The Effects of Ethyl Oleate on the Behavior of *Apis mellifera*

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Abstract:

The pheromone ethyl oleate was recently discovered in honeybees and has offered researchers the opportunity to further understand how division of labor is managed in honeybee (*Apis mellifera)* societies. Biologists recognize two types of pheromones: Releaser pheromones, which elicit immediate responses from receiving animals, and primer pheromones, which cause long-term changes in behavior without immediate behavioral change. The pheromone ethyl oleate used in this study acts as a primer pheromone in honeybees. The behavior of guard bees was examined after direct exposure to ethyl oleate, by recording the number of treated bees present in the hive. The number of bees present inside and outside the hive was recorded once daily for three days after exposure of guard bees to ethyl oleate. A control group was also maintained to compare the result to the treatment group. This research was conducted in the summer when bees are most active. No significant difference was seen in the number of control and treatment bees present, indicating no behavioral change after exposure to ethyl oleate. However, a significant decline in the number of bees occurred over time, with the lowest number of bees recorded on the third day. More control bees were found inside and outside the hive during early summer, while more treatment bees were observed in late summer. In the future, tagging more bees, conducting the entire study early in the summer and spending more time observing and tracking bees in the hive would improve such studies. Improved delivery methods of ethyl oleate to the bees and a means to measure the concentrations of ethyl oleate in bees would also be helpful.

Introduction:

Social insect are known for their complex behaviors. They maintain well-organized colonies and a use a highly decentralized system for division of labor. In honeybee societies, division of labor involves queen-worker interaction and worker-worker interaction (Johnson,

2010). Among workers, division of labor is divided into temporal and physical aspects, where temporal refers to age-related behaviors found in many insects and physical refers to specific castes designed for specific tasks as is common in some insects societies such as ants (Johnson, 2010).

Numerous studies (see, e.g., Seeley, 2002) have shown the benefits of adopting a decentralized system, based on self-organization of a large system without a central manager, as opposed to a hierarchal system for regulating division of labor. For example, in honeybees, a particular site is chosen for a colony by a group scouts (Stojmenovic and Vukojevic, 1991). This is the opposite of a single individual making a decision for a group. One of the advantages of decentralized systems of organization is protection from predators. Animals that live in big groups, such as bees, spend less time scanning for predators, while spending more time on feeding and reproducing (King and Cowlishaw, 2007).

Pheromones are one of the tools insects use to achieve efficient division of labor. In honeybees, two different classes of pheromones, i.e, primer and releaser pheromones, are used to influence of the behavior of the bees. A pheromone is a chemical, secreted from an exocrine gland of an animal, which "elicits a behavioral or physiological response in another animal of the same species and therefore acts as a chemical message" (Free, 1987). Releaser pheromones cause "rapid, transient changes in behavior, whereas primer pheromones cause more long-term changes in behavior and physiology" (Leoncini et al., 2004). Hundreds of releaser pheromones are known; in contrast, very few primer pheromones have been identified, primarily because they are much more difficult to assay. For example, the alarm pheromone, a releaser pheromone, was first recognized in honeybees in 1814 (Vander Meer et al., 1998) and alerts the colony. Nasanov pheromone attracts other bees when released from a gland on the dorsal surface of the abdomen

(Free, 1987). More information on primer pheromones is needed because they may play important roles in the regulation of behavior in many animal societies (Leoncini et al., 2004). *This thesis addressed how ethyl oleate, a primer pheromone, affects the behavior of guard bees.* Understanding how ethyl oleate is utilized by honeybees should enhance the understanding of how division of labor is utilized by honeybees.

Primer and releaser pheromones are also used in other social insects. In termites, trail pheromones are produced in the sternal gland, allowing fellow members to easily reach the site of food (Vander Meer et al., 1998). In ants, females produce sex pheromones (releaser pheromones) to attract males (Vander Meer et al., 1998). Researchers showed evidence for the presence of primer pheromone in ants. Vargo and Fletcher found that colonies with fewer queens contained fewer sexuals than colonies with single or no queens (Vander Meer et al., 1998). The researchers examined the corpses of queen bees to demonstrate the presence of primer pheromone involved in determining the behavior of workers towards the development of larvae (Vander Meer et al., 1998).

Pheromones elicit behavioral changes in many animals. They are best studied in eusocial insects such as bees and ants. Examples of releaser pheromones of eusocial bees are those concerned with sex attraction, alarm and aggression, trail production, clustering and mutual recognition (Free, 1998). Pheromones vary greatly in their chemical structure and in their function. For the most part, pheromones are well known for their role in helping animals find mates and protect territories. However, other functions have also been reported. For example, nursing mother rabbits release a pheromone to induce suckling in their pups (Wyatt, 2015). Therefore, pheromones require close study and need to be investigated not just in insects, but also in other animals, including humans.

In eusocial insects, such as honeybees, it is important for members of a colony to discriminate against outsiders. In general, animals use olfaction or contact chemoreception to distinguish between relatives and outsiders (Breed et al., 1990). In honeybees, members of a particular hive can successfully recognize their own nestmates. In particular, wax and cuticular compounds are relevant to honeybee nestmate recognition (Breed et al., 1990). Often it is not just one compound but a mixture of many compounds that determines the final makeup of bees' wax.

In honeybees, tasks in a nest are highly divided. The queen, nurses, drones, guards, and foragers all stick to their particular task (Breed et al., 1990). Furthermore, individual bees exhibit altruistic behavior and work for the benefit of the hive. Unique in this organization is the queen that is responsible for reproduction. Each bee has a crucial task in the survivorship of the hive as a whole.

I. Objective and Significance:

The objective of this study was to further investigate ethyl oleate, a primer pheromone in honeybees. Early work suggested that older bees control the maturation of younger bees via ethyl oleate (Leoncini et al., 2004). The presence of older bees apparently affects the behavioral development of younger bees, but the exact mechanism was not known until recently. Researchers, however, knew that bees exhibited a high degree of plasticity and responded to changing conditions in the hive (Huang and Robinson, 1992). One such plasticity is the tendency for younger bees to age more quickly than usual by releasing the juvenile hormone prematurely (Huang and Robinson, 1992). In my study, I assessed the behavior of guard bees after they were exposed to ethyl oleate. If the exposure to ethyl oleate delays the aging of younger bees, then I expected non-exposed guards to stay at the hive longer than usual. The study predicts control bees would mature at a slower rate compared to the treatment group. Consequently, I expected

the guards exposed to ethyl oleate (treatment group) to become foragers at a later age. The implication of this study might be that ethyl oleate could be utilized by commercial beekeepers to control the behavior of their bees and possibly increase yields of honey.

II. Background:

The subject of this study is the European honeybee that is is native to Europe, the Middle East and Africa. Honeybees were introduced into the Americas for their economic benefits related to pollination and honey production (Mortensen et al., 2013). More recently, the African honeybee, that can interbreed with the European subspecies, was introduced into the Americas (Mortensen et al., 2013). The most common and widely used subspecies of *Apis mellifera* is the Italian honeybee (*Apis mellifera ligustica)*. It stands out from the rest of the bee races in their high production of honey and their gentle nature, making them a favorite of beekeepers (Stone, 2005).

Ethyl Oleate:

The pheromone used for this study is ethyl oleate. Ethyl oleate is a fatty acid ester with the chemical formula $C_{20}H_{38}O_2$ (Sigma Aldrich, 2010). Previously, researchers reported evidence suggesting that older bees inhibited the aging of younger bees but had not discovered the mechanism. However, in 2004, researchers identified a substance, ethyl oleate, produced by older bees, which presumably delays the onset of foraging (Leoncini, et al., 2004). The previous hypothesis held that aging onset was regulated by worker-worker interaction (Huang and Robinson, 1992). In their study, Huang and Robinson (1992) found that 1-week-old bees reared in an isolated environment had the same levels of juvenile hormone levels as 24-day-old foragers. Higher levels of juvenile hormone production in younger bees are associated with behavioral development (Huang and Robinson, 1992).

Ethyl Oleate Transmitted Via Trophallaxis:

In 2004, Leoncini and colleagues found that foragers had 30 times more ethyl oleate in their stomach than nurses. The difference in the concentrations of ethyl oleate was most significant in the crop (Leoncini et al., 2004). This finding suggested that this pheromone is only found in older bees. These results suggest that worker behavioral maturation is modulated via trophallaxis, a form of food exchange that also serves as a prominent communication channel in insect societies. This discovery was significant as it identified a primer pheromone potentially responsible for the aging delay of foragers. The findings from this study propose a model that explains how older bees may inhibit the maturation of younger bees in the hive. The research of Leoncini and colleagues (2004) suggested that reduced exposure to ethyl oleate, caused by a lack of foragers, led to accelerated behavioral maturation by some younger bees, providing for an adaptive colony response. The absence of ethyl oleate in younger bees showed older bees controlling the behavior of younger bees by inhibiting the amount of ethyl oleate they produce. Perhaps the most significant finding from the Leoncini paper was the tentative identification of ethyl oleate as the primer responsible for the delaying the age at onset of foraging.

Ethyl Oleate Transmitted Via Olfaction:

Another group of researchers claimed to have shown the transmission mechanism of ethyl oleate (Muenz et al., 2004). The researchers showed that (i) ethyl oleate is most abundant on the cuticle, where it is received by olfactory receptors on the antenna, (ii) that information from ethyl oleate reception is processed in glomeruli of the antennal lobe, and (iii) that this learned information resides in olfactory centers of the brain (Muenz et al., 2004). These results suggest that the primer pheromone ethyl oleate is transmitted via olfactory methods between bees that are in contact with each other (Muenz et al., 2004). The researchers showed that the

amount of ethyl oleate in the head was significantly higher than in the thorax. The findings from Muenz et al. (2004) contradict earlier findings by Leoncini et al. (2004), who postulated a transmission via trophallaxis. It is possible that there are two methods for transmission of ethyl oleate. Perhaps, bees respond differently to ethyl oleate depending on the transmission process. Given the level of interest raised the papers by Leoncini et al. (2004) and Muenz et al. (2004), it is surprising that few follow-up studies have been published.

Thermoregulation In Honey Bees:

In addition to investigating effects of ethyl oleate on guard bees, I briefly looked into the effect of ethyl oleate on the thermoregulation behavior of honeybees. Thermoregulation was chosen due to previous findings showing that bees exposed to heat were less likely to fan if they were alone compared to bees in groups of 3 or 10 (Cook and Breed, 2013). Additionally, Cook and Breed (2003) found that group size influenced the temperatures at which the bees started fanning. The fanning study was added to the present study in order to address the effect of ethyl oleate on guards. The main goal was to see whether bees exposed to ethyl oleate fanned more frequently, in greater numbers, or at a lower or higher temperature than control bees. The heating trial study was the last phase of this project and was conducted in late summer. It is important to note that outdoors air temperatures in late September were very different from temperatures in late July. This may have altered the chemical levels of certain pheromones in the bees and may also have impacted their response times. Castillo and colleagues (2012) found that workers synthesized more ethyl oleate during the growing season (early summer) than during the fall and winter months. The results from my thermoregulation studies conducted in late summer/early may be different from those of studies conducted early summer.

Methods:

I. Lab Experiments

In the first phase of this project, it was determined whether honeybee behavior was affected by ethyl oleate. In July and August, small experiments were conducted using treatment and control groups to observe the behavior of the bees after they were exposed to ethyl oleate. Bees were fed 1 Molar sugar (34.23 g/100 mL) water or 1 Molar sugar water with ethyl oleate. Distilled water was used to minimize the influence of contaminants in water. Regular table sugar was used to make the sugar water. Only two drops of ethyl oleate were used for most experiments. Throughout the study, the concentration of ethyl oleate was not changed. High amounts of ethyl oleate exposure led to less movement among bees and higher mortality was observed with high ethyl oleate concentrations. Bees were placed in a Petri dish in small groups (5-10) and individual bees were later observed. In the dish, the bees were exposed to ethyl oleate through filter paper and a piece of cotton containing the sugar water. For the control group, the same conditions were applied without the ethyl oleate. The Petri dish experiments were conducted over several weeks in early summer. The behaviors of the bees were observed for 10-20 minutes. The observations were done on the first, second and third day after the bees were tagged. After observations, bees were released back to the apiary, while the filter paper and the cotton piece containing the ethyl oleate were discarded. The goal of these initial experiments was to determine whether exposure of ethyl oleate led to clear behavioral changes in guard bees. These early experiments were designed to test the minimum exposure of ethyl oleate needed to bring about behavioral changes.

II. Apiary Experiments:

These experiments were conducted on East Campus at an apiary owned by CU. The

second phase of the project involved tracking bees after they were treated with ethyl oleate. In this phase, bees were collected, divided into treatment and control groups (with paint) and treated with ethyl oleate. This was the easiest way to track the bees without interfering with their behavior or ability to fly. Each experiment took 3-4 days. After bees were collected and painted, they were tracked. Their behavior (whether they were guarding, fanning or nursing) was recorded. The location of the bees in the hive was also recorded. The bees were either guarding or fanning outside the hive or were found inside nursing or making food for the hive. This was the main experiment and most data were gathered from this phase. Data were collected as long as the bees were active (late September to October). Another experiment was conducted to supplement the main study and investigate how ethyl oleate affects the fanning behaviors of fanners. In this experiment, bees were divided into two groups of ten bees, each one treated with ethyl oleate and sugar water and the other group with just sugar water (control). Bees were then exposed to different temperatures and their behavior was recorded. The supplemental study lasted for as long as the weather permitted. In a past study, Chelsea Cook, a graduate student in the Breed lab found that fanners were most likely to fan, while foragers were least likely to fan (Cook and Breed, 2013). The advantage of conducting research outside compared to laboratory setting inside a building was that the bees were calm and continued doing their work after their release.

III. Thermoregulation Experiments:

Thermoregulation experiments were conducted after the main study involving ethyl oleate. The thermoregulation study was conducted at the end of the summer to see if bees exhibited different fanning behaviors after exposure to ethyl oleate. Five trials were conducted at the end of September. Bees were divided to two groups of ten bees each. One group was exposed

to ethyl oleate in sugar water solution while the other group was given just a sugar water solution. The thermoregulation experiments were conducted inside the lab on East Campus as opposed to the main study, which was conducted in the apiary. The apiary was located outside in a field and the bees felt more at home. Another disadvantage with the thermoregulation experiments was that the researcher had to wait for period of about 20 minutes in order for the bees to come down and settle in their new environment. The bees could only be observed after they were calm enough to be fed sugar water.

RESULTS

Hypothesis 1:

H_0: Guards treated with ethyl oleate will stay at the hive for the same amount of time as the control group

In this study, a total of 260 bees were tagged and divided into control and treatment groups. A total of 6 trials were run from late July to early September. I observed no difference in the number of bees of bees found in the hive between the treatment and the control group (X^2 = 1.83 and p = of 0.1761), i.e., ethyl oleate treatment of guards did not significantly affect their behavior. The number of control guards found outside and inside the hive did not differ significantly from the number of treated guards found outside and inside the hive. Therefore, I cannot reject the null hypothesis, which stated that guards treated with ethyl oleate stay at the hive for the same amount of time as the control group.

Results From X^2 Analysis

	Inside	Outside	Total
Treatment	58	133	191

Control	56	177	233

Table 1: a total of 260 bees were tagged throughout the summer. The bees were divided into two groups. The bees were inspected and their numbers recorded.

Chi Squared value	Df	P value
1.83	1	0.1761

Table 2: The results from the X^2 squared analysis of the data. The results showed no significant difference between the control and he treatment group, an indication that ethyl oleate did not affect the behavior of the bees.

Overall, more bees of either the treatment or the control groups were found inside the hive than outside the hive. Additionally, more of the control bees were recovered throughout the summer compared to the treatment group.

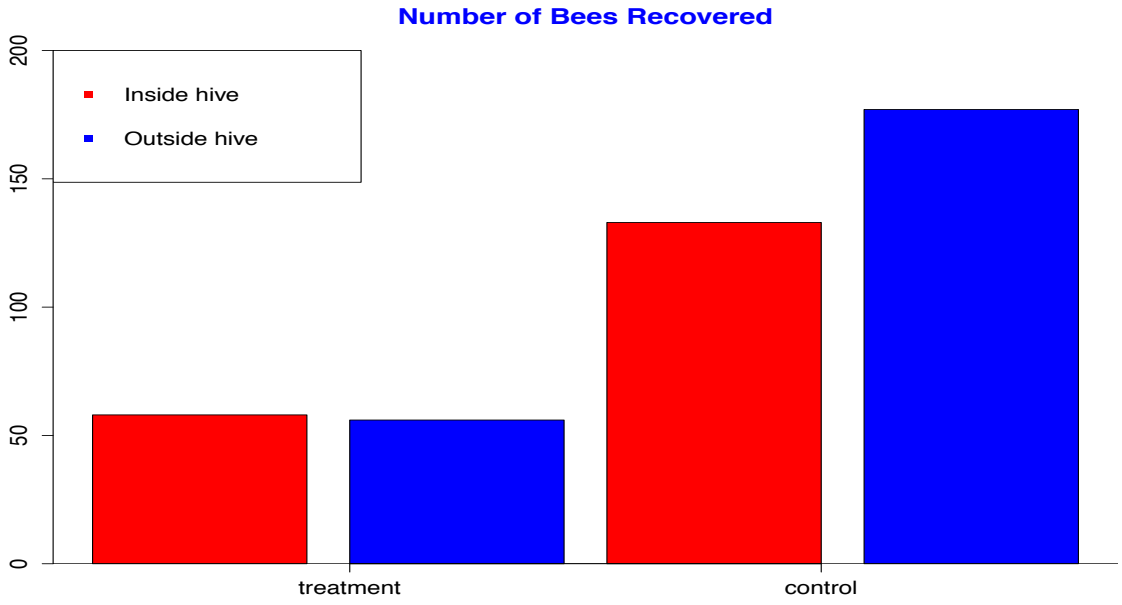

Figure 1: A bar graph showing the number of bees found inside and outside the hive for both the control and the treatment group. N=260. Data collected from late July to September. Number of trials = 6. The thoraces of the bees were painted using colors to allow discrimination of the control and the treatment group. Treatment = 191. Control = 233

Hypothesis 2:

H_0: The mean of number of bees found in the hive does not change over the course of 3 days.

Other aspects of the data were also analyzed. Specifically, the number of tagged bees recovered on the first, second and third day of inspection varied greatly. The decline in the number of bees as days passed was one of the most interesting aspects of the research. The tagged bees were observed in great numbers at the beginning of the inspection (first day), but very few of them were seen either inside or outside the hive by the third day. There are several possible reasons for the decline of the bees as days progressed.

A two-way ANOVA was conducted to analyze the effect of days on the number of tagged bees present inside and outside the hive. The number of bees found inside and outside the hive for all 6 trials was used to calculate the ANOVA. In addition, the ANOVA took into consideration the difference between the days as the data was divided into first, second and third day columns. The results from the two-way ANOVA showed that there was a significant association between days and the number of bees present in the hive.

Results from Two-Way ANOVA

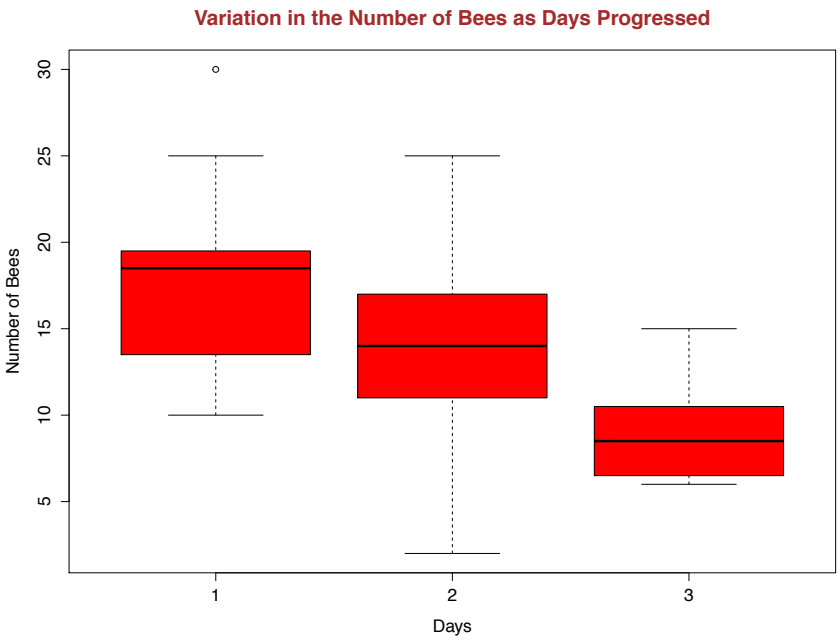

Figure 2: A boxplot showing the interaction between days and the number of tagged bees found in the hive. The horizontal lines on each box show the maximum value excluding the outlier, the bottom line shows the minimum value excluding outlier. The black line shows the median value, while the "whiskers" of the red box represent the upper and lower quartiles

Summary	**Df**	**F**	**P**
Day	**2**	**6.37**	**0.00542 ****

Table 3: Two-way ANOVA analysis of the effect of days on the number of bees found in the hive. With an F of 6.37 and p = 0.00542, the analysis showed a significant association between days and number of bees in the hive. ** = $P \leq 0.01$

Tukey multiple comparisons of means

	Difference	lwr	upr	P adj
2-1	**-4.033**	**-9.802**	**1.735**	**0.2113**
3-1	**-8.833**	**-14.982**	**-2.683**	**0.0038**
3-2	**-4.800**	**-11.190**	**1.590**	**0.169**

Table 4: Tukey post hoc analysis showing the main interaction between days and number of tagged bees in the hive.

A Tukey post hoc tested showed that the number of tagged bees present in the hive on the first was not significantly different from the number of bees on the second day ($p = 0.2113$. The number of tagged bees found in the hive on the second day was also not significantly different from the number of bees found in the hive on the third day ($p = 0.1691$). The analysis, however, revealed that the number of tagged bees found in the hive on the first day was significantly different from the number of tagged bees found in the hive on the third day.

ANOVA For Control and Treatment:

H_0: The mean of number of tagged bees found in the hive for control and treatment bees is the same on all three days

Another post hoc analysis showed the effect of the number of days on the number of tagged bees present in the hive for control and treatment bees. The interaction between days and number of tagged bees in hive was significant for control bees ($p = 0.0364$) but not significant for the treatment group ($p = 0.166$). Additionally, the boxplot showed that more control bees were found all three days compared to the treatment group over the course of the study.

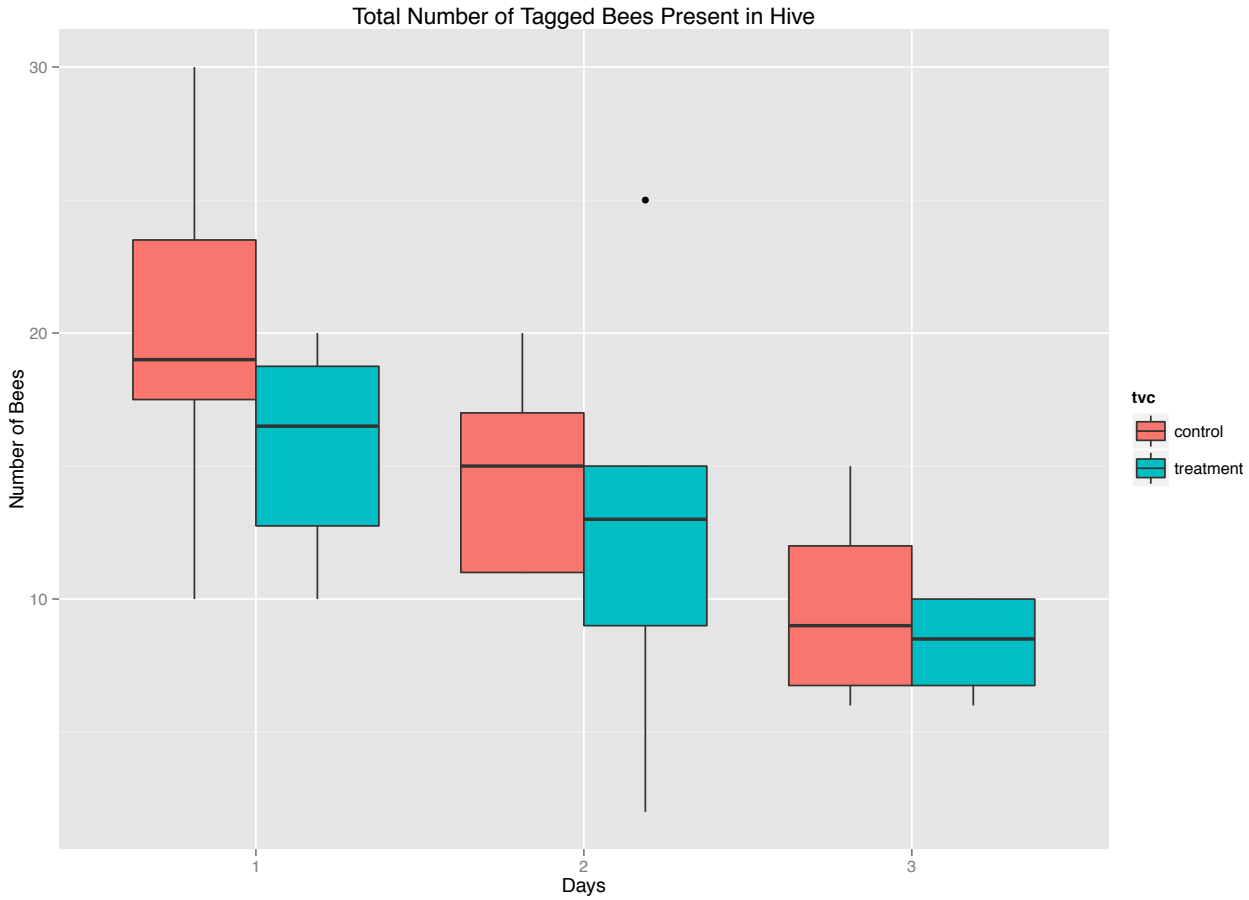

Figure 3: Post hoc analysis for control and treatment groups. The horizontal lines on each box show the maximum value excluding the outlier, the bottom line shows the minimum value excluding outlier. The black line shows the median value, while the upper and lower quartile are represented by the “whiskers” of the box

Summary	Df	F value	Pr>F
Day Control	**2**	**4.434**	**.0364***

Table 5: Two-way ANOVA results for the effect of days had on the number of tagged bees found in or outside the hive for the control group. The results show a significant interaction between days and the number of control bees in hive. The number of bees declined significantly as the days progressed (F = 4.434, p = 0 .0364). * = p-value ≤ 0.05

Summary	Df	Mean	F	Pr>F
Day Treatment	**2**	**66.03**	**2.091**	**.166**

Table 6: Two way ANOVA results for the effect of days on the number of tagged bees found in our outside the hive for the treatment group. The results show no significant interaction between days and the number of control bees in hive. The number of bees declined but not in a significantly way (F = 2.091, p = 0.166)

Thermoregulation Results:

The thermoregulation trials further supported the null hypothesis that the mean of number of tagged bees found in the hive for control and treatment bees is the same on all three days**.** There was no significant difference in fanning behavior of control and treatment guards after they were exposed to elevated temperatures. The temperature at which control and treatment bees fanned was also similar. Finally, the number of control and treatment guard bees that suffered mortality after exposure to elevated temperatures was the same. The thermoregulation trials were conducted late in September, when air temperatures were starting to dip. It took the bees around 20 minutes to calm down and get used to the environment inside the mesh. The bees stopped moving around 50ºC, when they ceased any activities.

9/21/2014	Heat Experiment	Trial 1		
type	T F	T d	number tested	number of fanners
treatment	41.3, 51.9	53.79, 54.44, 55.32, 55.92, 56.91, 57.22, 57.68, 58.95	10	2
control	40.5	50.9, 55.1 (all dead)	10	1
9/21/2014	Heat Experiment	Trial 2		
type	T F	T D	number tested	number of fanners
treatment	0	52.33 (4), 58.43 (rest)	10	0

control	41.9		10	1
9/27/2014	Heat Experiment	Trial 3		
type	T F	T D	number tested	number of fanners
treatment	44.55	44.12, 46.76, 48.08	10	1
control	41.22	54.44, 55.78, 59.12	10	1
9/27/2014	Heat Experiment	Trial 4		
type	T F	T D	number tested	number of fanners
treatment	45.59	56.77, 57.33, 58.1, 58.73	10	1
control	41.87	50.54, 51.01, 52.08, 52.38, 52.97, 54.11, 55.04	10	1
9/27/2014	Heat Experiment	Trail 5		
type	T F	T D	number tested	number of fanners
treatment		50.24, rest stopped moving after 55.88	10	0
control	42.9, 49.98		10	2

Table 7: Effect of exposure to elevated temperatures of fanning behavior and bee vitality. T = temperature in ºC, F = fanning, D = dead. The numbers indicate the temperatures at which the bees fanned or died.

Variation Across Summer:

The types of bees found in the hive during early versus late summer differed (Figure 4). In early summer (late July-mid August), more tagged controls were found inside and outside during data collection. However, more treatment bees were found in the hive in late summer/early fall (Late August-Mid September). This could not be easily explained, as equal number of treatment and control guards had been tagged. Moreover, the concentration of ethyl oleate used on the treatment group was the same both in early and late summer. The number of tagged bees found inside and outside the hive for both groups in late summer was consistent with findings from early summer.

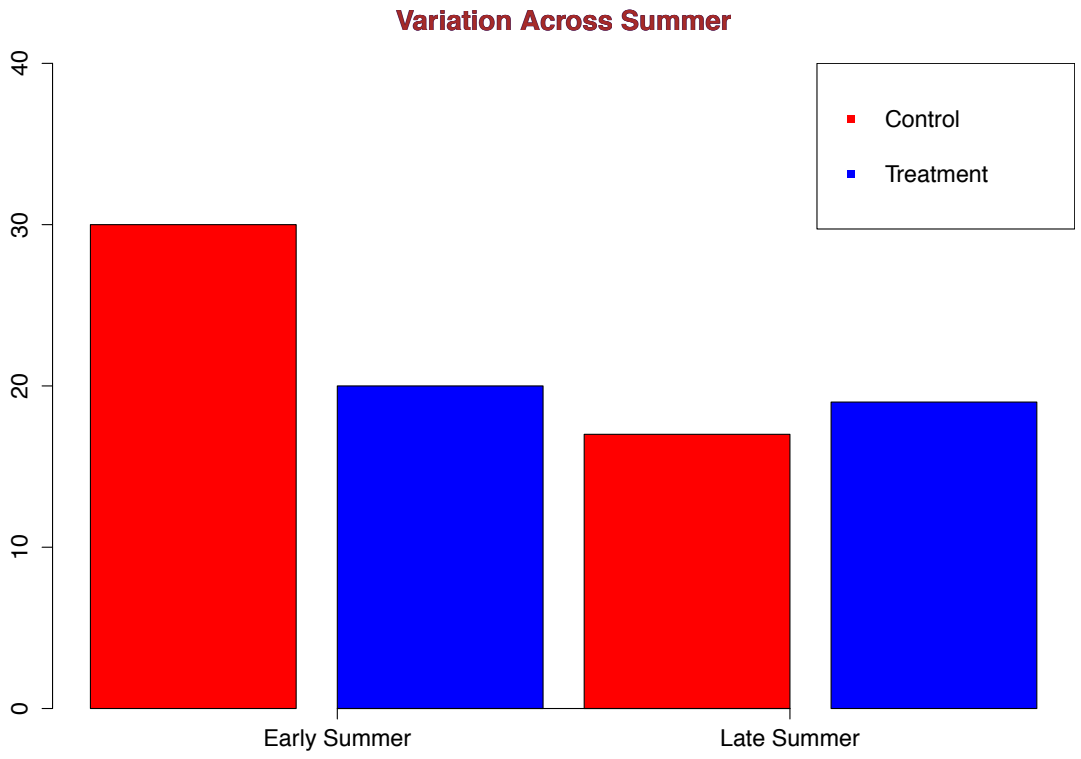

Figure 4: Variation in the number of tagged bees found in the hive in early versus late summer. More control bees were found in the hive during early summer while more treatment bees were found in the hive in late summer.

Manually exposing guarding *Apis mellifera* to ethyl oleate did not alter their behavior. The treatment guard bees behaved similarly to the control bees. We observed almost the same number of treatment guard bees inside and outside hive as the control. In addition, there was a trend over the summer in which more

control bees were found inside and outside the hive at the beginning of the summer while more control bees compared to the treatment were found inside and outside the summer at the end of the summer (Figure 4). Also, the same mean number of bees disappeared from the hive for both the control and treatment group. The X^2 analysis supports these conclusions (Table 1).

Discussion

The results of the present study did not support my hypothesis that treatment guard bees would mature more slowly compared to control bees or that more of the treated guard bees would be found inside and outside the hive, while control guards would mature naturally and become foragers. My findings are inconsistent with previous ethyl oleate studies, which indicated the pheromone's ability to delay the delay of age onset of foraging (Leoncini et al., 2004). Leoncini and colleagues (2004) reported that foragers had approximately 30 times more ethyl oleate in their crops than did nurses. In the latter study, the researchers used three cohorts (n = 500 per cohort) of different ages and found that exposure to ethyl oleate delayed the age of onset of two of the treatment groups (high and low dose) compared to the control group.

My results showed that the number of tagged bees of either the control or the treatment groups found inside or outside the hive significantly declined as days progressed with the most pronounced decline seen on the third day. The colors used to tag the bees had been alternated in the six trials to eliminate potential preference for a specific color by predators. Honeybees have known predators, including wasps and spiders (Bromley, 1948). It is possible that the ethyl

oleate did not have an effect on the bees in the present study and the bees matured naturally. The mean age of guard bees is believed to be 15 days (Breed et al., 1990). While there is a known interaction between guards and foragers, the present study did not observe foragers and only detailed the activities of guard bees (Johnson 2010). We did not monitor their other behaviors or their interaction with other castes in the hive. Only the presence or the absence of the guard bees was recorded.

The results of the present study also showed seasonal variation in the number of bees found in the hive. As discussed above, more tagged control bees found inside and outside the hive at the beginning of the summer, while more tagged treatment bees were found inside and outside the summer at the end of the summer (Figure 4). During the trials in early summer (late July to mid August), more control bees were observed guarding the hive. On the contrary, more treatment bees were found guarding the hive late summer (mid to late August). Furthermore, the overall number of bees counted declined in late summer for both control and treatment groups. This was unexpected; we had predicted that more treated bees would be found in the hive throughout the summer. The seasonal variation findings could suggest a seasonal response to pheromones. It could also suggest a seasonal variation in the behavior of the guard bees as they age. Castillo et al. (2012) found that endogenous ethyl oleate concentrations of foragers significantly increased during the growing season, reaching their maximum in August. Later, researchers also found that most of the synthesis of ethyl oleate took place in late spring and early summer compared to fall and winter (Castillo et al., 2012). Foragers are the closest cast to guards age-wise. The researchers in the latter paper studied how foragers interacted with nurse bees and not guards.

Another interesting observation made during the early stages of the present study was the absence of an apparent response of the bees to ethyl oleate. In experiments conducted in Petri dish, different behavior between control and treatment bees was not seen in response to exposure to ethyl oleate. Preliminary experiments determined the dosage of ethyl oleate suitable for bees. A high dosage of ethyl oleate (two drops) was determined to be lethal to most bees in the Petri dish experiments; most bees became disoriented and did not fly away after being released from the Petri dish. A few ceased all activities, while a small number of them remained active in the petri dish and flew away after being released.

Overall, the results of my study, therefore, did not fit in with findings from the literature. There was no explanation for the disappearing of my control and treatment bees. Possible explanations are natural progression of guards to foragers, disorientation after being exposed to ethyl oleate and thus abandonment of the hive, or increased visibility after being treated, which may have led to higher rate of predation. It is also possible that the bees in my study did not adequately take in the ethyl oleate. It was assumed that the pheromone would affect the bees if they came into contact with it in the Petri dish. In the present study, the amount of ethyl oleate present in the treatment and control guard bees was not determined. Most studies in the literature measured directly how much ethyl oleate was present in the crops or other regions of the bee's body. Additionally, the trials could have been conducted beginning early May and concluded in late June to mid July. As mentioned above, most bees are active in late spring to mid summer. Ethyl oleate is also synthesized in high amounts in late spring and summer, reaching peak production in late spring (Castillo et al., 2012). The time period of the experiment is crucial because it could indicate honeybees' higher sensitivity to ethyl oleate in late spring and summer than late summer/early fall.

Future studies should include larger numbers of bees to increase the likelihood of finding them during the observation period. Every trial should include close to 100 bees instead of the 30-50 bees that were tagged for the present study. Also, it should be ensured that the treated bees actually ingested the sugar solution. In this study, the bees were merely exposed to ethyl oleate and some of the bees failed to drink the sugar solution. The bees were in the Petri dish for about 3-5 minutes each and were not calm enough to feed. In future studies, the researcher should wait for the bees to adapt to their new environment in the Petri dish before providing them with the sugar solution. Also in future studies, more time should be spent observing the nest. Foragers should be examined by blocking the entry to the hive, which would allow the researcher to see if any of the guard bees transitioned to foragers. Furthermore, the tagged bees in the control and the treatment groups should be preserved for future analysis of their anatomical parts. This is important in order to measure the amount of ethyl oleate present in each group's crop, brain and other parts. With such additional data, the researcher could test whether the presence of ethyl oleate prevented the age progression of guards into foragers.

The findings of the present study and previous similar studies could be used to develop methods to regulate beehives for commercial reasons. Commercial beekeepers could apply ethyl oleate to their hives in order to delay nurses, workers and guards from aging and transitioning to foragers. However, more research is needed to develop a reliable method to ensure that bees take in the pheromone. Researchers would also need to evaluate to extent at which bees are affected by ethyl oleate and how to assess whether it delays aging. Early research suggested that bees exposed to ethyl oleate aged more slowly. Control bees became foragers at a later stage (around 20 days) compared to the control (around 17 days) (Leoncini et al., 2004). Such studies also help scientists understand how division of labor is regulated in honeybee societies. Honeybees behave

similarly to human societies by forming strict hierarchies and careful division of labor. Interestingly, bee societies do this by not forming top-down channels of communication. Bees use signals to send messages to other bees. For example, alarm pheromones are used to alert other bees of imminent danger. Using chemical signals has the advantage of faster task allocation compared to the usage of cues or other methods of communication, which can decrease the probability of response from the target receiver (Johnson, 2010). Ethyl oleate is a primer pheromone, for which little is known about its effect on, and usage by, honeybees. However, as a tool to delay the aging of younger bees by older ones (Leoncini et al., 2004), ethyl oleate could be crucial to the maintenance and organization of honeybee communities.

Conclusion:

The discovery of the primer pheromone ethyl oleate in 2004 took researchers a step closer to understanding how honey bees utilize division of labor in managing hives. Early research on ethyl oleate highlighted its potential importance in controlling the maturation of younger bees by older bees (Leoncini et al., 2004). The results from the present study did not fit in with earlier findings in the literature. I propose using larger number of bees in our future studies, recording data earlier and for longer period in the summer and examining the concentration of ethyl oleate in the anatomical parts of subject bees in order to get conclusive findings. I also suggest a more useful way to ensure subject bees absorb ethyl oleate without too much interfering.

While several lines of inquiries in the present study failed to show difference in the behavior of bees exposed to ethyl oleate (treatment group) versus control bees not exposed to ethyl oleate, I found a conspicuous lack of decline in bee numbers in late summer in the

treatment group. It is possible that the steady bee numbers in late summer in the ethyl oleate treated group may, in fact, be a result of a delay of aging by ethyl oleate.

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