12-2014

Relationship between Relative Hive Entrance Position and Dance Floor Location

Chelsea E. Corrigan
East Tennessee State University

Recommended Citation

Corrigan, Chelsea E., "Relationship between Relative Hive Entrance Position and Dance Floor Location" (2014). *Undergraduate Honors Theses.* Paper 474. https://dc.etsu.edu/honors/474

Introduction

The symbolic dance language employed by honey bee foragers to advertise profitable food sources conveys information about source distance and direction to other foragers inside the hive in order to recruit others to exploit the same source. To direct others to a source with the dance, a forager performs repeated waggling runs by moving in a straight direction on the comb while moving her abdomen from side to side. The orientation of a bee's body on the comb informs other bees of the direction of the food source from the hive and the duration of the waggle run conveys the distance of the food source. Following a waggle run, the dancer circles around (returning near the location she started the previous waggle run) and begins another. Waggle runs punctuated by a forager circling around to realign herself on the comb resembles a figure eight pattern. The dancer is surrounded by other bees that face toward the dancer and follow her movements. The dance followers observe several waggling runs and leave to exploit the source if recruited. This behavior is astonishingly sophisticated, well studied and well described (von Frisch, 1965; Boch, 1956; Dyer, 2002). This exchange of information, however, requires more individuals than dancing foragers alone. Unemployed foragers inside the hive participate as dance followers and receive information conferred by dancing bees. Also present on the dance floor, food receiver bees accept and relocate quantities of nectar and water provided by returning foragers. A congregation of dancing foragers, dance followers and food receiver bees result in the establishment of a specific, localized site of information and resource exchange known as the dance floor (von Frisch, 1967). Although the waggle dance is well elucidated, many aspects of the dance floor remain a mystery; specifically the criteria used by forager bees to establish and recognize this site.

The dance floor is typically reported to be within close proximity of the hive entrance. Von Frisch (1967) described the dance floor in glass-sided observation hives as a small, localized area of approximately 100 cm^2 located on the comb adjacent to the hive entrance. He reported observing bees dancing on comb near the hive entrance in commercial box hives as well. Seeley and Towne (1992) observed that 94% of recruitment dances in an observation hive were performed within 24 cm of the hive entrance, with the majority closer still, within 4–18 cm of the entrance. They suggested that congregating near the hive entrance reduced time and travel inside the hive for returning foragers by enabling them to enter the hive, offload or deposit resources and potentially elect to dance without having to seek out the dance floor in another part of the hive.

The hive entrance itself serves as a junction between the honey bee nest and the outside world. Seeley and Morse (1976) closely examined honey bee nest structure by dissecting nests of wild colonies. They found a nonrandom predominance of entrances to the nest cavities located at the bottom (as opposed to the middle or top). They suggested two plausible explanations. First, bees might actively select cavities with openings at the bottom; this presumably could accommodate removal of debris with greater ease. Second, bottom entrances may be common because tree cavities often result from fungal decay which usually expands upward from its entry point into a tree. Advantageously, it has been shown that a cavity with an opening at the bottom limits convectional heat loss compared to nests with entrances at the top (Budel, 1960).

Tautz and Lindauer (1997) examined the consistency of dance floor location within an observation hive. In a two-frame observation hive, they reversed the locations of the frames multiple times throughout days, removing the bees present on the frames prior to the manipulation. They found that during the day foragers sought out the frame previously supporting the dance floor, and did not simply re-establish a dance floor near the hive entrance. They anecdotally reported that foragers slowly searched the hive until they found the same area previously used for dancing and resumed dancing on that portion of comb despite its distance from the hive entrance. Regardless of where the dance floor was located at the end of the day, the dance floor was re-established near the hive entrance on the subsequent day. It was suggested that the dance floor is marked with a scent that foragers use to locate the dance floor within a single day, but that the scent dissipates overnight. This hypothesis is consistent with the finding that honey bees performing the waggle dance do, indeed, release chemical compounds (two alkenes and two alkanes) that non-dancing bees only produce in minute quantities (Thom et al, 2007).

Other factors, aside from hive entrance location, that could influence where a bee elects to dance could be light and the comb substrate. Light is introduced to the cavity through the hive entrance. Tautz and Rohrseitz (1998) found that artificially illuminated dances are more successful at recruiting dance followers than non-illuminated dances. The substrate on which the bees dance is highly variable, ever changing and potentially influential. The substrate varies by the contents of individual cells. Cells of comb can remain empty or they can be occupied by small eggs or larvae or by pollen or nectar. Also, cells may be capped over to encase brood or honey. These attributes of honeycomb substrate are transitory; they also affect the transmission of vibrations. Tautz (1996) showed that dances performed on open cells recruited three times as many followers as dances performed on capped brood cells, though a slight preference for capped brood was acknowledged. The waggle dance itself produces vibrations between 200–300 Hz. It is proposed that this vibration is used by potential dance followers to locate a forager advertising a source (Nieh and Tautz, 2000; Tautz et al., 2001).Vibrations produced by the waggle dance were seen to be transmitted further through open comb than through capped brood cells (Sandeman et al., 1996). Although bees may show preferences for certain substrates and substrate types may influence the efficiency of recruitment, it has not been determined if bees use substrate as a principal criterion to establish the dance floor. Furthermore, a wax substrate (empty or replete) is not a necessity for information transfer; honey bees perform waggle dances on top of other bees when temporarily congregated during a swarm to advertise and elect a new nest site (Seeley and Buhrman, 1999).

A fascinating aspect of honey bee foragers is that, although they act as sensory units of the colony as a whole (Seeley, 1994), they are also individual agents with their own predilections. It is important to consider that the dance floor is composed of many individuals and this could contribute to idiosyncrasies within one colony as well as among colonies. Individual differences, for example, are exemplified by sensory-physiological variation in the perception of sucrose among bees. Such sucrose response thresholds predispose a bee to collect water, pollen or nectar (Pankiw and Page, 2000). Individual bees also modulate their dancing behavior: an abundance of food sources results in a higher threshold for dancing where a lower dance threshold occur when forage is scarce (Dyer, 2002, Lindauer 1948; Seeley 1986). Foragers also modulate dancing behavior by performing more waggle runs for longer durations when advertising food sources perceived as highly profitable (Boch, 1957; Dryer, 2002; Farina, 1996; Seeley et al, 2000; von Frisch, 1967). Another example of individual differences within the population of workers is experience of an individual in the field and the influence of that experience on subsequent behavior. Moore and Doherty (2009) found that an individual’s time-accuracy of arrival at a food source increases with more rewards at an artificial feeder. Also, an individual with more days of experience at a feeder is more likely to investigate the feeder for several days following the last feeding event.

The location in which an individual elects to dance is certainly a relevant component of the dance floor; therefore, it is important to understand how individuals are choosing locations to dance. This question was addressed by Seeley (1994), by investigating the location of dances performed by individual bees. It was documented that a dancing forager performs dances in multiple areas of the dance floor as opposed to advertising in a single location repeatedly. When the bees were trained to two spatially distinct food sources, the recruitment dances for each site were not spatially distinct within the dance floor, suggesting that dance followers are exposed to a variety of dances advertising a variety of sources (Seeley, 1994).

The aim of this study was to investigate the intensity of the documented relationship between the hive entrance location and the location of the dance floor. Specifically, we sought to examine establishment of the dance floor in response to an altered hive entrance location. Do honey bees continue to establish their dance floor in close proximity to the hive entrance despite its relative position in the hive? An experiment was devised in which the location of the hive entrance in an observation hive could be manipulated and the dance floor location recorded. We also sought to assess bees' reaction to a physical inability to dance above the hive entrance, and document how individual bees react to hive entrance manipulation.

Materials and methods

The Hive

Honey bees (*Apis mellifera*) were maintained and observed in an observation hive that was modified so that the entrance could be changed with minimal disturbance to the colony. Five experiments were performed using five unrelated bee colonies. An eight-frame observation hive was adapted to contain three hive entrances resulting in three entrance conditions: bottom, middle and top (Figure 1a). A single entrance was accessible during a three-day observation period; the other two were obstructed with wooden plugs. To guarantee that nearly all waggle dances were performed on one side of the hive (left), a plastic barrier prevented incoming foragers to access the back (right) side of the frames. Wooden shims were placed between the four vertical right frames (closest to the hive entrances) to further impede bees from crossing between combs.

As the summer season progressed, ambient available food sources changed as certain sources began to bloom and others ceased. Weather conditions were variable, most notably the presence of rain. This was accounted for by collecting data for three days of favorable weather (i.e., not raining) before performing a manipulation. Furthermore, each colony examined contained a queen that was not related to the others studied; resulting in five genetically different colonies.

Observations: Waggle Scans

A grid consisting of 20 rows and 20 columns of 5cm x 5cm squares was drawn on the glass on the observed side of the hive. To detect changes in dance floor location and shape, the location of individual waggle dances were recorded on data sheets representing the grid drawn on the hive. To conduct these "waggle scans" a scan-sampling technique was employed in which an observer repeatedly scanned the hive row by row in a descending "zig-zag" fashion, starting from the top left corner and ending at the bottom of the hive. Each scan sample lasted 30 minutes. All of the dance locations for a single day were compiled into a single hive map using Adobe Illustrator.

Experimentation- displacing the hive entrance

For this study, five experiments were conducted. For Experiments 1, 2 and 3, waggle scans were performed at 11:00h, 12:00h, 13:00h and 14:00h on each day of the experiment. Unless interrupted by inclement weather, the experiments lasted twelve days. For the first three days, the bottom hive entrance was accessible. During the second three day period, the bottom hive entrance was obstructed with a wooden plug and the middle hive entrance above it was unplugged; observations continued as described. In the third set of three days, the top entrance was opened and the middle entrance was obstructed. Finally, the bottom entrance was re-opened for the last three days of observation.

Experiments 1 (August 2012) and 2 (September/ October 2013) consisted of 12 days of favorable weather. Experiment 3 (June/ July 2014) was performed over 15 days. An additional three days of observation were performed with the bottom hive entrance accessible.

For Experiment 4 (September 2012), the hive entrance displacement was performed again with some variation from the first three experiments. The observation hive was modified to support a half-frame adjacent to the top hive entrance along with a wooden barrier above it (Figure 1b), thus interfering with the bees' tendency to establish the dance floor above the level of the hive entrance. During this experiment rain interrupted observation for five consecutive days during observation of the dance floor under the middle entrance treatment. Observation period under the middle hive entrance treatment was extended to account for this; resulting in six days of observation.

To observe individual foragers' reaction to entrance displacement, individually marked bees were utilized in Experiment 5 (August 2014). To achieve this, a frame of capped brood was taken from a strong colony that occupied a Langstroth hive. It was placed in an incubator at 30^0C. As bees emerged, they were individually color coded with paint dots using Testor's Pl_2 Enamel and Posca pens. The individual codes ranged from three dots to five dots with one to two dots being placed on the thorax and one to three dots being placed on the abdomen. Powdered sugar was then applied to the bees and they were deposited into the Langstroth hive. This colony was later installed into the experimental 8-frame observation hive. Waggle scans were performed as described above. When an individually marked bee was observed dancing, her location and identity were recorded. The manipulation of entrance location was more extreme than in the previous experiments. After it was ascertained that a desirable number of marked bees were confirmed foragers (by being observed performing the waggle dance), the bottom entrance was obstructed and the top entrance was made accessible. Waggle scans continued as described above for four days. Initially, four waggle scans were conducted starting at 11:00h, 12:00h, 13:00 and 14:00h as previously detailed. On Day 12 of the experiment (8/21/14) seven waggle scans were conducted continuously for thirty minutes each from 11:00h- 14:40h.This protocol was adopted for the remainder of the experiment to maximize instances of observing marked foragers advertising their food sources (i.e., waggle dancing)

Results

The dances observed within each day of a single entrance treatment were examined collectively. The bees reacted to hive entrance displacement by establishing the dance floor near the hive entrance, regardless of hive entrance position (Figure 2). Despite differences in time of year, weather conditions, ambient food sources and genetics, the dance floor location was always displaced in response to displacement of the hive entrance. The dance floor location was then maintained on subsequent days until the entrance was again displaced.

One characteristic of the dance floor affected by variation in hive entrance location was its shape. Interestingly, dance floor shape exhibited colony to colony variation even when the hive entrance location was identical. For example, when the entrance was located in the bottom position, prior to any manipulation, the colony observed in Experiment 1 displayed an elongated dance floor extending upwards at an angle (Figure 2a), whereas the colony used in Experiment 2 exhibited a large oval dance floor with bees distributed relatively evenly across the two frames closest to the bottom entrance (Figure 2b). The shape of dance floor observed with the colony in Experiment 3 was oval and localized almost exclusively to the frame nearest the bottom entrance (Figure 2c). The shape of the dance floor was consistent among days of a hive entrance treatment within a single colony.

Upon entrance manipulation, the shape of the dance floor underwent changes in all colonies observed. Consider Figure 2a; dances are no longer observed on the frame adjacent to the bottom entrance after the middle entrance is made accessible. The shape of dance floor observed during the middle entrance treatment is more vertical in contrast with the angled dance floor observed under the bottom entrance treatment. The dance floor recorded during the top entrance treatment is more circular than the shapes documented using the bottom and middle entrance treatments. When the bottom hive entrance was made accessible for the last three days of the experiment, the shape of the dance floor is elongated and oval, resembling the dance floor observed previously under with the bottom entrance treatment. The shape of the dance floor was seen to change following entrance manipulation in all colonies (Figure 2). These new shapes were also colony-specific. Consider the differences in dance floor shape observed during the middle entrance treatment of Experiments 1, 2 and 3 (Figure 2). The bottom entrance treatment was observed at the beginning and the end of each experiment. Comparing the first and last three days of observations reveals that the return to the original entrance location restored the original dance floor shape in two colonies (Figure 2a and 2b) but not in another (Figure 2c).

Immediately following entrance displacement, the dance floor was re-established in the vicinity of the new hive entrance. The process of relocating the dance floor varied among colonies. Some colonies demonstrated an adjustment period following entrance manipulation (Figure 3). An adjustment period was characterized by a dispersed dance floor observed only on the first day following an entrance manipulation. The dance floors observed on subsequent days of an entrance treatment were seen to be more consolidated. An adjustment period was not a consistent finding as detailed in Table 1. The colony used in Experiment 4 displayed an adjustment period after both manipulations performed. The colonies observed in Experiments 2 and 3 only exhibited an adjustment periods after manipulation 1 (in which the bottom entrance was closed and the middle entrance was opened) and manipulation 3 (in which the top entrance was blocked and the bottom entrance was re-opened). The two colonies observed in Experiment 1 and 5 never displayed an adjustment period.

A consistent finding among colonies was a tendency to dance above the hive entrance level (Table 2). If an imaginary horizontal line was collinear to the floor of the hive entrance, over half of the dances associated with a particular entrance treatment were performed above the horizontal level of the entrance. This tendency was observed most noticeably in Experiments 1, 2 and 3; the majority (80% or higher) of dances were performed above the middle entrance and the majority (70% or higher) of dances were seen above the top entrance (Table 2). Furthermore, this tendency was most pronounced in Experiment 1 in which over 90% of dances were performed above any hive entrance. Dancing above the hive entrance was not observed during Experiment 4 (Table 2), in which the half-frame adjacent to the hive entrance was not danced upon (Figure 4). During Experiment 5, the top hive entrance was opened upon closing the bottom entrance (Figure 5). Over half (55.9%) of dances were recorded above the top entrance (Table 2).

Dance location information was collected for individually marked bees, in addition to un-marked foragers, during Experiment 5. To evaluate hive entrance manipulation on individual dance location, bees recorded dancing on multiple days throughout the experiment were identified. Bees that were only observed dancing prior to entrance manipulation were separated from bees with one or more sighting taking place after the entrance manipulation. If an individual was observed dancing on the same frame during all of her sightings she was classified as “frame consistent”. If a bee was recorded dancing on different frames she was classified as “frame inconsistent”. Notice in Figure 6, before the hive entrance was displaced 44.8% of the bees observed were recorded dancing on different frames (frame-inconsistent) while the other 55.2% of bees were recorded consistently on the same frame. The majority (93.3%) of bees observed dancing during at least one day following entrance manipulation displayed frame-inconsistency. A Yates Chi-Square test revealed that this difference was statistically significant (P= 0.005).

Discussion

Previously the location of the dance floor has been seen to correspond with the hive entrance location (von Frisch, 1967; Seeley and Towne, 1992). The correlation between dance floor location and hive entrance location was additionally supported by findings from experiments designed to observe and record the dance floor of multiple colonies and attempt to distinguish common attributes. These observations revealed that the dance floor location was always in close proximity to the hive entrance despite differences in year, weather and genetics of colony. The proximity of the dance floor to the hive entrance was variable among colonies; some colonies clustered closer to the hive entrance than others. In all cases, the dance floor was localized to three (or fewer) frames of the eight available frames, and was located adjacent to the hive entrance (Bryson, Corrigan, Reyes, Van Nest, Wagner, White, Yost, and Moore, unpublished). It has been convention, however, for the hive entrance to be located at the bottom of observation hives and Langstroth hives. Even in natural hives, the majority of entrances are found at the bottom of the hive (Seeley and Morse, 1976). Because the entrance in all previous accounts has been located at the bottom of the hive, evidence was needed to confirm that the dance floor was being established adjacent to the hive entrance; rather than established at the bottom of the hive. We show conclusively that bees deliberately establish the dance floor adjacent to the hive entrance despite its position relative to the rest of the hive. Upon hive entrance displacement, every colony, (regardless of year, time of year, genetics or available ambient food sources) re-established its dance floor near the newly accessible hive entrance (Figure 2).

Although dance floor establishment adjacent to the hive entrance was a consistent occurrence among all observed colonies, the shape of the dance floor was not (Figure 2). The aforementioned unpublished descriptive observations revealed that the mean dance location was consistent among days of observation as well as shape. However, throughout the day, the mean dance location and shape of the dance floor were more variable. Interestingly, the colonies observed produced uniquely shaped, distinct dance floors; each established near the hive entrance (Bryson, Corrigan, Reyes, Van Nest, Wagner, White, Yost, and Moore, unpublished). Because the influence of specific criteria used in dance floor formation remain enigmatic, it is difficult to draw conclusions from these findings; factors in addition to hive entrance location are likely affecting where foragers elect to dance. One such factor could be substrate preference. The influence of substrate on dance location appears to be subtle (if present at all) because of the ever changing contents of wax comb in juxtaposition with the consistency seen in dance floor location and shape of a colony across days of this study and previous unpublished results (Bryson, Corrigan, Reyes, Van Nest, Wagner, White, Yost, Moore, unpublished). Substrate is variable-pollen and nectar are consumed and relocated, eggs and larvae develop and the cells are capped over, mature brood emerge rendering previously capped cells empty and so on. Should a predominant substrate preference exist it would be expected that the shape and location would be highly variable. This is not the case. In fact, the location and shape of the dance floor are remarkably consistent across multiple days as substrate is not, as observed by our lab at ETSU (unpublished). Substrate is a candidate for influencing where a bee dances because it has been shown to influence transmission of vibratory signals (Sandeman et al., 1996) and dances performed on empty comb are more successful at recruiting others (Tautz, 1996). Despite the ability to enhance recruitment success, it has not been shown that bees establish the dance floor on particular types of comb. Further experimentation is required to investigate the cause of these differences seen in dance floor shape among colonies.

Another candidate source of differences in dance floor shapes seen among colonies, as well as different reactions to hive entrance displacement is individual idiosyncrasy. Genetic variety among foragers is introduced from the queen mating with multiple males. It has been established that foragers' physiological differences affect the resources they are inclined to collect (Pankiw and Page, 2000). Although the present study shows that the hive entrance is an important criterion used by all colonies observed to establish the dance floor, it is not the only criterion used. This was seen by Tautz and Lindauer (1997), as bees reacted to frame relocation by seeking out the frame previously used for dancing instead of electing to dance near the hive entrance. An olfactory cue has been proposed as another important criterion utilized in dance floor establishment (Tautz and Lindauer, 1997; Thom et al, 2007). Because honey bee foragers are individuals with physiological differences that influence their behavior, and because there are more than a single factor influencing where bees dance, it seems reasonable to hypothesize that some of these factors could influence individuals more significantly than others- which could account for the dissimilar dance floor shapes observed and different reactions to entrance displacement among colonies. To address this fully a greater understanding of how a forager elects a dance location is needed, followed by an exploration of physiological differences among colonies.

A consistency seen among colonies regarding the hive entrance was a tendency to dance above an imagined line extending from the floor of the entrance (Table 2). Because it is convention for the hive entrance to be located at the bottom of observation hives, previous accounts of the dance floor (Seeley and Towne, 1992; von Frisch, 1967) show all dances occurring above the hive entrance; however, dancing below it was impossible. Further experimentation has been performed to determine if this behavior is innate or a product of forager experience: entering the hive and not being able to travel below the hive entrance. There is preliminary evidence from our lab at ETSU suggests a tendency to dance above the hive entrance is seen in naïve foragers that have only experienced an entrance in the middle of the center frame of a three frame observation hive (Bryson, Corrigan, Reyes, Suich, Wagner, Welch, White, Williams, Yost, Moore, unpublished).

During Experiment 4 (Figure 4), the eight frame hive was modified to support six and one half frames; with a half frame adjacent to the top hive entrance and a physical barrier was placed to restrict bees’ ability to dance above the hive entrance (described in materials and methods). When the top hive entrance was made accessible the bees did form a dance floor below the hive entrance, but also avoided the half frame almost entirely. The possible influence of substrate could be relevant in this case. The half frame used was a frame from a honey super; the substrate contents were capped honey. Capped honey is bulbous and protrudes beyond the rest of the comb. The physical attributes of this substrate could have discouraged bees from dancing on the half frame, thus undermining the preference to establish the dance floor adjacent to the hive entrance. Furthermore, it has been documented that vibrations like those produced by the waggle dance were seen to be transmitted further through open comb than through capped brood cells; however, the impact of capped honey specifically was not examined regarding transmission of vibrations (Sandeman et al., 1996).

Before entrance manipulation in Experiment 5, it was observed that 44.8% of the bees observed were recorded dancing on different frames (frame-inconsistent) while the other 55.2% of bees were recorded consistently on the same frame. Frame inconsistency was also described in the literature. Bees were seen to perform dances across the dance floor, as opposed to advertising in a single location repeatedly (Seeley, 1994). The occurrence of frame inconsistency was seen to increase after manipulation a statistically significant amount (P=0.005) when 93.3% of bees displayed frame inconsistency. Although to thoroughly investigate consistency of an individual's dance location, a higher resolution is needed than identifying the frame danced upon; our results do suggest that frame inconsistency is increased by hive entrance manipulation. It has been seen that memory of experiences in the field influences a foragers behavior. Moore and Doherty (2009) showed that an individual's time-accuracy of arrival at a food source increases with more rewards at an artificial feeder. Also, an individual with more days of experience at a feeder is more likely to investigate the feeder for several days following the last feeding event. It could be hypothesized that memories associated with a dance location near the previous hive entrance could motivate a forager to return to the location to dance, as well as dancing near the new hive entrance location. Further experimentation is needed to investigate frame inconsistency and its relationship to hive entrance manipulation and honey bee memory.

References

Boch, R. (1956). Die Tänze der Bienen bei nahen und fernen Trachtquellen. *Z. Vergle. Physiol.* 38:137–167.

Boch R. (1957). Rassenm¨aßige Untershiedebei den T¨anzen der Honigbiene (*Apis mellifera* L.).*Z. Vgl. Physiol*. 40:289–320.

Budel A. (1960). Bienenphysik. In Budel (A.) and Herold (E.), Ed. : Biene und Bienenzueht, pp. 115-180. Ehrenwirth Verlag, publ., Miinehen.

Dyer, F. C. (2002). The biology of the dance language. *Annual review of entomology*, 47(1), 917-949.

Farina, W. M. (1996). Food-Exchange by Foragers in the Have: A Means of Communication among Honey Bees? *Behavioral Ecology and Sociobiology*, Vol. 38, No. 1, pp. 59-64.

Hrncir, M., Maia-Silva, C., McCabe, S., & Farina, W. (2011). The recruiter's excitement – features of thoracic vibrations during the honey bee's waggle dance related to food source profitability. *Journal Of Experimental Biology, 214*(23), 4055-4064.

Lindauer M. 1956. Uber die Verständigung bei indischen Bienen. . Z. Vgl. *Physiol:* 38:521-57.

Moore, D. and Doherty, P. (2009). Acquisition of a time-memory in forager honey bees. *J Comp. Physiol. A.* 195:741–751.

Nieh, J. C. and Tautz J. (2000). Behaviour-locked signal analysis reveals weak 200–300 Hz comb vibrations during the honeybee waggle dance. *J. Exp. Biol.* 203:1573–1579.

Pankiw, T., & Page Jr, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behavioral Ecology and Sociobiology*,*47*(4), 265-267.

Sandeman, D. C., Tautz, J. and Lindauer, M. (1996). Transmission of vibration across honeycombs and its detection by bee leg receptors. *J. Exp. Biol.* 199:3585–2594.

Seeley, T. D. (1994). Honey Bee Foragers as Sensory Units of Their Colonies. *Behavioral Ecology and Sociobiology*, Vol. 34, No. 1 (1994), pp. 51-62

Seeley TD, Buhrman SC. (1999). Group decision making in swarms of honey bees. *Behav. Ecol. Sociobiol.* 45:19–31

Seeley TD, Mikheyev AS, Pagano GJ. (2000). Dancing bees tune both duration and rate of waggle- run production in relation to nectar-source profitability. *J. Comp. Physiol. A* 186: 813-819.

Seeley, T. D. and Morse, R. A. (1976). The nest of the honey bee. *Museum of Comparative Zoology*. 4: 495-512.

Seeley, T. D. and Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? *Behav. Ecol. Sociobiol.* 30:59–69.

Tautz, J. (1996). *Honeybee* waggle dance: recruitment success depends on the dance floor. *J. Exp. Biol.* 199:1375–81.

Tautz, J. and Lindauer, M. (1997). Honeybees establish and maintain specific sites on the comb for their waggle dances. *J. Comp. Physiol.* 180:537–539.

Tautz, J. and Rohrseitz, K. (1998). What attracts honeybees to a waggle dancer? *J. Comp. Physiol.* 183:661–667.

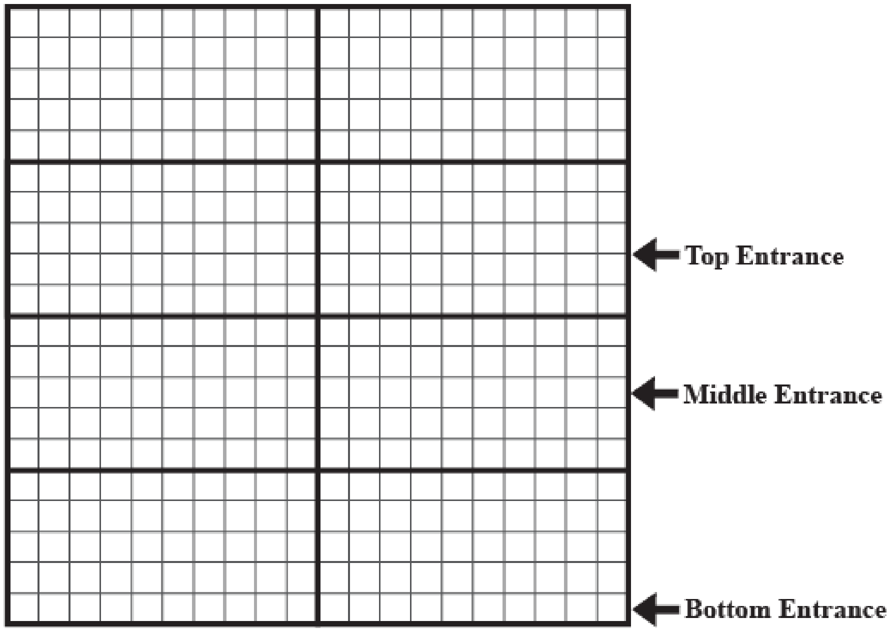
Tautz, J., Casas, J. and Sandeman, D. (2001). Phase reversal of vibratory signals in honey-comb may assist dancing honeybees to attract their audience. *J. Exp. Biol.* 204:3737–3746.

Thom, C., Gilley, D. C., Hooper, J., & Esch, H. E. (2007). The scent of the waggle dance. *PLoS biology*, *5*(9), e228.

von Frisch, K. (1965). *Tanzsprache und Orientierung der Bienen.* Berlin Heidelberg New York: Springer.

von Frisch, K. (1967). *The Dance Language and Orientation of Bees.* Cambridge (MA): Harvard University Press.

a) 8 Frame Observation Hive



b) 6 1/2 Frame Observation Hive

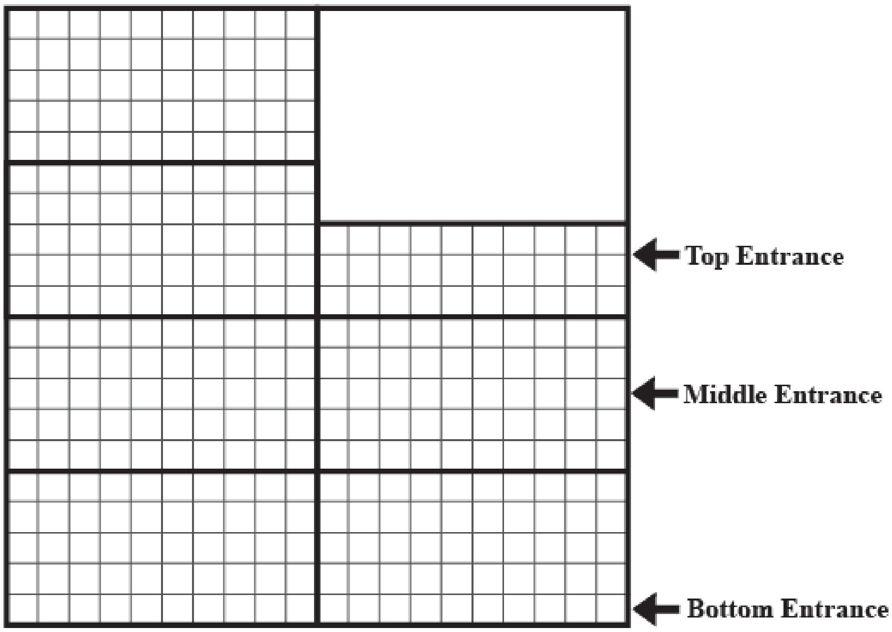
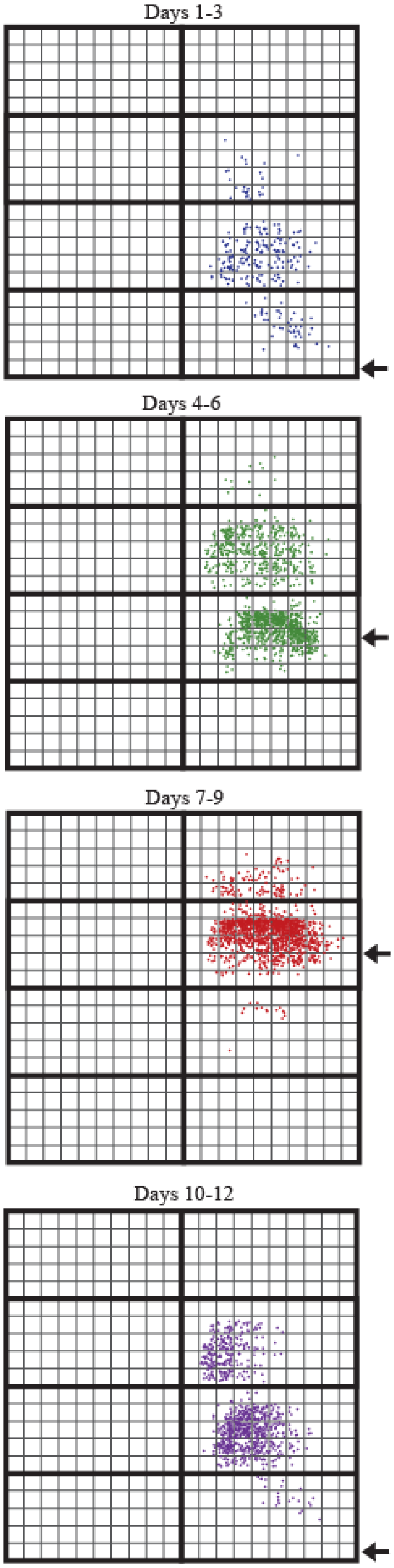
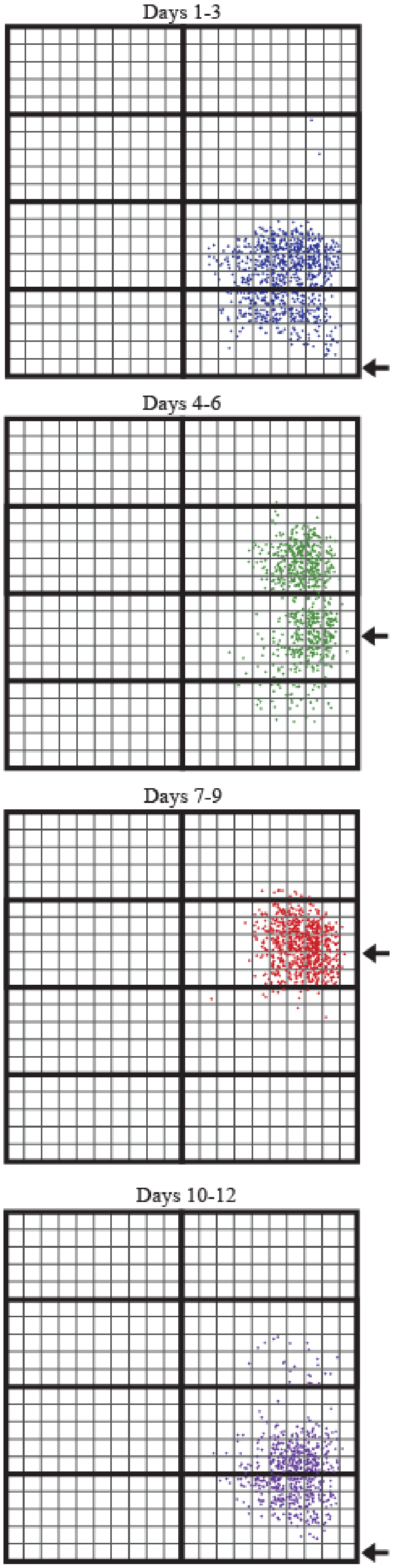


Figure 1: An eight frame hive was modified to contain three hive entrances (1a) at the bottom, middle and near the top of the hive. This hive was further modified with a barrier above the top-most frame on the right hand side (1b) preventing access to space above the top hive entrance.

a) Experiment 1



b) Experiment 2



c) Experiment 3

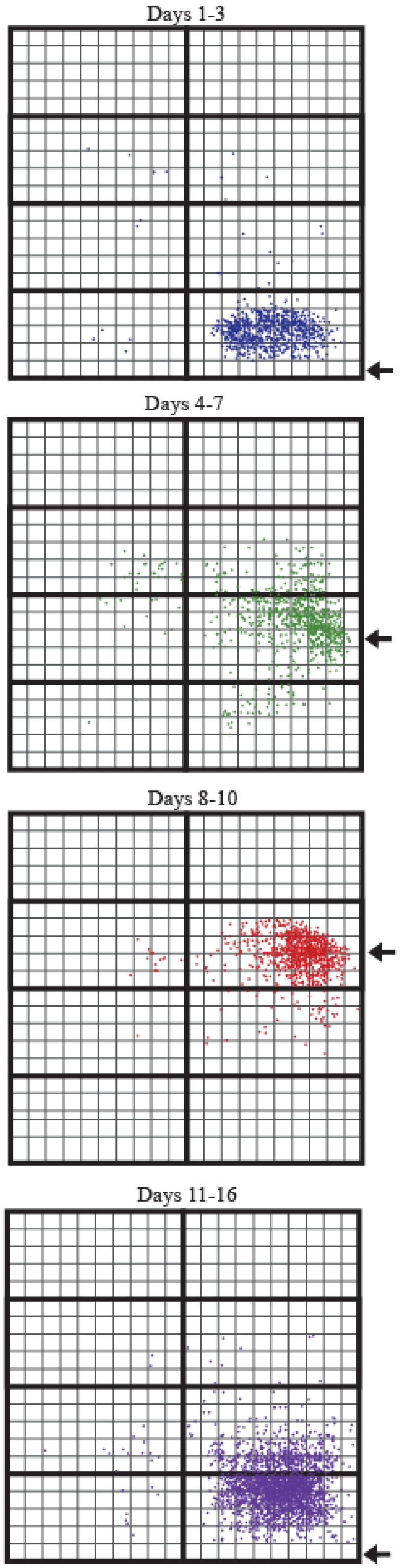
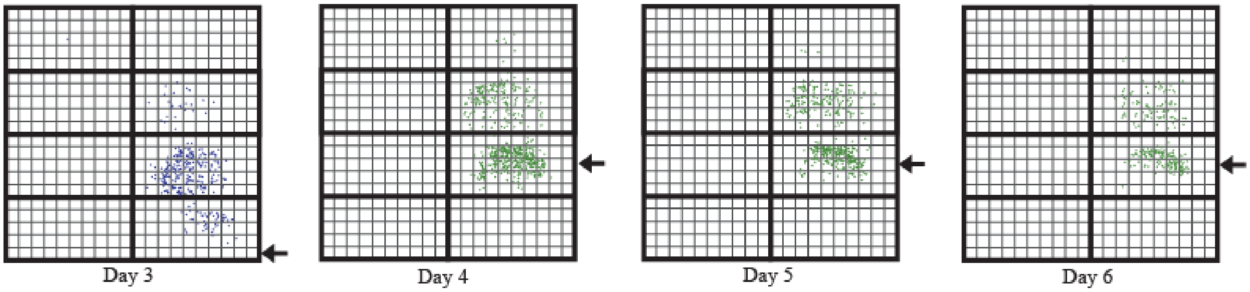


Figure 2: Experimental manipulation of the hive entrance was replicated three times. The hive entrance location is designated by a black arrow. Each colored dot represents the location of an observed waggle dance. Following each displacement of the entrance the majority of dances are performed adjacent to the newly accessible entrance and on frames above it; dancing on the frame adjacent to the previous entrance ceased or substantially reduced. As the locality of dances are altered, the shape of the dance floor is also altered. The dance floor shape observed among colonies is unique, even when entrance location is constant. **(2a)** Following the first entrance manipulation the dances are observed distinctly on the two frames adjacent to the newly open entrance- resembling two dance floors separated by the wooden frames surrounding drawn comb. Upon opening the top entrance, most dances are localized on the frame adjacent to the entrance with some occurring on the frame immediately above and below. When the bottom entrance is re-opened the dance floor changes shape drastically as bees begin dancing near the available hive entrance. The resulting dance floor extends upwards at an angle similar to that observed during the first three days, yet dancing does not cease on the frame adjacent to the previous entrance. **(2b)** When the entrance is first displaced upwards, dances are distributed across the frame next to the middle entrance and the frame above; the dance floor assumes a slender, vertical shape. Opening the top entrance induced a circular dance floor almost exclusively located on the frame closest to the top entrance. The dance floor observed during the last three days is roughly circular, like the first three days, but some dances are still performed on the frame previously containing the dance floor. **(2c)** The dance floor observed in the first three days was oval and horizontal. After manipulation the locations of dances become dispersed: the majority of dances occur next to the middle entrance but also on the frames above, below and to the left of it. When manipulated again, dancing is localized primarily adjacent to the top entrance in a circular manner, some dances are seen on the frame below and the left frame. Upon availability of the bottom entrance, the dance floor occupies the frame adjacent to the entrance and the frame above it, with some dances occurring on the left frames. This shape is maintained across six days.

a) Experiment 1



b) Experiment 3

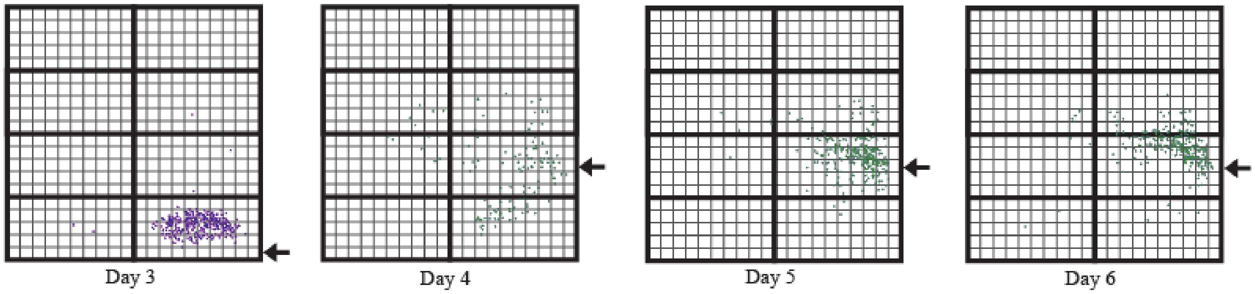


Figure 3: An adjustment period was observed in some colonies on the first day following an entrance manipulation and others did not. This adjustment period was characterized by a dispersed dance floor observed only on the first day after a manipulation followed by a consistent, more localized dance floor observed on subsequent days. The black arrow indicates the location of the hive entrance. **(3a)** No adjustment period was observed when the middle entrance was made accessible in Experiment 1. The shape and location of the dance floor on day 4 is nearly identical to the dance floor observed on days 5 and 6. **(3b)** An adjustment period was observed in Experiment 3. On the night of day 3 the bottom entrance was closed and the middle entrance was opened. The dance floor observed on day 4 is dispersed mainly across the three right frames near the middle hive entrance; many dances took place on the bottom right frame. The dance floor is less dispersed on the following two days; the majority of dances occur on the frame adjacent to the hive entrance and very few dances are performed on the bottom right frame.

Observation of Adjustment Period			
Experiment	**Manipulation 1** (middle entrance opened)	**Manipulation 2** (top entrance opened)	**Manipulation 3** (bottom entrance re-opened)
1	No	No	No
2	Yes	No	Yes
3	Yes	No	Yes
4	Yes	Yes	Not Observed
5	Not Observed	No	Not Observed

Table 1: The observation of an adjustment period following a manipulation was not consistent. The colony used in Experiment 4 displayed an adjustment period after both manipulations performed. This colony formed a dispersed dance floor on the first day following each manipulation, and then established a more localized consistent dance floor on subsequent days. The colonies observed in Experiments 2 and 3 only exhibited an adjustment periods after manipulation 1 (in which the bottom entrance was closed and the middle entrance was opened) and manipulation 3 (in which the top entrance was blocked and the bottom entrance was re-opened). The two colonies observed in Experiment 1 and 5 never displayed an adjustment period; the dance floor was in a similar shape and location on all days following each manipulation.

Percentage of Dances Performed Above Hive Entrance		
Experiment	**Middle Entrance Treatment**	**Top Entrance Treatment**
1	91.8%	95.9%
2	80%	72.5%
3	85%	70%
4	28.9%, 36.1%	1%
5	Not Observed	55.9%

Table 2: In all cases (excluding Experiment 4) over half of dances observed were performed above an imagined horizontal line extending from the floor of the hive entrance.

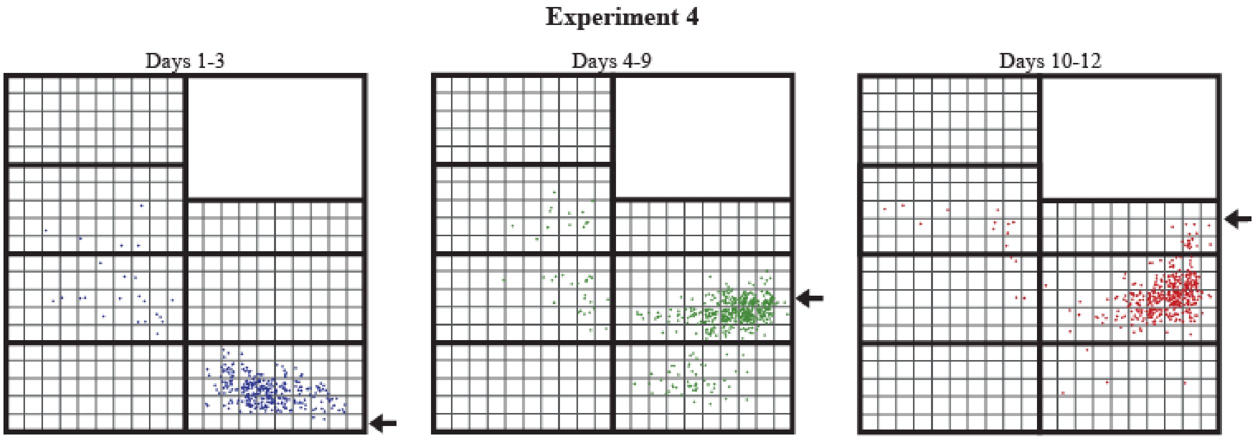


Figure 4: A barrier was placed above the top hive entrance physically preventing bees from dancing above the top hive entrance when made accessible. Here, the black arrow indicates the relative hive entrance location and the colored dots represent locations of individual waggle dances. After the middle entrance is made accessible, the majority of dances become localized on the frame adjacent to the entrance, though some are seen on the frame below and to the left. This location and shape was observed over six days due to weather. When the top entrance was made accessible the dance floor remains primarily located on the frame next to the middle entrance.

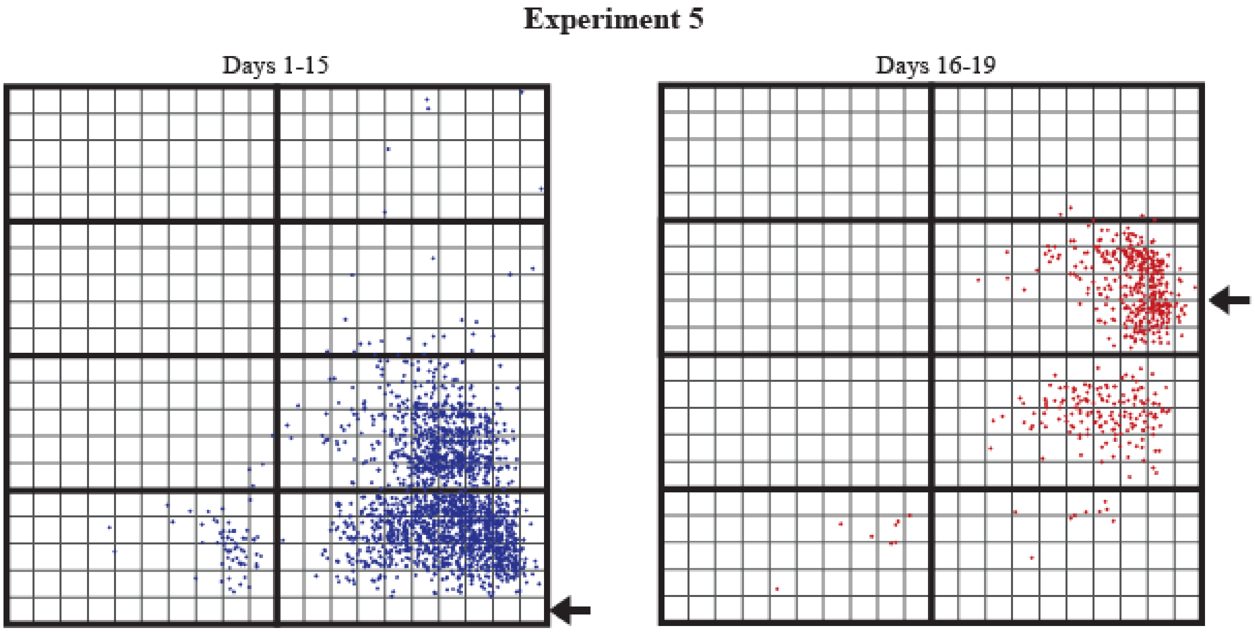


Figure 5: During this experiment, a colony containing individually marked bees was observed for 15 days with the bottom entrance accessible, each observed dance is shown by a dot and the entrance is designated by a black arrow. A single manipulation in entrance location was performed by opening the top entrance (with the middle entrance obstructed throughout). Despite a less gradual displacement of entrance location the bees discontinued dancing on the frame adjacent to the bottom entrance (almost entirely) which was utilized for dancing consistently for 15 days prior to the manipulation.

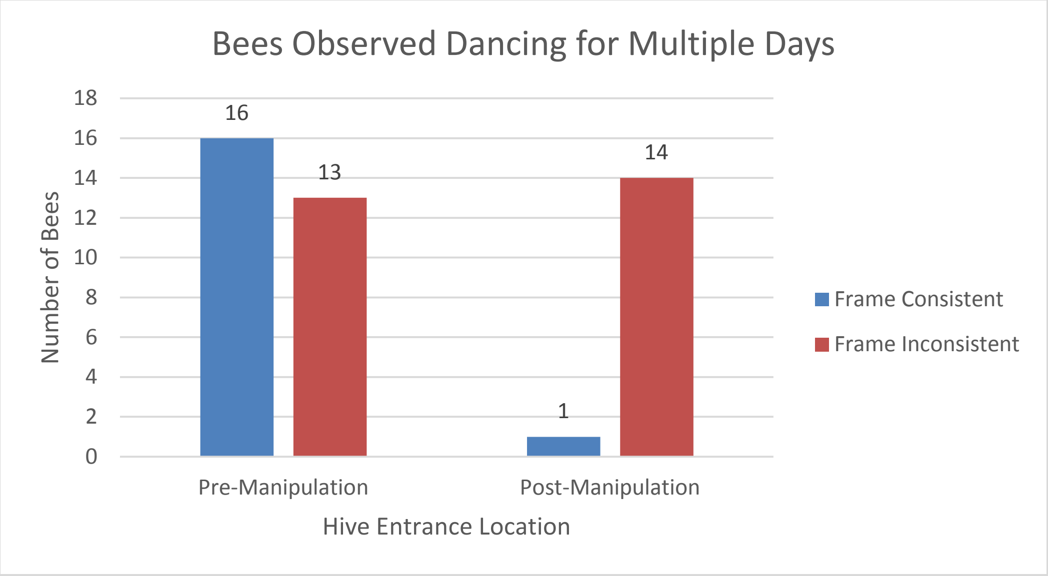


Figure 6: After being individually painted, honey bees recorded on multiple days of the experiment were examined. Bees recorded dancing exclusively before hive entrance manipulation were termed " pre-manipulation" Bees that were recorded on at least one day following the entrance manipulation were termed " post-manipulation". Frame consistency refers to the frame the bee was sighted on. If the bee was observed on the same frame during all sightings she is considered frame consistent. Before the hive entrance was displaced, 44.8% of the bees observed were recorded dancing on different frames (frame-inconsistent) while the other 55.2% of bees were recorded consistently on the same frame. The majority (93.3%) of bees observed dancing during at least one day following entrance manipulation displayed frame-inconsistency. A Yates Chi-square test was performed (χ^2=7.87; df=1) and the differences in frame consistency observed in bees prior to manipulation and after were statistically significant (P=0.005).