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The Effect of Conspecific Cues and Neighborhood Effects on Bee Foraging Behavior

Abstract

Foraging bees use social information (e.g. presence or absence of other bees) to assess the quality of flowers when choosing a flower to visit. My research tests how bees choose to visit a particular flower once they have been recruited to a flower patch. I tested if neighborhood effects, or the relative number of bees on neighboring flowers compared to a focal flower, affected to which flower a foraging honey bee visited. I also conducted a meta-analysis to test whether bees in the super-family Apoidea are more likely to visit a flower occupied by a con- or heterospecific bee or visit an unoccupied flower, and what circumstances lead to these two different foraging behaviors. My results suggest that visual cues, or relative abundance of bees in the neighborhood, impact the number of bee visits a flower will receive, thus highlighting potential implications for pollination services and plant reproductive output.

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The Effect of Conspecific Cues and Neighborhood Effects on Bee Foraging Behavior

A Thesis

Presented to

the Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Eva Sofia Horna Lowell

June 2019

Advisor: Dr. Shannon M Murphy

Author: Eva Sofia Horna Lowell
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ABSTRACT

Foraging bees use social information (e.g. presence of absence of other bees) to assess the quality of flowers when choosing a flower to visit. My research tests how bees choose to visit a particular flower once they have been recruited to a flower patch. I tested if neighborhood effects, or the relative number of bees on neighboring flowers compared to a focal flower, affected to which flower a foraging honey bee visited. I also conducted a meta-analysis to test whether bees in the super-family Apoidea are more likely to visit a flower occupied by a con- or heterospecific bee or visit an unoccupied flower, and what circumstances lead to these two different foraging behaviors. My results suggest that visual cues, or relative abundance of bees in the neighborhood, impact the number of bee visits a flower will receive, thus highlighting potential implications for pollination services and plant reproductive output.

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CHAPTER ONE: Small Scale Neighborhood Effects and Honey Bee Foraging Behavior

Introduction

The interaction between neighboring plants can affect their fitness and these neighborhood effects can either increase or decrease a focal plant's detection by herbivores or pollinators (Barbosa et al., 2009; Champagne, Tremblay, & Côté, 2016; Hahn & Orrock, 2016; Kim, 2017; Merwin, Underwood, & Inouye, 2017; Mesgaran, Bouhours, Lewis, & Cousens, 2017; Setiawan, Vanhellefont, Baeten, Dillen, & Verheyen, 2014; Stutz, Banks, Dexter, & McArthur, 2015; White & Whitham, 2000). In many different systems, including economically important crops, native plants, and non-native plants, neighborhood effects can result in pollination facilitation when the presence of one plant species increases the number of pollinators that visit a second plant species, or alternatively, neighborhood effects can result in competition between plants for pollinator visits (Albrecht, Ramis, & Traveset, 2016; Bruckman & Campbell, 2014, 2016; Feinsinger, Tiebout lli, & Young, 1991; Fitch, 2017; Hersch & Roy, 2007; Montero-Castaño & Vilà, 2015; Ye et al., 2013). Most research about neighborhood effects generally tests how plant traits or different communities of focal and neighboring plants affect plant-pollinator or plant-herbivore interactions (e.g. visual cues of plants: Finch *et al.*, 2003; plant architecture: Marquis *et al.*, 2002; different species of plants: Hersch & Roy, 2007; Lazaro *et al.*, 2009, 2014; Le Guigo *et al.*, 2012, density of plants: Feinsinger *et al.*, 1991; Garcia-Camacho *et al.*, 2009; Bruckman & Campbell, 2014; Hegland, 2014; Fitch, 2017; Mesgaran *et al.*, 2017). Just as neighborhood effects describe interactions between neighboring plants, neighborhood effects can also describe interactions among bees on neighboring plants. Thus, we considered neighborhood effects from the point of view of an insect that is pollinating or eating the plants and how the presence of their own conspecifics on the plants alters their perception of plant quality.

When testing neighborhood effects on pollinator foraging behavior, researchers generally do not test whether the number of pollinators on neighboring plants affects how many pollinators visit a focal plant. Instead, most researchers tend to manipulate the total number of pollinators foraging in an arena at the same time to determine what effect pollinator abundance has on the visitation rates to focal versus neighboring flowers and plants (e.g. Ye *et al.*, 2013; Geslin *et al.*, 2014; Lazaro *et al.*, 2014). Previous research has also manipulated plant density, composition of plants, or frequency of plants to test if these variables affect pollinator visitation rate to particular flowers (e.g. Feinsinger *et al.*, 1991; Bruckman & Campbell, 2014, 2016; Montero-Castaño & Vilà, 2015). For example, Ye *et al.* (2013) manipulated both pollinator abundance and the densities of conspecific and heterospecific flowers and found that when pollinator abundance was high, increasing the density of focal and neighboring plants significantly increased pollinator visitation rates to the focal flower. However, Ye *et al.* (2013) also found that when pollinator abundance was low, increasing the number of focal plants had no effect on pollinator visitation rates to the focal flower, but there was a significant decrease in pollinator visitation rates to the focal flower when they increased the number of neighboring plants. Pollinators such as honey bees frequently forage with conspecifics in nature, and therefore it is important to understand how they respond to the presence of conspecifics on flowers on a finer scale than plant to plant interactions reveal. To our knowledge nobody has yet tested whether honey bee foraging behavior is affected by the relative number of bees on neighboring flowers compared to a focal flower.

We used the European honey bee, *Apis mellifera*, to test whether small-scale neighborhood effects, in the context of the relative number of bees on focal and neighboring flowers, impact honey bee foraging behavior. Honey bees understand the numerical concepts of “greater than” or “less than” within a numerical continuum between one and six, and they understand the number zero is “less than” one, therefore learning the concept of zero relative to other numbers (Howard, Garcia, Greentree, & Dyer, 2018). We previously showed that honey bee foragers arriving at a flower array land more frequently on flowers that are occupied by more conspecifics compared to flowers occupied by few or no conspecifics (Horna Lowell *et al.* In

Review), adding further support to the idea that bees understand “greater than” and “less than”. However, our results were highly variable; for example, when there were six honey bees on a flower, anywhere from 0 to 10 honey bees landed on that flower. To try to explain some of this variation, we hypothesized that neighborhood effects created by the presence of conspecifics on neighboring flowers may alter a bee’s perception of the quality of a focal flower.

We formed three alternate hypotheses about how honey bee foragers assess the number of conspecifics on neighboring flowers relative to a focal flower when choosing to land on a focal flower (Figure 1.1). Our first hypothesis is the ‘direct comparison hypothesis’ (Figure 1.1; dotted line); if there are more bees on the focal flower relative to neighboring flowers, then more bees may land on the focal flower if the greater relative abundance of bees on the focal flower informs bees about the positive quality of that flower (e.g. presence of resource or lack of predators). Conversely, if there are more bees on neighboring flowers relative to the focal flower, then fewer bees would land on the focal flower because relative to the neighborhood, it would be viewed as poorer quality. Our second hypothesis is the ‘neighborhood attractiveness hypothesis’ (Figure 1.1; dashed line); if there are more bees on the focal flower relative to the neighboring flowers, then fewer bees would land on the focal flower if the relatively low abundance of bees on neighboring flowers confers information about the poor quality of the neighborhood generally. Thus, a flower with a lot of bees, which would otherwise be an attractive flower based on our prior research (Horn & Lowell et al. In Review), becomes unattractive if the neighborhood is viewed overall as being unattractive and would attract fewer bees than expected. Conversely, more bees on neighboring flowers relative to the focal flower would increase the attractiveness of an otherwise unattractive flower, and more bees would land on the focal flower because the greater relative presence of bees on neighboring flowers increases attractiveness of the neighborhood. Lastly, our third hypothesis is the ‘null hypothesis’ (Figure 1.1; solid line); the number of bees on a focal flower compared to neighboring flowers has no effect on honey bee visitation. Our results will provide information regarding the impact of neighborhood effects on honey bee foraging behavior, which affects pollination facilitation among plants.

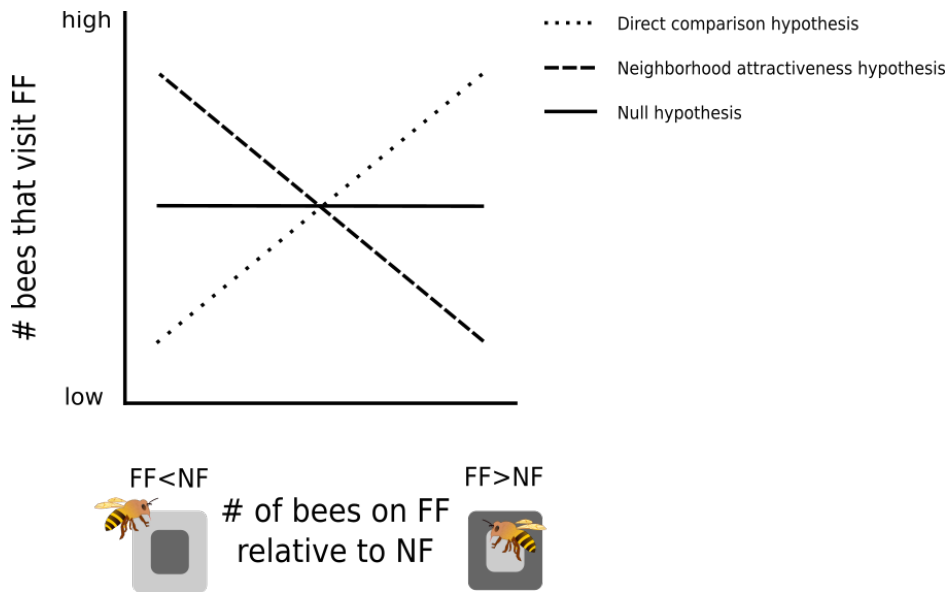


Figure 1.1: Predicted outcomes from our three hypotheses regarding how neighborhood effects may impact whether a honey bee forager lands on a focal flower. Our independent variable is the relative number of bees on the focal flower (FF) compared to the neighboring flowers (NF). If there are fewer bees on the FF compared to the NF ($FF < NF$, icon of the bee on the light grey outer NF ring), then these values fall on the left side of the x-axis. If there are more bees on the FF compared to the NF ($FF > NF$, icon of the bee on the light grey inner FF), then these values fall on the right side of the x-axis. Our dependent variable is the number of bees that visit the FF. The 'direct comparison hypothesis' (dotted line) predicts that honey bee foragers would directly compare the number of bees on FF to the number of bees on the NF and land more frequently on the FF when there are comparatively more bees on the FF than NF. The 'neighborhood attractiveness hypothesis' (dashed line) predicts that if there are more bees on NF, then bees would view the entire neighborhood as attractive; alternatively, if there are few bees on NF then bees would view the entire neighborhood as unattractive. Thus, this hypothesis predicts that honey bees would land on FF more frequently if the NF are occupied by a relatively high number of conspecifics. The 'null hypothesis' (solid line) predicts that the number of bees on the FF relative to the NF has no effect on the number of bees that visit the FF.

Methods

Study Design

We studied honey bees in the community garden on the University of Denver campus during July and August of 2018. We created two square flower arrays, each with 25 fake flowers (Figure 1.2). Each flower consisted of a 2.0mL Eppendorf tube that we filled with a sucrose solution (1kg sucrose:1L water), taped to a wooden skewer (25cm) that served as the flower "stem". We then placed a laminated piece of blue construction paper (5cm²) that served as the "flower" around the Eppendorf tube. To create each array, we inserted 25 flowers into a base

made of styrofoam and cardboard taped together. The laminated blue paper square on all 25 fake flowers stood 17cm above the base and each flower was 10cm away from any other flower or the edge of the board. Each time we repeated the experiment we randomly chose which of the two arrays to use, making sure to never repeat the same array more than twice in a row.

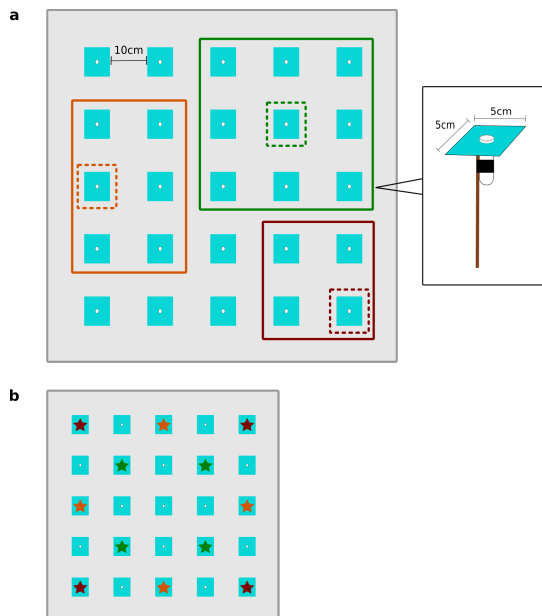


Figure 1.2: a) The flower array with the 25 fake flowers (blue squares); the magnified image depicts a single fake flower that we used in our experiment. For each experiment, the focal flower (FF) is outlined with the small dashed-line colored square (*Experiment 1*=green, *Experiment 2*=red, *Experiment 3*=orange) and the neighboring flowers are the surrounding flowers within the solid-line colored squares (square color again corresponds with experiment). b) The position of the four focal flowers that we randomly rotated among for each of the three different experiments (*Experiment 1* FF = green stars, *Experiment 2* FF= red stars, *Experiment 3* FF=orange stars).

Experimental Design

To test whether foraging honey bees consider the number of bees on neighboring flowers (NF) relative to the focal flower (FF) when choosing to visit a FF, we counted how many bees visited the FF based on the initial number of bees on the FF as well as the initial number of bees on all the NF. We performed three different experiments; each experiment had a different FF position in the flower array and thus different numbers of NF surrounding the FF.

For Experiment 1 each FF was surrounded by eight NF (Figure 1.2a; large solid-line green square). We positioned the FF (Figure 1.2b; flowers with green stars) in one of four different places on the interior of the board. For each replicate, we used a random number generator to determine which of the four FF to use and made sure to never repeat the same FF twice in a row. We repeated this experiment 220 times (53-56 times for each FF).

We collected data for Experiment 1 between July 3-31 on 10 different days. Each day that we collected data, we haphazardly placed the flower array somewhere inside or near the community garden (within 50m). We recorded the date, time of day, temperature, percent humidity, and percent cloud cover at the beginning and end of each time we performed the experiment. For each replicate, one person recorded data, one person kept track of the time, one person collected data on the FF (ESHL in all replicates), and at least four people collected data for the NF (in some replicates one person collected data on more than one NF). We filled the Eppendorf tubes with sucrose solution before every replicate until a meniscus formed at the top. As soon as the flowers were filled with sucrose solution, the time keeper started the timer and the replicate began (methods adapted from Horna Lowell et al. In Review). Our replicates were 50 seconds long and divided into two parts: 1) counting the initial number of bees on both the FF and NF, and 2) counting the number of new bee arrivals on the FF. Observers spent the first 30 seconds watching their assigned flower and keeping track of the number of bees on the flower. At exactly the 30 second time point the observers noted the number of bees on their flower and told the data recorder; this value served as the initial number of bees on the flower. For the remaining 20 seconds, the person assigned the FF (ESHL) used a tally counter to count the number of new bees that landed on the top of that flower; this value served as the number of bees that visited the FF. ESHL used a tally counter so that she could simultaneously report the initial number of bees on the FF at the 30 second time point to the data recorder and also count the number of new bees that visited her flower for the remaining 20 seconds. We did not count any bees that landed on the wooden stem of the flower or on the underside of the flower. After

each replicate, the observers filled the depleted Eppendorf tubes with sucrose solution, the data recorder assigned new flower numbers to the observers, and the next replicate began.

Previously we found that honey bee foragers visit the flowers on the edge of our array more frequently than flowers in the interior (Horna Lowell et al. In Review). To account for this apparent preference for edge flowers, we conducted two additional experiments. In Experiment 2 we positioned the FF on the corner edge of the array (Figure 1.2b; flowers with red stars) and for the Experiment 3 we positioned the FF as the middle flower on the edge of the array (Figure 1.2b; flowers with orange stars).

For Experiment 2 each FF was surrounded by three NF (Figure 1.2a; large solid-line red square). We positioned the FF (Figure 1.2b; flowers with red stars) in one of the four corners on the array. Similar to the Experiment 1, for each replicate, we used a random number generator to determine which of the four FF to use and made sure to never repeat the same FF twice in a row. We collected data for Experiment 2 from July 13 to August 10 on 8 different days and we repeated this experiment 221 times (54-56 times for each FF).

For Experiment 3 each FF was surrounded by five NF (Figure 1.2a; solid-line orange square). We positioned the FF (Figure 1.2b; flowers with orange stars) in one of four different spots on the center edge of the array. For each replicate, we used a random number generator to alternate between the four FF and their neighborhood making sure we never repeated the same FF and neighborhood twice in a row. We collected data for Experiment 3 from July 16 to August 10 on 10 different days and we repeated this experiment 220 times (55 times for each FF).

Statistical Analysis

To analyze if honey bee foragers consider the number of bees occupying the NF relative to the FF when choosing to visit the FF, we used a linear regression model for each of the three experiments. Our response variable for all models was the number of bees that visited the FF. Our independent variables were FF rank, sum number of bees initially on NF, initial number of bees on FF, and two PCs for weather. We included FF rank as a measure of the relative

abundance of bees on the FF as compared to the NF (see below for calculation). To account for the total number of bees per replicate, we included sum number of bees initially on NF. We included initial number of bees on FF in our model because our previous results show that it has a significant effect on the number of bees that visit the FF (Horna Lowell et al. In Review). In order to capture the inherent variation among the different days we collected data, we included two principal components (PC1 and PC2) that summarized our weather variables: time of day, temperature, percent cloud cover, and percent humidity. Together the first two principal components summarized 89.3% of the variation among these variables for Experiment 1 (Appendix 1.2), 87.5% for Experiment 2 (Appendix 1.3), and 81.9% for Experiment 3 (Appendix 1.4). Initially we included FF position as a random variable in our model because we previously found that whether a flower was on the edge of the array or in the center significantly affected the number of foragers that visited that flower (Horna Lowell et al. In Review). However, in all three experiments FF position never had a significant effect, likely because within an experiment our positions did not vary between edge and center, so we dropped this variable from all models. Because ESHL always counted the number of new bees that landed on the FF, we did not include observer ID as a random effect in our model. Thus, our final model for each experiment included our response variable (number of bees that visited the FF) and five fixed effects (FF rank, sum number of bees initially on NF, initial number of bees on FF, PC1 and PC2).

To calculate FF rank within each replicate we followed Siegel and Castellán (1988, p. 87-88) to rank the FF based on the number of bees on the FF compared to the number of bees on each NF. The FF rank depended on the total number of flowers in each replicate (Experiment 1: rank could be between 1-9, Experiment 2: rank could be between 1-4, Experiment 3: rank could be between 1-6). A FF with a rank above the midpoint (midpoints: 5 for Experiment 1, 2.5 for Experiment 2, and 3.5 for Experiment 3) indicates that the FF was occupied by more bees than half of the NF. Alternatively, a FF with a rank below the midpoint indicates that more than half of the NF had more bees than the FF. A FF that is ranked at exactly the midpoint indicates that half of the NF had more bees than the FF and half had fewer bees than the FF, or that all FF and NF

had the same number of bees. In some replicates, two or more flowers had the same number of bees at start, and therefore the same rank, resulting in a tie. In these instances, the rank assigned to each of the tied flowers was the mean of the rank positions the flowers occupied (following Siegel & Castellan, 1988; e.g. for one replicate in Experiment 2, the number of bees at start on all four flowers was 1, 1, 2, and 5; the flowers with 1 bee at start occupy rank positions 1 and 2, and we therefore assigned them both a rank of 1.5 with the flower with 2 bees ranking a 3, and the flower with 5 bees ranking a 4).

To visualize the effect that FF rank had on the number of bees that visited the FF, we created partial residual plots (Zuur et al. 2007). The partial residual plots demonstrate the relationship between the predictor variable, FF rank, and our response variable, number of bees that visited FF, while filtering out the effects of our other independent variables. We report the partial regression P-value and R-squared value. We used RStudio version 1.1.463 to create partial residual plots and we used JMP Pro 13.0.0 for all statistical analyses.

Results

We found that the number of bees on neighboring flowers relative to the focal flower (FF rank) affected the number of foragers that visited the focal flower, but only in the first experiment (Table 1.1). Therefore, in our first experiment, if a focal flower had fewer bees relative to its neighboring flowers, then foragers were more likely to land on what would otherwise be an unattractive vacant focal flower (Figure 1.3a; Table 1.1). Similarly, if a focal flower had more bees relative to its neighboring flowers, then foragers were less likely to visit what would otherwise be an attractive occupied focal flower. However, in our second and third experiment, the number of bees on neighboring flowers relative to the focal flower (FF rank) did not significantly affect the number of new bees that visited the focal flower in the complete model (Table 1.1). However, our partial residual plots show that when *FF rank* is considered alone, it significantly explained the *number of bees that visited the FF* in Experiment 1 (Figure 1.3a; $F_{1,218} = 59.2$ $R^2 = 0.2$

P<0.0001) and Experiment 3 (Figure 1.3c; $F_{1,218}=0.28$ $R^2=0.04$, $P=0.002$), but not Experiment 2 (Figure 1.3b; $F_{1,219}=9.5$, $R^2=0.0$, $P=0.59$).

Similar to our previous results (Horna Lowell *et al.* In Review), we found that the *initial number of bees on FF* significantly affected the number of bees that visited the focal flower in Experiment 1 and Experiment 2 (Table 1.1). Honey bee foragers landed on flowers occupied by conspecifics more frequently than flowers occupied by fewer bees or none (see Appendix A; *Experiment 1*: $R^2=0.5$; *Experiment 2*: $R^2=0.48$; *Experiment 3*: $R^2=0.46$). Notably, this pattern was even stronger than in our other work ($R^2=0.25$ in Horna Lowell *et al.* In Review). We also found that the *sum number of bees initially on NF* significantly affected the number of bees that visited the focal flower in all three experiments, indicating that bee abundance in the area is an important factor (Table 1.1). Lastly, weather had a variable effect; our two principal components were significant in Experiment 2 and had no effect in Experiment 1 or Experiment 3 (Table 1.1).

Table 1.1: Linear regression model summary for the effect of each variable on the number of bees that visited the focal flower (FF) for each of our three experiments. Bolded values indicate the corresponding variable significantly affected whether a bee landed on the FF.

Variable	Experiment 1			Experiment 2			Experiment 3		
	DF	F Ratio	P	DF	F Ratio	P	DF	F Ratio	P
Initial # bees FF	5,214	81.8	<0.0001	5,215	10.6	<0.001	5,214	3.4	0.68
FF rank	5,214	29.1	<0.0001	5,215	2.0	0.16	5,214	0.1	0.78
Sum initial # bees NF	5,214	5.5	0.02	5,215	16.4	<0.0001	5,214	29.4	<0.0001
PC 1	5,214	0.4	0.54	5,215	9.1	0.003	5,214	0.0	0.94
PC 2	5,214	2.8	0.1	5,215	11	0.001	5,214	2.3	0.13

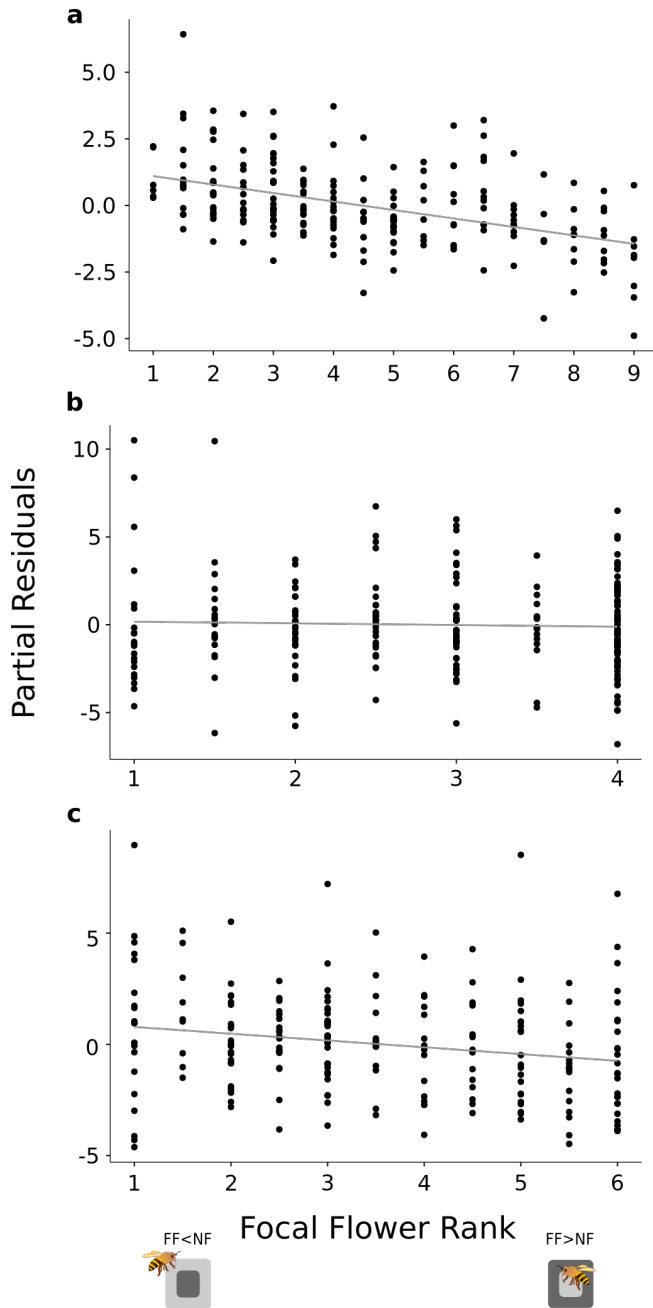


Figure 1.3: The partial regression for focal flower (FF) rank for our three experiments. The partial residuals plots demonstrate the pure effect that FF rank has on the number of bees that visited the FF after filtering out the effects of our other predictor variables. FF rank depended on the number of flowers in each replicate (*Experiment 1* = 1-9, *Experiment 2* = 1-4, *Experiment 3* = 1-6). A FF with a rank above the midpoint (midpoints: 5 for *Experiment 1*, 2.5 for *Experiment 2*, and 3.5 for *Experiment 3*) indicates that the FF was occupied by more bees than half of the neighboring flowers (NF). Alternatively, a FF with a rank below the midpoint indicates that more than half of the NF had more bees than the FF. A FF that is ranked at exactly the midpoint indicates that half of the NF had more bees than the FF and half had fewer bees than the FF, or that all FF and NF had the same number of bees. a) *Experiment 1*, b) *Experiment 2*, c) *Experiment 3*

Discussion

Our results demonstrate that small-scale neighborhood effects do impact honey bee foraging behavior on a small neighborhood scale, but only for one of our experiments. Previous studies have focused on manipulating the composition of neighboring and focal plants in a particular plant community (Bruckman & Campbell, 2014, 2016; Feinsinger et al., 1991; Fitch, 2017; Hegland, 2014; Lazaro & Totland, 2010; Mesgaran et al., 2017; Schmitt, 1983; Ye et al., 2013) or pollinator abundance in an area (Lazaro et al., 2009, 2014; Ye et al., 2013). We tested whether the number of honey bee visits to a focal flower is affected by the number of conspecifics on a focal flower relative to the neighboring flowers, which had not previously been tested.

Our significant results support the neighborhood attractiveness hypothesis; high numbers of bees on neighboring flowers increases the attractiveness of the neighborhood and low numbers of bees on the neighboring flowers decreases the attractiveness of the neighborhood. Thus, more bees on the neighboring flowers compared to the focal flower increases the attractiveness of the focal flower and foragers are more likely to visit what would otherwise be an unattractive vacant focal flower. Similarly, fewer bees on the neighboring flowers compared to the focal flower decreases the attractiveness of the focal flower and foragers are less likely to visit what would otherwise be an attractive occupied flower.

In agreement with our earlier findings, (Horna Lowell *et al.* In Review) honey bee foragers were more likely to land on flowers that were initially occupied by more bees compared to flowers that were occupied by few bees or that were vacant. When we compare the focal flower rank and the initial number of bees on the focal flower (Table 1.1) we find that initial number of bees is generally a better predictor of how many bees are going to land on the focal flower. Therefore, it seems that when choosing to land on a focal flower, honey bee foragers are primarily looking at the number of bees present on the focal flower and then to a lesser extent consider the abundance of bees on neighboring flowers.

The small-scale floral neighborhood we used in our experiments may explain why we found an impact of neighborhood effects on foraging behavior in our first experiment (interior focal

flowers) and no evidence in our second or third experiments (edge focal flowers). Our flower array was approximately 60cm² and thus our neighborhood was smaller compared to scales used in other neighborhood effects research. Previous research has found that the impact of neighborhood effects on plant-pollinator interactions can be scale-dependent (Albrecht et al., 2016; Bruckman & Campbell, 2016; Hegland, 2014; Ye et al., 2013), but there is no consensus about the scale at which (small, intermediate, or large) neighborhood effects will have the largest impact on plant-pollinator interactions. For example, Hegland (2014) found that at a small plot scale (5m²) an increase in intra and inter-specific floral density led to pollination facilitation on the focal flower, but on a larger scale (10m and 25m), an increase in intra and inter-specific floral density led to competitive interactions between flowers for pollinator visits. Conversely, Bruckman & Campbell (2014) showed that on a small-scale (2m²), increasing the density of a non-native plant had no neighborhood effect on pollinator visitation rate to the native, focal plant. Furthermore, Albrecht et al. (2016) suggested that on a landscape scale (hectares), honey bee pollinators made more visits to the focal plant in sites that were invaded by a non-native plant, irrespective of the spatial scale at which the invasive plant was present (hectares or m²). Many of these experiments are context-dependent and therefore it is difficult to find general patterns about the scale at which neighborhood effects begin to impact plant-pollinator or plant-plant interactions. We tested neighborhood effects on a smaller scale than what has been previously tested, which could explain why we didn't detect an effect on foraging behavior in some of our experiments. We encourage future research to look at whether there is a threshold in the scale at which neighborhood effects begins to impact plant-pollinator interactions.

The density and composition of pollinators that are available to pollinate plants can affect how neighborhood effects impact plant-pollinator interactions (e.g. pollination facilitation between plants or competition between plants for pollinator visits; (Hanley et al., 2011; Lazaro et al., 2014; Ye et al., 2013). For example, Hanley *et al.* (2011) observed twice as many bumble bees visiting native flowers when planted adjacent to a field of mass flowering crops (beans) then when planted adjacent to a field of non-mass flowering crops (wheat). This 'spillover effect' of bumble

bees visiting the native flowers when next to mass flowering crops can briefly enhance pollination services to the native flowers (Hanley et al., 2011). Similarly, a high abundance of honey bee pollinators has been shown to increase visitation rate to a focal flower, irrespective of increasing densities of the focal or neighboring flowers (Ye et al., 2013). However, when pollinator abundance was experimentally reduced, the effect of neighboring plants on the focal plant (competitive or facilitative interactions) was dependent on the response variable measured (e.g. seed set, pollen limitation, or flower visitation rate; Lazaro et al. 2014). Similarly, a recent meta-analysis that looked at the effect of honey bee density on crop yield also suggested that results can vary in patterns depending on the response variable measured, such as visitation rates or colony density (Rollin & Garibaldi, 2019). These studies further demonstrate that the impact of neighborhood effects on plant-pollinator or plant-plant interactions (competitive vs. facilitative) are contextual and vary depending on the density of plants and pollinators, and the response variable measured.

In conclusion, we found some evidence that neighborhood effects impact honey bee foraging behavior. Previous literature has shown that the type of impact neighborhood effects can have on plant-pollinator interactions (competition vs facilitation vs no effect) depends on the spatial scale of the floral neighborhood, the species of plants and pollinators present, and the response variable measured (Albrecht et al., 2016; Bruckman & Campbell, 2016; Hanley et al., 2011; Hegland, 2014; Lazaro et al., 2014; Rollin & Garibaldi, 2019; Ye et al., 2013). Therefore, neighborhood effects can alter pollination services and reproductive output of plants in contrasting manners. In the context of current pollinator decline (Biesmeijer et al., 2006; Goulson, Lye, & Darvill, 2008), it is important to consider how the relative abundance of pollinators can affect plant-pollinator interactions and ultimately, plant reproductive output. Our study demonstrates that on a small floral neighborhood scale, the relative number of pollinators present on focal and neighboring plants has a small, but significant effect on whether a pollinator visits the focal flower, highlighting the potential implications neighborhood effects can have on pollination services.

CHAPTER TWO: Local Enhancement or Local Inhibition? A Meta-analysis on Bee Foraging Behavior

Introduction

Foraging animals are frequently faced with decisions about where to forage and have developed a variety of strategies to increase foraging efficiency, such as applying previous experience or using social information (Avarguès-Weber & Chittka, 2014; Slaa & Hughes, 2009; Slaa, Wassenberg, & Biesmeijer, 2003). When foragers assess social information, such as the presence or absence of other foraging individuals in a resource patch, their foraging efficiency increases (Free & Williams, 1983; Galef & Giraldeau, 2001 and papers within; Kalmus, 1954; Slaa & Hughes, 2009). Animals may be attracted to forage near other individuals because the presence of foraging individuals may increase vigilance for predators, or indicate the resource is particularly safe or rewarding (Clark & Mangel, 1986; Tautz & Sandeman, 2003; Yokoi & Fujisaki, 2011). Conversely, animals may be deterred from foraging near other individuals to avoid competition for the resource or risk visiting an unrewarding resource (Giurfa & Núñez, 1992; Slaa et al., 2003).

Foragers at a resource patch can use information provided by visual and olfactory cues to identify a high-quality resource. These cues generally elicit two types of behavior: local enhancement or local inhibition. Local enhancement occurs when foragers are attracted to resources with recently deposited pheromones or resources currently occupied by either con- or heterospecifics (Free & Williams, 1983; Horna Lowell et al., In Review; Kalmus, 1954; Leadbeater & Chittka, 2007; Worden & Papaj, 2005; Yokoi & Fujisaki, 2011). Conversely, local inhibition occurs when foragers are deterred from visiting resources where foragers have previously deposited pheromones or that are currently occupied by other foraging individuals (Slaa &

Hughes, 2009; Slaa et al., 2003; Yokoi & Fujisaki, 2011). Both of these foraging behaviors are commonly observed among social insects, and particularly bees who frequently encounter other con- or heterospecific bees on flowers while foraging (Slaa & Hughes, 2009). For example, when honey bee foragers arrive to a flower array, they can exhibit local enhancement as they are more likely to land on flowers where there are already foraging conspecifics present (Horná Lowell et al., In Review; Kalmus, 1954; Kalmus & Ribbands, 1952). However, other studies have found that honey bees avoid landing on flowers already occupied by conspecifics, thus showing local inhibition (Yokoi & Fujisaki, 2011). We used a meta-analysis approach to synthesize the existing foraging bee behavior literature to test if bees show local enhancement or inhibition more often and what circumstances lead to these two different foraging behaviors.

It is important to understand under what conditions foraging bees exhibit local enhancement or inhibition because these behaviors can have contrasting effects on pollination and thus plant reproduction. Flower distribution, the species of bee and the foraging strategy (solitary vs. social), and whether the foraging bee interacts with con- or heterospecific bees can all influence bee foraging behavior (Baude, Dajoz, & Danchin, 2008; Rogers, Cajamarca, Tarpy, & Burrack, 2013; Slaa et al., 2003). For example, Baude et al. (2008) found that in randomly distributed flower patches with rewarding flowers occupied by a conspecific cue and unrewarding flowers unoccupied by a conspecific cue, there was no difference in which flower naïve *Bombus terrestris* landed on more frequently. However, in flower patches where rewarding and non-rewarding flowers were clumped together, naïve bees landed on rewarding flowers occupied by a conspecific cue more often than unoccupied, unrewarding flowers (Baude et al., 2008). Additionally, bumblebees seem averse to landing on flowers occupied by honey bees, whereas honey bees seem neither attracted nor repelled from landing on flowers with bumblebees (Rogers et al., 2013). Therefore, the conditions that lead foragers to show local enhancement or inhibition are contextual, and it has been difficult to draw general conclusions about the behaviors of different species without a quantitative analysis.

Using a meta-analysis approach, we tested whether bees show local enhancement or inhibition more often, and how the tendency for one behavior over the other depended on three factors: the genus of the bee that was tested, whether the visual cue was a con- or heterospecific bee, and the type of visual cue used (i.e. a model, dead, or alive bee). The genus of the bee that was tested could influence whether bees show local enhancement or inhibition because some bees are solitary foragers and others are group foragers, and foraging strategies are associated with different behaviors (Slaa et al., 2003; Yokoi & Fujisaki, 2011). We predicted that solitary bees that forage alone would be likely to exhibit local inhibition more whereas social bees would be more likely to exhibit local enhancement. We also tested whether a conspecific or heterospecific visual bee cue influenced the likelihood of local enhancement versus inhibition because existing literature suggests that different species (bumblebees versus honeybees, Rogers et al., 2013; Xie, Pan, Teichroew, & An, 2016; solitary versus social bees, Yokoi & Fujisaki, 2011) make different foraging decisions when they encounter a con- rather than a heterospecific cue on a flower. We predicted that the genus and species of the tested bee would influence whether a bee shows local enhancement or inhibition when presented with a flower occupied by a con- or heterospecific. Lastly, we tested the type of visual bee cue used: model, dead, or alive. If studies used dead bees as their visual cues, the behavior of the foraging bee may depend on how the bees were killed (e.g. freezing or by crushing) and which body part (if not the whole bee) was used as the cue. Dead bees can emit odors such as ethanol or alarm pheromones which can negatively affect the attractiveness of the flower to the live bees (Ferguson & Free, 1979; Yokoi & Fujisaki, 2011). Thus, we predicted that studies that used live bees or model bees as visual cues would have a stronger positive effect of local enhancement whereas studies that used dead bees as visual cues would have a weaker effect of local enhancement and potentially even a negative effect of inhibition if the bee was killed by crushing. Our meta-analysis tests which foraging behavior, local enhancement or inhibition, bees show most often, and which conditions lead to the various trends we find in the local enhancement and local inhibition bee behavior literature.

Methods

Data Survey

We conducted our literature survey in June-August of 2018 and searched across six different databases: Web of Science, Biological Abstracts, Zoological Records, Agricultural and Environmental Science, Google Scholar, and Academic Search Complete. We used the following search terms for each data base: (*Bee OR honeybee OR bumblebee OR Apis OR Bombus OR hymenoptera*) AND (“*decision making*” OR “*social attraction*” OR “*local enhancement*” OR “*local inhibition*” OR “*social cue*” OR “*associative learning*” OR “*social learning*” OR “*inadvertent social information*” OR “*flower visitation*” OR “*flower constancy*” OR “*foraging choice*”) AND (*forag* OR demonstrators OR observers*) AND *flower*. Our searches provided us with a total of approximately 6,500 publications. We read through all the titles and only kept the studies that appeared to test whether foraging bees landed more frequently on occupied flowers compared to unoccupied flowers. After this review process, 151 publications remained. We then read the abstracts of these 151 publications to ensure the study was relevant. We excluded studies that did not address whether foraging bees were more likely to land on flowers that were occupied by other bees or unoccupied flowers. Some studies tested whether foraging bees could associate a social cue with a reward, such as a sucrose solution, and we included these studies if they also collected data on whether foraging bees landed more frequently on occupied or unoccupied flowers. We also only kept studies that tested bees in the superfamily *Apoidea*. After reading all of the abstracts, 74 studies remained and for all of these we read the entire paper to ensure that they tested the response of a foraging bee to either a visual cue or both visual and olfactory cues at the same time. After this review process 31 studies remained, however many of these did not report mean values for both the treatment and control group, and therefore we could not create effect sizes for these studies. Thus, we excluded studies that did not report both the number of bees that visited flowers with a visual cue (treatment group) and the number of bees that visited an unoccupied flower (control group). Additionally, many studies did not include standard measures of error (or variance) with their treatment and control values, which are necessary to weigh the

effect of individual case studies in the meta-analysis. We contacted several authors from studies that did not originally report their standard error, and some were able to provide us with this information. After our final review process, we had found a total of 10 publications that we included in our meta-analysis. Most of the studies consisted of multiple individual case studies that each measured the number of visits on occupied versus unoccupied flowers under different conditions. We used each individual case study as an independent effect size in our meta-analysis and thus we had a total of 30 cases (Table 2.1).

Data Collection and Effect Sizes

For each case study, we assigned the treatment variable as the choices, visits, or landings on flowers that were occupied by a visual cue. We assigned the control variable as the choices, visits, or landings on flowers that were unoccupied. If the study collected the number of bee visits on flowers occupied by a visual cue and an unoccupied flower over a time span of multiple trials, we selected the first and last trial as two different case studies. Thus, our treatment and control variables often were not what the authors considered as their own treatment and control variables in their study. For each case study we recorded the mean and measures of error for both the treatment and control when it was reported in the text, and we used PlotDigitizer to obtain the values when they were only reported on graphs.

To create our effect sizes we used the standardized mean difference calculation (SMD, or Hedges' *d*) because we were comparing the difference between two means (Koricheva & Gurevitch, 2014; Nakagawa, Noble, Senior, & Lagisz, 2017). Our effect sizes represent the difference between bees that visit flowers occupied by a conspecific or heterospecific bee (treatment), and bees that visit unoccupied flowers (control). A positive effect size means that foraging bees are more likely to visit an occupied flower compared to an unoccupied flower (local enhancement). Conversely, a negative effect size means that foraging bees are more likely to visit an unoccupied flower compared to an occupied flower (local inhibition). An effect size that

does not differ from zero means that occupied and unoccupied flowers are equally attractive to foragers and neither flower type is more likely to receive more bee visits.

We collected a variety of information about how the study was performed from each case study that we thought could influence whether a bee shows local enhancement or local inhibition (Table 2.1). We recorded information about the genus and species of the bee that was tested, and whether the bee species was social or solitary. We recorded whether the bee cue on the flower was a visual cue or both a visual and olfactory cue. We recorded whether the visual bee cue used was a model bee, a dead bee, or a live bee. If the visual bee cue was a real bee (dead or alive) and not a model, we recorded the genus and species, and whether it was a con- or heterospecific to the foraging bee that was tested. Lastly, we recorded whether we found support for local enhancement or inhibition for the cases within each study.

Table 2.1: Publications that tested if foraging bees land more frequently on flowers occupied by other bees (local enhancement) or unoccupied flowers (local inhibition). For each study we report the genus and species of the tested bee, whether the species is social or solitary, whether the authors tested visual cues or both visual and olfactory cues, whether the visual bee cue was a conspecific (C) or heterospecific (H) bee, whether the visual bee cue was a model, live, or dead bee, and whether we found support for local enhancement, inhibition, or neither. We also report the authors and year of the study as well as the number of cases per study. The five filled and five un-filled symbols on the far right are unique to one of the 10 studies we included in our analysis and they correspond to the symbols in Figure 2.1 The three different symbol colors indicate which of the three types of visual cues were used: a black symbol indicates a dead bee, a pink symbol indicates a model bee, and a blue symbol indicates a live bee was used as the visual cue.

Genus	Species	Social/Solitary	Olfactory/ Visual Cue	Cue Type		Support For Local Enhancement or Inhibition	Study (# of cases)	
<i>Apis</i>	<i>mellifera</i>	Social	Both	C	Live	Enhancement	Horna Lowell et al. In Review (n=1)	■
<i>Bombus</i>	<i>impatiens</i>	Social	Visual	C	Dead	Enhancement, Inhibition, neither	Dunlap et al. 2016 (n=9)	□
				C	Dead	Enhancement, neither	Plowright et al. 2013 (n=3)	★
				C	Live, Model	Enhancement, neither	Worden & Papaj 2005 (n=4)	☆ ☆
<i>Bombus</i>	<i>terrestris</i>	Social	Visual	C	Model	Enhancement	Avergués-Weber & Chittka 2014 (n=3)	●
				C	Live	Enhancement, neither	Baude et al. 2008 (n=4)	○
				C	Dead	Enhancement	Leadbeater & Chittka 2007 (n=2)	◆
<i>Bombus</i>	<i>vagans</i>	Social	Visual	C, H	Dead (crushed)	Inhibition	Abbot 2006 (n=2)	◇
<i>Heriades</i>	<i>fulvohispidus</i>	Solitary	Both	C	Dead	Enhancement	Sugiura et al. 2007 (n=1)	▲
<i>Trigona</i>	<i>amalthea</i>	Social	Visual	C	Live	Inhibition	Slaa et al. 2003 (n=1)	△

Statistical analysis

We used a multi-level model for our statistical analyses and we included publication identity as our random factor, our moderators (genus of foraging bee tested, conspecific or heterospecific cue, and type of bee cue used) as fixed effects, and SMD (Hedges' *d*) as the response variable. Initially we also included the genus of the bee tested as a random variable to consider phylogenetic relatedness in our model (as suggested by Koricheva & Gurevitch, 2014; Nakagawa et al., 2017), however the variance was almost zero so we dropped it from the final model. We used the variance from the effect sizes to weigh our model. We used the *metafor* package to perform our analysis (Viechtbauer, 2010). To calculate the SMD values for our effect sizes, we used the *escalc* function and then we ran our multi-level model using the *rma.mv* function. To obtain our overall model, we first used a model with no moderator as a fixed effect (a null model). We then tested if our moderators could explain some of the differences in the effect sizes in our model (as suggested by Nakagawa et al., 2017). We tested the influence that the genus of the tested bee, whether the visual cue was from a con- or heterospecific bee, and the type of bee cue used (model, dead or alive), had on whether the bee landed on the occupied or unoccupied flower.

The distribution of the individual effect sizes calculated can influence the overall effect size of meta-analyses. The calculated effect sizes can either have similar values, or there can be a lot heterogeneity or asymmetry in the effect sizes (i.e. a variety of negative and positive effect sizes of different magnitudes) such that when you average all the weighted effect sizes, the overall effect size is insignificant. Thus to test if our effect sizes were asymmetrical (i.e. if the distribution of effect sizes was skewed in one direction) we used a funnel plot and trim and fill analysis (Duval & Tweedie, 2000), and the Eggers test, for which we used a *rma.mv* model with the variance as a moderator (mod="vi"). Using the Eggers' test, we found significant asymmetry in our data set ($z=5.02$, $n=30$, $p<0.0001$; see Appendix E for funnel plot, and Appendix F for effect size distribution) suggesting that we may have some publication bias (Nakagawa et al., 2017). We also found a considerable amount of unexplained heterogeneity, or large differences between

the effect sizes, in our data set (Trim and Fill analysis: $\tau^2=3.89$, $I^2=78.3\%$; Complete model: $Q_E=495$, $df=29$, $p<0.0001$).

We performed three sensitivity analyses where we re-analyzed our dataset without a particular study or individual effect sizes to determine how sensitive the results from our meta-analysis are towards particular studies or effect sizes. First, we removed data from Abbot (2006) because they used dead, crushed bees as their visual cues and bees killed this way release alarm pheromones that deter other bees from landing near the dead bee (Dukas, 2001). The other studies in our meta-analysis that used dead bees killed them by drying or freezing them rather than crushing them (Dunlap, Nielsen, Dornhaus, & Papaj, 2016; Plowright et al., 2013). Therefore, we would expect there to be a negative effect in which bees are deterred from landing on flowers occupied by the dead crushed bee and land more frequently on unoccupied flowers. Secondly, we removed data from Dunlap et al. (2016) because 9 out of the 30 effect sizes in our meta-analysis came from this paper. For our last sensitivity analysis, we removed data from Horna Lowell *et al.* (In Review) because this study had a much larger sample size compared to the others. We performed all statistical analysis in R Studio version 1.1.463 (R Core Team, 2017).

Results

From the 10 publications that we collected, we obtained 30 effect sizes to use in our meta-analysis. We did not find statistically significant support for foraging bees' preference to visit an occupied flower compared to an unoccupied flower, or vice versa ($z= 1.18$, $n=30$, $p=0.24$). Although we found no significant support, there was a positive trend that foraging bees prefer to land on occupied flowers with 13 significantly positive effect sizes and only 6 significantly negative effect sizes (Figure 2.1). When we grouped effect sizes by genus, our results were still statistically insignificant, but both means showed a trend towards a positive effect size (Figure 2.1). Furthermore, none of our moderators explained the heterogeneity in our data set. Neither the genus of bee tested ($Q_M=5.32$, $df=3$, $p=0.15$), the type of bee cue that was used ($Q_M=3.43$,

df=3, p=0.3), nor whether the cue was a conspecific or heterospecific ($Q_M=1.29$, df=1, p=0.26), significantly explained the heterogeneity in our model.

For our sensitivity analysis, when we removed Abbot (2006) from the data set we found that there was still no overall significant effect of what type of flower, occupied or unoccupied, foraging bees preferred to visit ($z= 1.66$, $n=28$, $p=0.1$, estimate=0.55, lower c.i.=-0.1, upper c.i.=1.19). Although there was no significant effect, the overall effect size slightly increased from 0.4 to 0.55 (towards local enhancement) after removing the data from Abbot (2006) in which bees avoided visiting flowers occupied by dead crushed bees, presumably because of the alarm pheromone. Additionally, our fail-safe number calculation indicated that the number of non-significant unpublished studies that we would need to negate our results increased from 558 to 729 when Abbot (2006) was not included in the dataset. For our second sensitivity analysis we removed Dunlap *et al.* (2016) because almost one third of the effect sizes we used came from this study. There was still no significant positive or negative effect size, and therefore there was still no evidence that bees are more likely to land on either occupied or unoccupied flowers ($z=0.92$, $n=21$, $p=0.36$, estimate= 0.34, lower c.i.=-0.39, upper c.i.=1.06, fsn=216). Our last sensitivity analysis showed that even without the effect size from the study with the largest sample size (Horna Lowell *et al.*, In Review), bee foragers do not prefer to visit occupied flowers relative to unoccupied flowers or vice versa ($z=0.91$, $n=29$, $p=0.36$, estimate=0.34, lower c.i.=-0.39, upper c.i.=1.06, fsn=189). These results from our sensitivity analyses show that our overall analysis is robust to possible effects from individual studies.

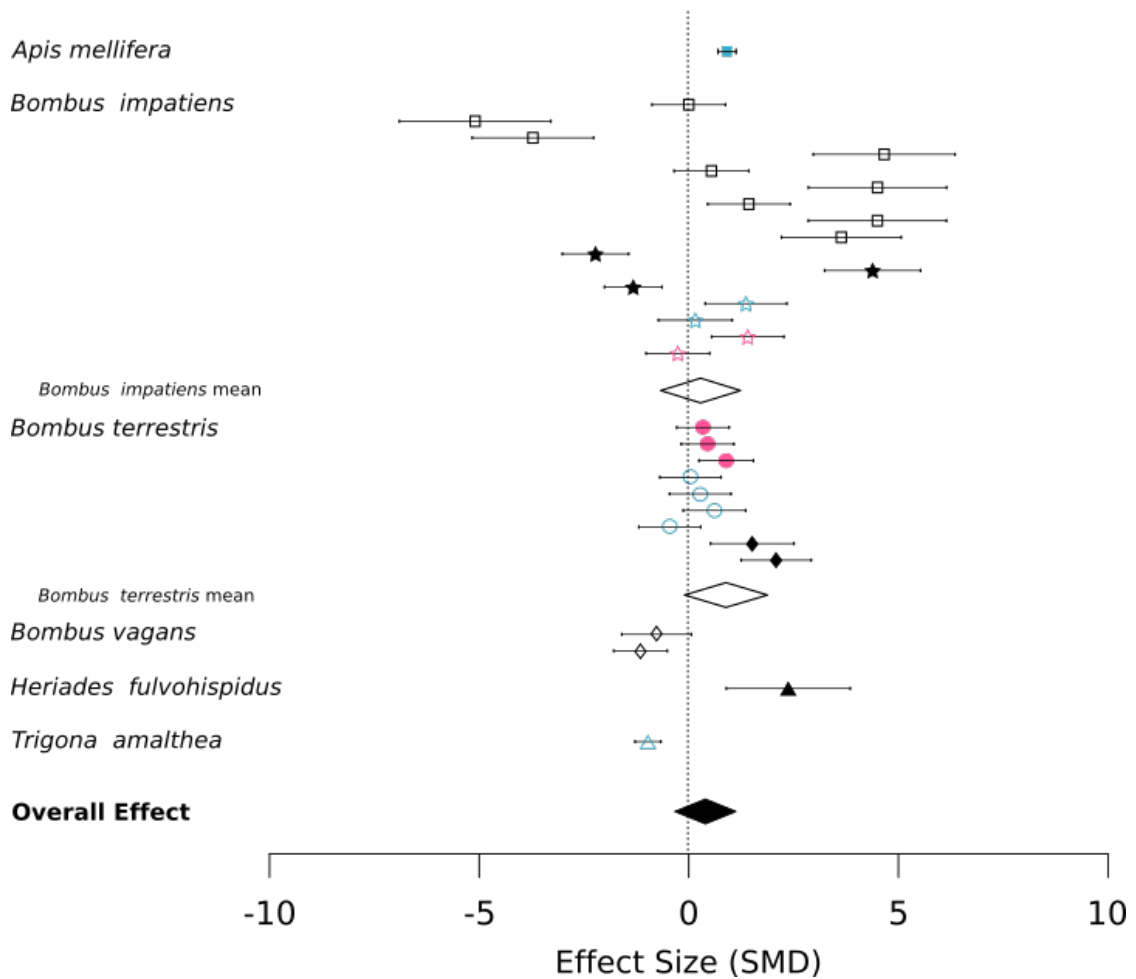


Figure 2.1: Our effect sizes from studies that tested whether bees show local enhancement or local inhibition more frequently ($n=30$). Our effect sizes represent the difference in visitation between bees that visit flowers occupied by a bee (dead, alive, or model bee), and bees that visit unoccupied flowers. The dotted vertical line represents a value of zero. If an effect size crosses the dotted line, then it is not different from zero and hence bees are no more likely to visit an occupied flower compared to an unoccupied flower. A positive effect size means bees tend to show local enhancement and a negative effect size means bees tend to show local inhibition. The effect sizes are organized and labeled by genus and then species. There are ten different symbols for the effect sizes that correspond to the papers in Table 1. The three different symbol colors indicate which of the three types of visual cues were used: a black symbol indicates a dead bee, a pink symbol indicates a model bee, and a blue symbol indicates a live bee was used as the visual cue. We found multiple measures for *Bombus impatiens* and *Bombus terrestris*, and thus we were able to calculate a mean effect size for these species. The mean effect sizes for both *Bombus* species (filled-in diamonds) as well as the overall effect size with all 30 cases (unfilled diamond) are represented by wide horizontal diamonds (*B. impatiens*: estimate=0.28, lower c.i.=-0.67, upper c.i.=1.23; *B. terrestris*: estimate=0.81, lower c.i.=-0.18, upper c.i.=1.80; Overall: estimate=0.40, lower c.i.=-0.26, upper c.i.=1.05).

Discussion

When bees forage for nectar and pollen, they can encounter other con- or heterospecific bees on the same flower. In our meta-analysis we quantified whether bees were more likely to show local enhancement or local inhibition when presented with flowers occupied by other bees and unoccupied flowers. We found no overall significant positive or negative effect size, suggesting that foraging bees do not exhibit local enhancement or local inhibition more frequently (Figure 2.1). We did find a lot of variability in the effect sizes, even within species. For example within a single paper, some cases had a large negative effect size (local inhibition) while other cases had a large positive effect size (local enhancement) (Dunlap et al., 2016; Plowright et al., 2013). Although our results are not significant, we found more than twice as many significant positive effect sizes (n=13) as significant negative effect sizes (n=6) and our overall effect size was slightly positive, which suggests that bees may land on flowers occupied by other bees more than unoccupied flowers (local enhancement), but more research is needed. For some of the species we tested, only a single study included a measure of error for their means and could therefore only be included in our overall meta-analysis. There were two *Bombus* species that had multiple measures and for which we could group effect sizes by genus; while our results for both *Bombus impatiens* and *Bombus terrestris* were not significant, both mean effect sizes trended positively, and thus showed local enhancement (Figure 2.1).

We found that most of the negative effect sizes (local inhibition) came from studies that had a few variables in common with each other; these studies either tested the behavior of bees in response to cues over time (over many trials), used a crushed dead bee as their visual cue, varied how reliable the visual cue was at indicating a rewarding resource, or varied the frequency of visual cues on flowers. The studies that tested if bees visited flowers occupied or unoccupied by other bees more often over time found that initially bees were more likely to exhibit local enhancement, but in later trials bees tended to show local inhibition (Baude et al., 2008; Slaa et al., 2003). One potential explanation could be that foraging bees are initially naïve when they arrive to a resource patch and thus benefit from landing on flowers occupied by conspecifics

because the presence of another bee can indicate a safe and rewarding place to forage (Slaa et al., 2003). However, as bees continue to revisit the area and visit more types of flowers, they learn more information about rewarding versus unrewarding flowers. As foragers become more experienced, they do not benefit as much from the information provided by the visual cue and thus avoid landing on flowers with these cues, perhaps due to competition. A similar result was demonstrated with Siskins (*Spinus spinus*) that suggests transient birds naïve to an area are more likely to show local enhancement and forage with conspecifics whereas resident birds that are familiar with an area are less likely to forage with other conspecifics (Senar & Metcalfe, 1998). Alternatively, the more time a bee spends foraging in a resource patch, the more depleted the floral resources become, and so bees may start to exhibit local inhibition once the resource decreases in value. Dunlap et al. (2016) directly manipulated the quality of the conspecific cue by varying how reliable the cue was at indicating a rewarding flower. Foraging bees only showed local inhibition when half of the conspecific cues were on a rewarding flower and the other half were on unrewarding flowers (Dunlap et al. 2016). However, when the conspecific cues were placed on rewarding flowers 83% or 100% of the time, then bees showed no preference or they exhibited local enhancement (Dunlap et al., 2016). Lastly, Plowright et al. (2013) varied the frequency of conspecific cues on flowers and showed that when the frequency of conspecific cues was low, foraging bees exhibited local inhibition, but when the frequency of conspecific cues was high, bees showed a slight trend towards local enhancement. These findings demonstrate that the presence or absence of conspecific cues not only impact foraging behavior, but also the density of conspecific cues at a resource patch. All these studies illustrate how context is important in determining whether a bee exhibits local enhancement or local inhibition. When visual con- or heterospecific cues are reliable and abundant, and foraging bees are at a novel flower patch, they are more likely to show local enhancement. However, as visual bee cues become less reliable and abundant, or foragers gain experience at a flower patch, bees become less likely to show local enhancement and more likely to show local inhibition.

We encountered a few limitations in the literature while conducting our meta-analysis. First, we found that the majority of studies that tested our question did not report standard measures of error or variance. We were not able to create effect sizes for these studies and as a result our meta-analysis does not include them. We encourage authors to report their measures of error or variance to ensure that meta-analyses are a more comprehensive synthesis of the existing literature on a particular topic. Second, about 63% of the studies we included in our analysis used bumblebees as their study system. Bumblebees are relatively easy to manipulate and are thus the most common study system, but we have to remain conservative in speculating how our results may apply to other bee species. Notably, several species only had a single study in our analysis, including a major pollinator, the European honey bee (*Apis mellifera*). Although initially when we included genus as a random variable in our model there was no effect, it is possible that if we increased the diversity of bee species used in our analysis, the overall effect size could change. Even with these limitations our sensitivity analyses confirm the robustness of our results. Nonetheless we encourage future bee foraging behavior studies to test a larger variety of bee species and to include standard error and variance.

Our meta-analysis provides a foundation to understand broad scale patterns of local enhancement or local inhibition in bees. Although we did not find any significant overall patterns of local enhancement or inhibition across bee species, we did find a slight trend towards local enhancement. Our results suggest that when a visual con- or heterospecific cue is reliable, abundant, and provides novel information, bees tend to land on flowers occupied by the cue. However, when the visual cue is less abundant or no longer provides reliable information, bees no longer visit occupied flowers more than unoccupied flowers. Our results provide information about how bees generally forage in the presence or absence of other conspecifics and heterospecifics, which can have large implications for pollination, an essential ecosystem service.

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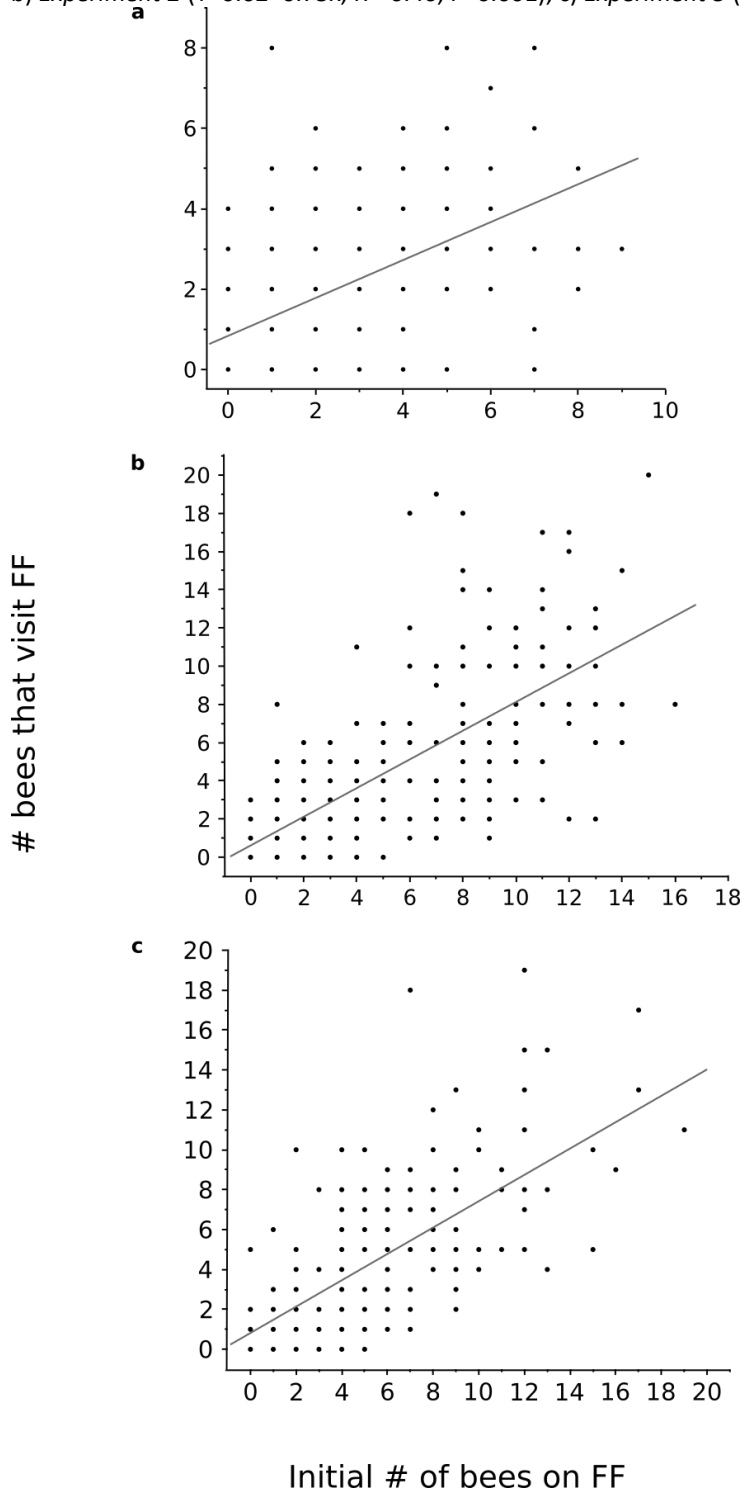
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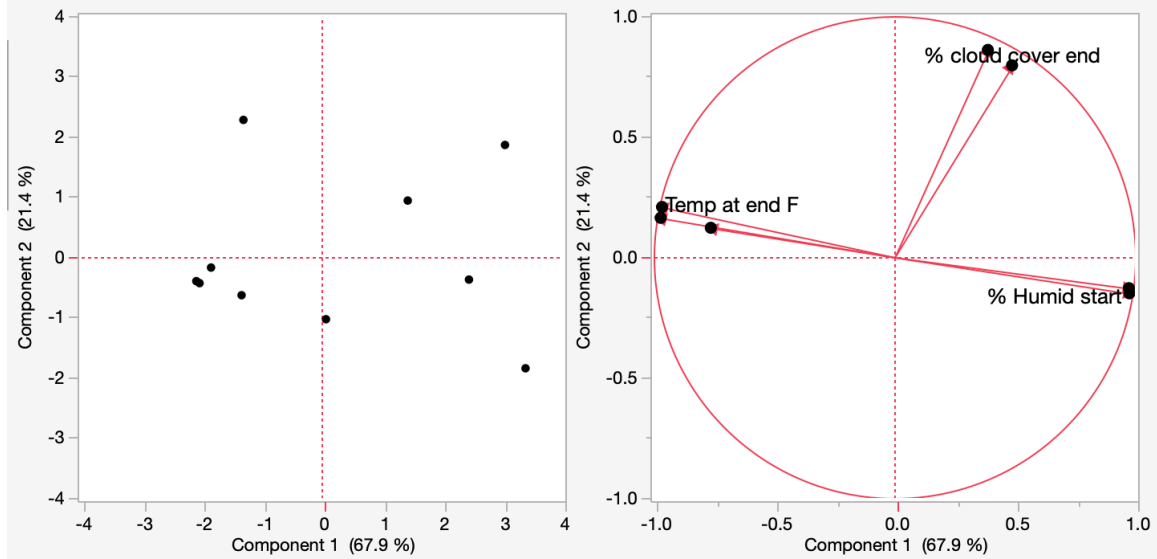
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APPENDICES

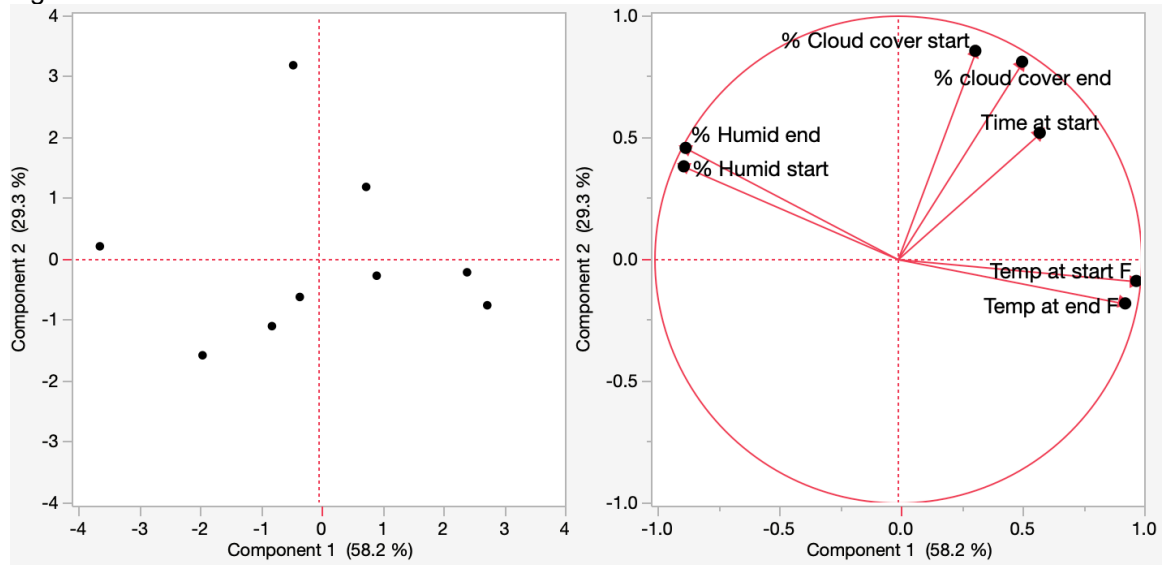
Appendix A: The linear relationship between initial number of bees on the focal flower, and the number of bees that visited the focal flower in 20 seconds for a) *Experiment 1* ($Y=0.85+0.5x$, $R^2=0.31$, $P<0.0001$), b) *Experiment 2* ($Y=0.62+0.75x$, $R^2=0.46$, $P=0.001$), c) *Experiment 3* ($Y=0.81+0.7x$, $R^2=0.48$, $P=0.07$)



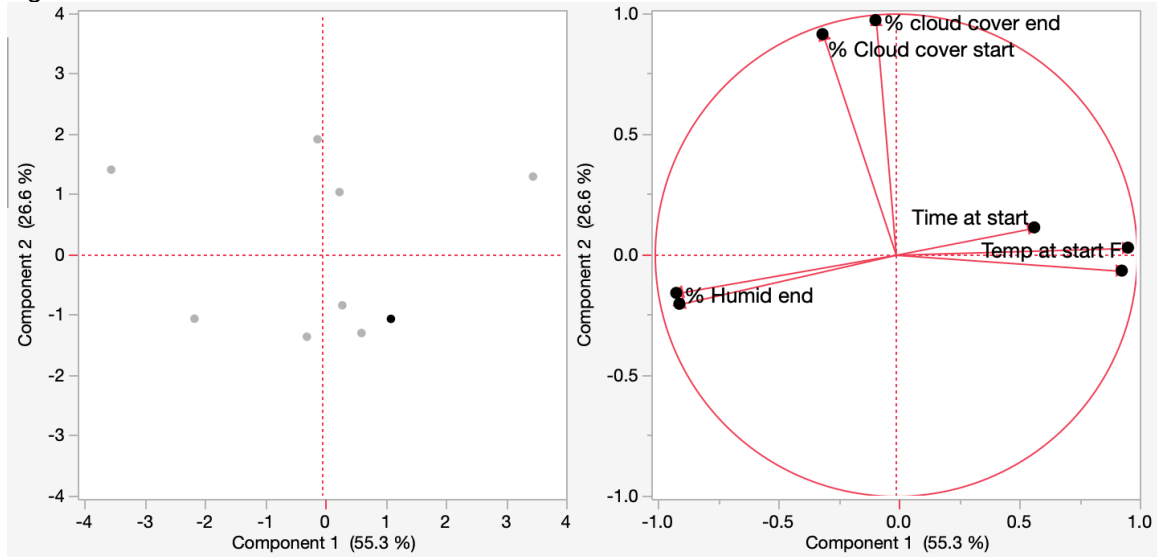
Appendix B: The two principal components ($PC1=67.9\%$ and $PC2=21.4\%$) for *Experiment 1* that summarized our climactic variables, which were percent cloud cover, percent humidity, temperature, and time of day. The figure on the left shows the eigenvalues for each individual data point that we collected of our climactic variables. The figure on the right shows the average eigenvalues for each of our climactic variables.



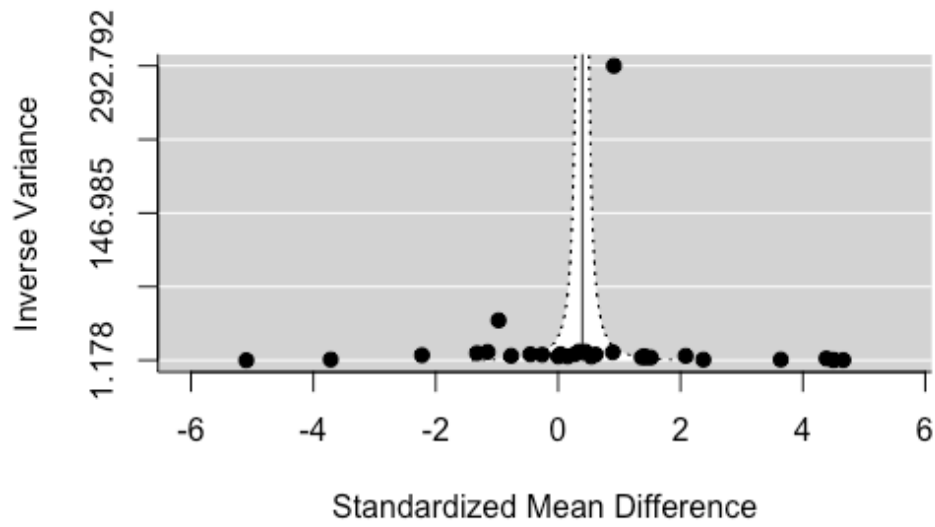
Appendix C: The two principal components ($PC1=58.2\%$ and $PC2=29.3\%$) for *Experiment 2* that summarized our climactic variables, which were percent cloud cover, percent humidity, temperature, and time of day. The figure on the left shows the eigenvalues for each individual data point that we collected of our climactic variables. The figure on the right shows the average eigenvalues for each of our climactic variables.



Appendix D: The two principal components ($PC1=55.3\%$ and $PC2=26.6\%$) for *Experiment 3* that summarized our climactic variables, which were percent cloud cover, percent humidity, temperature, and time of day. The figure on the left shows the eigenvalues for each individual data point that we collected of our climactic variables. The figure on the right shows the average eigenvalues for each of our climactic variables.



Appendix E: A funnel plot with the trim and fill method (Duval & Tweedie, 2000) we used to test our effect sizes for asymmetry (Trim and Fill analysis: $\tau^2=3.89$, $I^2=78.3\%$).



Appendix F: A histogram of our 31 effect sizes.

