely because our sample size was smaller, thus outliers will cause the data to be skewed and not significant.

Our class results support the results of Chen et al. (4) and Fei et al. (5) and the review of Costa-Leonardo (1). We found that termites would remain on the ballpoint pen ink box longer than they would remain on the Sharpie ink box because of the presence of 2-phenoxyethanol present in the ballpoint pen ink. Chen et al. (4) also found that ballpoint pen ink caused trail-following behavior in termites (4). Fei et al. (5) found that when 2-phenoxyethanol was present, there was more trail-following and trail constructing behavior than in the absence of 2-phenoxyethanol (5). The review by Costa-Leonardo et al. (1) described the way that 2-phenoxyethanol and trail-following pheromones are structurally similar and this similarity causes termites to respond with trail-following behavior (1).

Our study did not measure how many times the termites would walk around the squares, a behavior that they performed often. Replications of this experiment should include a way to measure this, possibly by drawing a border box a half centimeter around the perimeter of the original box and separately timing the amount of time they spend walking in the perimeter. There is also likely an effect of reaction times between saying to start or stop the timers and pressing the start and stop buttons on the timers. Our study also did not consider what else may be

affecting the termite trail-following behavior in trails that they design themselves rather than experimentally designed trails. This could include species-specific trail-following pheromones or additions of other pheromones to the trail (2,3). Future experiments should compare the ballpoint pen ink trail with a trail made of the species-specific pheromone and with a trail that termites make themselves. This would involve isolating the species-specific pheromone of the termites (3) and allow the termites to create their own trail (2), showing if there is a difference between the 2-phenoxyethanol trail-following behavior and that of the termites' pheromones or trail.

We also questioned if there was an effect on the termites from the Sharpie fumes, since some of the termites would stay on the Sharpie box for extended periods of time. Also, some termites needed to be corralled in from the sides of the paper and other termites did not move when placed on the paper. This caused substantial outliers in our data. To solve this problem, replications of the experiment should use a larger sample than our experiment. There may be an additional effect of trail-following pheromones that the previous termites left behind on the paper or on the squares, which became stronger as the trials progressed due to other termites leaving more pheromones. This means that the termites that participated in the later trials may have been more attracted to the squares because of the previous termites leaving pheromones (2). To judge this, replications of this experiment should separate the termites that are run on one of the squares into two groups – the first group (first half of the termites to be put on the square) and the second group (second half of the termites to be put on the square). They should then run the termites individually as our experiment did, and run two *t*-tests, one to compare average times between the squares and one to compare average times between the first and second groups of each square. This would show if there is an effect on the second group of termites due to the pheromones of the first group of termites. Additionally, we do not believe that there would be an

effect due to the age of the termites, since trail-following is an innate, rather than a learned, behavior (1,4). Finally, the paintbrushes used to corral the termites may have picked up the scents from the squares or the termites' pheromones and caused the termites to wander in search of the trail made by the paintbrush. This would cause an effect of termites being attracted to the a different trail rather than being attracted to the 2-phenoxyethanol in the ballpoint pen ink square.

Future experiments should look for the specific trail-following pheromone in *Reticulitermes flavipes* and see how it relates to the chemical compound of 2-phenoxyethanol to reexamine why termites will follow ballpoint pen ink (3). This could be done using the isolation and identification method that Tokoro et al. described with the species *Reticulitermes flavipes* (3). The isolation process begins with removing and drying the sternal glands of the termites. Then there are multiple fractionations: silica gel column chromatography, argentation silica gel column chromatography, high performance liquid chromatography, and gas chromatography. The pheromones are then identified with capillary gas chromatography-mass spectrometry and capillary gas chromatography-Fourier transform infrared spectrometer (3). This identified pheromone could then be compared to related species' trail-following pheromones and to the chemical composition of 2-phenoxyethanol to better understand why termites are attracted to and follow the 2-phenoxyethanol.

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