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Fatty Acids as a Potential Foraging Cue in Bumblebee Pollen Detection

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by

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Abstract

Bee populations, both wild and commercially supplied, are on the decline. A diminishing availability of required nutritional components may be a major contributing factor. Understanding how bees are able to both detect and remember the locations of these dietary macromolecules will be a valuable contribution to sustainability efforts worldwide. To learn more about how bees detect and assess necessary pollen fats, I explored the potential of bees to form associations between these nutrients and visual stimuli. I found strong evidence that bees can detect pollen based fatty acids and that the presence of these compounds increases bee learning and perhaps memory. Additionally, I found evidence suggesting that fatty acid consumption may increase bee longevity. My research suggests that pollen fats are a chemical attractant potentially important to pollinator species.

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Introduction

Fatty acids are the building blocks of lipids, one of the four biological macromolecules necessary to all living organisms. These molecules are found in incredible variety, but their properties generally depend upon their hydrogen saturation status, including completely saturated, monounsaturated and polyunsaturated (Kiran, Panickar, Bhathena, 2010). Fatty acids play a wide range of roles within biological systems. Their potential inclusion into amphipathic compounds makes them a major component of cellular membranes (Chapman, 1974). Fatty acids also act as the major reservoir of potential usable energy in animals. They can be broken down via the beta oxidation catabolic pathway to yield seven kilocalories of energy per gram, three more kilocalories than an equivalent amount of carbohydrates (Harwood, 1988). The potential anti-inflammatory effects and neurological benefits of some fatty acids have represented significant areas of research within the past few decades (Mattson & Grundy, 1985; Grundy, 1986; Kiran et al., 2010).

Fatty acids play interesting biological and ecological roles across a variety of species. Some wasp species (*Cotesia glomerata*) utilize fatty acid signals in the determination of suitable host plant locations for egg laying (Horikoshi, Takabayashi, Yano, Yamaoka, Ohsaki, and Sato, 1997). Cockroaches utilize fat-based pheromones in communication of alarm signals (Rollo, Borden, Casey, 1995). The nest structures (e.g. honey pots and brood cells) many bee species create are formed from a fatty acid-rich waxy substance secreted by glands on the bee abdomen (Tulloch, 1970). Some bee species produce fatty acid containing pheromones utilized in intra-hive signaling and

communication (Breed, 1998). Fatty acids even play a role in bee mating, as male bumblebees (*Bombus terrestris*) utilize these molecules in the formation of a "mating plug" which prevents the queen from repeated mating (Baer, Morgan, Schmid-Hempel, 2001).

Fatty acids are thought to play a major but under-explored role in bee nutrition. Bees obtain their fatty acid requirements from pollen. Foraging bees collect pollen (their major source of protein) from flowers and return with it to the hive. There the pollen is stored and consumed by both adult workers and developing larvae (Roulston & Cane, 2000; Smeets & Duchateau, 2002). The fatty acids contained within this pollen represent an essential dietary component to bees. From a purely energetic standpoint fatty acids are capable of providing a significantly higher Caloric equivalent per mass than carbohydrate or protein alternatives. This high energy storage potential makes them particularly valuable to these pollinating species which must expend many kilocalories of energy to sustain flight (Kammer, 1978).

Fatty acids are not found within pollen necessarily, but rather in the substance that surrounds pollen grains. Pollen is covered in a lipid-dense protective coating known as pollenkitt (Pacini, Hesse, 2005). The high hydrophobicity of the fat molecules and free fatty acids within pollenkitt allow for pollen's water resistant properties. It is speculated that the fatty acids found in pollenkitt may also provide attractive cues to pollinators, thus increasing the likelihood that a pollen store would be remembered and returned to (Dobson, 1988; Lepage, Boch, 1968). Recent evidence indicating the ability of *Drosophila* to detect fatty acids (Masek, Keene, 2013) raises the question of whether bees

may possess a similar ability. The existence of a chemical cue to drive pollen collection is so far understudied. Knowing how bees respond to fatty acids would help elucidate the chemical basis of bee-pollen attraction and shed light into what role pollen rewards play in learned preferences for certain flower species. This is of particular significance to those species such as tomato plants and poppies which utilize only pollen as an incentive to attract pollinators.

The bumblebee represents an excellent model organism to examine the potential attractive quality of pollen fats. Bumblebee colony organization, which includes specific foraging workers, allows for ample subject selection to test the potential effects of a pollen-based reward on learning (Jandt, Dornhaus, 2009). Bumblebees have also been shown to possess a very strong potential for rapid associative learning of multiple stimuli. The proboscis extension response (PER) is one method of testing this learning potential (Giurfa, 2007). The PER method is a form of classical conditioning which involves the training of a bee subject to a conditioned stimulus such as color or odor. The subject extends the proboscis to receive an unconditioned reward (generally sucrose solution) while being exposed to the stimulus. Once an association is formed between this stimulus and the reward, the subject will extend the proboscis to the conditioned stimulus alone as indication of learning (i.e. the conditioned response). This method has provided a very successful gauge of learning in honeybees (Giurfa, Sandoz, 2012), and has recently begun to be used in bumblebee testing with similar success (Riveros, Gronenberg, 2009).

Bumblebee populations around the world, both natural and commercially supplied, are on the decline. These species are subjected to an increasing number of barriers to survival, many of which are human imposed (Williams, Osborne 2009). A likely cause of this population loss is the expansion of human development and the accompanying change to the natural environment. Diminishing availability of required dietary resources, or alteration to the naturally available supplies could pose substantial threat to these pollinators. Bumblebee pollination sustains many wild and cultivated plants, and as a result sustains human agriculture and food production (Kevan, 1999). The loss of this vital organism would thus have significant and lasting consequences to human life and the sustainability of this planet's ecosystem (Potts, Biesmeijer, Kremen, Neumann, Schweiger, Kunin, 2010). A major player in the decline of both wild and managed bee populations is the decline of the nutritional quality of pollen available (Goulson, 2008). A better understanding of how bumblebees detect and remember the locations of vital dietary components such as pollen would therefore be a significant contribution to sustainability efforts worldwide.

I explored four questions relating to bumblebees and pollen fatty acids. 1.) Can bumblebees detect fatty acids at all? 2.) If possible, does detection occur primarily via pre-ingestion mechanisms such as taste and/or smell, or by post-ingestion mechanisms which would be dependent on consumption of the compound? 3.) Does the presence of fatty acid within a reward increase associative learning by bumblebees? 4.) Does consumption of fatty acid solution increase longevity? I performed three independent experiments including a PER learning assay utilizing blue light as the conditioned stimulus, a fatty acid preference assay and a longevity test. Oleic acid was selected as the

fatty acid for testing due to both its significant presence within pollenkitt (Dobson, 1988) (Manning, 2001) and its prominence within the bee body (Cvacka, Horvorka, Joros, Kindl, Strandsky, Valterova, 2006).

Materials and Methods

Animals

I maintained four queen right colonies of *Bombus impatiens* at 25 ºC for two months, May-June, 2014. I added a total of four additional colonies which I maintained under similar conditions, July-September, 2014. All colonies experienced a natural 24 hour photoperiod. The nest boxes were commercially supplied (Koppert Biological Systems, MI, USA) and connected to a single foraging and flight arena (99cm x 96cm x 91cm), to which bees were given constant access. Foragers were allowed *ad libitum* access to artificial feeders containing a 15% sucrose (w/w) solution and I supplied approximately 0.60 g of honey-bee collected pollen directly into colonies every second day. In an attempt to mimic the stimulatory effects of the natural environment I placed blue, yellow and red artificial flowers into the flight arena.

Experiment 1. Proboscis Extension Response Assay

Harnessing

I used a proboscis extension response (PER) harnessing technique, protocol and apparatus as described by Riveros and Gronenberg (2009). I collected a total of 195 subjects from artificial feeders using a low powered ‘Bee-Vac’, insect aspirator device. Only females were selected for testing, as male bumblebees do not forage for pollen.

Subjects were selected at random and it is assumed that an equal representation of subjects across all colonies were tested. I tested twelve subjects per training bout. After collection, I placed the subjects on ice for 20-25 minutes to induce the short-term paralysis necessary to harness safely. I then mounted subjects in plastic harness tubes (7-8 mm diameter) utilizing a "yolk" which supported their head securely forward while allowing for the full range of proboscis extension (Figure 1). I allowed the subjects to acclimate to the harness for two hours at room temperature without access to feeders.

Training Apparatus

The apparatus consisted of a circular rotating platform suspended 28 cm above the tabletop (Figure 1). Twelve testing chambers created from plastic cylinders were glued to this platform, approximately 6 cm apart, and each with an open window (3cm x 1.5cm) facing outward which allowed access to the test subject. Except for a thin mounting platform, the bottom of each testing chamber remained open, allowing light to project in from below. The interior of each cylinder was lined with aluminum foil, providing reflection necessary for an even distribution of light throughout the chamber. Light was provided by a platform positioned below the testing chamber, onto which were mounted three blue LED bulbs (peak wavelength 470 nm). I chose blue light was as the associative stimulus as it is the most effective at eliciting PER learning (Riveros, 2009). Light only entered the chamber positioned directly above the LED bulbs and trials were conducted in an otherwise dark room. I controlled the timing of visual stimuli via a switchboard adjacent to the training vessel. A constant 6 volts of power was provided to the system.

Training Protocol

Prior to the start of testing, I presented subjects with a small droplet of a 30% sucrose solution via a syringe to ensure that bees would exert their proboscises in the presence of a reward. The solution was presented within detection distance of the subject's antennae and, after palpation of the solution, the subject would extend its proboscis to drink. Once a successful PER was elicited I transferred subjects to the training apparatus and allowed them to acclimate for 5 minutes prior to testing. If a subject failed to show a PER at this time it was removed from further testing. This ensured all subjects were somewhat equally motivated and responsive to the reward.

Subjects then entered the "training phase" during which they received their first exposure to the stimulus. Timing of the stimulus and reward was constant for each subject and occurred in the following sequence (Figure 2a): First the conditioned stimulus (blue light) was turned on for 10 seconds absent reward (stimulus period). The reward was then introduced to the subject for 5 seconds during which the stimulus remained present (associative period). Rewards were presented via two syringes attached in parallel (Figure 2b), one of which was available for palpation by the antennae but not consumed (pre-ingestion reward), and the other consumed but not palpated by the antennae (post-ingestion reward). First I presented the "pre-ingestion" syringe for palpation by the antennae, and once PER was elicited I quickly replaced it with the "post-ingestion" syringe to allow for consumption of reward. Within this five second period the subject was allowed to drink from the reward for up to 3 seconds. Finally I removed both the blue light and the reward simultaneously, resulting in a total trial period of 15

seconds. The "training phase" represents the first opportunity for the subject to form an association between the stimulus and the reward, and while this was never observed, any subject exhibiting PER prior to the first introduction of reward would have been removed from the experiment.

Eight "trial phases" then followed, each of which consisted of a testing period and a reinforcement period. The testing period describes the initial ten seconds of each trial during which blue light is present without reward. I interpreted any PER occurring during this time as evidence of associative learning, and immediately provided three seconds of reward. The reinforcement period describes the final five seconds of each trial, occurring only if the subject failed to respond with PER in the initial ten second testing period. During the reinforcement I again presented the reward in the presence of blue light, thus increasing the potential for associative learning by the subject. I tested subjects every five minutes for a total of eight trials per subject.

Finally I performed a long term memory test thirty minutes after the last (eighth) trial of each testing bout. During the interim time subjects were maintained in harnesses and in a dark room. The subjects were exposed to a final ten seconds of blue light exposure, representing the last testing period of the session. PER occurring during this time was interpreted as evidence of long term memory of the association between the color and the reward.

Treatments

Bees were randomly assigned into one of six treatments. On each day of training, all six treatments were represented. All treatments included one or both of the following

rewards: “Sucrose”- 30% reagent grade sucrose in water solution with a 1:200 ethanol addition (S), and “Fatty Acid”- 30% reagent grade sucrose solution with a 1:200 dilution of equal parts oleic acid dissolved in ethanol (F). Treatments are notated as follows: (pre-ingestion)//(post-ingestion), with the left bracket indicating what was present in the “pre-ingestion” syringe (i.e. presented to the antennae) and the right bracket indicating what was present in the “post-ingestion” syringe (i.e. what the bee actually drinks via its proboscis) (Table 1).

I also carried out two unpaired treatments where the reward presentation occurred prior to the conditioned stimulus, as opposed to after it. As such no association between the reward and the stimulus should be formed by subjects. This provides a control for external variables other than learning influencing PER in the presence of a stimulus, such as fatty acid presence itself altering the overall responsiveness to blue light.

Table 1. Representation of the six treatment types utilized in the Proboscis Extension Response Assay.

Treatment	**Reward Direction**	**Pre-Ingestion**	**Post-Ingestion**
(S)//(S)	Paired	Sucrose	Sucrose
(F)//(S)	Paired	Fatty Acid	Sucrose
(S)//(F)	Paired	Sucrose	Fatty Acid
(F)//(F)	Paired	Fatty Acid	Fatty Acid
(S)//(S)	Unpaired	Sucrose	Sucrose
(F)//(F)	Unpaired	Fatty Acid	Fatty Acid

Experiment 2. Assessing Fatty Acid Preference

I selected subjects for testing as described in Experiment 1. I placed subjects in translucent acrylic cylindrical chambers (13.5cm length x 2.5cm diameter) and allowed them to acclimate to the testing environment for 24 hours while being provided *ad libitum* access to 15% sucrose w/w. Two hours prior to testing, the sucrose was removed from the subjects to ensure sufficient and consistent motivation for the preference test. Preference for the two solutions described in Experiment 1 ("Sucrose" and "Fatty Acid") was tested by placing 50 µl of each solution into separate capillary tubes which were previously packed with a small wad of cotton from which bees could drink. The initial amount of solution present in each tube was marked to use as a baseline for comparison. Both solutions were presented simultaneously to each subject at "minute 0" and I measured the amount of consumption every 30 minutes until 90 minutes time then took one final measurement at 180 minutes time. I determined consumption via millimeters of solution missing from the "minute 0" solution mark, with one millimeter equivalent to approximately 12 microliters of solution consumed (Figure 3).

Each testing bout consisted of 12 subjects for a total of 22 subjects, and I monitored their activity continuously throughout the trial to ensure that solution loss from the capillary tubes occurred only through consumption and not via accidental loss. If solution loss occurred other than from consumption, for instance via spillage, data was excluded from analysis. Evaporation controls accompanied each training bout by setting up preference chambers with solution filled capillary tubes, however absent a bumblebee subject (Figure 3). The amount of solution lost from each capillary tube in these controls

estimates the evaporative contribution to fluid loss in each of the testing chambers. I subtracted this amount from the total amount of fluid missing from each capillary tube at the measurement times, and thus the remainder of the missing fluid is assumed to due to consumption alone.

Experiment 3. Longevity Assay

I selected subjects for testing as described above, with twelve total subjects being selected per testing bout for a total of 34 bees. I placed subjects into PER testing harnesses as described in Experiment 1, and they were allowed to acclimate to the harness for three hours without access to feeders. I then randomly assigned the twelve subjects to one of two treatment groups, ensuring that both groups were represented equally in each testing bout. The first treatment group was fed to satiation on the same sucrose solution described in Experiment 1 ("Sucrose"). The second treatment group was fed to satiation on the same oleic acid-containing solution described in Experiment 1 ("Fatty Acid"). I defined satiation as the refusal of a subject to consume more of the solution following vigorous feeding. Any subject that failed to consume solution to satiation was excluded from the experiment. I then monitored subject longevity at 10 hour marks from the time satiation was achieved. If a subject had expired between two 10 hour points, it is assumed that the subject lived up until that last 10 hour mark.

Data Analysis

In Experiment 1, I compared the percentage of subjects per treatment group exhibiting PER to the blue light across 8 trials. Non-responders, subjects who failed to respond to reward presentation (extend the proboscis and drink) two or more times, were excluded from all summary statistics and data analysis. I measured the total percentage of PER across all trials by excluding all subjects who exhibited less than three conditioned PER responses in 8 trials. General linear models were used in order to compare learning curves of the four paired treatment types. In all cases the response is the percentage of subjects expressing PER, and the explanatory variables are the trial number (1-8) and the fixed treatments (both "pre" and "post" ingestion rewards). A maximal model was first employed and all non-significant interactions were then removed in a step wise fashion resulting in a minimal model. Analysis was performed by R v.3.1.0 (R Development Core Team 2010). In Experiment 2, for fatty acid preference analysis a paired T test was performed comparing solution loss at the point of largest difference between the two provided solutions. In Experiment 3, for analysis of longevity, a Gehan Breslow survival analysis was performed comparing survivorship between the two treatments. All analyses were conducted in consultation with Dr. Felicity Muth and Dr. Anne Leonard.

Results

Experiment 1: Proboscis Extension Response Assay

Bees which consumed rewards containing oleic acid were more likely to learn than those which consumed only sucrose-containing rewards. This is represented by the significantly higher proportion of conditioned PER exhibited by subjects in both treatment groups which consumed oleic acid-containing rewards ([F]//[F], [S]//[F]) when compared to subjects in treatments which did not ([S]//[S], [F]//[S]) (*GLM: trial:* $F_{1,28} = 75.709$, $p < 0.001$, Figure 4). I found no significant effect on learning as attributable to oleic acid presence in pre-ingestion rewards.

An improvement in long term memory is strongly suggested in bees which consumed oleic acid-containing rewards. I found a very strong trend toward more subjects continuing to exhibit PER after a thirty minute interval if they had consumed oleic acid compared to subjects which had not (z test: $n = 141,\ z = 1.934,\ p = 0.053$).

Experiment 2: Preference Assay

Bees showed no preference for oleic acid-containing sucrose solution over a plain sucrose solution when tested in isolated preference tubes. The amount of either solution consumed by subjects did not vary significantly after 180 minutes time, suggesting that subjects did not prefer one over the other (t test: $n = 22, t = 1.320\ \ p = .20$).

Experiment 3: Longevity Assay

It is strongly suggested that bees which were fed to satiation with oleic acid-containing sucrose solutions lived longer than did bees fed to satiation with a plain sucrose solution. Of the 17 subjects tested in each of the two treatments, a very strong trend exists toward a larger proportion of oleic acid-fed individuals surviving longer than individuals fed only sucrose (GB Test: 3.718, $p = 0.054$, Figure 5). The average survival time for subjects which were fed oleic acid-containing sucrose was 42.9 hours (SD ± 3.181) compared to subjects which were fed plain sucrose and had an average survival time of 33.5 hours (SD ± 3.424).

Discussion

This experiment attempted to mimic natural stimulatory cues provided by pollen fatty acids, a vital resource to bumblebees. I sought to determine if bumblebees are capable of detecting individual fatty acids via either pre or post ingestion mechanisms, and if so, what the consequences might be for associative learning potential and/or longevity. I found evidence to support the existence of a bumblebee fatty acid detection mechanism which appears to be dependent on actual consumption. Bumblebees which consumed fatty acids were significantly more likely to exhibit associative learning and showed a strong trend toward increased long term memory. However, bumblebees showed no significant preference for a fatty acid and sucrose solution over a plain sucrose solution when presented in a free-moving preference assay. Finally, bees that consumed a fatty-acid containing solution showed a strong trend toward increased longevity.

The PER testing apparatus represents a high stress environment for bee test subjects which are constantly attempting to escape harnessing. In order to elicit learned PER the associated reward must be of sufficient detectable quality to compensate for this stressed setting; previously demonstrated by testing with different sucrose concentrations (Laloi, Sandoz, Picard-Nizou, Marchesi, Pouvreau, Tasei, Poppy, Pham-Delegue, 2003). In this experiment the concentration of sucrose was held constant throughout treatments, but only subjects which consumed fatty acid-containing solutions exhibited significantly more learned PER. The absence of a similar increase in learning by bumblebees which were allowed only pre-ingestional exposure to the fatty acid is suggestive of a post-ingestion-dependent detection ability which both increases reward potential and associated likelihood of learned PER.

Two possible mechanisms, both dependent on consumption, could help explain oleic acid's observed enhancement of visual learning: oral detection and physiological detection; neither of which are necessarily mutually exclusive. Both bumblebee and honey bee chemosensation occurs primarily via three organs- the tarsi, antennae and proboscis/mouth (Sanchez, 2011). Differences in olfactory versus gustatory receptor ratios between these organs remains highly under-studied in bumblebees, yet this could represent a plausible explanation for consumption-dependent fatty acid detection. The proboscis and mouthparts may contain the receptors necessary to perceive fatty acids within nutrient supplies and thus facilitate learned attraction toward those stores. But even the presence of these receptors within the proboscis and oral region may not constitute all detection requirements. In rats, fatty acids have been shown to activate certain gustatory receptors in the posterior oral region, yet only once these fatty acids

have actually been consumed. The mechanism for this action exists via fatty acid activation of delayed rectifying potassium (DRK) channels which work to inhibit efflux of potassium from taste receptor cells. The result is speculated to be an actual sensation of fatty acid presence in the consumed food source (Gilbertson et al., 2010). Certain ruminant species have also been shown to exhibit preference for more nutrient-dense food sources based on post-intestinal feedback mechanisms uninvolved with gustatory or olfactory sensation (Provenza, 1995). Some similar post-ingestional cue may exist in bumblebees either independent of or in conjunction with chemosensors in the proboscis/oral region.

The absence of a significant preference for fatty acid-containing sucrose solution over a plain sucrose solution (Experiment 2) may be further evidence of a post, rather than pre-ingestional perception capability. In the presumably low-stress testing environment of the preference tubes, and with both solutions presented in close proximity to one another, differentiation between which solution provided the post-ingestional benefit might be less apparent. Other researchers have noted dramatic differences in bees' consumption preferences when comparing free-moving vs. harnessed assays (Ayestaran, Giurfa, Sanchez, 2010). Additionally, the trend toward increased longevity associated with fatty acid consumption (Experiment 3) provides substantial evidence for the benefit of fatty acid recognition within a food source. Adult bee workers which are starved of pollen have been shown to have decreased survivorship compared to bees allowed ad libitum access to pollen stores (Smeets, 2003). As was previously mentioned, foraging bees possess an extremely high metabolic rate necessary to sustain flight, and thus require large stores of readily available energy to power their activity. Lipids provide almost

twice the kilocalorie yield compared to equivalent amounts of carbohydrates and thus would represent a beneficial component to the bee diet (Kammer, 1978). My results indicate an average of approximately 7 hours of increased survivorship in bees which were fed to satiation with the fatty acid solution compared to the plain sucrose solution. This apparent benefit was found following only one feeding event and with fatty acids present in very low concentrations. Similar significant differences in survivorship have been previously documented following only one feeding event, however utilizing very low concentrations of harmful compounds as opposed to potentially beneficial ones (Ayestaran et al., 2010). More significant findings of fatty acid benefit to individual longevity may result from further testing with repeated feedings and higher fatty acid concentrations.

No learned PER was elicited in subjects of the unpaired treatment groups, therefore the potential for the observed increased in conditioned responses to be associated with factors other than the conditioned stimulus (blue light) is unlikely. For example, bees did not simply become more responsive to the blue light after consuming fatty acids (in the absence of learning). That being said the PER protocol involves some unavoidable but un-natural variables including the induction of temporary paralysis necessary for harnessing, and the restraining of the harness itself. A further caveat in interpreting the findings of Experiment 3 is that in feeding bumblebees to satiation prior to measuring their longevity, it is assumed but unknown that subjects consumed relatively similar amounts of their respective solutions. Further experimentation could examine the effects of fatty acid sucrose versus plain sucrose consumption when both solutions are administered in the same amount, however this would entail some subjects

being fed to satiation and others not which could also complicate interpretation of findings.

Bumblebees would benefit in multiple ways from the ability to detect fatty acids. As pollen is necessary for the survival of both the individual worker and the colony as a whole, another mechanism of attraction to this vital resource could represent an important foraging cue. Bumblebees are known to utilize nectar quality as a basis for flower selection (Wright, 2009). A mechanism for fatty acid detection would allow foragers to further evaluate and select pollen based on nutrient density as well. Pollinators have been shown to selectively visit flowers providing higher pollen protein content (Rasheed, 2003), however the exact mechanism of this detection is as of yet not understood. Pollen proteins are concealed deep within the pollen grain, and therefore are not immediately accessible to detection by pollinating species. As was previously mentioned, pollen fats are located on the surface of the grain and are thus a much more likely chemically attractive candidate.

Previous research has also shed light on the neural plasticity of honeybees, specifically with regard to learned associations with reward. Neural connections are strengthened in associations involving higher reward quality, leading to improved long term memory and thus the ability to remember sources of a reward (Menzel, 1993). In this experiment, the strong trend toward increased long term memory in bumblebees which consumed fatty acid could provide basis for improved foraging ability, with pollinators better able to locate nutrient rich flowers based on the memory of fatty acid presence within their pollen. This would presumably benefit plant species that reward

bees with pollen alone, in terms of increasing the chances that bees will make subsequent visits to members of their own species, transporting enough pollen to conspecifics to result in reproduction for the plant.

Pollen has long been recognized as the bee's protein source, however it represents a nutrient supply far beyond that due to the fatty acids it contains. The results of this experiment provide evidence for the presence of another attracting force to pollen, and shed light into the mechanisms by which pollinators selectively visit certain flower species. A decline in the number of pollen producing plant species has been previously linked with pollinator decline (Kleijn, 2008). Understanding what nutrients bees seek when pollen foraging, and how they assess the presence of these nutrients, is vital to bee population success. Bees may prefer and/or require certain pollen types which they are able to detect based on the consumption of the fatty acids present within specific pollenkitts.

This work provides a significant basis for further research on fatty acid detection potential by bees, as well as the potential effects that fatty acids have on these species. More fatty acids should be tested within a PER setting, perhaps in combination in solution versus plain sucrose. Further examining of potential preference for one fatty acid over another would provide greater evidence for the ability to distinguish between pollen species, and a greater analysis of the fat content of pollen collected by wild foragers would also prove beneficial. Finally the implications of fat presence within the bee diet could be further explored by altering the fatty acid availability to subjects over a longer period and measuring associated effects on longevity at the individual or colony

level. The benefits of this continued exploration would be of considerable benefit to human agriculture and bee population sustainability.

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Figures

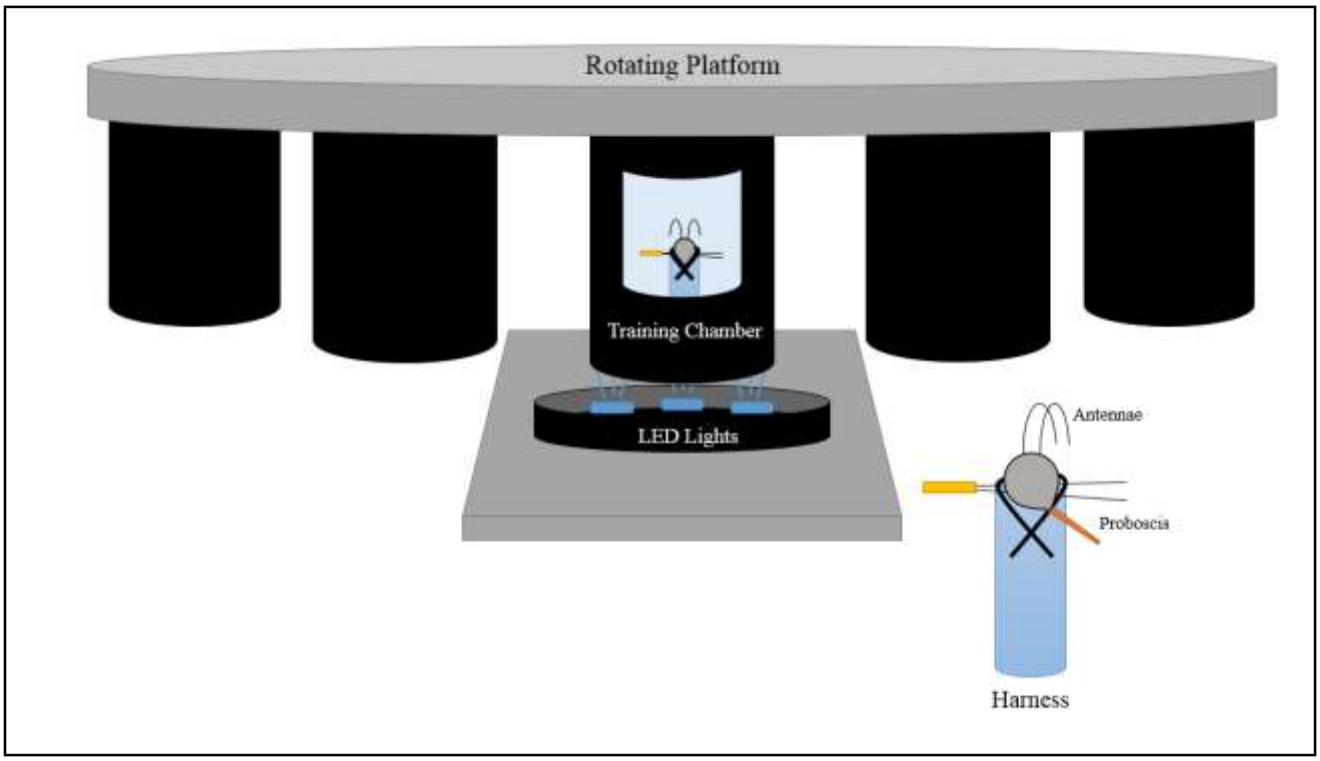

Figure 1. Illustration of the bee harness and PER training apparatus.

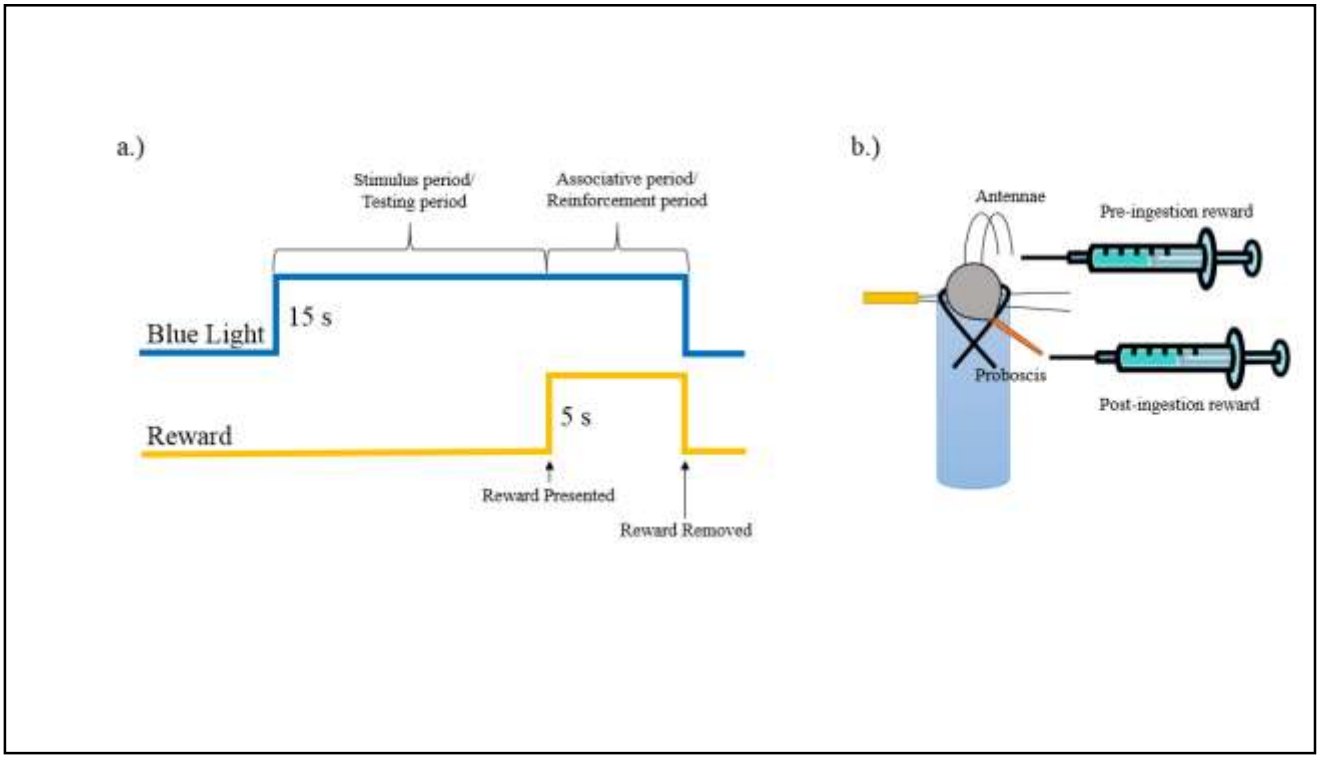

Figure 2. a.) Illustration of the training cycle. b.) Illustration of reward presentation to bees.

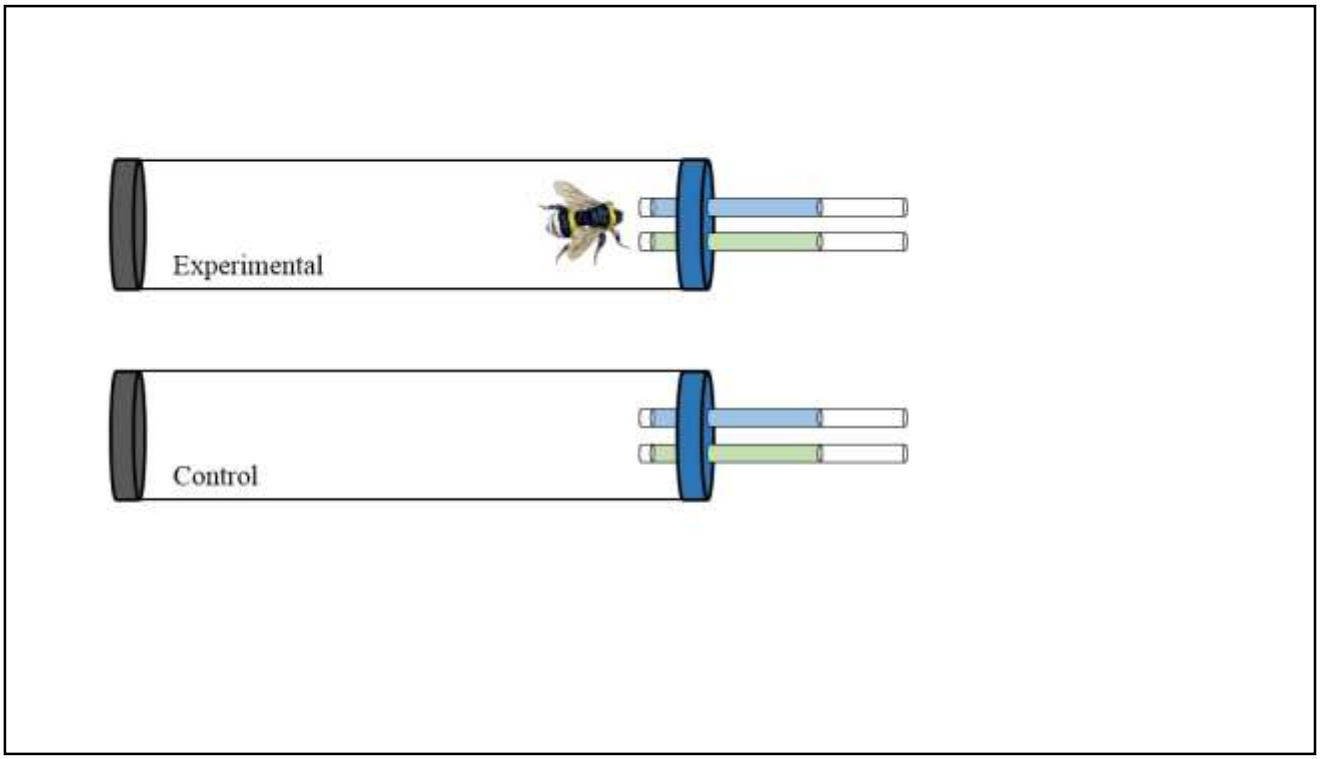

Figure 3. Illustration of the preference tube assay with both experimental and control groups.

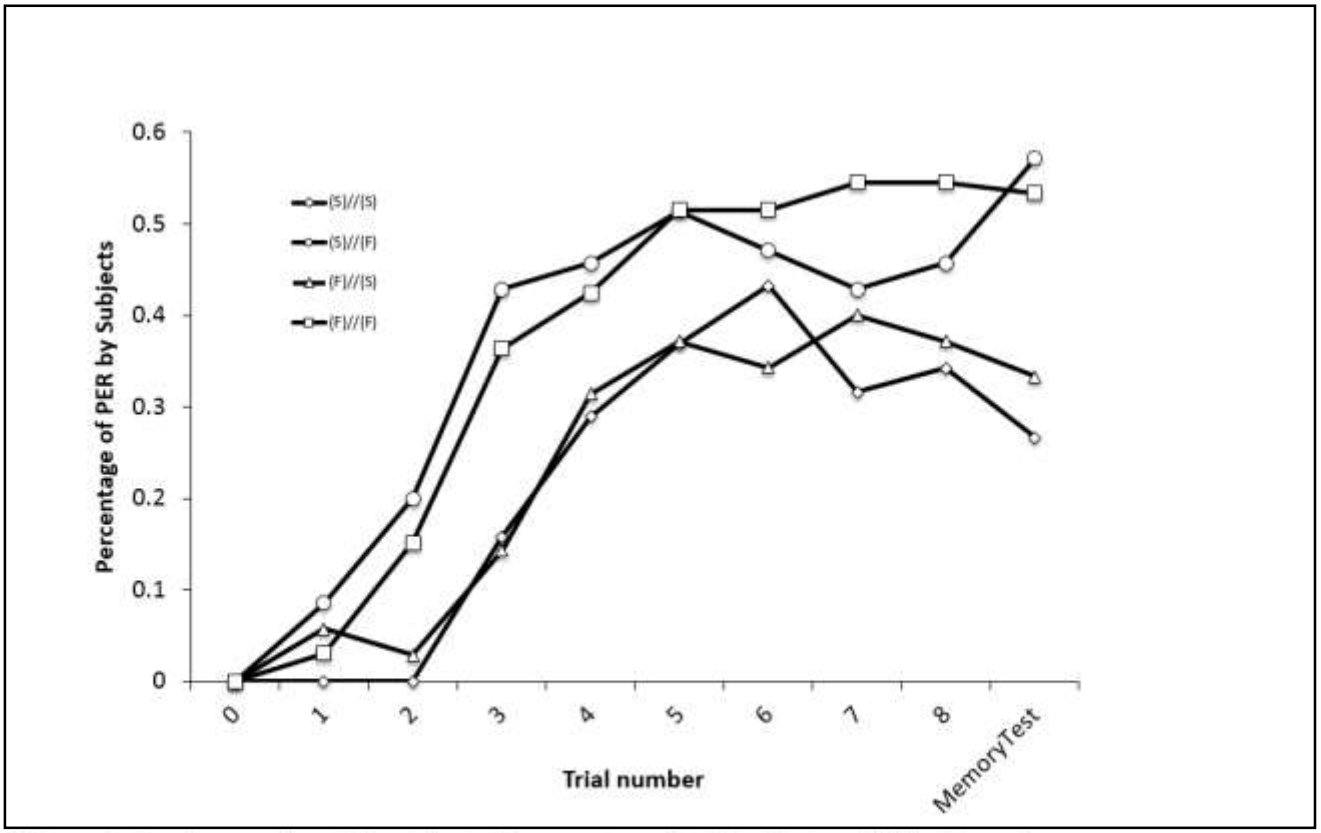

Figure 4. Graph comparing trial number to the percentage of total subjects exhibiting learned PER across four treatment groups.

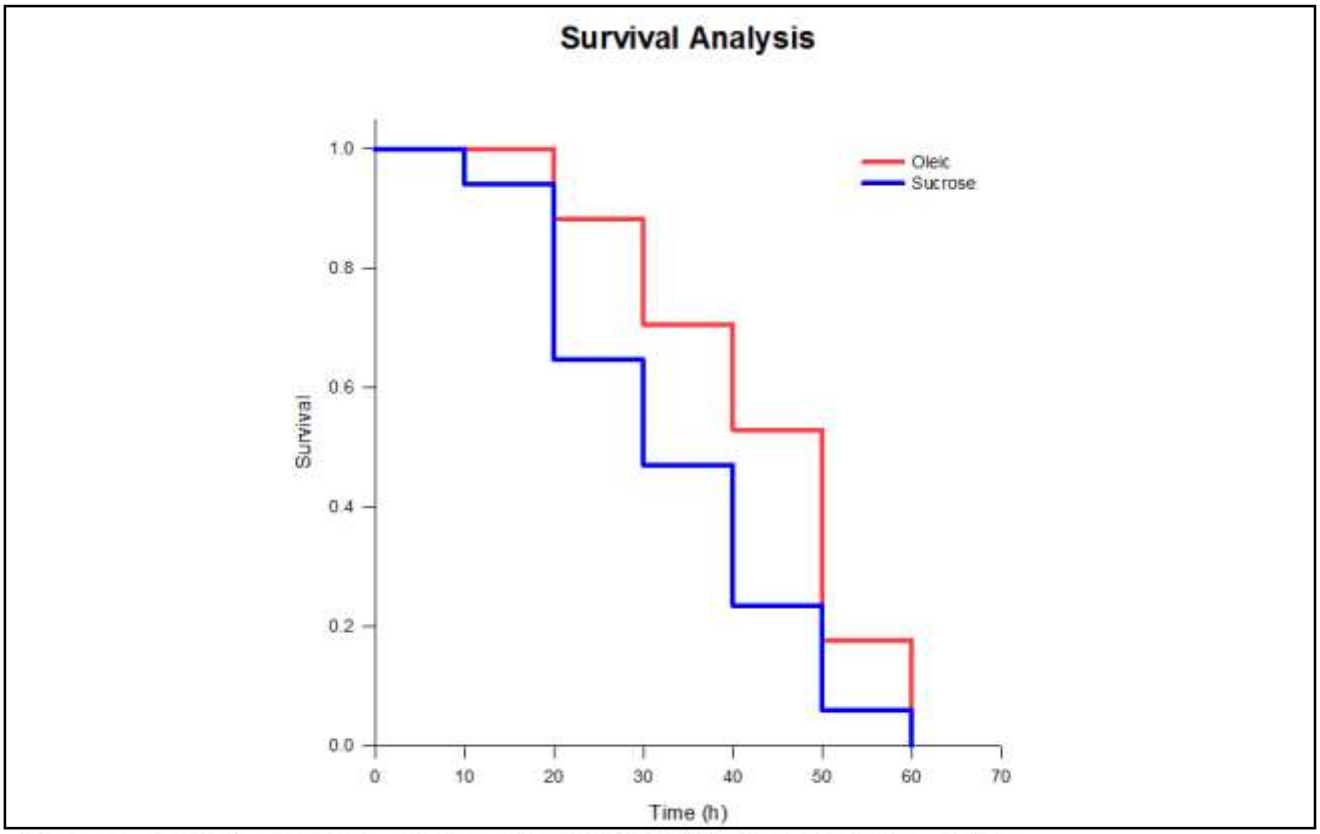

Figure 5. Survival curve comparing longevity of subjects fed either oleic acid containing sucrose (Oleic) or plain sucrose (Sucrose) solutions.