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The Waggle Dance and the Anti-Waggle Dance: Communication in Foraging Honey Bee Colonies

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The Waggle Dance and the Anti-Waggle Dance: Communication in Foraging Honey Bee Colonies

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Parry Macdonald Kietzman

June 2015

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DEDICATION

To Ben,
For sharing this beautiful life
with thousands of bees.

ABSTRACT OF THE DISSERTATION

The Waggle Dance and the Anti-Waggle Dance: Communication in Foraging Honey Bee Colonies

by

Parry Macdonald Kietzman

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, June 2015
Dr. P. Kirk Visscher, Chairperson

Many of the activities within honey bee (*Apis mellifera* L.) colonies rely on the use of communication signals for organization. A classic example of this is in foraging, which is regulated through the use of the waggle dance, a positive feedback signal that recruits other foragers to the advertised food source, the tremble dance, which recruits bees to unload food from incoming foragers, and the stop signal, a negative feedback that acts as a counter to the waggle dance. During the waggle dance, observers touch the dancer with their antennae and follow her through one or more iteration of the dance. Though the results of previous research stated that the followers must be located to the rear of the dancer to receive the information encoded in the dance, I found that bees following from any location relative to the dancer succeeded in locating the advertised food source. In an experiment where half of the bees visiting a feeding station experienced a simulated attack via a pinch

with forceps, the pinched bees produced more stop signals and danced fewer waggle dances than bees that had not been pinched. Most of the stop signals observed, however, came from bees that had not visited the feeding station at all. These may have been unloader bees attempting to decrease foraging due to an unmanageable influx of food from the feeding station or foragers that had visited other food sources and were not being unloaded promptly. When the amount of storage space available for food in a colony was manipulated between ample storage space and no storage space, significantly more stop signals and tremble dances were observed when there was no available storage space. This suggests the bees had assessed the lack of storage space and were attempting to decrease their colony's foraging efforts.

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CHAPTER 1: INTRODUCTION

THE ANTI-WAGGLE DANCE: USE OF THE STOP SIGNAL AS NEGATIVE FEEDBACK

Abstract

Numerous activities within honey bee (*Apis mellifera* L.) colonies rely on feedback loops for organization at the group level. Classic examples of these self-organizing behaviors occur during foraging and swarm nest site selection. The waggle dance provides positive feedback, promoting foraging at a specific location or increased scouting at a potential nest site. Rather less well known than the waggle dance is the stop signal, a short vibration often delivered while butting against a dancing bee. It is currently best understood as a counter to the waggle dance, offering negative feedback towards the advertised foraging location or nest site. When the stop signal is received by a waggle dancer she is more likely to terminate her dance early and retire from the dance floor. Bees that experienced danger or overcrowding at a food source are more likely to perform the stop signal upon their return to the colony, resulting in an inhibition of foraging at that location. During a swarm's nest site selection process, scout bees that visited a different site than the one being advertised are more likely to stop-signal the waggle dancer than are scouts that had visited the same site. Over time, the scout bees build recruitment to a single site until a quorum is reached and the swarm can move to it. The balance between the positive feedback from the waggle dance and the negative feedback

from the stop signal allows for a more sensitive adjustment of response from the colony as a unit. Many of the processes associated with the feedback loops organizing a honey bee colony's activities are in striking parallel to other systems, such as intercellular interactions involved in motor neuron function.

Introduction

Honey bees (*Apis mellifera* L.) employ numerous chemical, tactile, and vibratory communication signals to coordinate their activities. Here, we will focus on a few of the vibratory ones including the well-known waggle dance, which signals the distance and direction of a resource such as a food source or a potential nest site to nestmates (Von Frisch 1967). We will also discuss the tremble dance, which is performed by foragers who have experienced delay in unloading, and recruits more bees to assist in unloading food from incoming foragers (Seeley 1992). An additional signal, and the focus of this article, is the stop signal. Sometimes referred to as the "brief piping signal," (e.g., Seeley and Tautz 2001, Thom *et al.* 2003) in addition to "peeps" (Esch *et al.* 1965), "squeaking" (Von Frisch 1967) and "short squeaks" (Kirchner 1993a), the stop signal is an acoustic signal produced by a bee briefly vibrating her wing muscles (with little wing movement), often while butting her head against another bee (a video of honey bees performing the stop signal can be found in the online supplementary material).

Here we review what is known about the stop signal and its uses. The focus will be on exploring the balance among communication signals used by individuals and the resulting adjustment of response by the colony as a unit.

Early work on the stop signal

The first recorded observations of the stop signal did not find clear uses and meanings for it. Esch (1964) observed bees attending waggle dances and noted that they occasionally emitted squeaking sounds, after which they sometimes received food samples from the dancer. Wenner (1962) reported that disturbed bees emit short bursts of sound, similar to the stop signal. Von Frisch (1967) also observed it in use by bees interacting with waggle dancers, and agreed with an interpretation by Esch (1964) that it was a begging call for food.

The sounds made by these bees were later identified as vibrations of the comb made by pressing the thorax briefly to it and pulsing the wings (Michelsen *et al.* 1986), or by a bee butting her head into a dancer and pulsing the wing muscles (Nieh 1993). Michelsen *et al.* (1986) described these sounds as typically lasting approximately 100 ms at ~380 Hz. The results of Schlegel *et. al* (2012) averaged 407 Hz for 147 ms. Honey bees also make a similar-sounding acoustic signal known as worker piping, but this can be differentiated from the stop signal by its much longer duration, approximately 602 ms , and a higher and upward sweeping frequency, (451 to 478 Hz, Schlegel *et al.* 2012).

The term “stop signal” seems an appropriate name for the signal, since Von Frisch (1967) reported that the dancer and surrounding bees are “paralyzed” by the sound. Similarly, Michelsen *et al.* (1986) found that artificial signals made by vibrating the comb caused bees in the area immediately surrounding the point of vibration to briefly freeze their movements. When Nieh (1993) observed bees on the dance floor and recorded the behavior of individuals before and after sending or receiving the stop signal he found that the sender very seldom receives food (once out of 576 stop signals delivered to waggle dancers), discrediting the idea that the stop signal is a begging call. The most common occurrence after a waggle dancer received the stop signal was to leave the dance floor (Nieh 1993). Similarly, in a later study Pastor and Seeley (2005) investigated the behavior of waggle dancers and dance followers. They found that dancers that received the stop signal were more likely to stop dancing and they never observed an instance of food exchange between a stop signal sender and receiver.

Which bees produce the stop signal?

In an effort to determine which bees within a colony produce the stop signal and which receive it, Nieh (1993) trained foragers from an observation hive to visit an artificial feeding station filled with sugar water and made observations on the bees populating the dance floor. Recently-returned foragers were observed with a video camera and microphone. The study focused on classifying the stop signal senders and receivers, and found that tremble dancers are the most likely individuals to perform the stop signal, although they can occasionally be performed by waggle dancers and dance followers (Nieh 1993). Waggle dancers and tremble dancers were the most common stop signal receivers, although food exchangers, dance followers, and "other" bees not dancing or observing dances were also targeted (Nieh 1993).

Pastor and Seeley (2005) revisited the question of which bees send and receive the stop signal after noting that the bees in Nieh's (1993) study may not have been behaving normally due to the large influx of food they were receiving from the feeding station. When they observed a colony that was foraging on naturally-available food resources with no access to a feeder, most of the waggle dance followers that used the stop signal had not previously been tremble dancing (Pastor and Seeley 2005). Additionally, though Nieh (1993) found that dance followers occasionally use the stop signal on waggle dancers, in Pastor and Seeley's (2005) results the majority of stop signalers were dance followers.

When waggle dancers receive a stop signal they are more likely to leave the dance floor (Nieh 1993, Pastor and Seeley 2005) and their average dance length is shorter (Kirchner 1993b). These factors, combined, likely result in an inhibition of recruitment to that food source and an overall decrease in foraging.

It is possible that this effect was also observed by Wenner (1962), as he described waggle dancers being interrupted in their dances by other bees or abruptly halting their dances, sometimes even in the middle of a waggle run, for unknown reasons. He also mentioned the short sounds made by disturbed bees, which may have been stop signals. Unfortunately, insufficient information was given to determine if these short sounds were stop signals.

What elicits the stop signal?

Aside from its effect of halting waggle dances, the stop signal can also be seen in use by bees not located on the dance floor and received by bees that are not waggle dancers. Thom *et al.* (2003) observed colonies both when they had access to a sugar water feeding station and when they were foraging under natural conditions.

Stop signaling increased when a feeding station was available (Thom *et al.* 2003). Most of the stop signaling activity was by tremble dancers, although non-waggle dancing nectar foragers also performed the stop signal (Thom *et al.* 2003). Tremble dancers that used the stop signal ended up staying in the hive for longer than those that did not use the stop signal (Thom *et al.* 2003). Foragers that performed the stop signal tended to spend less of their time within the colony on the dance floor, and often continued performing the stop signal outside of the dance floor (Thom *et al.* 2003). Tremble dancers that performed the stop signal tremble-danced for longer than non-stop signalers and traveled deeper into the hive (Thom *et al.* 2003). Also, bees that used the stop signal sometimes inspected cells by entering them up to the thorax, which was a behavior not exhibited by non-stop-signaling bees (Thom *et al.* 2003).

It can be inferred that by inhibiting the waggle dance, the stop signal strengthens the nectar-receiver-recruiting effect of the tremble dance (Figure 1.1), but this does not account for the bees observed using the stop signal outside of the dance floor. Thom *et al.* (2003) suggested that the off-dance-floor stop signaling could be an effort to modulate the recruitment of more nectar receivers by lowering potential nectar receivers' response thresholds to the tremble dance.

Role	**Evidence**	**References**
Stop other bees' movements	Bees surrounding a stop signaler briefly freeze movements	Von Frisch 1967; Michelsen *et al.* 1986
Inhibit waggle dancing	Waggle dancers receiving the stop signal leave the dance floor	Nieh 1993; Pastor and Seeley 2005; Kirchner 1993b
Modulate the tremble dance	More stop signaling by tremble dancers when a feeding station is present	Thom *et al.* 2003
Decrease recruitment to a food source	More stop signaling when a feeding station is crowded; more stop signaling when there is danger or competition at a feeding station	Lau and Nieh 2010; Nieh 2010
Provide cross-inhibition during a swarm's nest site selection process	Waggle dances are shorter when stop signaling occurs; stop signalers target dancers advertising nest sites not visited by the stop signaler	Seeley *et al.* 2012

Table 1.1: A summary of the roles of the stop signal in honey bee colonies.

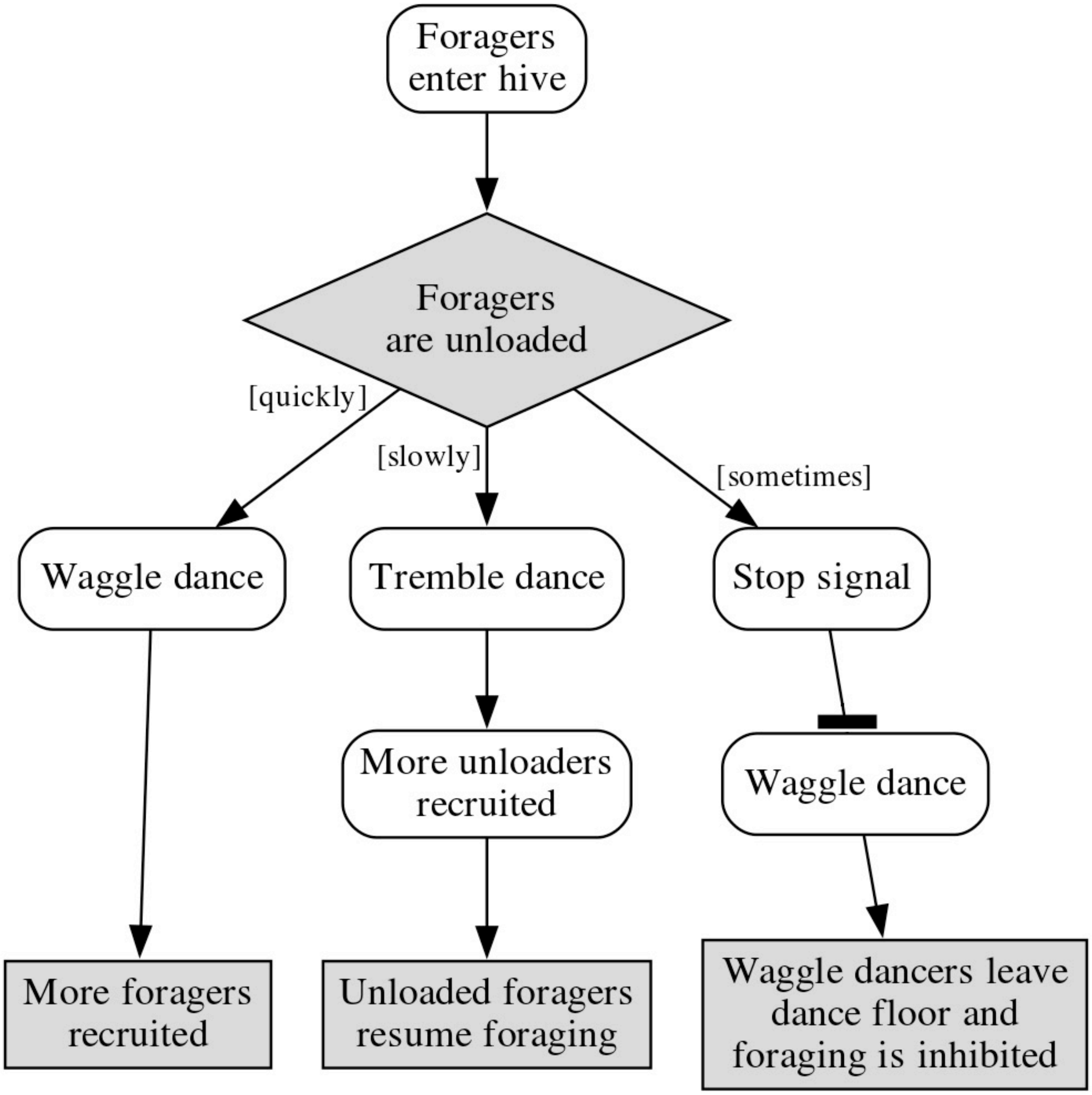

Figure 1.1: Feedback loops in a foraging colony, showing the effect of the stop signal on waggle dances and forager recruitment.

An interesting finding of this study (Thom *et al.* 2003) was that many of the tremble dancers that also performed the stop signal began signaling as soon as they entered the colony, i.e., before having an opportunity to be met by a nectar receiver and be influenced by the time-delay cue described by Seeley (1992). Thom *et al.*

(2003) suggested they may have been acting based on their experiences from prior foraging trips or by cues sensed outside of the hive. One source of such cues may have been scramble competition at the feeding station (Thom *et al.* 2003). This conclusion seems possible, especially since it has been shown that rich food resources such as feeding stations lead to a spike in tremble dancing and stop signaling within the colony (e.g., Nieh 1993, Pastor and Seeley 2005). Thom *et al.* (2003) might have been able to determine the effect of the feeding station on the timing of tremble dancing if they had compared the behavior of the tremble dancers in their colony foraging under natural conditions to that in their colonies that had access to the feeding station. However, they scanned the natural conditions colony for communication signals but did not closely examine the behaviors exhibited by the individual signalers, thus, an effective comparison can not be made using their results.

Proximate stimuli that elicit the stop signal

Lau and Nieh (2010) hypothesized that a food source that is declining in quality, for example running out of food or becoming overcrowded, may provoke the use of the stop signal by foragers. Supporting this, they found that as a feeding station grew more crowded, the rate of stop signaling within the colony increased. The foragers that had to wait to access the feeder were not more likely to perform the stop signal themselves, but they were more likely to receive stop signals from other bees that had visited the feeder and also from individuals that had not been observed there. This may be because some bees were not able to access the feeder at all due to overcrowding and returned to the colony without feeding. Thus, feeder crowding probably increases the number of foragers within the nest, thereby increasing the number of opportunities for them to use the stop signal (Lau and Nieh 2010). Lau and Nieh (2010) conjectured that it is likely that the stop signal is present in the colony at low levels at all times, but does not have a colony-wide effect until some threshold level is reached. Additionally, using the stop signal may enable bees to rapidly adjust foraging efforts in response to shifts in their environment's nectar flow.

The stop signal is also used when foragers experience some form of danger or competition at a food source (Nieh 2010). Under natural conditions this would likely be a response to being attacked by other bees or a predator while foraging. Nieh (2010) observed fights between conspecifics at a feeding station, and he simulated a predator attack by pinching bees visiting a feeder on the metathoracic femur with forceps. Both the bees that had experienced intraspecific competition and those that were attacked by forceps were more likely to perform the stop signal toward other foragers waggle-dancing for the same food source than they were toward dancers for other locations. Bees that had not had the negative experiences were much less likely to use the stop signal at all (Nieh 2010).

The intraspecific competition that the bees experienced at the feeding station was probably somewhat artificial. When bees forage on natural food sources such as flowers, these are usually spread across a patchy landscape, individually offer small amounts of food, and are seldom simultaneously visited by more than one bee. In contrast, a feeding station is a very rich food source found only at a single location. Nieh (2010) acknowledged this and suggested that competition at the feeding station may be more similar to the competition experienced when bees rob food from other colonies (though Johnson and Hubbell 1974, and others, have reported competitive interactions at floral sources.). In a paper modeling a hive-robbing event, Johnson and Nieh (2010) showed the stop signal in use to rapidly shut down robbing by countering the waggle dance. Aside from this model, however, an actual assessment of the signaling that occurs during an actual robbing or dense-flower situation and comparing it to the signaling used while foraging at a feeding station is an area of research that has not yet been explored.

Stop signals as cross-inhibition

Another observed use of the stop signal is during the swarm nest site selection process. When honey bees swarm (reviewed in Visscher 2007), thousands of workers and the original queen leave the hive and settle in a cluster a short distance away. From there, scout bees depart and search for potential new nest sites that the colony could inhabit. When a scout locates a favorable site, she returns to the swarm and advertises its location using the waggle dance. Over time, multiple sites maybe be advertised by many different dancers, with each group competing to recruit additional scouts to their site. Support for the different sites will wax and wane until a threshold number of scouts, or quorum, is reached at one of the sites, after which recruitment declines, and the swarm can be mobilized to move to its new home. This deadlock avoidance is of key importance to the nest site selection process because unlike during foraging, a decision for a single site must be reached.

The stop signal is used to provide cross inhibition in the form of negative feedback during this decision making process (Seeley *et al.* 2012). This study made video recordings of waggle dancers on the surfaces of swarms and recorded stop signals performed on the dancers by following bees on video with audio from a microphone held close to dancing bees. The dancers stopped dancing soon after receiving stop signals, and their dances were shorter than those of dancers not receiving stop signals When swarms simultaneously scouted two identical nesting boxes, dancers for either box received more stop signals from bees that had visited and been marked at the other site (contra-signalers) than bees that had visited the same site (ipsi-signalers). After a decision was reached about which nest box to occupy (inferred from the initiation of worker piping, which prepares the swarm for takeoff), the stop signalers no longer selectively targeted dancers advertising the opposing site and dancers received contra- and ipsi- signals equally. Seeley *et al.* (2012) inferred that negative feedback from the stop signal was provided cross inhibition between the two potential nest sites while the swarm was still making a decision, and that after a decision had been reached it contributed to shutting down waggle dancing. This contributes to having nearly all the swarm's bees at the swarm cluster when it takes off for cross-country flight, which will be guided by the scouts that know the way to the chosen site.

Negative feedback in other social insect systems

It is of interest to note that negative feedback is present in other social insect systems. Trail pheromones, which are also used by termites, stingless bees, and social wasps, are used to recruit other individuals to food sources and nest sites (Czaczkes *et al.* 2015). These can encode complex information as a result of having varying chemical blends, concentrations, and operating synergistically with other factors (Czaczkes *et al.* 2015). Positive feedback from trail pheromones can cause groups of ants to focus inflexibly on a single food source due to the strong, non-linear response of recruits to the trail, even when other potentially better options exist (e.g. reviewed in Camazine *et al.* 2001). This effect can be countered by negative feedback from overcrowding at a food source, which results in an equal distribution of foragers across multiple food sources or the quick reallocation of the majority of foragers to a superior food source (Grüter *et al.* 2012). Negative feedback can also come from encounters with other foragers on a trail, where greater crowding leads to less trail pheromone deposition (Czaczkes *et al.* 2013), or from repellant trail pheromones used as “no entry” signals marking unrewarding paths (Robinson *et al.* 2005).

Discussion

Decision-making by groups of animals has received increasing recent attention in part because of recognition of its significance to other systems, in particular complex nervous systems and human engineered systems. Mechanisms of coordination discovered in social insect colonies have provided models for human-engineered systems in computing and robotics, because in both kinds of systems there is a need for reliable, robust decision-making based on simple interactions among components (e.g. Bonabeau and Meyer 2001, Tsuda *et al.* 2006) Also, recent discoveries in decision-making mechanisms of vertebrate brains and swarms of honey bees have revealed striking parallels in their mechanisms (Passino *et al.* 2008, Marshall *et al*. 2009).

In all such systems, individual units are able to use a relatively small repertoire of behaviors or actions to achieve a complex task as a whole. Each unit, be it an insect, a robot, or a neuron, accumulates evidence until some threshold is reached and a decision can be made. The stop signal reviewed here provides negative feedback that can help modulate achieving that threshold and tune the behavior of honey bee colonies during foraging and swarming. The findings reviewed here suggest that the stop signal has diverse uses and effects. It is quite likely that not all of these have yet been described.

For example, the question of stop signaling during swarming is still not well understood. The results of Seeley *et al.* (2012) support the idea that cross-inhibition during the decision-making phase by contra-signalers provides negative feedback from scouts that had visited a different nest site. This, however, does not explain the lower-level occurrence of the ipsi-signaling that was also present. In a follow-up study using two nest boxes of differing volumes, much of the stop signaling observed was ipsi-signaling rather than contra-signaling, and some of stop signalers had not visited either nest site (Visscher, Schlegel, and Kietzman, unpublished data). These are puzzling results that beg the immediate questions of what might have been motivating the bees to signal and what the signals' effects were on the decision-making process. There is clearly more to learn about the uses and effects of the stop signal during swarming.

Another not-yet-explored avenue is the idea that tremble dancers that use the stop signal outside of the dance floor may be modulating the recruitment of more nectar receivers by lowering potential nectar receivers' response thresholds to the tremble dance (Thom *et al.* 2003). This could be tested by assessing whether or not non-dancers that received the stop signal were more likely to become nectar receivers after being contacted by a tremble dancer than individuals that did not. If so it would be a novel use of the stop signal within the context of a foraging colony.

A variety of conditions external to the colony have been explored to determine their effects on the communication signals used by bees, but few have considered the factors within the hive that may influence the bees' communication. We now know that a lack of nectar receivers stimulates tremble dancing, which results in the recruitment of more nectar receivers (Seeley 1992). We also know that stop signaling inhibits foraging and is also associated with tremble dancing (e.g., Thom *et al.* 2003). A useful line of research would be to determine what factors, if any, within the hive might help drive bees' decisions to tremble dance or stop signal. For example, decreasing the food storage space available to the bees might be expected to result in an increase in tremble dancing, as the nectar receivers would be unable to store the food brought in by foragers. There would likely also be in an increase in stop signaling as the colony's nectar-handling capacity would be exceeded and foraging would need to be shut down.

While there are unanswered questions about the use of the stop signal, most of what has been discovered fits a picture of the stop signal as a negative-feedback component in recruitment, a sort of anti-waggle dance. The use of such a signal allows the bees to tune their recruitment more accurately and quickly in response to changing conditions, and in a variety of contexts.

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CHAPTER 2

DOES FOLLOWER POSITION MATTER TO WAGGLE DANCE INFORMATION TRANSFER?

Abstract

It is known that the honey bee waggle dance communicates the distance and direction of some item of interest, most commonly a food source, to nestmates. Previous work suggests that in order to successfully acquire the information contained in a dance, other honey bees must follow the dancer from behind. We revisit this topic using updated methodology, including a greater distance from the hive to the feeder, which produced longer, more easily-read dances. Our results are not congruent with those of earlier work, and we did not conclude that honey bees must follow from behind a dancer in order to obtain the dance information. Rather, it is more likely that a follower can successfully acquire a dance's information regardless of where she may be located about a dancer.

Introduction

The honey bee (*Apis mellifera* L.) waggle dance has been a topic of great interest to biologists for over 50 years, and although it has been well-researched during that time, certain aspects related to it remain contested. The waggle dance communicates the distance and direction of some item of interest, most commonly a food source, to other bees within the hive (Von Frisch 1967). When a returning forager performs the waggle dance to alert her nestmates to the existence of a useable food source, other bees crowd around her, touching her with their antennae and at times following her movements through portions of the dance. Judd (1995) examined whether there were specific locations relative to the dancer that a follower must occupy in order to successfully receive the information from the dance. He concluded that in fact bees must follow from the rear of a dancer for information transfer to occur.

However, we have not observed following bees vying for the limited positions available at the rear of dancers (Kietzman and Visscher, personal observation). Additionally, bees following from the side of a dancer remain in contact with the dancer for longer periods of time than bees following from other positions (Bozic and Valentincic 1991), which would give those bees a greater opportunity to interpret the dance. Based on our observations, dance followers appear satisfied in any position where it is possible to have antennal contact with the body of the dancer. Also, Judd (1995) described a type of "lunging" movement that was of importance to the dance following experience, and it was unclear what this movement entailed. It is possible that the methods he employed did not produce data that were easily interpreted, as the feeding station he employed was located at a mere 150m from the hive. At this distance bees perform very short waggle runs with much turning during the dance. Due to such constant turning it would be difficult to determine where the followers were located relative to the dancer. Additionally, it would be difficult for the followers to position themselves in a precise location for any length of time.

This study was conducted using a feeding station located further away from the hive. Data analysis was structured to address the hypothesis that bees would follow more dances from the rear of a dancer, as compared to the null hypothesis that they would follow dances from random positions.

Methods

Overview

This experiment was conducted over the course of three days, 20 September 2010 to 22 September 2010, in agricultural fields belonging to the University of California, Riverside. Our approach, like that of Judd (1995), was to mark an entire observation colony of honey bees, train scouts to a feeding station, and, with video, observe the dance following behavior of those bees that were successfully recruited.

Colony marking

All the bees used in this experiment were marked with numbered tags following the method described by Seeley *et al.* (1991). To do this, approximately 2500 worker bees were shaken from a large hive into a wire mesh box. From there we shook small groups of approximately 10 bees each into plastic bags and refrigerated them briefly to sedate them. We then transferred them to a cold-well, an open-topped foam box with “blue ice” covered with paper towels at the bottom, so that they would remain chilled. We marked the bees with colored plastic number tags adhered to their thoraxes and dots of colored paint applied to their abdomens with paint pens. Using this system of marking all the bees in the colony could be individually identified. After they were marked we moved the bees to a different cage to recover. Once all the bees had been marked they we established them as a new colony in an observation hive together with their original queen.

Training

The bees were allowed to fly and forage freely during an acclimatization period that lasted one week. Following this, we trained foragers to visit a feeding station baited with 2M sucrose solution scented with anise. The feeding station was first placed directly outside the entrance to the hive and gradually moved further away as a group of foragers began visiting it repeatedly. By the end of the training period the feeding station was located at 400m from the hive. This was the furthest distance used in the experiment and was visited consistently by several foragers. Data collection began directly after the last day of training had been completed. We recorded the identities of all the visitors to the feeder during the training period.

The feeding station was located at 400 m from the hive on the first day of the experiment, and then moved back to 350 m on the second day, and 150 m on the third day. We used the 400 and 350 m distances so that the dancers would make longer waggle runs, which would consequently be easier to analyze. We used the

150 m distance for one day so that the resulting shorter waggle runs would be more like the dances observed by Judd (1995).

Dance following

During the experiment, three trained foragers that reliably danced promptly upon arrival to the hive were allowed to fly freely between the feeding station and the hive. An observer at the feeding station (PKV and undergraduate assistants) captured all other bees that visited the feeder (others familiar with the feeder, and new recruits), recorded their identities, and retained them in a cooler until the end of the experiment so that they could not return to the hive.

At the observation hive, an observer (PMK) recorded the waggle dances that each of the three focal foragers performed, and the bees following the dances, using a digital video camera. Other bees that had visited the feeder during the training period were considered non-naïve and therefore were not included in any data collection when we found them following dances in the video footage.

Video analysis

The list of bees that had arrived at the feeder was used to locate those bees following dances in the video footage. Because many of the bees that followed dances were not recruited to the feeding station, only those that did arrive at the station were considered in this study. We considered these to have had a successful dance following experience. We defined a follower as any bee facing a dancer and within antennal contact and counted the number of waggle runs that each follower attended. For each waggle run, the position that the follower was in relevant to the dancer was recorded. There were three possible positions (Figure 2.1) available to the followers, corresponding to the front of the dancer (F), the side of the dancer (S), and the rear of the dancer (R). During the original video analysis, each of these positions was split into three smaller positions, each of a size to be occupied by a single follower bee. This allowed for greater precision during the video analysis, but to make our statistical analyses more robust we then pooled the values for the smaller positions into the larger zones pictured in Figure 2.1.

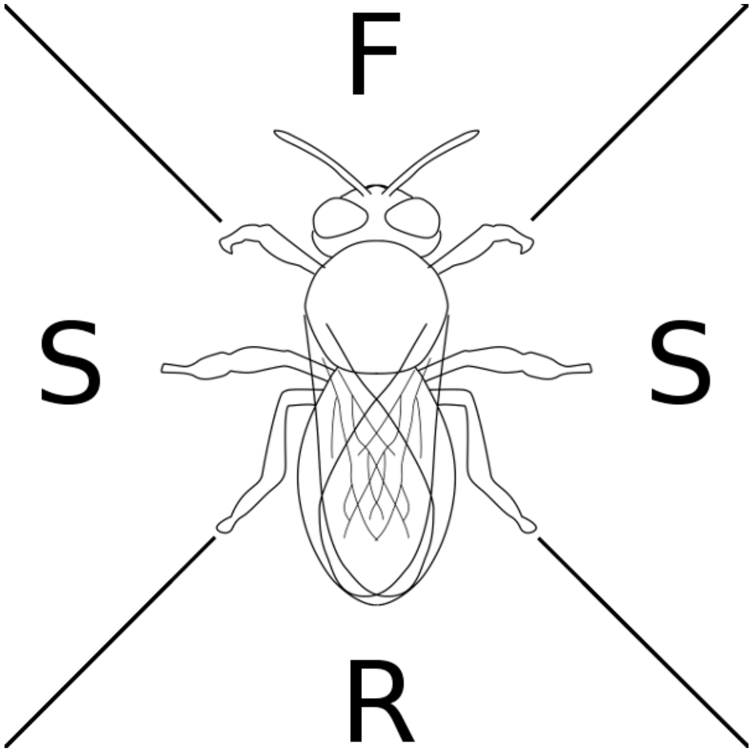

Figure 2.1: The possible positions available to a bee following a waggle dancer (pictured at center). F=the front of the dancer; S=the side of the dancer; R=the rear of the dancer.

Analysis

We first analyzed the data to determine the relationship between the following position used and the number of waggle runs followed by each bee. Because the rear position was of particular interest, the likelihood that a bee following exclusively from a single position would use the rear position as compared to the other possible positions was also addressed. Additionally, in the event that the final following experience was of key importance to successfully arriving at the feeding station, the final waggle run followed by each follower was examined and the position used by each bee was recorded. Finally, the most efficient followers, or those that only needed to follow 10 or fewer waggle runs before arriving at the feeding station were analyzed to determine whether they had a position preference.

Results

On Day 1, 47 bees arrived at the feeder and we were able to identify 16 of them following dances in the resulting video footage. On Day 2, 34 bees arrived at the feeder and 22 were in the footage. Day 3 had the lowest number of visitors to the feeder, 29, and 17 of them were also in the footage. The number of waggle runs followed by each bee varied widely, ranging from as few as a single run to as many as 131. To address this difference, in a later portion of this analysis we assessed the following experiences of the most efficient bees (those that had followed fewer than 10 waggle runs).

Figure 2.2 summarizes the total number of waggle runs followed in each position per day of the experiment. We used a generalized linear mixed model to assess the effect of position on the number of waggle runs followed. Because there was no effect of the day on the outcome of the model (p-value=0.0755), the results from each of the three days were combined into the same model. The number of waggle runs followed from the rear position was significantly different from the number of runs followed from the front position (p-value$<2\times10^{-16}$). The number of waggle runs followed from the front was also significantly different from the number of dances followed from the side (p-value=3.86×10^{-12}). A post-hoc Tukey test assessed the differences among all pairwise combinations of positions and found significant differences between each. These data are summarized in Table 2.1.

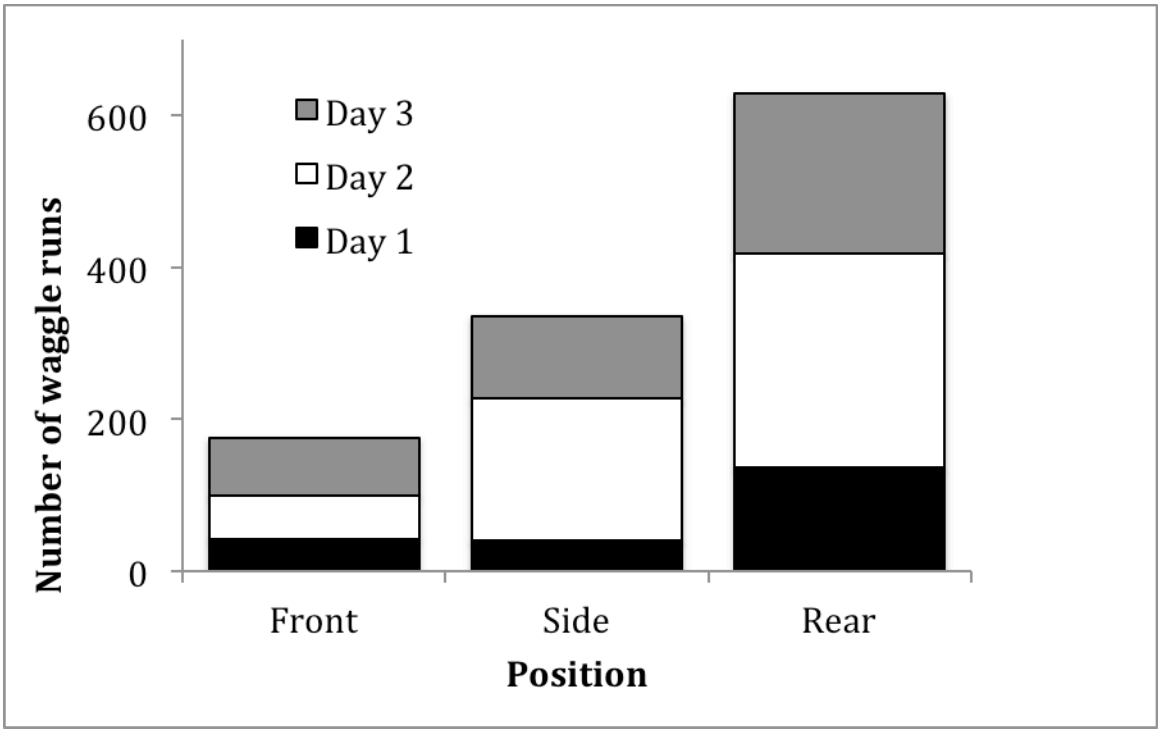

Figure 2.2: The total number of waggle runs followed in each position per day. Each bar pools all of the runs followed in that position for all the days of the experiment.

Pair	SE	z	p-value
R-F	0.08536	14.921	$<1 \times 10^{-10}$
S-F	0.09314	6.942	$<1 \times 10^{-10}$
S-R	0.06764	-9.270	$<1 \times 10^{-10}$

Table 2.1: Summary of the differences among all pairwise comparisons of the positions using a post-hoc Tukey test.

Of the 55 bees that were observed following dances, 47 followed from at least two of the possible positions. For the question of whether particular positions are more effective for information transfer, recruits that had followed from just a single position are the most informative. To evaluate Judd's assertion that following from the rear was essential in order for followers to successfully interpret the dance information, we performed a Chi-square test on these 8 individuals (Figure 2.3).

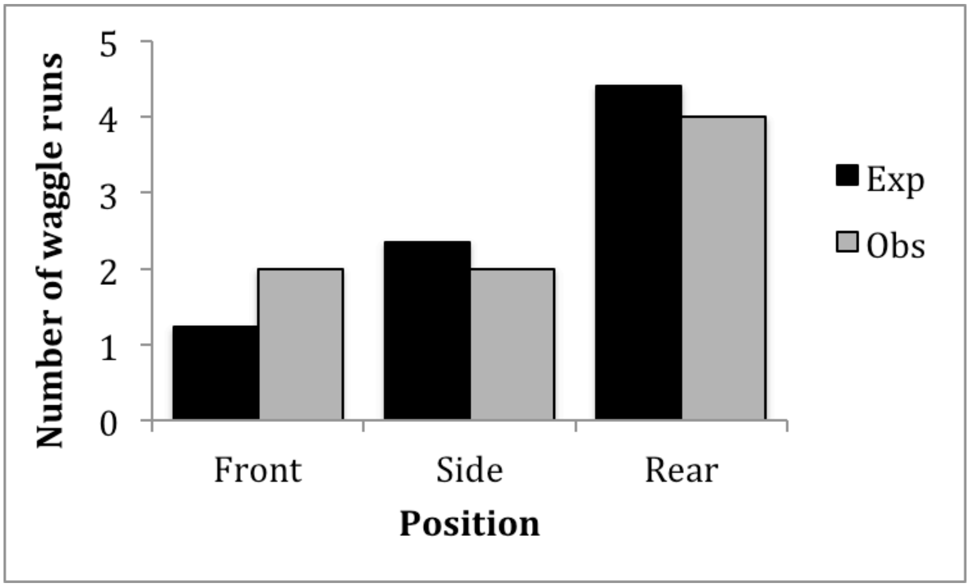

Figure 2.3: The observed and expected number of waggle runs followed in each of the possible positions by bees that followed from a single position.

To determine whether a follower's final position prior to departing the hive was of particular importance to her dance following experience, we recorded the positions each of the successful followers used during the last waggle runs they watched (Figure 2.4). We then compared the number of waggle runs followed in each position using ANOVA. There were no significant differences found among the positions (p-value=0.3496, F=1.2586).

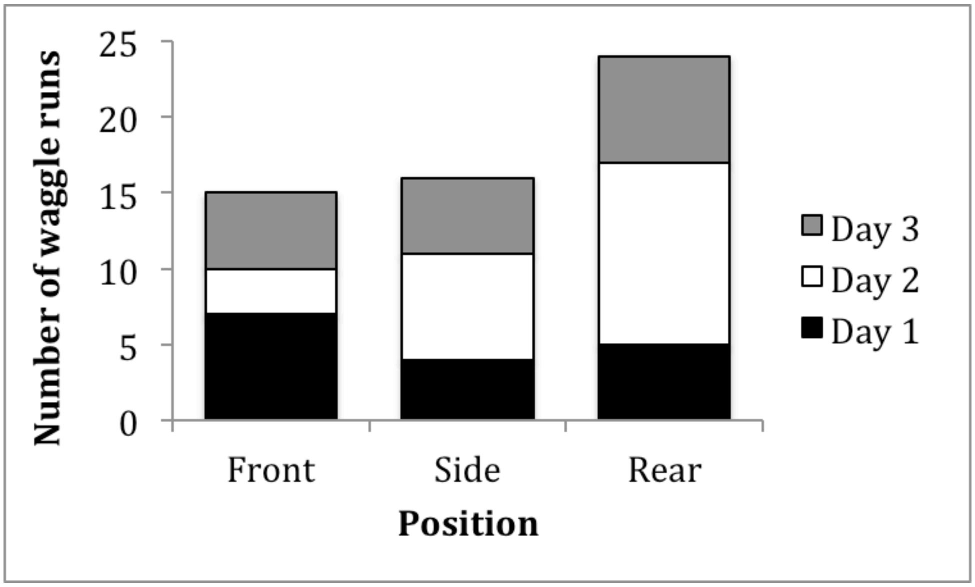

Figure 2.4: The final position followed by each of the successful followers that arrived at the feeding station. Each of the bars pools all of the runs followed in that position for all of the days of the experiment.

There was a very wide range in the number of waggle runs followed by each bee. Of key interest were the individuals that were highly efficient in locating the feeding station. This efficiency was measured in the number of waggle runs observed before departing for the feeding station. We considered individuals that followed 10 or fewer waggle runs to be the most efficient, and analyzed their following experiences. Because Day 1 of the experiment had the most efficient recruits of any of the days, we used the efficient followers from that day for this analysis. A summary of the waggle runs followed in each position by each of the efficient bees from Day 1 is found in Table 2.2. We compared the number of runs followed per position for the bees using ANOVA. There were no significant differences among the positions (p-value=0.07822, F=2.839).

Bee	F	S	R
1	0	3	0
2	2	1	6
3	2	5	1
4	0	1	5
5	0	0	1
6	2	0	2
7	0	3	6
8	1	0	0
9	1	0	6

Table 2.2: The waggle runs followed in each position by the most efficient followers of Day 1.

Discussion

Far more bees arrived at the feeder than were observed following dances in the video footage, particularly on Day 1. A likely reason for this was that it was often difficult to discern the numbers on the bees' identification tags in the video, and thus, those bees would not end up being counted. Also, there was no way to control for bees that followed dances during the training period but did not actually land on and feed from the feeder. Such bees would not have been completely naïve with regards to the feeder location but not identified. Regardless, the 55 bees that were possible to analyze in the video provided an adequate sample size from which to draw conclusions.

Although a significantly greater number of individual waggle runs were followed from the rear of the dancer, it should be noted that the overwhelming majority of the followers used at least two positions before departing for the feeding station. Furthermore, of the few bees that followed from a single position, there was no greater likelihood that the rear position would be the position used. If one considers the number of dances followed to equal the number of dances needed to receive the information, it could be concluded that following from the rear was actually less efficient than the other positions because bees that followed from this position followed for many more waggle runs (Figure 2.2).

Next, the analysis of the final waggle run observed before arriving at the feeding station showed that any of the positions could be used. The bees were not specifically following from the rear, rather, it seemed that the positions were selected at random based on what part of the dancer they could conveniently access.

The final component of the analysis involved only the most efficient followers. It is conceivable that if a specific following position was required to receive the dance information it would clearly be used with the greatest frequency by the bees that only needed to follow a small number of waggle runs before locating the feeding station. The results of this analysis showed, however, that there was no single position that was used significantly more times by the most efficient bees.

In his 1995 paper, Judd described a type of “lunging” movement that he believed to be of particular importance to the dance following experience. Throughout the video analysis we carefully studied this movement whenever it was present, and it seems best described as simply a maneuver performed by bees following from the side of the dancer to help them get into position as she turns for the return phase of the dance. Because the dancer moves forward rapidly during the waggle run and then turns quickly to begin the return phase, bees attempting to remain in contact with her must dart forward or be left behind.

A consideration of the dynamics of waggle dancing and following is important here. In a well-populated colony of bees there is little available space around a dancer. Interested followers, therefore, must crowd in against the dancer wherever space allows. As the dancer moves forward during a waggle run an empty space is created in her wake, and can readily be occupied by followers much more conveniently than the areas next to or ahead of her. We propose that this is the reason for the greater number of dances followed from the rear, and not some need that the followers have to position themselves at the rear in order to successfully acquire the dance information.

In conclusion, these results do not support the earlier assertion that dance followers must be positioned to the rear of a dancer in order to receive the dance information. When considering the crowded conditions of a beehive, where followers must cram themselves in next to a dancer wherever it is possible, it is much more likely that the position itself should not matter, and rather that the factor of greatest importance to followers should be physical contact with the dancer.

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CHAPTER 3

STOP SIGNALING BY FORAGERS NOT ENCOUNTERING DANGER AT A FOOD SOURCE

Introduction

Honey bees (*Apis mellifera* L.) regulate their colony's foraging through a repertoire of signals. The best known of these is the waggle dance, a key discovery of animal behavior, described by von Frisch (1967), which is used to communicate the distance and direction to food and other resources. The waggle dance is, however, part of a suite of other signals involved in recruitment (see Kietzman and Visscher 2015).

One such signal is the stop signal, a brief acoustic signal that was originally interpreted as a begging call (Esch 1964) and involves a worker bee butting her head against another individual and briefly pulsing her wings to emit a brief chirping sound. It has since been reinterpreted as a negative feedback signal, because it has been shown to cause worker bees to stop waggle dancing significantly more often than could be ascribed to chance alone (Kirchner 1993; Nieh 1993; Pastor and Seeley 1995). By employing the stop signal, bees that have had a negative experience at a food source can decrease waggle dances advertising that location and consequently inhibit foraging (Lau and Nieh 2010; Nieh 2010).

This note reports unexpected results found during an experiment employing a method similar to that used by Nieh (2010) of pinching foragers at a feeding station to simulate a predator attack.

Methods and Results

We conducted this experiment in January 2013 at the University of California, Riverside's Agricultural Operations, a research farm located directly southwest of main campus. We established a two-frame observation hive on 15 January and started our four-day training period on 17 January. We trained the bees to visit a feeding station containing a 2M solution of sucrose lightly scented with anise by first placing it directly outside the entrance to their hive and then gradually moving it farther away as foragers began visiting it consistently. Its final distance from the hive was 100 m. Data collection commenced on 21 January and lasted two days. Conditions were sunny and breezy with temperatures from 19-24°C, with the wind picking up more on the second day than the first. Data on the first day was collected from 9:30 to approximately 16:30, and 10:30 to 14:30 on the second day.

Bees in the control group were gently caught in a small insect net by an observer at the feeding station (JKL on Day 1, PKV on Day 2), marked on the thorax with a Sharpie© paint pen, and released. Those in the treatment group were additionally squeezed with forceps on the rear tarsus to simulate a predator attack and marked with a different color of paint. Treatments were randomized in pairs to avoid temporal bias and to ensure equal numbers (64 each) in the treatment and control groups. It is possible that some bees visited the feeder and left before they could be marked; however, much care was taken to ensure that this number was as small as possible. Marked bees that returned to the feeder were treated correspondingly with their color, i.e., bees that had previously been pinched were pinched again and bees that had not been pinched were left untouched.

Dances by marked bees were video-recorded in HD and the footage was later analyzed to record the duration of dances and the occurrence of stop signals performed by dance followers. We recorded 173 dances by marked bees and 317 stop signals by both marked and unmarked bees. There were 140 waggle dances by bees from the control (not pinched) group and 33 dances by bees from the treatment (pinched) group. We also observed stop signals performed by unmarked bees. A summary of the dances and stop signals performed by each group of bees is found in Table 3.1.

Overall, significantly fewer pinched bees than unpinched marked bees performed dances (Z test: $Z = 11.5047$, p-value < 0.0001). Furthermore, the mean dance length was significantly longer among bees that were not pinched, (Z-test: $Z < -1000$, p-value < 0.0001). A significantly higher proportion of dances were done by unpinched bees on Day 1 (2-proportion Z test: $Z = -3.0977$, p-value $= 0.002$), but there was no significant difference between days in the proportion of stop signals performed by the two groups (2-proportion Z test: $Z = -0.8323$, p-value $= 0.4052$). We found no significant difference in the number of stop signals performed on bees of either treatment group (Z Test: $Z = 1.1992$, p-value $= 0.2305$).

On Day 1, many more bees in the control group performed waggle dances than those in the treatment group (Z test: Z = -9.4391, p-value << 0.0001). On Day 2, bees in the treatment group did not perform any recorded waggle dances. This may have resulted from a difference in the researcher performing the treatment, the smaller data set because of the shorter collection time, the windier weather, or other unknown factors.

The bees in the treatment group (pinched) produced 21% of the total number of stop signals recorded, whereas bees in the control group (not pinched) produced only 8%, and this was a significant difference in proportions (2-proportion Z test: Z = -4.149, p-value< 0.0001). However, about 70% of the stop signals observed in this experiment were performed by unmarked bees. These bees probably did not visit our feeder (very few may have, but not been marked). When compared to the percentage of stop signals produced by bees in the study, there were significantly more stop signals performed by bees that were not part of either experimental group (2-proportion Z test: Z = -8.6162, p-value << 0.0001).

This analysis assumed independence of events. But, since the bees were not uniquely marked, the same individual may have performed either multiple dances or stop signals. Furthermore, some bees visited the feeder multiple times. However, the differences were sufficiently strong that we do not think lack of independence accounts for the observed effects. There was some evidence of differences between the two days in the proportion of dances performed by either treatment group, possibly due to handling differences between the two observers at the feeder.

Treatment	**Waggle Dances**	**Mean Dance Duration m:ss**	**Stop Signals**
Not Pinched (control)	140	2:35	26
Pinched	33	0:23	68
Unmarked (no feeder visit)	--	--	223
Total	173	--	317

Table 3.1: The number of waggle dances and stop signals performed by 3 groups of bees. Only waggle dances of marked bees were recorded.

Discussion

Our observations on stop signaling confirmed the results of Nieh (2010): a statistically significant higher number of pinched bees used the stop signal upon their arrival to the colony than bees that had not been pinched. However, we found that about 70% of stop-signaling bees were unmarked and therefore were unlikely to have visited the feeder.

Several explanations exist for the large number of stop signals produced by unmarked bees. One is that the unmarked bees may have been foraging at some other location and were not getting unloaded promptly due to the influx of food from the feeding station. It is common for stop signaling to increase when a feeding station is present (Nieh 1993; Thom et al. 2003; Pastor and Seeley 2005), and most of the stop signaling comes from tremble dancers (Thom *et al*. 2003). Tremble dancing occurs when foragers are not met quickly by unloader bees that receive food from incoming foragers and store it elsewhere in the hive (Seeley 1992). It follows that if the unloader bees were overwhelmed by the amount of food brought in by bees visiting the feeding station they would not have been available to unload bees that had foraged at other locations. Alternatively, the stop signaling could also have been coming from unloader bees themselves in an attempt to inhibit foraging due to an unmanageable number of foragers.

A further explanation pertains to the idea that having unfettered access to a rich food source such as a feeding station is more similar to a honey-robbing situation than to foraging on natural food sources such as flowers, which offer a small nectar reward and are located across a patchy landscape. Johnson and Nieh (2010) used a model to show how the stop signal might be used to rapidly shut down a robbing event by countering the waggle dance. The ability to quickly stop a robbing event would be important if the colony being robbed was very strong and many robbers were being killed by guard bees (Johnson and Nieh 2010). To date, an experimental assessment of this model has not yet been conducted; however, it is possible that the bees interpreted the presence of the feeding station as an unfavorable robbing situation and started using the stop signal accordingly.

Lau and Nieh (2010) conjectured that low levels of stop signaling exist in the colony at all times, and that the stop signal does not have a colony-wide effect until some threshold frequency is reached. After the threshold level, which is currently unknown, is reached, the stop signal would have a measurable effect shown by the decline of visitors to a food source such as the feeding station used in this experiment. The stop signal is a versatile communication tool and it is clear that all of its uses and meanings may not yet have been described.

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CHAPTER 4

DO HONEY BEES SIGNAL WHEN THEY ARE OUT OF STORAGE SPACE?

Abstract

Numerous activities within honey bee (*Apis mellifera* L.) colonies rely on feedback loops for organization at the group level. Many of the processes associated with the feedback loops organizing a honey bee colony's activities are in striking parallel to other systems, such as intercellular interactions involved in motor neuron function. This study looked at the signals honey bees employ while provisioning their nest with food and assessed whether or not the bees employ signals related to whether their hive has no more available storage space. The storage space was alternated each day between no available space and ample space. The communication signals used by the bees were counted during each treatment and compared. When the hive was full, significantly more stop signals, which inhibit foraging, and tremble dances, which recruit more bees to unload incoming foragers, were observed. This suggests that the bees had assessed the absence of storage space and modified their communication accordingly.

Introduction

Honey bees employ a variety of signals to communicate with their nestmates while provisioning their nest with food. The well-known waggle dance communicates the distance and direction of a food source and recruits foragers to that site (von Frisch 1967). Unloader bees take food from incoming foragers and store it in open cells of the wax combs in the colony. If foragers are not met quickly by unloader bees, they transition to tremble dancing, which apparently recruits more individuals to be unloaders (Seeley 1992).

The stop signal is an acoustic communication signal produced when a honey bee presses her thorax to the wax comb and pulses her wings briefly (Michelsen *et al.* 1986) or butts her head into the side of another bee and pulses her wings (Nieh 1993). It has been measured to average 407 Hz and last an average of 147 ms (Schlegel *et al.* 2012). The stop signal is best understood as a negative feedback signal that shuts down waggle dancing more rapidly and as a result inhibits foraging (reviewed in Kietzman and Visscher 2015). In foraging it has been observed in use when an influx in food to the colony renders unloader bees unavailable to many incoming foragers, though it is still unclear if the unavailability of unloaders elicited the stop signal in this context (Thom *et al.* 2003). It is also used to alert nestmates to peril or overcrowding at a food source (Nieh 2010; Lau and Nieh 2010).

The use of signals such as the waggle dance and stop signal are of key importance to bees as they forage for food because this is a cooperative act that involves thousands of individuals, each having only a small amount of information about the colony's nutritional needs and the locations of profitable food sources. By using communication signals individuals can influence other bees' decisions to forage or cease foraging, which over time can change the colony's behavior as a whole.

It would be advantageous to the bees if they were able to detect whether or not their hive has enough storage space available for incoming food, and if they could shut down foraging if space were not available. The aim of this study was to investigate the bees' response to changing amounts of storage space by assessing their levels of negative feedback signaling under different conditions. We measured their response by counting the number of stop signals, tremble dances, and waggle dances observed when their colony had no storage space available and compared it to when the colony was empty with plenty of storage space available.

Methods

General Methods

This experiment was conducted from February 2014 through May 2014. It was replicated three times using a different colony of approximately 2,000 honey bees each time. Each queen-right colony was installed in a two-frame observation hive several days in advance of the start of data collection. The hive was housed in a shed at UC Riverside's research farm, Agricultural Operations.

The bees had access to the outdoors via a plastic tube and they were allowed to forage freely on naturally available food. A wooden shunt at the entrance of the hive directed incoming bees to a single side of the lower frame so that a single observer was able to see almost all of the of the waggle dances occurring. On the side viewed, a wire mesh window screen covered the lower frame of the hive so that stop signals could be heard. The lower frame of the hive was the brood nest, and contained a mix of eggs, developing larvae, capped brood, and some pollen and honey stores. Data collection occurred from 7-12 February 2014 (Colony 1), 7-12 March 2014 (Colony 2), and 2-7 May 2014 (Colony 3).

Treatments

Two treatment frames were used. The frame for the Full treatment was completely filled with stored honey and had no empty cells available for further food storage. Approximately 90% of the Full treatment frame contained honey that had been previously stored and capped by bees. The remaining empty cells in the comb were filled with honey by hand prior to the frame's placement in the hive. The frame for the Empty treatment had fully drawn wax comb but contained little or no stored food and therefore had ample space available for food storage.

For the duration of the experiment, the top frame in the hive was changed between the Empty and Full treatment frames each day. Each colony began with the Empty treatment the first day and the frames were exchanged each evening after data collection was completed. Observations were made for six days for each colony for a total of three days of each treatment per colony. A diagram of the experimental setup is found in Figure 4.1.

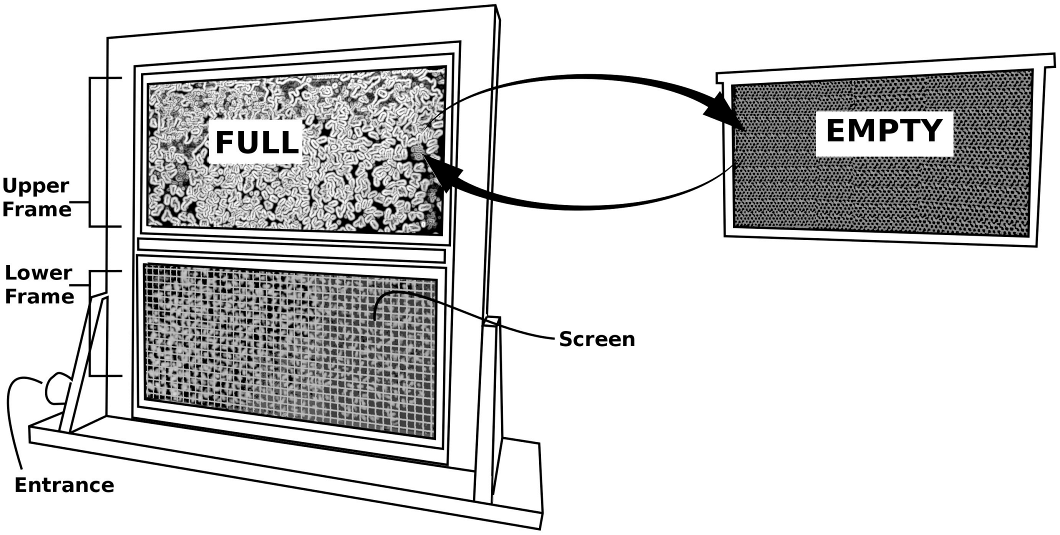


Figure 4.1: experimental setup showing two frame observation hive and the location of the treatment frames.

Data Collection

Observations were made every half hour from 9:00-11:30 and 12:30-16:00 for a total of twelve observations per day. During each observation, waggle dances, tremble dances, and stop signals were counted for five minutes. The number of bees entering and exiting the colony was also counted for five minutes to compare the bees' activity levels from day to day.

Waggle dances and tremble dances were observed by visual inspection of the bees on the side of the lower frame that was covered by a screen. Stop signals were detected using a lapel microphone on a holder stick, as described by Schlegel *et al*. (2012), swept slowly across the front of the lower frame.

Statistical analysis

The data were analyzed using RStudio version 0.98.501 for Macintosh OS X 10.7.5. The number of stop signals, waggle dances, and tremble dances per treatment and time of day were checked for normality of distribution using the Shapiro-Wilkes test. The Kruskal-Wallace rank sum test was used to assess differences in the numbers of signals used during each treatment.

Results

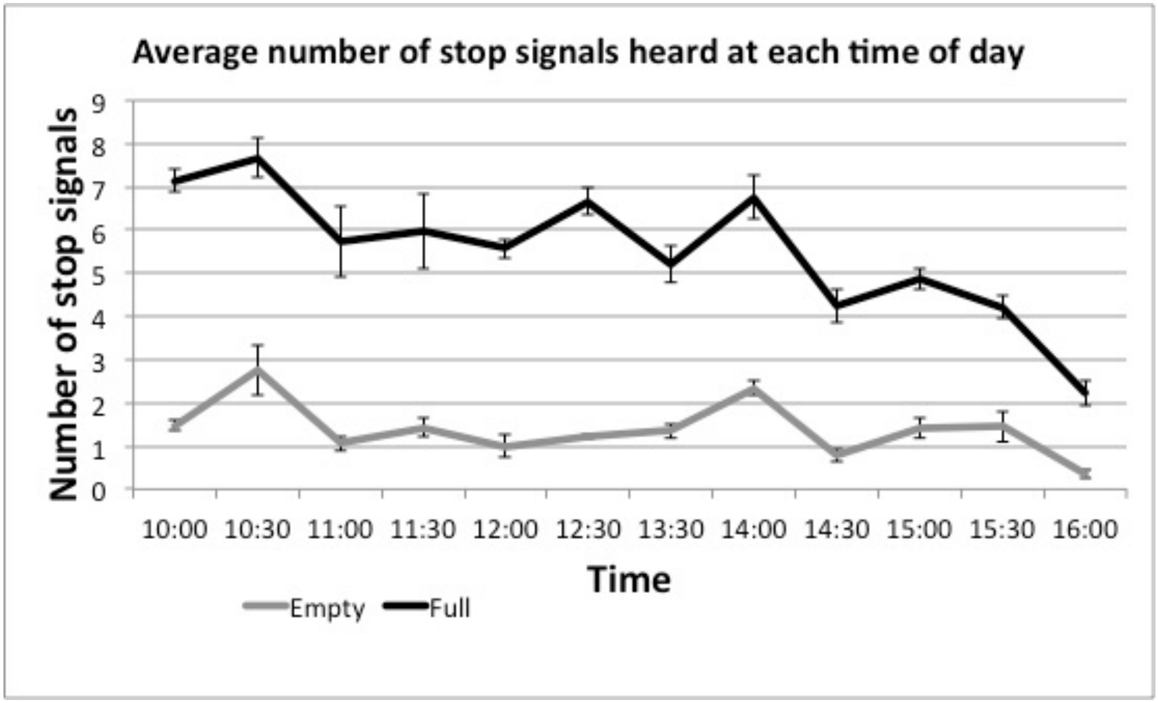
None of the data were distributed normally ($p<0.001$ for all signals and treatments).

Table 4.1 shows the total number of stop signals, waggle dances, and tremble dances observed during each treatment for all three colonies. Significantly more stop signals were heard during the Full treatment than during the Empty treatment for all three colonies ($p <0.001$, Kruskal-Wallace rank sum test, for all colonies). Significantly more tremble dances were also observed during the Full treatment than during the Empty treatment for all three colonies ($p <0.001$, Kruskal-Wallace rank sum test, for all colonies). There was no consistent treatment effect on waggle dances among the three colonies.

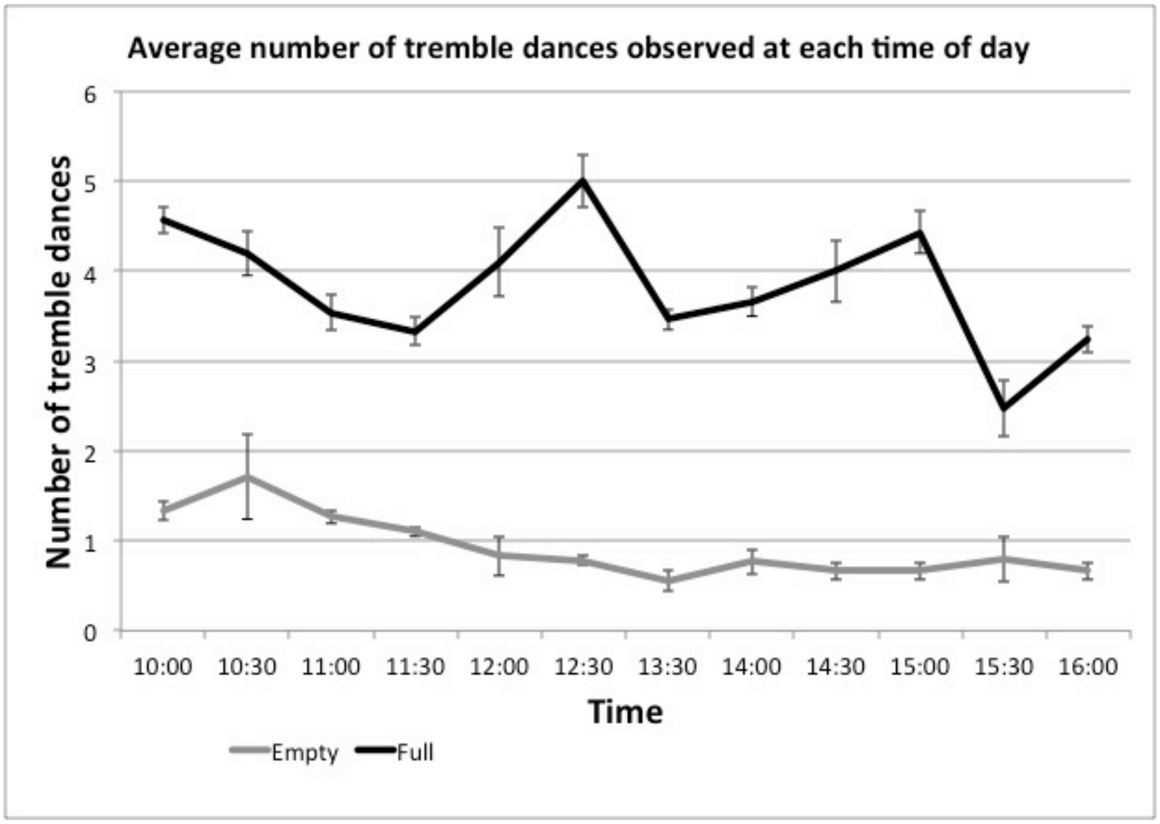
Colony	Treatment	Stop Signals	X^2	p-value	Waggle Dances	X^2	p-value	Tremble Dances	X^2	p-value
1	Full	117	22.2	<0.001	43	2.4	0.12	153	35.6	<0.001
	Empty	39			62			27		
2	Full	140	27.3	<0.001	19	6.1	0.013	125	40.1	<0.001
	Empty	44			55			33		
3	Full	190	37.3	<0.001	88	0.33	0.56	136	41.3	<0.001
	Empty	59			99			32		

Table 4.1: summary of the total number of stop signals, waggle dances, and tremble dances observed during the Full and Empty treatments for each colony. The results of the Kruskal-Wallis rank sum test are also reported for each colony and communication signal.

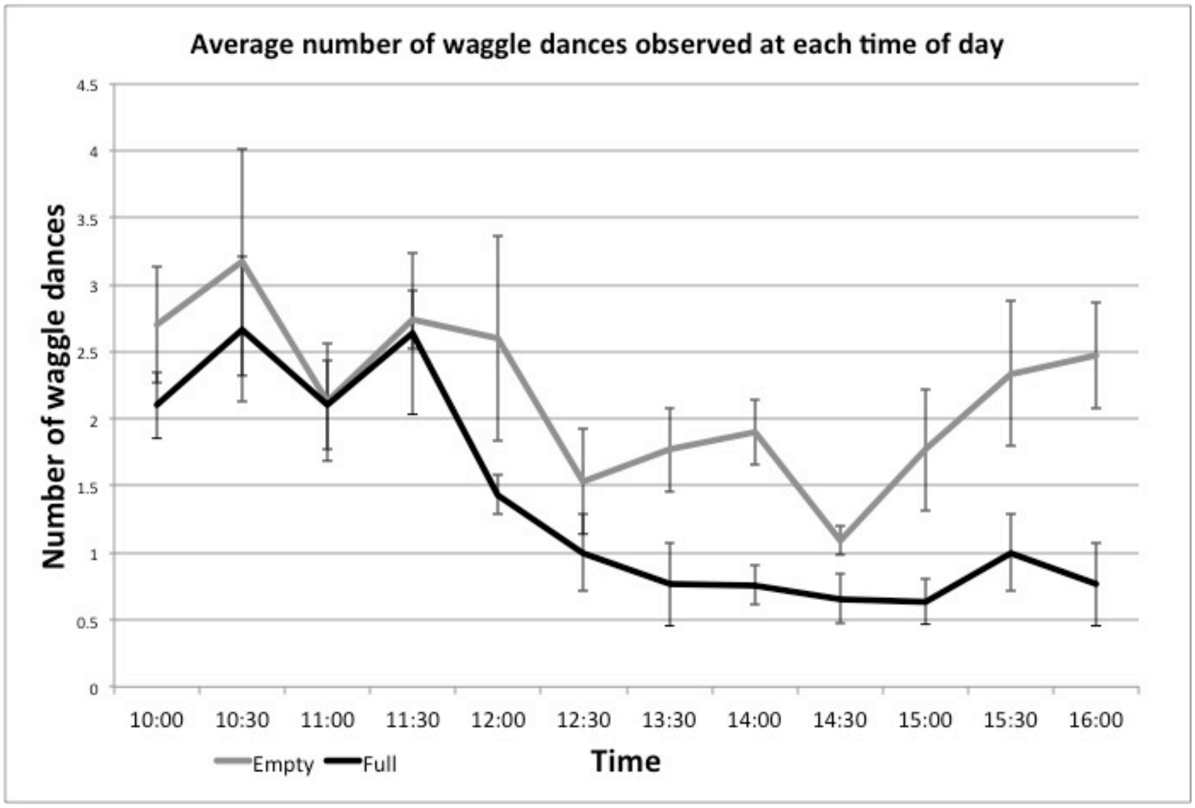
The clear differences between the number of stop signals and tremble dances observed between the treatments are shown in Figure 4.2 (a and b), which display the mean number of signals counted at each five minute observational period for all three colonies. The number of waggle dances differed more slightly between treatments (Figure 4.2 c).


Average number of stop signals heard at each time of day
Number of stop signals
0
1
2
3
4
5
6
7
8
9
10:00
10:30
11:00
11:30
12:00
12:30
13:30
14:00
14:30
15:00
15:30
16:00
Time
Empty
Full

(a)


Average number of tremble dances observed at each time of day
Number of tremble dances
6
5
4
3
2
1
0
10:00
10:30
11:00
11:30
12:00
12:30
13:30
14:00
14:30
15:00
15:30
16:00
Time
Empty
Full

(b)



(c)

Figure 4.2: The number of (a) stop signals, (b) tremble dances, and (c) waggle dances observed at each five minute observational period, averaged over all three colonies.

Discussion

The bees consistently used more stop signals and tremble dances during the Full treatment than during the Empty treatment, which suggests that unloader bees had experienced the lack of space, and, being unable to store nectar they had taken from foragers, were not able to unload further incoming foragers, and subsequently used the stop signal to inhibit further foraging. Alternatively, the foragers may have produced the stop signals because they were unable to be unloaded. We were not able to identify which individuals were producing the stop signals during this experiment; thus, it is not possible to know if they were primarily unloader bees or foragers.

Thom *et al.* (2003) suggested that stop signaling might modulate the recruitment of more unloader bees by lowering their response thresholds to the tremble dance. The increase in tremble dancing during the Full treatment may therefore have increased the colonies' nectar handling capacities and account for the similarities in the amount of waggle dancing between the treatments.

The communication signals employed by social insects such as honey bees are of increasing interest to scientists due to the robust decision-making processes they enable. In a honey bee colony, each individual worker operates using a simple repertoire of behaviors, however, as a group the bees can efficiently execute complex tasks such as regulating foraging or selecting a new nest site (reviewed in Visscher 2007). These processes are strikingly similar to the decision-making mechanisms of vertebrate brains (Passino *et al.* 2008; Marshall *et al.* 2009), and can also provide models for computing and robotics systems (e.g. Bonabeau and Meyer 2001; Tsuda *et al.* 2006). For example, there is a need for groups of robots that can work together to complete complex operations based on simple algorithms used by individuals in the group. Such algorithms can be developed from principles derived from social insect behavior, of which honey bee decision-making is of particular interest.

The results of this study present a new example of self-organizing behaviors within a social insect system. Each individual bee may play a single role, however, as a group they succeed in the complex task of regulating their colony's food intake based on information available to a small percentage of individuals.

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