VISITATION HABITS OF THE HONEY BEE (APIS MELLIFERA) IN RESPONSE TO SUGAR CONTENT OF FLOWER NECTAR

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Abstract. Flowers often provide a reward for their pollinators, most often in the form of nectar filled with sugars. Pollinators alter their behavior in response to different concentrations of nectar, often preferentially visiting some flowers over others on the basis of nectar sugar content. This study investigated the floral visitation habits of the honey bee (Apis mellifera) on the island of Moorea, French Polynesia, and how those bees responded to increased sugar concentrations in the base of flowers. Bees were dissected and found with the fungal gut parasite Nosema sp., which could have an impact in determining bee behavioral patterns. Bees had a preference for visiting flowers with added sugar both more often and for longer periods of time. While there was no correlation between higher concentrations of sugar and more visits, there was a significant positive relationship between higher concentrations of sugar and increased visit duration. Bees were also observed sharing flowers in response to elevated sugar concentrations.

Key words: honey bee; Apis mellifera; Nosema; pollination; Moorea, French Polynesia; nectar

INTRODUCTION

Due to their immobility, flowering plants require specialized reproductive modes for spreading genetic material across distances (Mason et. al. 2011). Male genetic material spreads using pollen, often by way of wind or other organisms (Mason et. al. 2011). The process by which pollen gets to the stigma of a flower is referred to as pollination (Mason et. al. 2011). This process occurs in many different ways, but pollination using other organisms is the most efficient method found in flowering plants (Kevan and Eisikowitch 1990).

"Pollinators" is a group composed of many different groups of organisms, with insects and birds comprising the majority (Mason et. al., 2011; Olesen and Valido 2003). Insect pollinators are commonly beetles, flies, and bees (Mason et. al. 2011). These insect pollinators often are more inclined to visit flowers based on the specific shape, color, or nectar of the flowers (Fenster et. al. 2004). For generalist pollinators such as bees, studies show that the shape and color of the flower may have less of an impact on pollinator visitation than factors such as nectar content, and that the morphological component of bee choice may simply be a factor of differing nectar content (Erickson 1975; Gonzales et. al. 1995; Hingston and McQuillan 2000; Waser et. al. 1996).

Nectar content of the flower is a huge draw for insect pollinators, as it provides the pollinator with much-needed nutrition in the form of sugars (Kaur et. al. 2013). Bees spend hours a day foraging for these sugars, filling their crop with nectar and pollen that will be brought back to the hive and made into honey (Wolf et. al 1987). Since flight in insects is metabolically very costly (Wolf et. al. 1987; Waddington and Holden 1979), efficiency in nectar and pollen collecting is vital. Bees have developed complex systems for selecting flowers based on nectar content, some of which involve floral morphological traits or odors, but often relying more on communication from other foraging bees (Seeley et. al. 1991; Fewell and Page 1996; Camazine and Sneyd 1991).

As stated, foraging bees bring nectar back to the hive, where it is processed into honey. This process takes 4-5 days for less sugary nectar, and 2-3 days for more sugary nectar (Wolf et. al. 1987). This means that it is more energetically efficient not only for the individual bee, but also better for the hive as a whole to acquire nectar with higher sugar content (Cox and Myerscough 2003).

Honey bees, specifically the species Apis mellifera, are widely distributed across the globe; often it is introduced for agricultural purposes (Mansora and Zakbah 2011). This may be the case on the island of Moorea in French Polynesia, where little work has been done on honeybees on this island. The work that has been done has simply been surveys of species, not investigative into bee behavior (Rejas 2008).

Preliminary observations of both Moorean and Tahitian bees have shown the presence of parasitic microsporidian fungi (Hopper unpublished data), which could alter their foraging behavior (Mayack and Naug 2009). This island provides an ideal study site for observing the behavior of microsporidian-infected bees because all of Moorea's hives are infected. This allows a good estimate of parasite prevalence that is not present in mainland studies.

Since nectar content is so important not only for individual bees, but also for the hive's wellbeing, it raises the question of how much sugar is too much. What specific concentrations of sugar will bees prefer in nectar, and how will they respond when they are exposed to these higher concentrations of sugar in nectar?

METHODS

Study organisms

The organisms involved were the Asian honey bee, Apis mellifera ceranae, a white shrub flower, Portulaca grandiflora, and the fungal microsporidian parasite Nosema sp.

Study Site

All observations were made at the Atitia Cultural center on Moorea, French Polynesia.

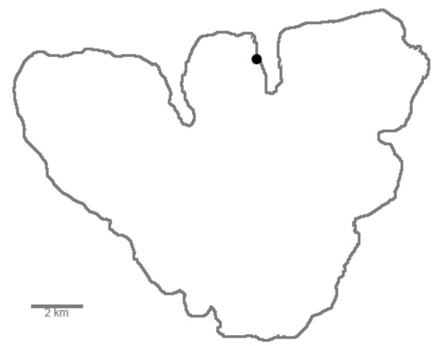

FIGURE 1: Study site (17°29'34.79"S 149°49'36.17"W, 72 feet elevation) on Moorea

The weather during this study was monitored with an altimeter, and ranged from around 21⁰C to 35⁰C, and the humidity around 50-70%. The average temperature for Moorea during the months of September, October, and November was 24, 25, and 26⁰C respectively. The average precipitation was 62mm, 92mm, and 148mm for the months of September, October, and November (Morice et. al. 2012).

Experimental design

Six flowers were chosen at random within a small area of the flowering patch. Three of these were used as controls and filled with one drop of a zero percent sugar solution (pure distilled water). The other three had one drop of sugar solution placed at the base of the stamens, simulating a flower with sweet nectar. Five sugar concentrations were tested each day (20%, 30%, 50%, 60%, and 70% by weight) for forty minute observation periods. I created the sugar solutions using the methods found in Roubik and Buchmanns' (1984) study. Only one concentration was tested at a time, and the order in which these were tested was randomized to attempt to control for differing activity levels of the bees at different times of the day. If after a bee visited there was no more nectar remaining in the flower, I replaced it with another drop of sugar solution after the bee left the flower.

Data collection

All bee observations were all made between 0800h and 1200h, between November 3 and 22, 2013. As mentioned, each trial was 40 minutes long and the time at start and finish was noted for each trial. I recorded the number of bees that visited the flower and how long they visited the flower. A visit was defined as a landing, however short, on the flower. If a visit was less than one second long, I recorded it as lasting one second. I took data on weather conditions (temperature, humidity, cloud cover and wind speed) for each day of observation. I captured twenty five bees in glass vials to use as vouchers and for dissection. Bees used for dissection were left to die naturally at 4^0C, in order to prevent killing any parasites that may be present. Dissection data was used to determine parasite prevalence in the bees that were pollinating the observed flowers.

Data analysis

All statistical analyses are done using R (R Development Core Team, version 3.0.2, 2013). Both the relationship of visit number and visit duration to sugar concentration were compared using general linear models (GLMs). The models originally included the time of day and the date as variables, but stepwise simplifying X2 tests deemed these insignificant.
The number of visits for each sugar concentration and the duration of visits were compared to the control solutions using two separate Wilcox signed-rank tests.
For all tests used the cutoff p value for determining significance is .05.

RESULTS

Visitation to flowers

Bees were also observed visiting flowers that were filled with sugar solution significantly more frequently (Wilcox signed rank test, $p<.001$), but there was no general trend relating higher sugar content flowers with higher visitation rates (GLM, quasipoisson, x2=1606.3, df = 79, $p=.25$). Table one shows a summary of the average visitation rates of the varying concentrations of sugar. Of the sugary flowers, the 50% flower was least visited with an average visitation of 16.000.

The bees did not have a preference for higher sugar concentrations over low sugar concentrations. A GLM shows a relationship with a P value of .25, meaning the relationship between increased sugar solutions and increased numbers of visits was insignificant. Fig 2 demonstrates this relationship.

TABLE 1. Average number of visits for each concentration of sugar solution

Concentration	Average Visits
0%	12.675
20%	34.375
~~30~~%	39.875
50%	16.000
60%	21.000
70%	18.500

Visitation duration

Honeybees preferred to visit for a longer period of time on flowers filled with sugar solution than flowers that did not have the solution. (Wilcox signed rank test: $p<.001$,). This can be seen in Table 2. The GLM showed that the bees did have a preference to land for a longer period of time on flowers with higher sugar concentrations added. This relationship was deemed significant using a generalized linear regression model (GLM, quasipoisson, x2=1382, df=79, $P<.001$), shown in figure 2.

TABLE 2. Average visit time in seconds for each concentration of sugar.

Concentration	Average Visit Duration (s)
0%	3.198
20%	22.123
30%	18.011
50%	30.357
60%	28.722
70%	39.984

Behavioral observations

Observations of the bees showed that they share resources when exposed to high sugar content plants. Multiple bees (most often two, sometimes three) would land on one sugary flower at the same time. This was not observed in flowers with 0% sugar solution added in any instance. The bees also tended to circle around the sugar-filled flowers and visit multiple times in a short span.

When bees landed on a 50%, 60% or 70% sugar flower, they would remain there until the nectar was gone. This was a rare occurrence on the 20% and 30% flowers. When the sugar flowers were damaged by frequent visitation, the bees would continue to land on the flowers. Feeding on nectar was visible (protrusion of the tongue into the nectar water).

Microsporidian parasite presence

Dissections showed that out of pollinating bees, 91.7% contained microsporidian (Nosema sp.) fungal spore parasites in gut tissue.

DISCUSSION

Floral visitation

These results did show that bees will prefer to land on flowers that have sugar content added to their nectar over flowers that have no added sugar; however, the bees did not show any significant preference to land on flowers that had higher sugar content over lower sugar content flowers (Fig. 2). This suggests that perhaps it is not the nectar itself that draws foraging bees to flowers. The methods used in this study did not alter any morphological aspect of the plant, and it has been suggested in previous literature that morphology is often a cue for the nectar content of a flower (Courcelles et. al. 2013; Hingston and McQuillan 2000; Gonzalez et. al. 1995), so it is entirely possible that the bees higher visitation rates at sugar filled flowers was not due to a sensory perception of the sugar content, but maybe of chance meetings of sugar filled flowers coupled with communication at the hive.

Insignificant differences between visitation rates of low and high sugar content flowers demonstrate that bees do have a preference for ingesting excess carbohydrates, but again suggest that higher levels of sugar in nectar aren't necessarily more attractive to bees on their own. The bees' preference for sugar filled flowers in general is based on general foraging patterns in line with previous literature (Wolf et. al. 1989; Harano et. al. 2013). If we consider foraging as described by Wolf et. al. (1989), which detailed the high metabolic costs of flight in insects and how those costs are compensated by nectar content, it is logical that honeybees will prefer to land on a flower that has sugar over one which does not. We are then left with the question of why the bee does not have a preference for high concentrations of sugar over low ones.

The bees' visitation at all concentrations of sugar can be explained from a morphological standpoint: since all flowers appear the same (there are no chemical or physical cues from the flower to indicate otherwise), to the bee it is equally rewarding to visit any of them. This is in line with work done by Gonzalez et. al. (1994), who found that honeybee visits were correlated not with the content of nectar within a species of flower, but to the larger sized flowers and the female flowers on a plant.

This then poses the question: how can bees seemingly preferentially land on flowers with sugar added, but not be able to differentiate between those concentrations of sugar? The findings in this study show that bees cannot differentiate between sugar

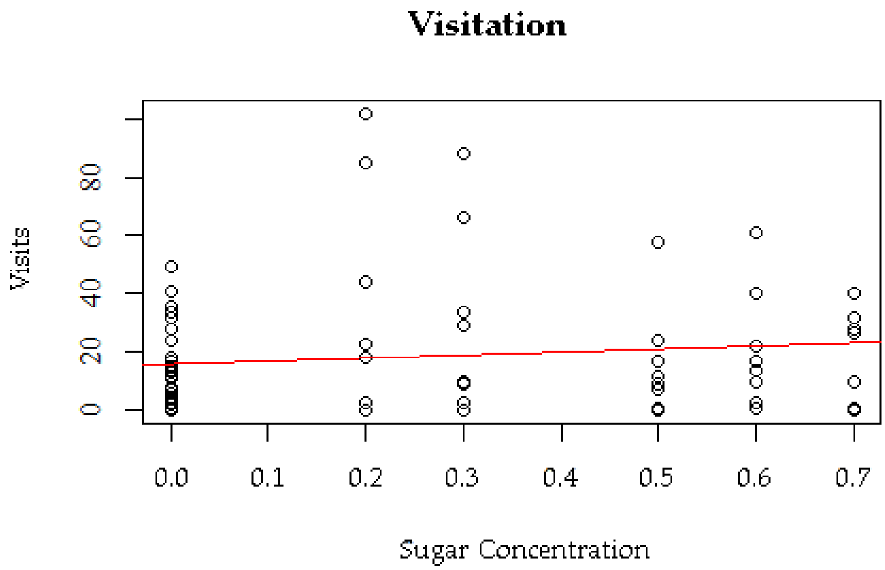

FIGURE 2. Scatterplot showing the number of visits in relation to the concentration of sugar in the flower. The red line represents the generalized linear model fit to the data. P=.247

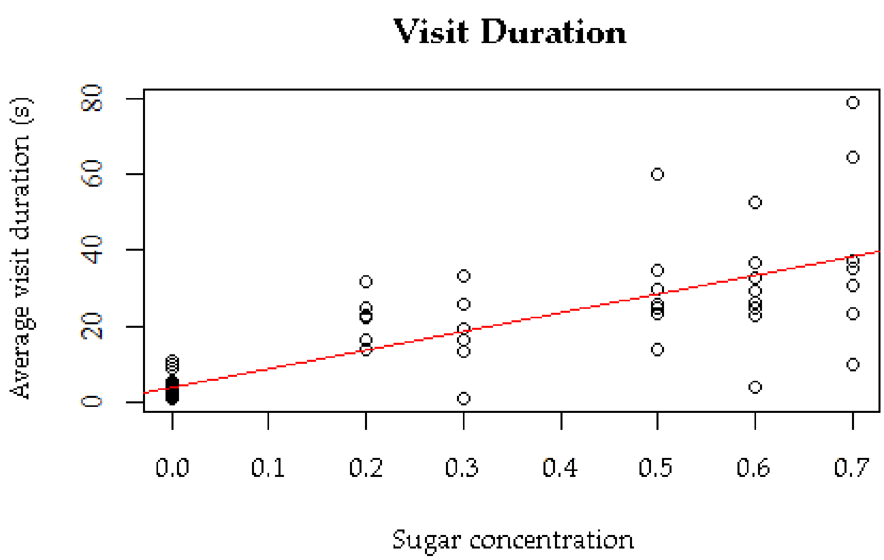

FIGURE 3. Scatterplot showing relationship between average visit duration and sugar cncentration. Red line represents the GLM fit to the data. P<.001.

solutions until landing, and can only tell the difference between a flower that has extra sugar compared to one that does not. This opposes Waddington and Holden's research (1979), which showed bees detecting 5% differences in sugar concentration. It is apparent that further investigation is necessary.

Floral visit duration

The significant relationship between sugar content of flowers and visitation duration reflects what bees require in the nectar of the flowers they visit. The bees not only landed longer on flowers that contained sugar, but of those sugary flowers they also remained longest at the higher concentrations of nectar. This again reinforces the need for bees to have efficient flights (Wolf et. al. 1979) by demonstrating that while bee may not be initially attracted to higher sugar concentrations, once they encounter highly sweet nectar they remain on that flower to ingest all that they can. This could be the result of needing more sugars to replenish the ones lost during the foraging flight, as well as needing to ensure that those foraging flights remain as efficient as possible.

When we consider this foraging efficiency model while looking at bee behavior, Harano et. al.'s (2013) study explains why the bees would prefer to drink higher sugar content nectar. Their research details forager bees' adjusting crop content before leaving the hive for a nectar-collecting flight. The amount of space allotted for flight resources (honey and water) is determined based on the distance to the flower and the nutritional content of the flower. With this in mind, the results found here can be looked at with metabolic efficiency as the main driving force for imbibing high concentrations of sugar. The bees contain limited space in the crop (or "honey stomach") used to transport nectar back to the hive. In order to ensure that they do not waste valuable energy making multiple unnecessary trips, the bees preferentially drink more of the higher sugar content solutions. This would ensure that more carbohydrates would get back to the hive over fewer trips, because more sugars can take up less space in highly concentrated solutions.

Behavior

The bee's behavioral patterns reflect this high demand for sugar in the bees' diet, but also relate this demand for sugar back to the hive as a whole. The bees were observed sharing floral visits, with multiple bees often landing on the same flower. This behavior in bees may suggest that gathering nectar has less of an individual payoff than a colonial one (Seeley and Tovey 1992), because the bees are willing to share high payoff floral resources when they are available.

Microsporidian parasite presence

Dissections showed that the Atitia hive was infected with an extremely high prevalence of parasites. Some of the bee behavior witnessed in this study may then be a direct link to this infection. A recent study found that Nosema microsporidian parasites may affect the feeding behavior of their hosts, making them more voracious feeders by consuming resources from the bees' midguts (Mayack et. al. 2009). When considering this, the behavioral patterns of the bees in this study are much more sensible. The parasitic fungi increases demand for sugars, but so far no literature has documented a change in bee senses due to the parasite. This means that while bees do not have any initial draw toward flowers with higher sugar content and visit sugar filled flowers randomly, when they encounter a sugar-filled flower they will remain there, capitalizing on this valuable food resource.

Future research

Much more work needs to be done involving the relationship between A. mellifera and Nosema sp. While the virulence of the parasite is well documented (Higes et. al. 2007; Paxton et. al. 2007), very little exists documenting the effects of the parasite on the bee's physiology or foraging behavior.

Future research could investigate at which point, if any, the sugar concentration of nectar in the flower becomes concentrated enough to reduce pollination effectiveness. Looking at the results from this study, it seems that flowers producing 70% sugar nectar have no apparent benefit (no increased visitation) while investing a good portion of their resources into producing highly potent nectar. How pollinator choice affects floral evolution of morphological traits has been thoroughly studied (Courcelles et. al. 2013; Hingston and McQuillan 2000; Fenster et. al. 2004; Gonzalez et. al. 1995), but the field would benefit from analyses of cost of producing nectar to benefit of attracting pollinators.

There is room also for more comparison between sites of bees to test whether the bees have the same visitation preferences in different environment types (for example, agricultural-use bees in comparison to recreational/garden use bees). This also could be linked to a study on pesticide effect on bees, a growing problem within apiculture worldwide.

Broader implications

This study relates primarily to bees infected with the microsporidian Nosema sp., and so would be useful background information for those wishing to do comparative studies of non-infected bees in order to increase our understanding of how these parasites affect bee behavior. These fungal spores affect survivorship of hives (Paxton et. al. 2007), and understanding the effect of Nosema sp. on A. mellifera pollination could be a key factor in understanding colony collapse. This would help at a conservation level as well as an economic one. With more understanding of the biological relationship between Nosema sp. and A. mellifera, researchers and apiculturists will be able to find efficient, economic methods of keeping hives healthy globally.

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