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Behavioral Syndromes in Individual Honeybees

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Behavioral Syndromes in Individual Honeybees (*Apis mellifera)*

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Abstract

Behavior can be defined as a response to a stimulus due to an individual's unique genotype and environment. As behaviors are expressed across contexts and over time it becomes personality. While personality has been studied extensively in vertebrates, it is only beginning to be investigated in insects. Considering the ecological importance of honeybees (*Apis mellifera*), it is important to learn whether or not, and how behavior influences their personality. In light of current concern regarding the welfare of honeybees, studies regarding their behavior may provide vital information about their responses to environmental stressors. In my study, I used a series of assays to quantify behavioral patterns. A shy-bold continuum of personality is recognized in many animals. I measured behaviors along this continuum in honeybees to determine if there are behavioral differences among individuals within a colony. I found correlations that suggest that individual bees may display behavioral differences. I was able to investigate differences among bees, but not behavioral trajectories over time. This study provides an initial look into how behavioral syndromes differ between individual honeybees within the same colony. Potential future research that follows bees from their first day as adults and throughout their lives will be necessary to augment the data collected here and provide information that may lead to the idea of personality in honeybees. As we continue to learn more about behavior and personality in honeybees it may become possible to provide ecosystem services that increase the fitness of the colony.

Background: Honeybee Biology

The western honeybee, *Apis mellifera*, evolved in Africa, Europe, and the Middle East and has been transported by humans to nearly all temperate and tropical terrestrial habitats (Breed, 2010). In their native range, bees are important pollinators and food sources for a variety of animals. In the habitats to which they have been introduced, bees have displaced native pollinators and are critical in the pollination of fruits and vegetables (Breed, 2010). Honeybees generally live in colonies of about 30,000 bees, but in mid-summer colonies can reach populations of 100,000 bees (Winston, 1987). Each colony contains a single queen and a large number of female workers. Males, or drones, are specifically raised to mate with the queen and do not contribute to the work within the colony. During the first few days of a queen's life, she mates with 10-15 drones and stores the sperm for the rest of her life (Winston, 1987). This generates genetic diversity among the workers as they are still all half- or full sisters. Full sisters are sometimes called super-sisters as they are, on average, related by 75% as all sperm from one male are identical. After new queen eggs are laid, the old queen departs from the hive with half of the workers in a process called colony fission or swarming (Winston, 1987). This allows for the spread of bees and may be repeated as new queens hatch until the parent hive reaches a low population.

Honeybees generally forage in a circular range with a 2-3 km radius around their hive (Winston, 1987). When a single worker bee finds an area that is rich in pollen or nectar it returns to the hive and very efficiently gathers recruits to help harvest the food source. Bees do this through a waggle dance that portrays angle relative to the sun and distance (Winston, 1987). Floral odor is also important and is carried from the flowers. Recruited worker bees then follow the directions from the dances to the feeding site. Bees consume a diet of carbohydrates,

provided by nectar, and protein, provided by pollen (Winston, 1987). During the summer, food sources wax and wane as weather changes interact with the plants' natural growing seasons. During times of low food supply, bees become less picky about the sources from which they harvest. Bees also stockpile honey for use during the winter when it is too cold to fly. Bees are able to create a microclimate within the hive during these cold seasons by using their flying muscles to "shiver" in a giant ball with an interior temperature of about 30° C and that warms up the outside bee to at least 10° C (Winston, 1987).

Efficient work within the colony is achieved by dividing labor according to age and genetically determined thresholds for performing particular tasks (Page, 2013). Throughout their lives, honeybees differentiate into different tasks according to age group. During their first few days of adult life, a bee primarily works as a cell cleaner of brood cells (Seeley, 1995). At this time, a worker will spend approximately 20% of her day resting, and 20% walking through the combs. By day three of adult life, a bee develops fully functioning hypopharyngeal glands which secret brood food. For the next ten days, the adult bee functions as a nurse. During this time, a bee also develops a sting reflex that will aid her as she ventures outside the nest (Breed et al., 2004). At an age of approximately 12 days, the adult bee leaves the broodnest to begin work in food storage. Here, she will use evaporation to turn nectar into honey. She also packs pollen, ventilates the hive, helps with guarding the hive entrance, and builds comb (Winston, 1987). At an age of approximately 20 days, a bee begins the dangerous work outside of the hive as a forager involved in gathering pollen, nectar, water, and resin. This work continues until death, which usually occurs after about four weeks of adult life (Winston, 1987). Bees are also able to transition through the worker castes depending on colony need. When the hive is functioning normally, bees may become elite in their role. Elitism can be defined as a situation where a

small proportion of workers perform a large proportion of specific work for the colony. When a disturbance wipes out these elite members, the hive is able to quickly recover by rearranging the division of labor to accommodate for the loss (Tenczar, 2014).

While bees progress through castes based on age, they can also differentiate based on colony need; at other times genetic behavioral differences may dictate the caste a particular bee belongs to. A study by Whitfield et al. (2003) showed that nurses and foragers differ in gene expression for 39% of DNA. Based on these differences, scientists were able to identify the job of a bee with up to 92% accuracy regardless of age, colony, or genetic source. Using the researcher's definition of behavior (an individual's unique product of genotype and environment), it is impossible to tell if the genotype of the bee determines which job it will move into, or if the environment the bee encounters (either before moving to a new task, or once assigned to a certain role) determines which genes will be expressed.

While all bees pass through some jobs, such as nursing larvae, only some bees will participate in guarding (Winston, 1987). Temporal polyethism, or the division of labor between morphologically similar individuals in an insect community, has long interested scientists (Johnson, 2008; Tofts, 1993). In other eusocial insects, such as ants, there seems to be an algorithm for division of labor that has three assumptions: (1) individuals seek out opportunities to work; (2) tasks exist both within and outside of the nest but are organized "centrifugally," radiating from the center of the colony; (3) workers start work at the center point of the nest and move to outer positions as they age. This creates a model in which a colony displays a pattern of age polyethism (Tofts, 1993). While the second and third assumption are easily seen in honeybees, as workers emerge in the center of the hive, immediately become a nurse bees, and then start working outward until finally becoming foragers, the first assumption is less certain. It

is not yet understood if workers switch task in response to the environment or due to some internal cue such as age, genetics, or personality (Seeley, 1995).

Introduction: Personality in Animals

Personality can be defined as a consistent set of behaviors across contexts, over time. Within the eusocial insect world, personality has been studied extensively. An individual's personality may affect its ability to survive (Jandt et al., 2013). Because of this, it may be easy to assume that one personality type would be favored over another. However, if a personality does not exhibit some plasticity, an individual may not be able to adapt to new situations, leading to a decreased ability to survive (Jandt et al., 2013). In these eusocial insects, it is sometimes easier to observe the personality of a colony rather than an individual (Jandt et al., 2013).

Because colonies reproduce by swarming when additional queens are reared, natural selection works at the colony level rather than on variation among workers. While selection at this larger level might create differences in personalities between hives as each hive employs different strategies to survive, it is still the sum of individual contributions that determines which hive will be successful enough to undergo colonial fission (swarming). Individuals and the colonies in which they live may vary in their personality across situations (Pinter-Wollman, 2012). When studying honeybees, it is important to note not only the obvious difference among colonies, but also the differences within a colony that contribute to its success over another colony.

A broad range of studies suggest that individual animals differ in their behavior across contexts and in response to social and environmental variation (Bergmuller et al., 2010; Whitfield et al., 2003; Jandt et al., 2013). Studies of *Apis mellifera* have shown that genes related to behavior help to determine specific tasks in the colony that the bee may perform

(Whitfield et al., 2003). While personality can be seen easily in various contexts, it is still unknown if the bee exhibits a predictable personality across its lifespan (Jandt et al., 2013).

For workers to make the greatest contribution within their hive, it would seem that unique personalities among the individuals could be important in facilitating division of labor. Bees of the same age often differentiate across jobs such as guarding and hive maintenance; additionally, colonies encounter changing situations during their active season. To be adaptable, differing personalities among workers should exist in order to allow hives to respond appropriately to new environmental stressors. To measure personality on the hive level, scientists have used a myriad of experimental designs. The majority of these look at easy to recognize traits such as shy-bold or aggression. In honeybees specifically, behaviors such as a "defensive response" has been correlated to aggression (Wray et al., 2011). If these behavioral responses were to be studied over the lifespan of a colony, an investigator could estimate personality of the colony.

Wray et al. (2011) assessed collective personality across hives on honeybees. The results of this study found two principal components for behavior: the first being composed of defensive response, activity level, and comb repair; the second being composed of defensive response, foraging activity, and undertaking. The first component corresponded with more excitable bees that were warier of disturbances and less likely to repair their hives. The second component related to more flexibility but also riskier behaviors.

This study provides another look at behavioral syndromes in honeybees. While most studies choose to look at differences between the hives (as selection acts at the hive level), this experiment is one of the beginning steps in observing how individual differences might create the behavioral plasticity that is necessary for a hive to survive. Previous studies have looked at

how environmental and genetic changes may affect colony personality, but it has remained difficult to relate an individual's behavior to that of the group (Bengston and Jandt, 2014).

Previous studies have explored the phenomenon of individual personality in other social insects. In *Myrmica* ants, it was found that colonies that were bold in responsiveness were made up of highly social individuals. Furthermore, within a colony, certain behavioral traits were correlated with task allocation (Chapman, 2011). In the social spider *Anelosimus studiosus*, within group variation has been found to aid fitness. In this species, task specialization and behavior have a positive association with individual and group level task efficiency (Pruitt, 2011). In paper wasps (*Polistes dominulus)*, aggression creates hierarchy as individual bees compete to gain breeding status (Cant et al. 2006). With other social insects displaying such differences in personality, honeybees may also benefit from individual differences within the colony.

In this study, I examined individual honeybees of the same age range and recorded time to calm, alarm response, and simulated predation response in order to determine how individuals differ from each other behaviorally. I arrived at these assays after extensive library research, field observation, and preliminary experiments. I hypothesized bees which took longer to calm and were more active after exposure to alarm pheromone could be seen as more active and this trait could be correlated with nervousness (Wray et al., 2011). The final assays explored how shy or bold a bee was by determining how quickly they were able to regain composure after a disorienting experience.

Background Information for the Behavioral Assays

While some eusocial insects divide labor based on fixed morphological differences, honeybees remain uniform in body shape and size throughout their adult life and have more

temporary jobs within the hive (Seeley, 1995). One such job is "guarding." Generally, bees in this group are between the ages of 12 and 25 days (Winston, 1987). Guard workers are easily distinguishable by the posture the bee assumes when on the entrance, or porch, of the hive. With their wings up, antenna alert, and forelegs lifted, these bees patrol the porch and ensure that any bee entering the hive is a member of the colony by looking at their odor and behavior (Winston, 1987). By the time bees have reached the point of engaging in guarding behavior, they are producing the maximum amount of alarm pheromone they will ever produce and their mandibular glands have switched from producing brood food to producing a second type of alarm pheromone, 2-heptanone (Winston, 1987).

Isopentyl acetate (also called amyl acetate) is one of two alarm pheromones used by honeybees. It is emitted either when a bee takes a stinging posture and fans, or when the stinger is ripped from the body of the worker (Winston, 1987). This acts as a chemical signal that can coordinate a defensive response against danger (Seeley, 1995). When pheromone is placed on the porch of a hive, bees will emerge from inside the hive to provide an army against any potential enemy (Boch et al., 1969). Even though a defensive group is gathered, bees will still refrain from stinging unless the potential target moves (Boch et al., 1969). This allows the bees to be prepared to defend themselves without losing members of the group unnecessarily. When bees are exposed to alarm pheromone, this causes an increase in activity. In order to determine if different bees have different personalities, it is necessary to see if activity levels in response to alarm pheromone vary among individuals. Because it would not be fortuitous to have an entire hive place itself in danger, I aimed to test whether there would be differences among individuals.

In *A. mellifera*, workers attempt to mitigate the risks of predation by flying away from the colony to sting and bite vertebrates. They have a small defensive perimeter that extends just 50

meters away from the nest (Breed et al., 2004). In a colony level test that measured the speed with which bees ran across the comb (runniness) following a brick drop, Wray et al. (2011) found significant differences between hives. I attempted to run a similar experiment with the hypothesis that if colonies differ, individuals within the hive probably also differed.

Methods

From July to August of 2014, worker *A. mellifera* were tested from hives located on the East Campus of the University of Colorado at Boulder. Two hives that were not involved in any other experiments were sampled in to ensure that hive-wide personality traits were accounted for. Tests alternated frequently between the two hives in order to ensure that times of year were not a factor. In an effort to standardize any personality that may come about from assigned jobs, only guard bees were tested. Guard bees were determined by their presence on the porch of the hive and their guarding stance (Winston, 1987). Bees were collected and placed in individual glass petri dishes with filter paper on the bottom during the morning hours and were run through a series of 3 assays. The average length of time a bee spent in captivity was just over an hour.

Time-to-Calm

Immediately upon collecting guards from the porch, bees were transported inside to a temperature controlled environment. The petri dishes were placed on a flat surface and a timer was started. In this assay, calm was defined as a period of non-movement that lasted a period of two minutes or more. Bees that were not calm after a period of 45 minutes were considered to have never calmed and were sent on to the next assay. This was to ensure that any lack of activity was indeed due to calmness and not due to a lack of calories.

Alarm Pheromone

After the time to calm experiment, bees were transported outside for an experiment using alarm pheromone. Alarm pheromone is the scent that bees emit when they believe they are in danger. When a person gets stung by a bee, a small amount of pheromone is released. After being transported outside, bees were observed for a period of two minutes. During this assay, the amount of time in activity was recorded. In this assay, activity was defined as any moment in which the bee's wings would not be seen. This is exhibited in either attempted flight or Nasonov fanning, a behavior displayed when a bee is attempting to gather a swarm (Winston, 1987). Attempted flight might indicate that the bee is attempting to flee from the situation, and Nasanov fanning is a behavior that emits a pheromone which causes swarm clustering and alerts the colony that a support system is necessary (Winston, 1987). After the initial period was over, a small drop of pheromone was added using a pipette. Activity levels were again observed and recorded over a period of two minutes. Two minutes was chosen due to the fact that alarm communication generally reaches a certain concentration and then disperses relatively rapidly. Due to this, bees generally recover rapidly from exposure so they can better respond to a second event (Collins & Rothenbuhler, 1978). As soon as this trial was completed, bees were transferred into clean petri dishes and allowed a ten minute calming period.

Escape Time

The final assay was a simulated predatory event. While still in lidded petri dishes, the bees were shaken for 5 seconds. They were then placed on level ground and the lid of the petri dish was removed. The bees were then timed until they were no longer touching the petri dish in any way. This was defined as a complete escape. If a bee had not escaped after a period of 200 seconds, it was deemed to have never escaped. Generally, if a bee had not escaped at this point, there had been no movement of the bee in any capacity.

Results

Time-to-Calm

For 64 individuals tested, the mean amount of time needed to calm was 2060 seconds with a standard deviation of 766 seconds. Although the majority of individuals appeared to have never calmed, the columns previous to that indicate that there were differences between individuals (Figure 1). A second figure was made that excluded bees that did not calm (Figure 2). This figure depicts the beginning of a histogram of individual behaviors that could be expected if bees had been given unlimited time to calm. In this scenario the average was 1530 seconds with a standard deviation of 670 seconds.

Alarm Pheromone

Bees were introduced into the pheromone assay following the time to calm experiment. Because of this, the majority of bees had very low activity before the pheromone was added (mean=9.48 seconds of activity, standard deviation=21.20 seconds) (Figure 3). Once the pheromone was added, mean activity level increased to 16.31 seconds with a 25.06 second standard deviation (Figure 4). A correlation between these two assays revealed significance (p=0.00001) (Table 1). A linear model showed a positive relationship between the two assays (Figure 5) ($p \leq 0$) (R^2=0.351).

Escape Time

Escape time of the 64 bees was found to vary among individuals. Some bees never escaped, while others flew off immediately. The mean amount of time it took a bee to escape was 95.90 seconds with a standard deviation of 84.00 seconds. With 25 bees taking longer than

150 seconds to calm (max amount of time allotted = 200 sec), and 28 taking less than 50 seconds, individual bees varied widely within this assay.

Assay Comparisons

After running a series of correlations (Table 1), it became clear that the results of a majority of the assays were not correlated. The two exceptions to this were the activity level before and after pheromone (p=0.00001), and the correlation between the activity level before pheromone and the effects of pheromone (the activity level after pheromone minus the activity level before pheromone) (p=0.0003). Additionally, there was a nearly significant association between the activity level before the pheromone and the escape time (p=0.059).

Using r as a platform for my statistics, I used the significant correlations that had been found to create linear models and run regressions. Looking at escape time versus activity level before pheromone yielded a p-value of 0.079 with an R^2 of 0.049 (Figure 6). Because a regression between initial activity level and the change in activity following the addition of pheromone was highly significant (p=0.019, R^2=0.086) (Figure 7), I wanted to follow up by also looking at how activity level after pheromone related to escape time. I modeled this using linear models and regressions and found p=0.270 with R^2=0.020 (Figure 8). This indicates that the data does not fit into a line very well and is not statistically significant.

Discussion

The most significant finding from this study was the difference in activity level before and after exposure to alarm pheromone. As mentioned above, alarm pheromone serves to initiate a defensive response from a group. This assay showed a significant correlation between the activity before the addition of pheromone and the activity level following treatment with the pheromone. Furthermore, a positive trend on the linear model indicates that bees do become

more active following treatment with pheromone. A paired t-test also shows that individuals do vary significantly from each other, indicating a difference in behavioral expression at a given point in time.

The relationship between pre-pheromone activity level and escape time also produced a strong correlation. Although not quite significant, it is possible that the number of trials is the only preventative factor.   The negative regression seen in this figure indicates that bees that were more active before the pheromone also tended to escape more quickly. This is especially striking because bees that had been more active before the pheromone already burned more calories in their activity, and yet were able to exert the energy necessary to remove themselves from danger. This may be indicative of a strong defensive response.

The histogram (Figure 1) denoting time to calm also provides an interesting look into differences among individual honeybees. While the data became truncated in the "2500 sec to never calm" range, there were many bees that varied in their timing. Once the bees that never calmed were removed from the data pool, the resulting histogram (Figure 2) depicted the differences between the remaining bees. Some bees seemed to quickly reach a level of docility, while others remained agitated for longer. If these behavioral differences were consistent throughout a lifespan, it would be reasonable to begin to look toward personality as a cause. It is clear that there are differences between individuals of the same sub-caste in this behavioral assay.

I attempted to control for some variables in this study, but there are a vast number of additional factors that could affect a bee's behavior. I ran the majority of my tests between the hours of 0900-1400, and bees are most active between 1100-1200 hours (Tenczar, 2014). Future studies should further narrow the window of time in which bees are tested to control for

differences based on time. Additionally, in order to define personality, it is necessary to look at behavioral symptoms across contexts and over time. My study assessed how behavioral symptoms varied among bees, but did not account for "over time." As summer progressed and food sources dwindled, it also became evident that bees could not be captured for an hour without experiencing the effects of starvation. In future studies, controlling for nutrition would also be essential. It is also necessary to consider the weather. Boulder summers generally have patterns of hot mornings and rainy afternoons. Weather has been found to significantly affect bees by causing more activity during high temperature and humidity (Southwick et al., 1987). While it is not possible to control weather, it would be interesting to record air temperature and humidity each day; these parameters could then be mathematically controlled for in statistical tests. Additionally, the hive temperature experienced during pupal development can lend to differences in the waggle dances performed by foragers, learning time (Tautz et al., 2003). Keeping frames of pupae in an incubator would further cut down on outside factors that may be affecting the behavior exhibited by a honeybee.

Beyond these factors that require attention, it would be important to note that a high concentration of pheromone is associated with faster, more intense, and longer lasting responses (Collins & Rothenbuhler, 1978). It has also been found that, when alarm pheromone exceeds a certain threshold, it actually repels bees (Boch et al., 1969). During the development of the alarm pheromone assay, a glass pipette was used to deliver a small drop of pheromone into the petri dish. Glass pipettes are less than perfect to drop similarly sized amounts of liquid. Since the average concentration of the drops was never measured it is possible that the data I collected was somewhat skewed.

Future Studies

If I were to have the opportunity to further develop this study in a Master's degree, I would first test each hive to determine which apparent personality component it displayed, before looking at individual bees.

Tests would be run between June and August on the east campus of the University of Colorado at Boulder. Single cohort hives would be established with approximately 2000 1-day old bees as done in Tenczar (2014). Magnetic numbered tags would be affixed to approximately 120 individuals. These tags would allow for continued normal behavior in bees and would allow for the capture of individuals as they move through the hive with the help of magnets (Hagler & Jackson, 2001). Of the tagged bees, 60 would become part of the experimental group, while 60 would remain as controls. Behavioral assays would begin on day one of adult life and would continue until the bee had not been found by a magnet in 24 hours. This period would be considered to be the death event of the bee. During the lifespan of the bee, members of the experimental group would be collected every two days. Three members of the control group would be collected and tested every three days. These would be bees that had not previously gone through any testing in order to ensure that the stress of the tests was not skewing the data. Bees would be collected each day between the hours of 1000 and 1300. Tests would be completed inside of a temperature controlled building. Each bee would be run through a series of three assays as follows:

Immediately upon being captured, bees would be placed in a petri dish and placed on a flat surface. Dividers would be used to create "rooms" for each petri dish so that visual input was standardized.

After the initial feeding and adjustment, initial activity level would be recorded. A small weight would then be dropped onto the surface of each petri dish. It would be necessary to run

preliminary tests in order to determine the behaviors exhibited by an individual bee without the support of the hive. Upon the results of preliminary tests, amount of time spent in "activity" would be recorded.

Conclusion

This study provides an initial look into how behavioral syndromes differ between individual honeybees within the same colony. My research shows variation between individuals during a fixed point in life and gives preliminary evidence for differing behaviors between members of the same caste. Potential future research exploring how behaviors change throughout an individual's life will augment the data collected here and provide information that may lead to the idea of personality in honeybees. Because of the ecological importance of the honeybee, it is necessary to understand as much as possible about these creatures. With selection acting on a colony wide basis, it is interesting to see how individuals sculpt the behaviors of a hive. As we continue to learn more about behavior and personality in honeybees it may become possible to provide ecosystem services that increase the fitness of colonies.

Figures:

Correlation	P-Value	Significance
Time to Calm vs. No Pheromone	0.131934	NO
Time to Calm vs. Pheromone	0.375475	NO
Time to Calm vs. Escape Time	0.501893	NO
Pheromone vs. No Pheromone	0.00001	YES
No Pheromone vs. Escape Time	0.058811	NO
Pheromone vs. Escape Time	0.904053	NO
No Pheromone vs. Effects of Pheromone	0.000305	YES

Table 1: *Correlations between assays*: This table looks at the associations between two variables. Significant results were found between activity levels pre and post pheromone treatment. This indicates that the activity level that bees display before being treated with pheromone is linked to the activity level that bees exhibit after being treated with alarm pheromone. Additionally, the activity level before treatment with the pheromone shows near significance. It is possible that with more trials, this would reach significance. This would be interesting because it would generally show that activity level of a non-treated bee is predictive of how quickly a bee tries to escape after a disturbance.

Time to Calm

Mean = 2060.23 sec
Standard Dev = 765.87 sec

Frequency

30
25
20
15
10
5
0

0   500   1000   1500   2000   2500   3000
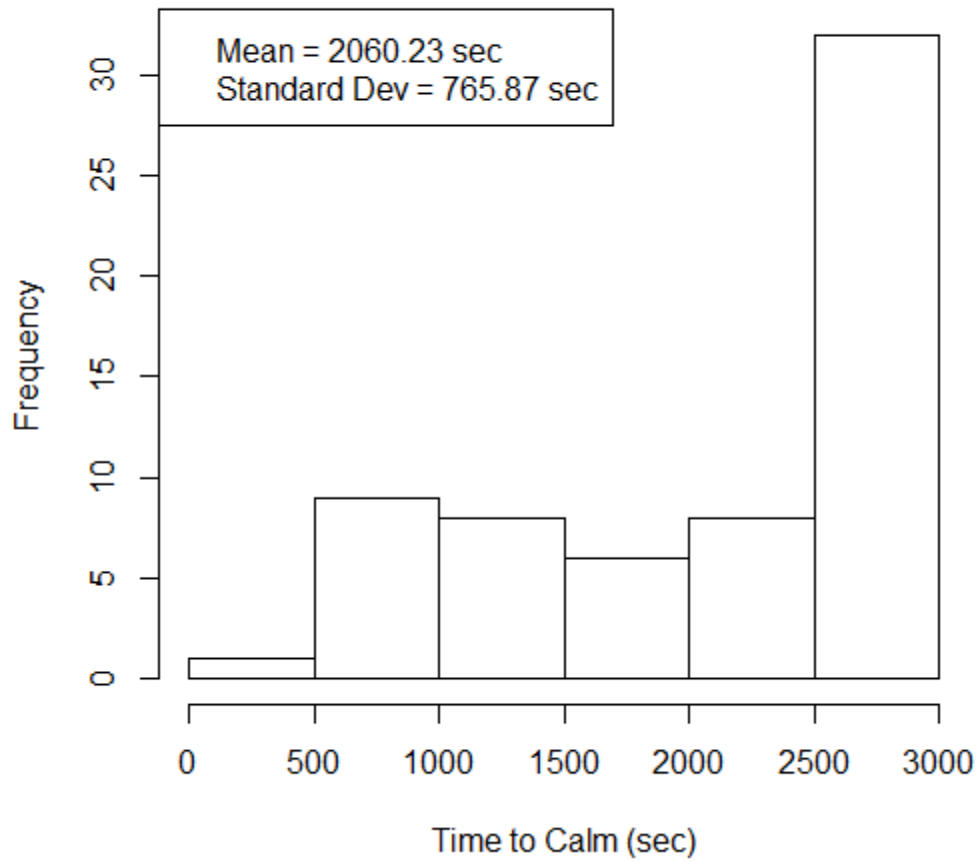
Time to Calm (sec)

Figure 1: *Histogram of time to calm:* This histogram shows the differences between individual bees in how long it took for them to calm. Based on the data, it can be seen that bees varied in the time they spent agitated.
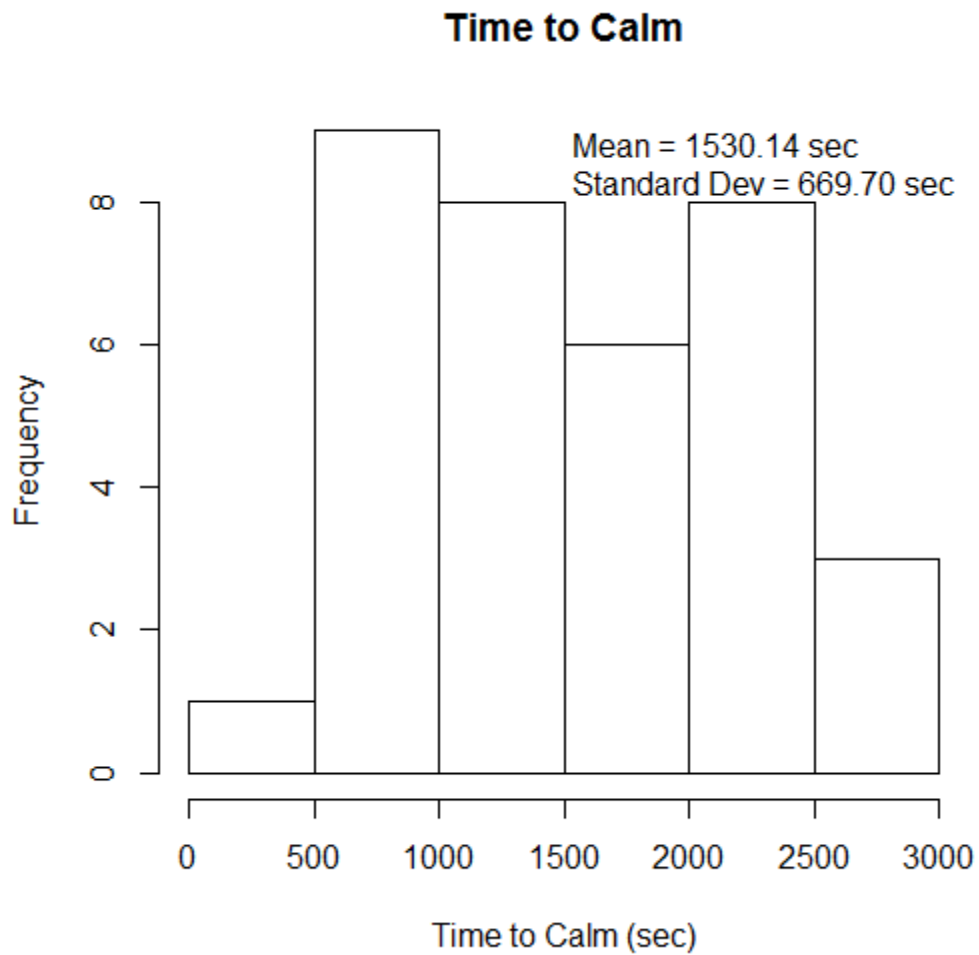
Figure 2: *Modified histogram of time to calm:* With the removal of data points that indicated that a bee had never calmed, a clearer picture of the variation among individuals is seen. While it is not acceptable to exclude the data entirely, this graph shows what the first part of a graph could look like if bees were given an unlimited amount of time to calm.
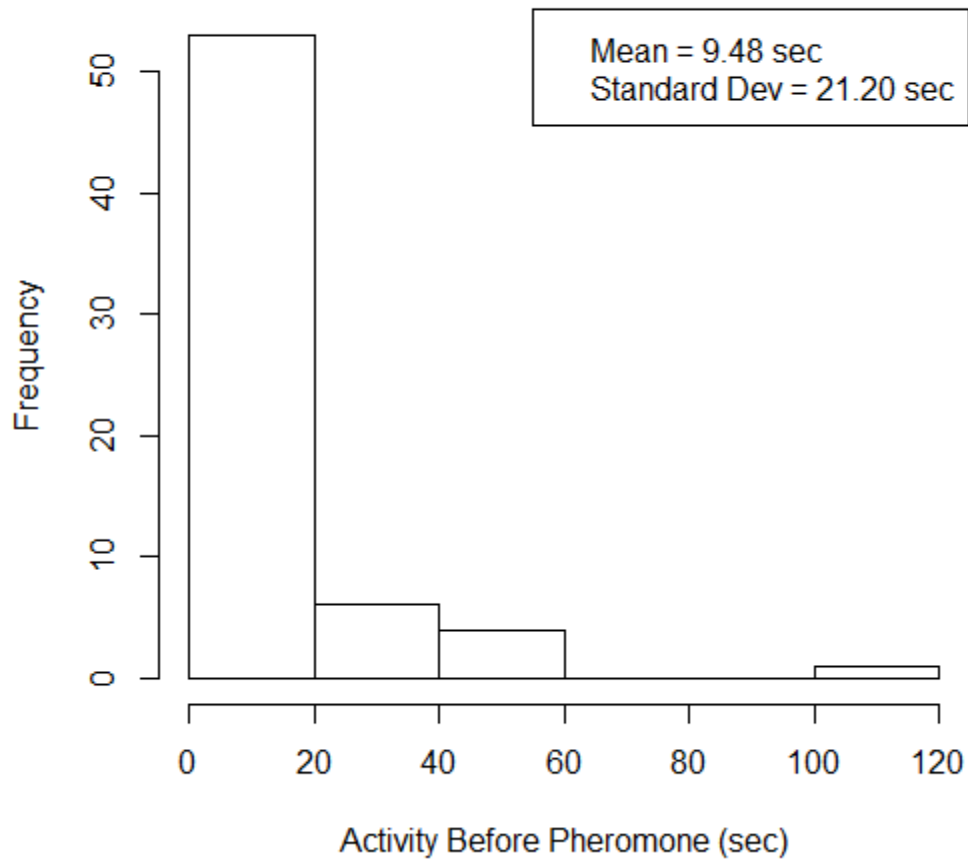
Figure 3: *Activity Level Before Pheromone Treatment:* **This histogram depicts the amount of time each bee spent in activity prior to treatment with alarm pheromone. Because this trial was immediately following the "time to calm" assay, the majority of bees fall into the first column. Even with that period, some bees became or were still active.**
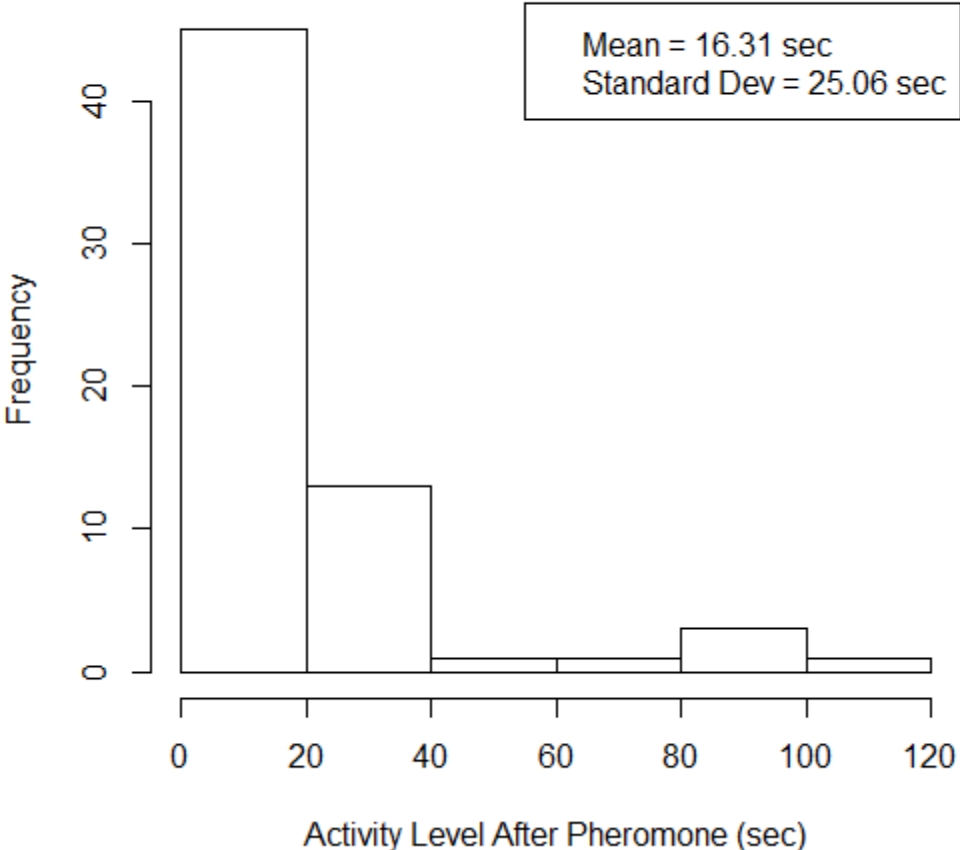
Activity Level After Pheromone

Figure 4: *Activity Level Following Pheromone Treatment*: This histogram depicts the amount of time each bee spent in activity after being exposed to alarm pheromone. The average from this figure compared to figure 4 indicates that bees generally became more active following exposure. A t-test also shows significance between these two numbers (p=0.011).

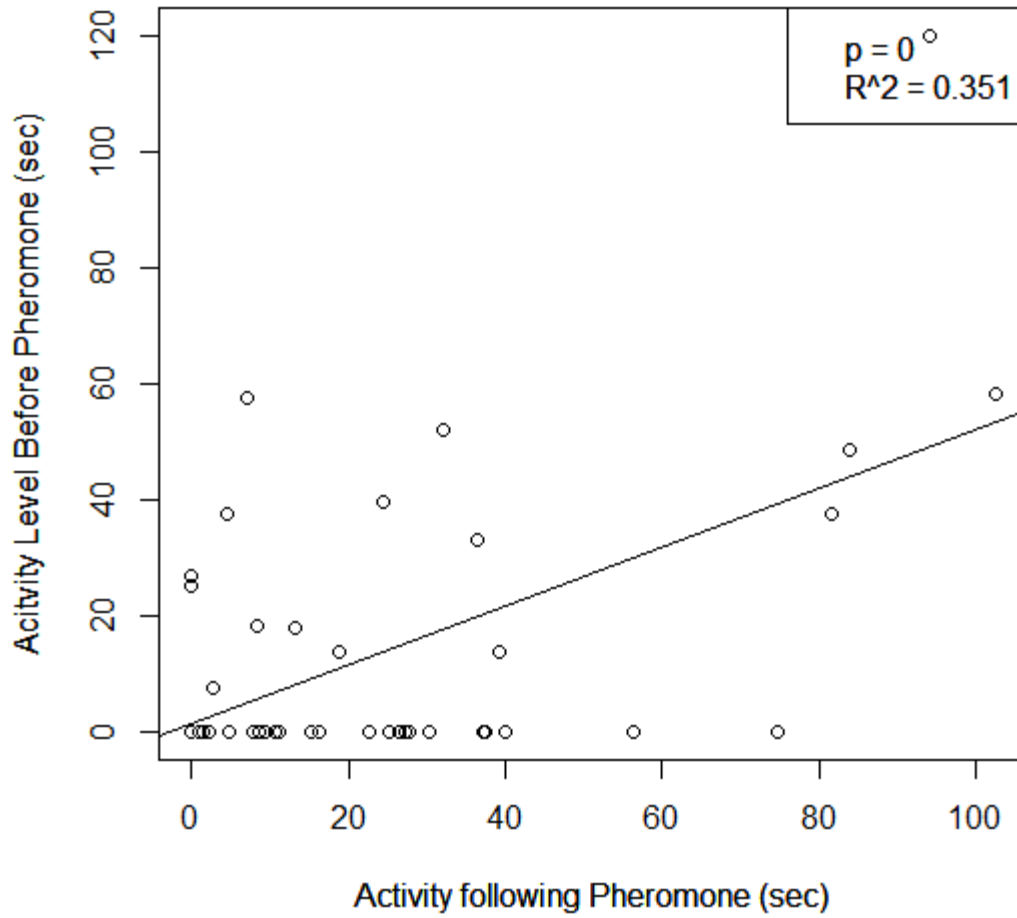**Activity Levels Before and After Pheromone**

p = 0
R^2 = 0.351

Acitvity Level Before Pheromone (sec)

Activity following Pheromone (sec)

Figure 5: *Activity Levels Before and After Treatment with Pheromone:* **This graph depicts the activity level of bees before and after treatment with alarm pheromone. It can be seen that there is a significant difference between the sets of numbers which indicates that individual bees significantly vary in their activity level after exposure to pheromone.**
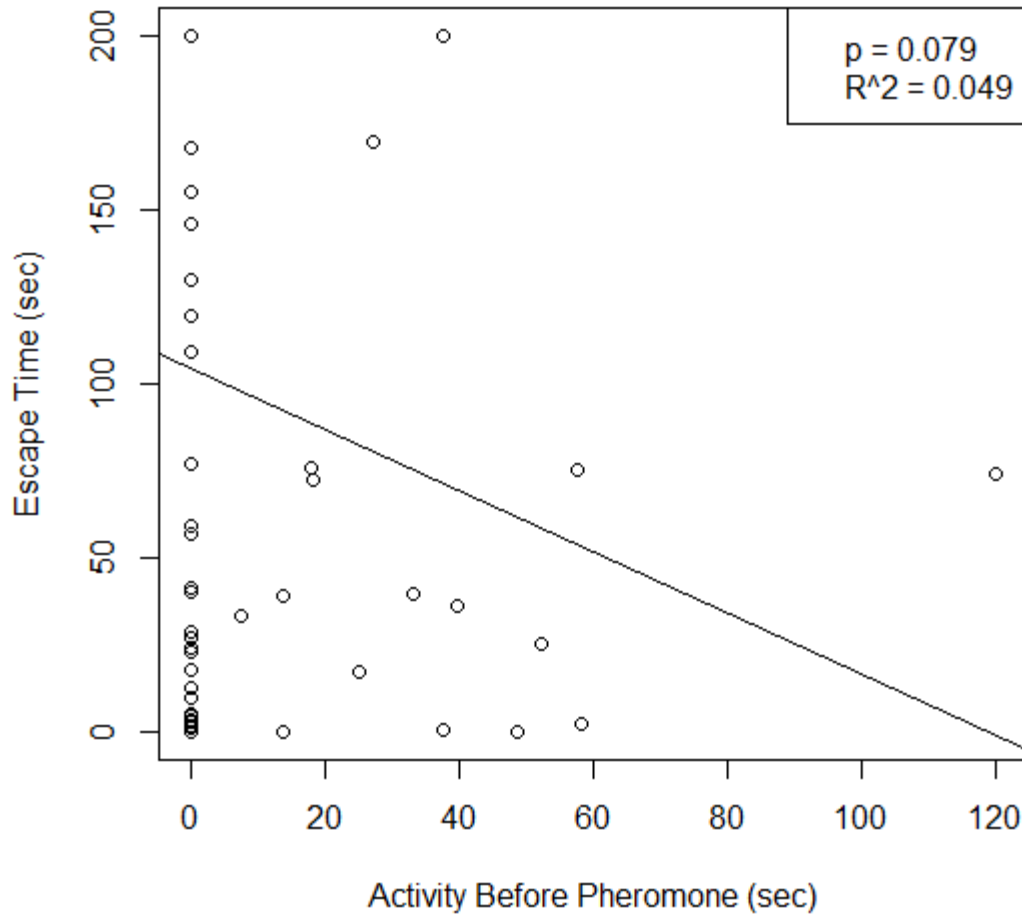
Figure 3: *Pheromone vs. Escape Time*: Because the activity level prior to treatment with pheromone correlated with the amount of time it took bees to escape (p=0.059), a regression was run in order to observe trends in the data. In this graph we can see a significant relationship between the activity level prior to treatment with pheromone and the amount of time it took a bee to escape following a disturbance.
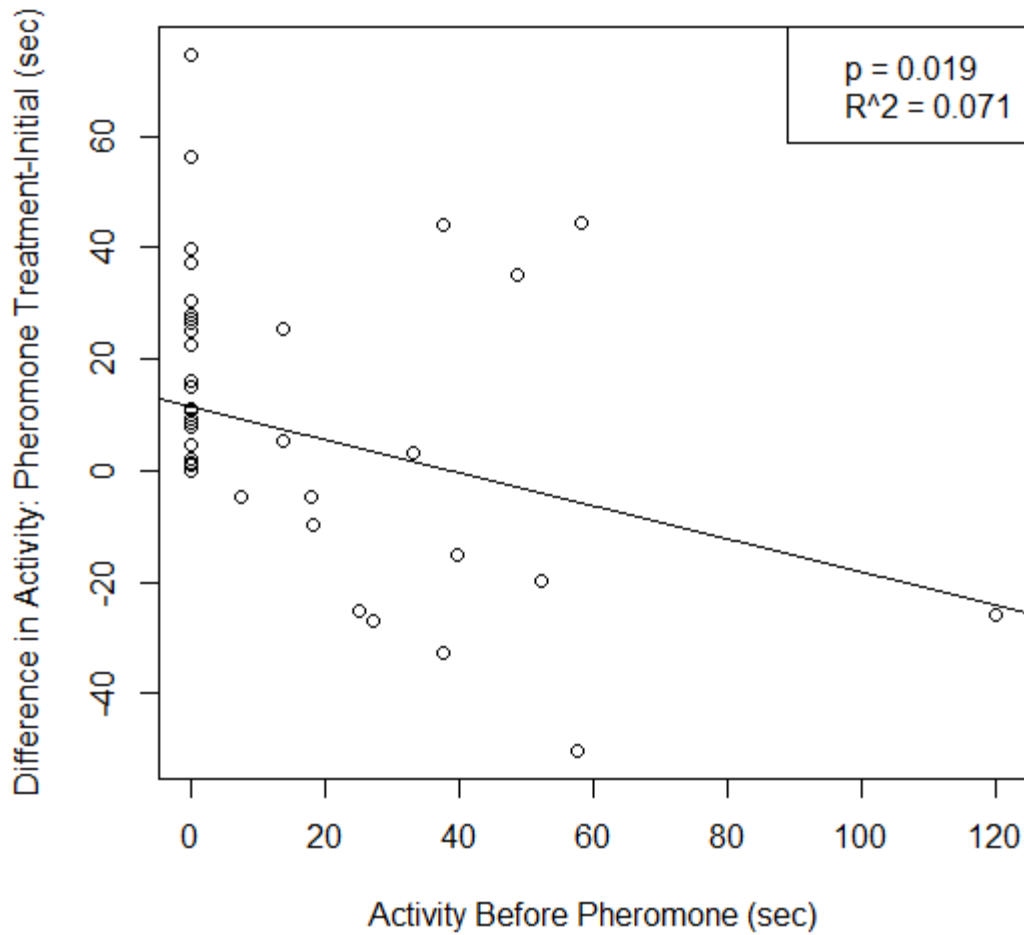
Figure 4: *Activity Level Prior to Pheromone Treatment vs. the Effects of Alarm Pheromone:* **This graph depicts a negative trend between the activity level prior to pheromone treatment and the effects of alarm pheromone. This would indicate that overall bees tended to be move less following treatment with pheromone.**
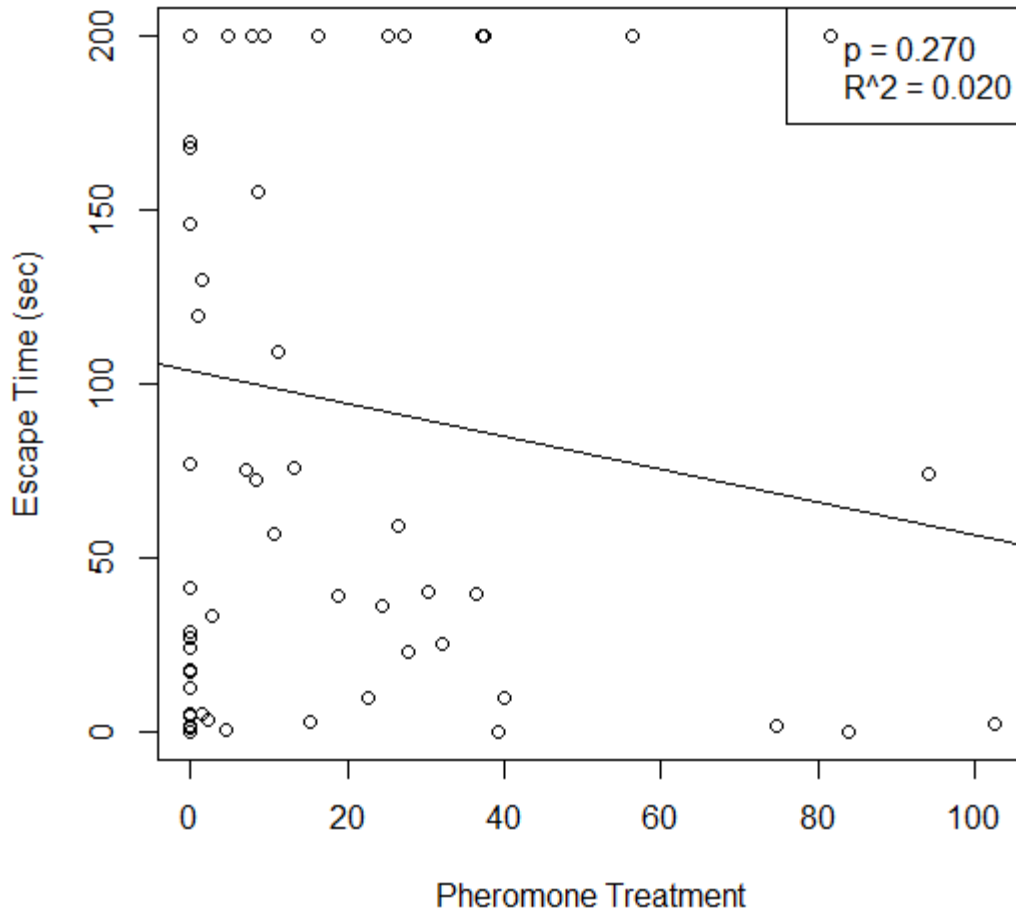
Figure 5: *Pheromone Treatment vs. Escape Time:* This graph shows the relationship between escape time and activity level following treatment with alarm pheromone. Although not significant, it is interesting to see the negative trend for data points that did not max out at 200 seconds. If there had been no maximum, it is possible that there would have been a stronger negative trend indicating that the less reactive you are to alarm pheromone, the quicker you are able to recover from a disturbance.

References:

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