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An efficient method for decoding the honey bee waggle dance and its use in determining the effects of hive relocation on foraging efficiency.

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A thesis submitted to the University of Sussex, department of Life Sciences for the Degree of Master of Philosophy.

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Supervisor: Francis L.W Ratnieks

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature:………………………………………

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Abstract

Our understanding and uses of the honey bee waggle dance have come a long way since its discovery by Karl von Frisch in the 1940s. It is now used as a powerful research tool for investigating various features of honey bee biology. Manual dance decoding is time-consuming. It is impractical to decode all circuits of a dance to determine foraging location; however, intra-dance waggle runs vary, so it is important to decode enough to obtain a good average. In chapter 2 I examine the variation among waggle runs made by foraging bees to devise a method of dance decoding. The first and last waggle runs within a dance are significantly more variable than any waggle run in between. We recommend that any four consecutive waggle runs, not including the first and last runs, may be decoded.

This thesis also examines how artificially moving hives affects the foraging efficiency of colonies that are moved and whether it has any effect on resident colonies (Chapter 3). We moved three colonies (in observation hives) onto the University of Sussex campus from a site more than 20km away and compared their foraging efficiency to three similarly sized resident colonies. Foraging distance, forager effort, nectar concentration, percentage of successful nectar foragers were the factors used to quantify foraging efficiency. We found that bringing new hives onto the apiary site had no effect on the foraging efficiency of the resident colonies that moved colonies were able to match the foraging efficiency of resident colonies immediately after the move.

Table of Contents

Chapter 1. Thesis Introduction and an Introduction and a Brief History of the Waggle Dance

A brief history of the waggle dance

Discovery and introduction to the waggle dance

The honey bee "waggle dance", or "Tanzsprache" (dance language) in German, was discovered and decoded by Austrian scientist Karl von Frisch (1946), a discovery for which he received the Nobel Prize for Physiology or Medicine in 1973. von Frisch set up artificial feeders and observed the dances of foraging bees who had located these feeders. He noted that more bees located the feeder after a dance had been performed (von Frisch, 1923). We now know that bees also perform dances to recruit to water, resin, and nest sites as well as to nectar and pollen sources. In a sense, the returning forager re-enacts the flight path to a resource.
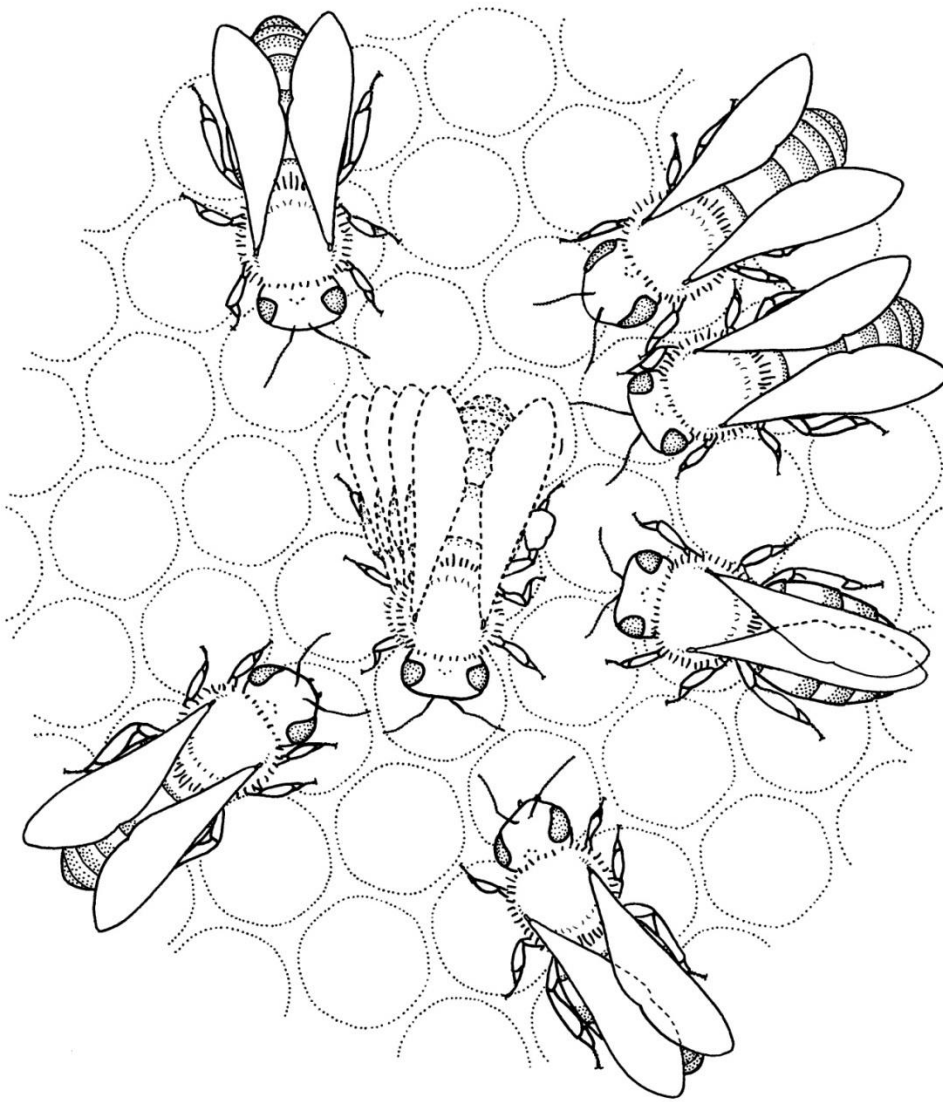
Figure 1. A honey bee performing the "waggle dance". The bee is currently performing the waggle run and will return to her start point via a figure of eight to begin again.

There are two main parts to the waggle dance; the waggle run and the return circuit. The waggle run is the information rich component of the dance and encodes both the directional and distance components. The dancing bee will vibrate her abdomen laterally as she advances on the comb (see Figure 1). The duration of this advancement symbolises the distance of the food source from the hive. It can last from <1-11 seconds. In *A. mellifera* this is done in the darkness of the hive so the distance information is communicated via an acoustic signal picked up by the Johnson organ of the dance followers (Dreller & Kirchner, 1993). Longer waggle runs have longer acoustic signals. The directional component is expressed in the angle the bee holds her body during the waggle phase. Gravity is used as a reference point for

the position of the suns' azimuth and the angle produced by the angle relative to gravity and the bee's body represents the angle of the resource from the sun. The dancing bee will then return to her starting point after each waggle run, alternating between returning via the left or right after each waggle run producing a figure of eight shape. A dancing bee may make 1 to as many 100 circuits, with more waggle runs representing a more profitable resource (Seeley *et al*, 2000). Not all bees dance, only a small proportion those with knowledge about above-average feeding sites perform dances (Seeley, 1995).

Sherman and Visscher (2002) showed that honeybees performing dances that were orientated correctly in terms of angle and duration would recruit foragers to the food source more effectively. They disrupted the light (diffuse lighting or a distinct light source) and gravity (by altering the orientation of the hive) to alter the accuracy of the dances the bees would perform. Significantly more recruits found the food source when the dancers had been able to orientate themselves and therefore produce informative dances.

There has been some scepticism on von Frisch's' theory on the waggle dance. Some scientists believed that the information in the dance was too complex for the bees to decode and that odour sharing was the reason for dancing (Wenner & Johnson 1967). It was also taking the bees longer than expected to locate the resource after following a dance. However, any remaining doubts about the information content of the waggle dance were laid to rest in 2005 when Riley *et al* fitted foragers with radar transponders and tracked their flight paths. After following a dance, naive foragers flew directly to the location of the advertised feeder on leaving the hive. To make sure the recruits were not simply following a known route or an odour trail left by the dancer, feeders were positioned downwind from the hive and some recruits were experimentally displaced 250m from the hive entrance and released. These displaced bees then flew in the direction and distance predicted by the dance and so did not find the feeder.

However, honey bees do not always use the dance in the same way. Biesmeijer and Seeley (2005) showed that novice foragers depended on information in dances 60% of the time, but more experienced foragers who had had a break in foraging, due to weather or nightfall, were only re-recruited to foraging at the previous location by dances 37% of the time. Experienced foragers that had not had a break in foraging only used waggle dances of other bees 17% of the time. In these circumstances it is likely that foragers were re-confirming that the previously visited locations were still profitable by the odour on the dancing bee before continuing to forage.

Grüter and Farina (2009) argued that the distance information conveyed in the waggle dance is only important if the dance follower does not have her own private information on the location of a resource. The waggle dance is very important to novice foragers with no information on foraging locations and it serves as a re-activation mechanism for bees with some foraging information. It will also serve to guide bees to a new resource, once their own resource is depleted, but some bees find new sources on their own by scouting.

The round dance and dancing constraints

von Frisch described the round dance as distinct and separate from the waggle dance, with round dances being a general signal for food within approximately 50m of the hive, and the waggle dance being for greater distances and containing vector (direction and distance) information (von Frisch, 1967). However, Kirchner et al (1988) revealed that the acoustic component in the "round dance" also encoded distance information. When performing shorter dances, bees are less able to provide accurate direction information, although they still produce discernible direction information for food sources as little as 1m away (Kirchner *et al* 1988). Round dances are now considered part of a continuum in the waggle dance, rather than a separate dance (Gardener *et al*, 2007).

Dances for greater distances have more variability in the duration component than shorter dances (Tanner & Visscher, 2010; Couvillon, Riddell Pearce *et al* 2012). However, the angle component is more accurate for greater duration dances than shorter ones. The greater imprecision for further away resources was once thought to be beneficial to the bees as it would spread recruits over similar sized area regardless of the distance from the hive and referred to the phenomenon as the 'Tuned error hypothesis' (Towne & Gould 1988). Later, Beekman *et al* (2005) showed that physical constraints were a more likely reason for the lower angular precision in dances for nearby locations as the dancing bee cannot turn her body quickly enough. Honeybees do increase their precision when dancing for a nest site entrance (small opening) rather than a large food patch (Beekman *et al*, 2008) but are still met by similar constraints over distances. The tuned error hypothesis was weakened further by Couvillon *et al* (2012a) who showed that dancing bees performing waggle runs horizontally provide less accurate angle information than bees dancing vertically, suggesting physical constraints limit the accuracy of the dancing bee. The variability in a dance will fluctuate as the as the solar azimuth changes throughout the day.

How bees use the dance

Bees follow dances in the dark of the hive and depend on sound information to obtain the distance component (Dreller & Kirchner, 1993). They receive the direction component by comparing the pressure on antenna during the dance to determine direction (Dreller & Kirchner, 1993; Tsujiuchi *et al* 2007). Bees follow and average multiple circuits (Tanner & Visscher, 2008) which is also the process we perform when decoding the dances. However, bees do not compare different dances before embarking on a foraging trip (Towne & Seeley, 1992).

It was originally proposed that the honey bees measured distance to a resource after following a dance by estimating their energy expenditure (Heran, 1956). Optic flow is now known to be the mechanism for determining distance flown (Srinvasan *et al*, 2000). Optic flow is the rate of movement of image across the visual field, bees flying in an enclosure or tunnel where there is a large amount of optic flow perceive the distance as greater then flying the same distance in an open environment (Srinvasan *et al*, 2000).

Stop signal exists to stop bees going to a dangerous location (Nieh, 2010) this is an important negative feedback signal that prevents the loss of workers. The stop signal is a short sound pulse that, if played into the nest can reduce recruitment by 60%. This signal can also be used to redirect effort away from an unprofitable location or to cut back foraging effort when the wait time for unloading reaches a certain threshold (Nieh, 2010). These stop signals are part of an overall system in which the foraging of a honey bee colony is regulated by a variety of signals among bees, and other processes within individual bees, so that the colony sends its foragers to where the resources are (Seeley, 1995; Anderson & Ratnieks, 1999).

The waggle dance is a useful tool to research the foraging behaviour of honey bees. It has been used extensively in studies of foraging and bee biology, such as: how bees follow the dance (Biesmeijer & Seeley, 2005); how they perceive the information in the dance (Dreller & Kirchner, 1993; Tsujiuchi et al 2007); how they measure distance (Srinivasan, 2000); how they regulate their foraging efforts (Seeley, 1995; Anderson & Ratnieks, 1999) and locate new nest sites (Seeley, 2011). Now, with the methods developed in Chapter 2 of this thesis, honey bee dances can be decoded quickly and accurately allowing for further investigations on this most fascinating animal. We use these methods to investigate the effects of hive relocation of honeybee foragers in Chapter 3.

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Chapter 2

Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding

Summary

Noise is universal in information transfer. In animal communication, this presents a challenge not only for intended signal receivers, but also to biologists studying the system. In honey bees, a forager communicates to nestmates the location of an important resource via the waggle dance. This vibrational signal is composed of repeating units (waggle runs) that are then averaged by nestmates to derive a single vector. Manual dance decoding is a powerful tool for studying bee foraging ecology, although the process is time-consuming: a forager may repeat the waggle run 1- >100 times within a dance. It is impractical to decode all of these to obtain the vector; however, intra-dance waggle runs vary, so it is important to decode enough to obtain a good average. Here we examine the variation among waggle runs made by foraging bees to devise a method of dance decoding. The first and last waggle runs within a dance are significantly more variable than the middle run. There was no significant trend in variation for the middle waggle runs. We recommend that any four consecutive waggle runs, not including the first and last runs, may be decoded, and we show that this methodology is suitable by demonstrating the goodness-of-fit between the decoded vectors from our subsamples with the vectors from the entire dances.

Introduction

In the decades since Karl von Frisch discovered that honey bee workers (*Apis mellifera* spp.) communicate foraging locations to nestmates via the waggle dance (von Frisch, 1946; von Frisch, 1967), researchers have studied many aspects of the dance language. Some of these aspects have included mechanisms and evolution of message production (Seeley et al., 2000; Dornhaus and Chittka, 2004; Couvillon, submitted); message reception (Riley et al., 2005; Tanner and Visscher, 2008; Tanner and Visscher, 2009); the role of odour, memory, and acoustics (Kirchner et al., 1988; Reinhard et al., 2004; Grüter and Ratnieks, 2011); and how honey bees measure distance (Srinivasan et al., 2000; Esch et al., 2001; Chittka and Tautz,

2003; Tautz et al., 2004). Additionally, the dance has been used as a tool to investigate honey bee foraging ecology (von Frisch, 1967; Visscher and Seeley, 1982; Waddington et al., 1994; Seeley, 1995; Beekman and Ratnieks, 2000; Steffan-Dewenter and Kuhn, 2003; Beekman et al., 2004; Seeley and Visscher, 2004). In this last field, biologists use the waggle dance to determine where bees collect food or find new nest sites.

In the waggle dance, a worker bee vibrates her abdomen from side to side as she advances linearly in one direction (the waggle run), then turns either to the left or right to circle back to the start (the return phase), at which point she usually repeats the waggle run with the return to the opposite side. The dancer is communicating a distance and direction vector from the nest site to a useful resource (nectar, pollen, water, propolis, or a new nest site). Distance and direction are encoded in the waggle run duration and orientation relative to vertical, respectively (von Frisch, 1946; von Frisch, 1967). The circuit of waggle run and return phase is made a variable number of times (1- >100) in a single dance bout, depending on resource quality (Seeley et al., 2000). Variation in angle and duration exists within these runs (Dyer, 2002; De Marco et al., 2008). Dance followers (recruits) take an average to derive a single distance and direction (von Frisch and Jander, 1957; Tanner and Visscher, 2008).

Quantifying and decoding waggle dances present certain experimental challenges, and the methodology used has changed with technological advances. von Frisch mostly analysed dances in real time but sometimes used motion film cameras. He used a clock to obtain duration, usually of complete dance circuits (waggle phase + return phase), and a protractor (accurate to 5°) to obtain orientation (von Frisch and Jander, 1957; von Frisch, 1967). Live dance decoding with timers, protractors (accurate to $2\text{-}11^{o}$), and the use of complete dance circuits as a duration measurement remained common practice in the decades after von Frisch (Visscher and Seeley, 1982; Waddington et al., 1994; Seeley, 1995; Steffan-Dewenter and Kuhn, 2003). The advent of video recording made it possible to review footage easily, allowing for after-the-fact dance decoding, the decoding of simultaneous dances, and more accurate measurement of orientation. The use of digital video and computers makes it technically easy to analyse dances frame by frame, improving resolution. However, even with these improvements, dance decoding is time-consuming: a single forager bee may make waggle runs for the same location for over an hour in real time. Therefore, there is a need for protocols to optimise dance decoding. Specifically, how do we select the waggle runs to

decode or not within a dance, and how many decoded waggle runs are necessary to obtain a good estimate of the location vector?

In this study we made a detailed analysis of intra-dance variation in waggle run duration and angle for dances to natural food sources in order to design time-efficient protocols for dance decoding. This involved the decoding and analysis of n = 3753 waggle runs for 273 dances. We found consistent and significant trends in waggle run variability. These we use to design a new, systematic protocol for dance decoding. Lastly, we demonstrate that our sub-sampling technique generates angle and duration means that are good estimates of the entire waggle dance angle and duration mean, confirming the suitability of our recommendations.

Materials and Methods

Study Organism and Hives
We used 3 honey bee colonies of mixed European race, predominantly *Apis mellifera mellifera*, housed in glass-walled observation hives located at the laboratory. Each colony had a queen and contained approximately 5000 workers on 1 deep and 3 medium Langstroth frames. Care was taken to maintain similar-sized patches of brood (roughly one side of one medium frame) and stored honey (1 medium frame) in each colony to control for foraging motivation via food storage quantity and brood nutritional needs. When supplemental feeding was needed, colonies were given sucrose solution on Friday afternoons. Data collection (videoing dances) was not done over the weekends, and the sugar solution was consumed by Monday morning. A 3cm diameter x 30cm plastic tube gave foragers access to the outside.

As bees dance relative to vertical, we placed plumb lines made of nylon fishing line with heavy metal washers at the ends at 5cm intervals across the outer glass wall. These appeared as thin white lines in the video. Additionally, we placed a digital clock (radio controlled for accuracy) on each observation hive within the video area. We repositioned the clock as needed so that the time was visible within the video. Time of day is needed to determine the solar azimuth for each dance, as the sun moves approximately 15 degrees per hour. We adjusted the zoom of each camera so that a "dance floor" area c. 25cm wide by 20cm deep was recorded. This included most of the dances on one side of the frame but still gave enough definition to quantify individual dances with ease from the video.

Data Collection

Video data were collected from 11 August 2009 to 13 October 2009 on days when the bees were foraging. We filmed each observation hive for one hour per study day using camcorders (Canon Legria HV40, HDV 1080i) and mini-DV tapes. The camera was set back 1m from the hive to reduce parallax effects. Our goal was to film all three hives simultaneously around the same time each day (10-11am GMT), although sometimes one of the hives was inactive or bad weather prevented foraging. We uploaded tapes to external hard drives connected to iMac computers running Final Cut Express (Version 4.0.1). Videos were played until we observed a bee making a waggle dance. In this study, we decoded every dance we saw for as many waggle runs as were made. As it takes several minutes to decode each waggle run, dances with many waggle runs could take several hours to decode.

To decode dances, it is necessary to extract two pieces of information per waggle run: the duration of the waggle run (von Frisch, 1967; Seeley et al., 2000), which is a function of distance to the advertised location, and the direction of the waggle run relative to vertical, which gives the direction from the nest relative to the solar azimuth. Previous work on waggle dance decoding used the duration of the entire circuit (waggle run + return phase) or the several entire circuits that is then averaged (von Frisch, 1946; von Frisch, 1967; Waddington et al., 1994; Beekman and Ratnieks, 2000). However, the waggle run is actually the information-rich portion of the dance (Michelsen et al., 1992), while the return phase (or more specifically, the quickness of the return phase) depends on resource quality (Seeley et al., 2000) and can be noisy. Therefore, we only used the waggle run, not the entire circuit, in our dance decoding.

We measured duration by noting the beginning and end of each waggle run, which was determined by the start and end of the blurring of the dancer's body and wings (start of vibration), which could easily be seen on a still frame of the video. Beginning and end times (hour, minute, second, frame) were taken from the timer of the video software, which provides a temporal resolution of 0.04 sec (1 frame). Thus, our maximum measurement error was approximately 0.08s. To determine foraging locations, duration must be transformed to distance. However, this transformation was not necessary in this study, as our aim was to investigate variation among waggle runs and not actual foraging locations. Therefore, all analysis is done on duration, not distance.

To determine the angle of the dancer's body relative to vertical during the waggle run, we taped acetate sheets on the computer screen and made dots above the centre of the dancing bee's thorax twice during the waggle run. Usually we found it best to make these dots during the middle portion of the waggle run, as sometimes the bee began to waggle when it was still turning its body. A line was drawn to connect the dots and was extended to the nearest vertical plumbline on the video. Using a 360° protractor, the angle of this line clockwise from vertical was determined to the nearest degree. Thus, our maximum measurement error was approximately 1 degree. We determined the azimuth using the Excel Macro (© W.F. Towne) Sun2007 and the time (GMT in minutes) for each waggle run. Ultimately, to determine foraging locations the dance angle is added to the azimuth to get a final direction. However, this was not necessary in this study, as our aim was to investigate variation among waggle runs and not actual foraging locations. Therefore, all analysis is done on measured angles, not angle + azimuth.

Data Analysis

While decoding the dances we had the impression that the first and last waggle runs were more variable than those in the middle of the dance. To test this possibility, we first calculated the mean and standard deviation for both duration and angle for all waggle runs within a bout of dancing (i.e., for all repeated waggle runs in a single continuous period of dancing). Then, we determined how many standard deviations each individual waggle run deviated from the mean (e.g., [(individual waggle run duration in a dance – mean waggle run duration for that dance)/standard deviation for that dance]. Lastly we took the absolute value of this number. In this way, we compared how the first, last, and the middle run deviated from the mean. For dances with an even number of waggle runs, and therefore two middle runs, we randomly selected one of these.

We also investigated whether waggle run variation increased or decreased for dances with more total waggle runs. Do bees, for example, become more consistent the more repetitions they make? To test this we calculated, as above, the mean and standard deviation for both duration and angle for all waggle runs within a bout of dancing. Then, we determined how many standard deviations each individual waggle run deviated from the mean for each run from second to penultimate. We averaged this across all dances to obtain a single value for second through penultimate waggle run variability. As our dances had a variable number of

waggle runs (range 4-72 waggle runs, n = 273 dances), the sample size decreased with circuit number.

We determined whether waggle run duration was correlated, either positively or negatively, with both duration standard deviation and with angle standard deviation. We also investigated whether replacing an "errant" waggle run (as measured by its difference from the mean for that dance) improves the fit of the mean of four waggle run sample compared to the overall mean of all waggle runs in a dance. To do this, for both angle and duration, we took the mean ($\bar{x}_1$) of waggle runs 2-5, which does not include the more variable first and last waggle runs. Within these four waggle runs, we replaced the most deviant waggle run, relative to the mean, with the next waggle run on the same side, left or right. Therefore, this analysis was only done on dances with >6 waggle runs (n=186). Then we calculated the mean of these four waggle runs ($\bar{x}_2$) and the total mean of all the waggle runs ($\bar{x}_t$). We took the absolute value of ($|\bar{x}_t - \bar{x}_1|$) and subtracted the absolute value of ($|\bar{x}_t - \bar{x}_2|$). If the resultant number is positive, this indicates that there has been an improvement, as $\bar{x}_2$ would be nearer than $\bar{x}_1$ to $\bar{x}_t$. If the resultant number is negative, this indicates that there has been a worsening of the fit, as $\bar{x}_1$ would be nearer to $\bar{x}_2$ than $\bar{x}_t$. If the resultant number is zero, this indicates neither an improvement nor a worsening. This was done for angles and durations and was plotted against the SD for that dance.

Lastly, we compared the goodness-of-fit of four waggle runs ($\bar{x}_1$) which did not include either the first or the last waggle run with the total mean ($\bar{x}_t$). The better the correlation, the stronger the fit between our estimated mean based on four waggle runs and the total mean of all the waggle runs in a dance. This was done for both angle and duration.

Results

The first and last waggle runs are significantly more variable than the middle run
Within a dance, the deviation in duration among the first, middle, and last waggle runs differed significantly (First circuit = 0.98 SDs; Middle circuit = 0.65 SDs; Last circuit = 0.86 SDs; One-way ANOVA, F=17.01, $p < 0.001$; Figure 1). This significance was maintained using non-parametric statistics (Kruskal-Wallis, H = 34.6, $p < 0.001$). The first and last waggle runs did not differ significantly in deviation (Tukey's Post Hoc Test, $p = 0.510$) but both had significantly higher deviation than the middle circuit ($p < 0.001$).

Within a dance, the deviation in angle among the first, middle, and last waggle runs also differed significantly (First circuit = 0.90 SDs; Middle circuit = 0.57 SDs; Last circuit = 0.77 SDs; One-way ANOVA, F=20.39, $p < 0.001$; Figure 1). This significance was maintained using non-parametric statistics (Kruskal-Wallis, H = 41.79, $p < 0.001$). The first waggle run angle was the most deviant, followed by the last waggle run (Tukey's Post Hoc Test, p = 0.02 for first vs. last). Both had significantly higher deviation than the middle circuit ($p < 0.001$).
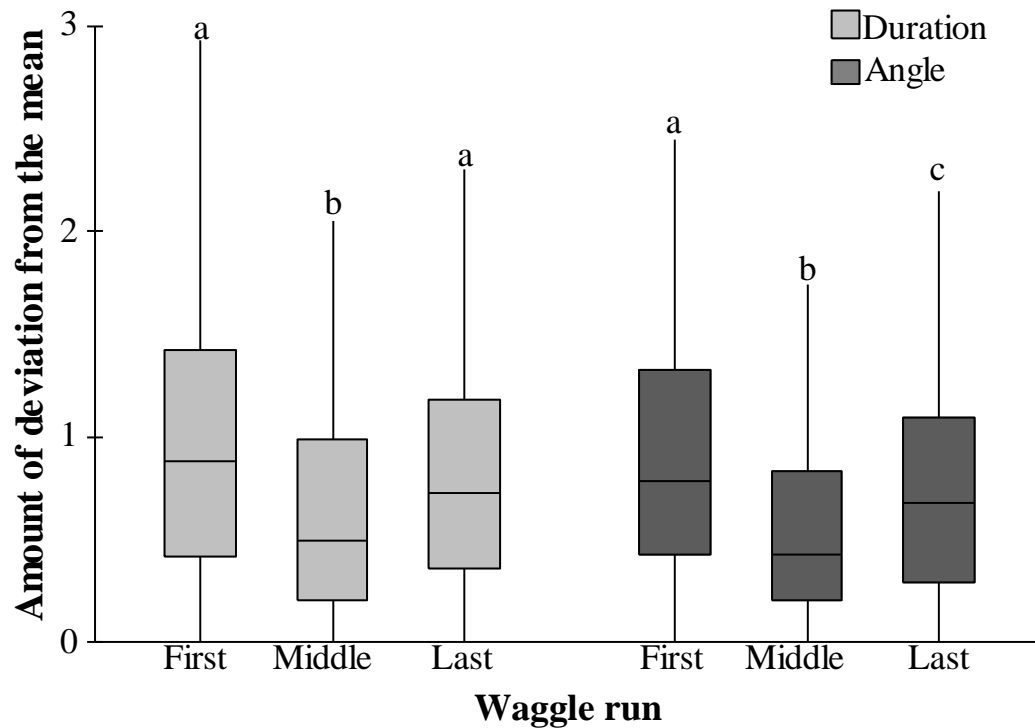
Figure 1

Figure 1: For both duration and angle, the middle waggle run within a dance is significantly less variable than either the first or last waggle run. The deviation shown is the absolute value of [(individual waggle run duration/ or angle in a dance – mean waggle run duration or angle for that dance) /standard deviation for that dance]. The amount of deviation from the mean for the whole dance, was calculated for each circuit within a dance, and the absolute value of these was compared across all dances (n=273 dances).

Variability across waggle runs, excluding first and last, is constant throughout a dance

There was a slight trend towards reduction of deviation with the order of the waggle run within a dance, but no significant decrease in waggle run deviation in duration with waggle run order within a dance from second to penultimate (Regression analysis [residuals were approximately normally distributed], $R^2 = 2.9\%$, p = 0.21) (Figure 2). Similarly, there was no significant decrease in angle but a slight, non-significant trend. (Regression analysis, $R^2 = 4.6\%$, p = 0.11; Figure 2). Therefore, as long as one excludes the first and last waggle runs, there is no significant trend in the remaining waggle run variability.
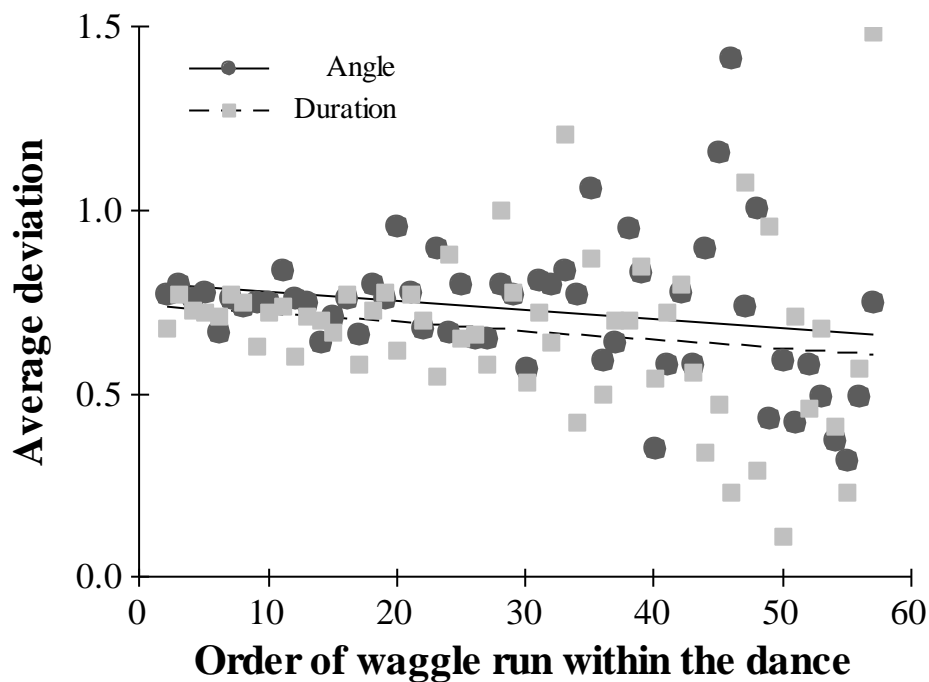
Figure 2

Figure 2: For both duration and angle, there is no significant increase or decrease in variability, in units of standard deviation, was a slight (non-significant) trend towards reduction of deviation with the order of the waggle run within a dance. The deviation shown is the absolute value of [(individual waggle run duration/ or angle in a dance − mean waggle run duration or angle for that dance) /standard deviation for that dance]. Each data point shows the average absolute value of the deviation for the second to penultimate waggle run, for all dances with that made that number of waggle runs (i.e., the sample size reduces) This

means that the data points around x = 2 represent an average of 273 dances, data points around x=20 represent an average of c. 70 dances, and data points x = 40 represent an average of c. 7 dances.

Duration variability is positively correlated with waggle run duration; angle variability is negatively correlated with waggle run duration

Across all dances, mean waggle run duration was positively correlated with the standard deviation of duration within a dance and mean waggle run duration was negatively correlated with the angle standard deviation (Duration: Spearman's Rank Correlation, $\rho = 0.58$, $p < 0.001$; Angle: Spearman's Rank Correlation, $\rho = -0.36$, $p < 0.001$; Figure 3). That is, dances for greater distances (higher mean duration) were more variable in their intra-dance waggle run durations. Dances for greater distances (higher mean duration) were less variable in their intra-dance waggle run angles, confirming what has previously been shown for shorter durations (see discussion).
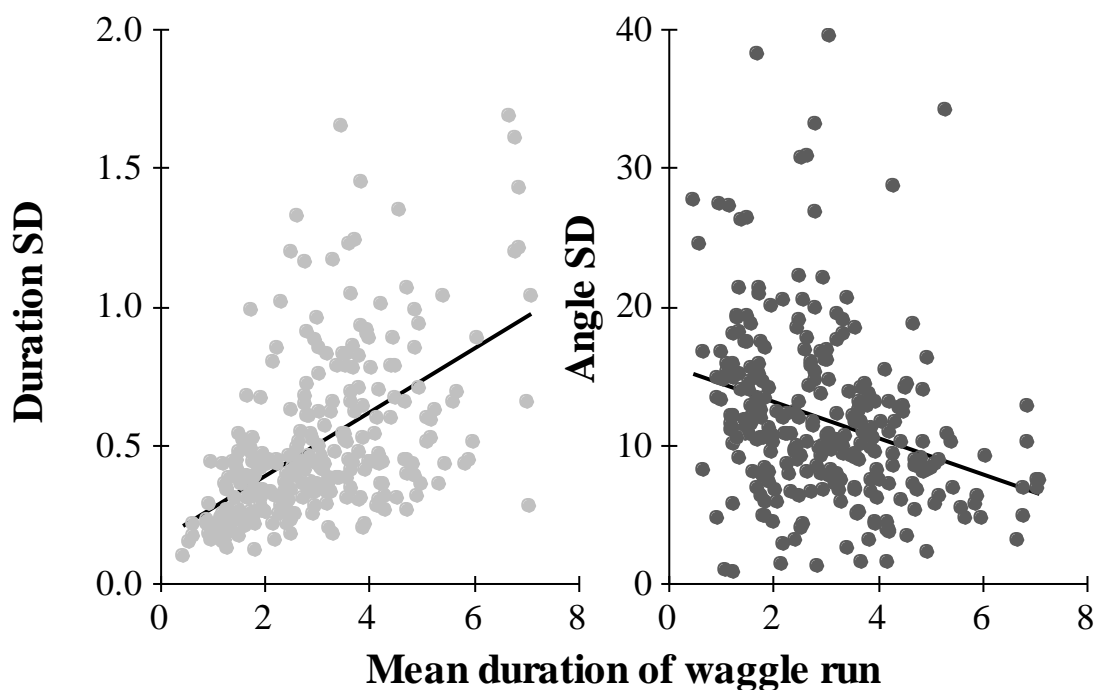
Figure 3

Figure 3: Within a dance, mean waggle run duration (seconds) is positively correlated with duration standard deviation (Spearman's Rank Correlation, $\rho = 0.58$, $p < 0.001$) and

negatively correlated with angle standard deviation (Spearman's Rank Correlation, $\rho = -0.36$, $p < 0.001$). That is, waggle dances describing resources located at a greater distance are more variable in the duration but less variable in angle.

Replacing an errant waggle run does not improve fit between the four waggle run sample and all the waggle runs of a dance.

There was no significant improvement when the most deviant waggle run was replaced with the next waggle run from the same side (to prevent left/right bias) (Regression analysis: Angle: $R^2 = 0.06\%$, $p = 0.31$; Duration: $R^2 = 1.5\%$, $p = 0.10$; Figure 4). This indicates that highly errant waggle runs (not including the first and last waggle run), whose substitution would have an effect on the mean, are rare.
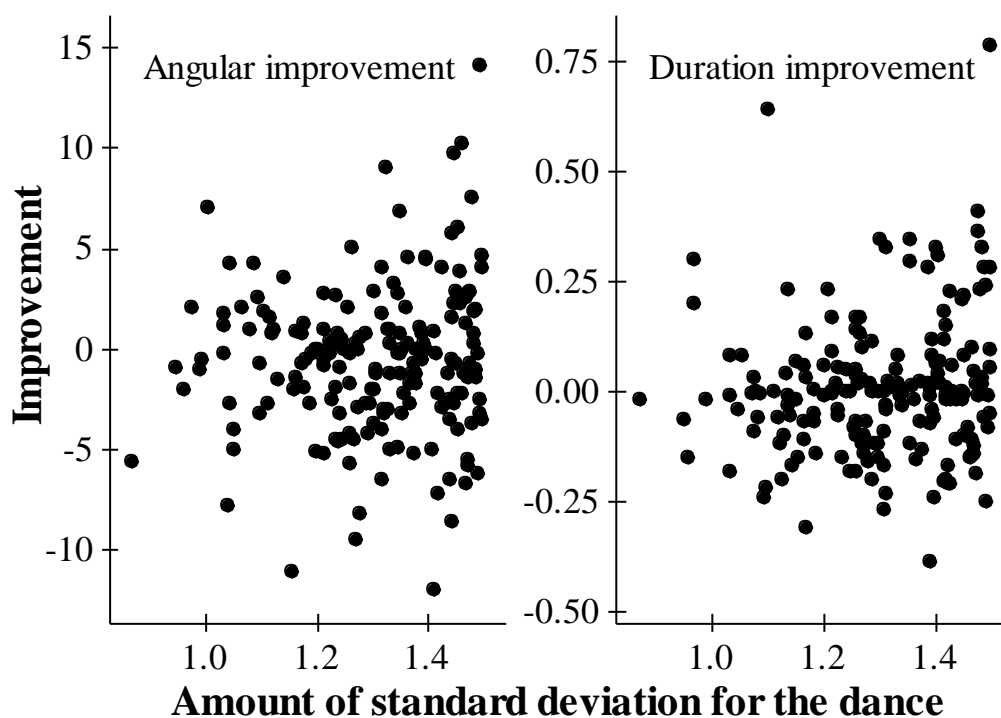
Figure 4

Figure 4: For both angle and duration, replacing the most deviant waggle run of the four waggle run sample does not significantly improve the fit of the sample mean to the overall mean of all waggle runs in a dance. The Y axis represents whether or not replacing an errant run improves the fit and is in units of degrees (panel 1) and seconds (panel 2). A positive number is an improvement, and a negative number is a worsening. There was no significant

trend in either direction for both angle (Regression analysis: Angle: $R^2 = 0.06\%$, $p = 0.31$; Duration: $R^2 = 1.5\%$, $p = 0.10$.).

Our methodology generates angle and duration means that are good representation of the mean for the whole dance

For both angle and duration, there was a highly significant correlation between our four waggle run sample ($\bar{x}_1$) and the mean of all the waggle runs within a dance ($\bar{x}_t$) (Regression analysis: Angle: $R^2 = 99.7\%$, $p < 0.001$; Duration: $R^2 = 97.3\%$, $p < 0.001$; Figure 5).
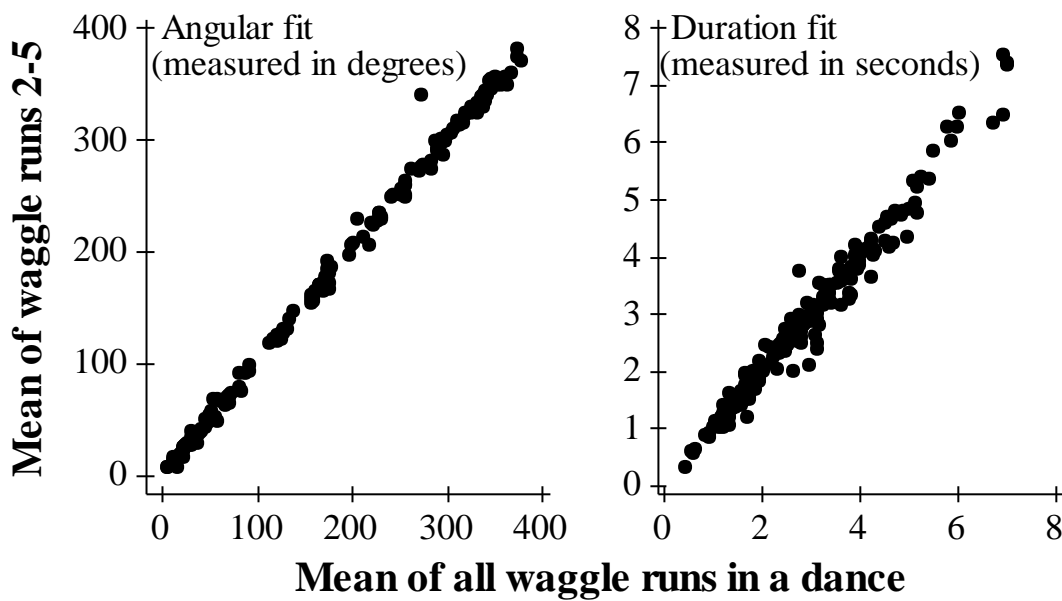
Figure 5

 Figure 5: A sample of 4 waggle runs, not including the first or the last run, from a dance generates a mean that is highly significantly correlated with the mean of all the waggle runs. This is true for both angle ($p < 0.001$, $R^2 = 99.7\%$), which was measured as degrees, and for duration ($p < 0.001$, $R^2 = 97.3\%$), which was measured in seconds.

Discussion

Our results clearly show consistent trends in the variability of waggle runs within a dance that are relevant to waggle dance decoding. In particular, the first and last waggle runs are more variable than the middle run. However, there was no significant trend in variability as a function of waggle run order for both waggle run duration (distance) and angle (direction) when the first and last runs were excluded. In addition, our results show that waggle dances for more distant locations (with longer average waggle run durations) are significantly more variable for duration, even as they are significantly less variable for angle.

What recommendations for dance decoding protocols arise from our results? The fact that the first and last waggle runs are more variable means that these should ideally be avoided. In contrast, the lack of any trend in variability from the second to the penultimate waggle run shows that any of these should be suitable for decoding. Previous studies on angular variability have shown the importance of decoding an even number of waggle runs because angles within a dance cluster around the left and the right of the average direction (Towne and Gould, 1988; Weidenmuller and Seeley, 1999; Gardner et al., 2007; Tanner and Visscher, 2008). These left and right-sided clusters result from alternating waggle runs in which the bee makes its return to the left or right (Towne and Gould, 1988). Therefore, when decoding dances, it is recommended to have equal numbers of left and right-hand circuits to even out any angular biases.

How many waggle runs should be decoded given that a bee may perform from 1- >100 waggle runs within a dance? There is a trade-off between the number of waggle runs per dance and the total number of dances that can be decoded in a given time period. Sometimes it will be more important to decode more dance locations and sometimes it will be more important to obtain more accuracy per dance, even at the expense of total numbers of dances decoded. Ultimately, this decision depends on the type of question being addressed.

For studies that require the decoding of many dances, we recommend that four consecutive waggle runs be decoded. We selected four because the confidence interval of the mean, which is important for both duration and for angle, depends on the square root of the sample size. Therefore, decoding four compared to two runs halves the estimated deviation from the mean. However, to halve it again, sample size would need to increase from four to sixteen. This not only results in a fourfold increase in the time it would take to decode the dance but

also decreases the proportion of dancing bees that make this many waggle runs in a single bout. For example, in our 273 dances, 206 of them (75%) made at least 6 waggle runs and would therefore be suitable for our methodology (i.e., ignoring first and last waggle runs to decode middle four). However, only 64 of 273 dances (23%) had the 18 or more waggle runs necessary to sample 16 runs. Four, being an even number, also takes into account the alternating angular biases. Therefore, decoders should find a bee performing a minimum of six waggle runs, ignore the first and last, and decode the middle four. Duration and angle are then averaged to obtain a single duration and a single angle.

Although we did observe some errant waggle runs that were not the first or the last within the dance, these occurred in less than 2% of the dances. For example, in the first panel of Figure 5, there is one point that does not fall on the line for angular goodness-of-fit. This dance possessed an errant middle run in our subsample (waggle run 2-5 measured angles: 352°, 247°, 350°, 337°), and therefore our sample average angle did not represent the overall average angle. Usually the reason for these truly errant middle waggle runs was because a bee was bumped by a nestmate during the dance. When this happens it is immediately clear to the researcher who is quantifying the waggle run. Hence, the run can be excluded prior to decoding and replaced with a subsequent run on the same side. Here we verify the relative rareness of these errant waggle runs by demonstrating that replacing the most deviant waggle run of four with the next subsequent waggle run on the same side does not improve the fit between the sample mean and the total mean (Figure 4). As it does not significantly help to replace the most deviant run, we recommend against any systematic detection and discarding of waggle runs.

Waggle runs for greater distances tended to be more variable in duration but less variable in angle (Figure 3). This effect of decreasing angular variability with increasing waggle run duration has previously been reported (Beekman et al., 2005) and is attributed to a physical constraint (Beekman et al., 2005; Tanner and Visscher, 2010a; Tanner and Visscher, 2010b). A bee dancing for a nearby location makes short duration waggle runs (<1 sec), which necessitates turning her body almost immediately to begin the next waggle run. This results in less angular precision (more variability) for nearby resources. In contrast, previous reports did not find any effect of waggle run duration on variability of waggle run duration (Beekman et al., 2005), whereas we found increasing variability with increasing duration. The probable reason for this discrepancy is that the previous study used feeders at distances

under 233m, whereas we studied dances that described locations up to c.12km distant. In general, the idea that a bee making longer duration waggle runs has greater difficulty with durational precision makes logical sense: estimating 10 seconds is easier than estimating 70 seconds. Lastly, we show here that our methodology, in addition to increasing the efficiency of dance data collection, also provides an estimate of the resource location that is not significantly bettered when the entire dance is decoded (Figure 5).

That there is intra-dance variation is not surprising, as communication systems sometimes possess a form of error in information transfer (Shannon, 1949; Levin and Miller, 1996; Maynard-Smith and Harper, 2003; Dussutour et al., 2009). As variation seems therefore to be inevitable in waggle dances, it is interesting to note that bees, especially recruits, have evolved some compensatory features. Dance followers take the successive, scattered waggle runs and "average", which is a common method of noise reduction (von Frisch and Jander, 1957; Tanner and Visscher, 2008). Averaging is also what biologists do to determine foraging locations. Here we have just streamlined the process of collecting the data to average.

The decoding of waggle dances can be a powerful tool for studies of honey bee conservation, pollination, and ecology. However, existing technology does not yet allow for automated dance decoding, although this may eventually come (Mitchell, 2005). For now, measurements must be made manually, which is time-consuming. Nevertheless, the honey bee is the only animal that tells a researcher where it has been foraging. To gather comparable ecological data from other species to help in their conservation requires the use of animal-borne radios (Jouventin and Weimerskirch, 1990; Rutz et al., 2007; Burger and Shaffer, 2008) or GPS loggers (Gagliardo et al., 2007; Schofield et al., 2007; Cagnacci et al., 2010), the trackers of which are too big for insects (Wikelski et al., 2007). The use of harmonic radar (Riley et al., 2005; Menzel et al., 2011) with honey bees is limited in the distance it may transmit ($< 1km$) and the terrain over which it must be used (flat). As honey bees may collect food 12-14km from the hive, their foraging range is $> 450 \ km^2$, making harmonic radar and extensive markings/field observations untenable. Here we have shown that the dance decoding process may be streamlined, and we provide a method that is quicker and cheaper than previously used methods.

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Chapter 3

The Effects of Hive Relocation on Honey Bee Foraging

Abstract

Honey bee colonies face major challenges including diseases and reduced food availability due to agricultural intensification. Other challenges have been suggested. Migratory beekeeping subjects colonies to moving stress, both from the move itself and, after the move, by having to forage in a novel environment where they have no knowledge of flower locations. This study investigated whether moving hives into a new foraging area affects colony foraging performance. We moved three colonies housed in observation hives onto the University of Sussex campus from a site 26km away and compared their foraging to three similarly-sized colonies at the same location that had not been moved. Hives were moved carefully and at the end of the foraging day to minimize any stress other than that caused by having to locate new flower patches. We obtained data on: foraging distance by decoding waggle dances, entrance activity by counting departures from the hive, sugar concentration of crop contents or returning foragers, and the proportion of bees returning to the nest entrance with nectar in their crop. We repeated this 3 times (August 2010, October 2010, June 2011) to encompass any seasonal effects. The data show no consistent difference in foraging performance of moved versus resident hives. For example, the mean waggle dance durations of the moved versus resident hives in August, October and June were 2.3s, 1.2s & 1.3s versus 1.8, 1.1s & 1.9s. Overall the results suggest that moving to a new location causes little stress in terms of reduced foraging.

Introduction

Beekeepers routinely move honey bee hives, *Apis mellifera*, to pollinate crops and to take advantage of asynchrony in nectar flows. For example, in the UK some hives are moved into apple farms for pollination in early April and onto heather moors in late July for nectar. In the USA, hives are moved from as far away as Florida to pollinate California almonds in February, a distance of 3000 miles that will take a minimum of 2 to 3 days by truck. Bees are also moved extensively in many other countries (e.g., Turkey, Kandemir *et al*, 2000). Moving hives has been suggested as an aggravating factor in the decline in colony numbers in the

United States (NAS Committee on Pollinators, 2007; Oldroyd, 2007; Ratnieks & Carreck, 2010).

After the WW1, honey bees were increasingly in demand to increase yields of fruit, nut and seed crops via pollination (Olmstead, 1986). The demand for commercial pollination has continued to rise and honey bees are now the most important commercial crop pollinator (NAS Committee on Pollinators, 2007; Delaplane & Mayer, 2000). Although the number of managed hives has increased by about 45% globally since 1961 (Aizen & Harder, 2009), the rate of expansion of pollinator-dependant crops is greater than the increase in the number of managed hives, creating increased demand for pollination. In addition, the number of managed hives has actually declined in Europe and N. America, at an average of 1.79% annually since 1961 (Aizen & Harder, 2009). This potential pollinator shortage is most serious in the USA, where insect pollinated crops are widely grown. For example, the California almond crop, which currently uses over 1 million bee hives for pollination, is worth over $1.6 billion annually (NAS Committee on Pollinators, 2007).

Honey bee diseases (Cox-Foster *et al*, 2007) and the reduction of available bee forage due to agricultural intensification (Goulson, 2003) are two important reasons for the decline in managed bee hives. Other causes have been suggested and include insecticides (Henry *et al*, 2012), climate change (Brown & Paxton, 2009), mobile phones (Sharma & Kumar, 2010), GM crops (Morandin & Winston, 2005), and nanotechnology. Bee colonies can also suffer from various stresses that may exacerbate diseases and other problems. Colonies suffering from European foul brood, for example, often recover fully when provided with adequate nutrition (Crane, 1990). Beekeeping practices such as hive relocation may also cause stress and other undesirable consequences. Close contact of colonies during transport can increase the likelihood of horizontal transfer of pathogens and pests among colonies, and hive movement can spread any newly-introduced pathogen in a new geographic area (Crane, 1990). The process of transport may directly cause stress leading to brood mortality (CCD Steering Committee, 2009), and being moved to a novel environment requires the bees to discover new foraging locations. Nelson and Jay (1989) showed that relocation of hives to a new apiary site can cause significant increase in the disorientation and loss of bees via drifting, particularly on the first day after the move. Moving a colony of bees from a familiar landscape into one they have never experienced should put the bees' ability to rapidly locate food to the test.

The aim of this study was to investigate stress on honey bee colonies caused by having to resume foraging in a new location. To do this we measured four indicators of

foraging: number of bees leaving the hive, proportion of returning bees with nectar, waggle run duration (indicative of foraging distance, von Frisch, 1967), and nectar concentration in relocated versus control hives. The results show no consistent effect of relocating hives on the foraging performance of moved colonies versus resident control colonies.

Materials and Methods

Study colonies and experimental setup

Each trial used six *Apis mellifera* colonies each housed in a glass-walled observation hive with three deep Langstroth frames covered in bees. Each hive was connected to the outside via a tube through the laboratory wall. Colonies were set up in the Laboratory of Apiculture and Social Insects (LASI) several weeks or more before a trial. Each had a laying queen, two frames of brood each with patches covering approximately half the frame, and sufficient worker bees to cover the frames. Each hive had half a frame of capped honey but also had space for further food storage.

Three weeks or more before a trial, three "moved" colonies were relocated from LASI to an observation hive shed at Wakehurst Place (OS Ref: TQ 348 089 GB) near Ardingly, West Sussex and allowed to forage naturally. This is 26km in a straight line from LASI, which is twice the maximum foraging distance of *A. mellifera* (Ratnieks, 2000). The three weeks is sufficient for foragers with experience of foraging around LASI to die (Winston, 1991).

Data were gathered from the 6 hives (3 moved and 3 resident) for 2 days (foraging days b1 and b2) to establish baseline data on foraging performance before the moved colonies were brought to the laboratory. Hives were carefully loaded and transported (<1h) by car in the evening, to avoid losing foragers, and set up that evening at the laboratory. Observation hive entrances were 0.9m or more apart, each with a distinctly coloured and patterned surround (50x50cm) to aid learning of nest location and reduce drifting.

Choice of foraging performance indicators and trial seasons

We chose four indicators of foraging performance. Waggle run duration, crop nectar concentration in nectar foragers, returning bee success, and departing bees per minute.
In terms of departing bees, one possible outcome would be that colonies with no information on local foraging locations (moved colonies) would send a greater proportion of bees into the field to locate resources. Alternatively we could predict that moved hives would show lower departure rates as they do not know where flowers are to have less foraging. Resident

colonies with clear information on available forage should send out fewer workers to reduce risk and forage more efficiently.

Waggle run duration is an indicator of foraging distance (von Frisch, 1967) and is useful in measuring efficiency as flying is costly in terms of energy expenditure and risk. Travelling 8,000m for food when equivalent food is available at 2,000m is inefficient and so honeybees only tend to forage at greater distances when food is in shortage (Beekman & Ratnieks, 2000; Couvillon *et al*, in prep). It may however benefit the colonies to forgo low quality food that is closer to the colony in favour of high quality food further afield. We anticipated that moved colonies would spread their foraging efforts over a wider range of distances in an attempt to locate the most profitable resources whereas the resident colonies would have located these resources and be foraging over a narrower distance range.

Nectar concentration is a good measure of foraging efficiency as sugar is the main energy source for a honey bee colony and honey bees are very sensitive to nectar concentration in foraging (Seeley, 1995). A crop full of sugar-rich nectar is worth more to the colony than the same volume of low-sugar nectar. Honey bee colonies should therefore aim to collect nectar with high sugar content. We predicted that resident colonies would initially bring back higher quality nectar than moved colonies until the moved colonies discover better quality nectar. During the late summer (Late July- early September) (Beekman & Ratnieks, 2000; Couvillon *et al*, in prep), flowers are less abundant than in spring. We, therefore, predicted that during the August trial, foraging bees would take longer to adjust to the new foraging site than in June when weather is normally conducive to foraging and flowers are more abundant. During October, flowering ivy is present and widespread as a food source in the study area and is the major source of forage for honey bees (M. Garbuzov & F. Ratnieks, unpublished data). In addition, however, temperature and weather conditions may have more of an impact on foraging (Couvillon *et al*, in prep). Sherman and Visscher (2002) showed that honey bee colonies lose weight in the autumn and gain in the spring due to differences in nectar availability.

Collection of performance indicator data

Departing bees per minute

Bees leaving the entrance were counted for 30 minutes per day per hive. 30-minute counting periods were initiated at 0900, 1200 and 1500, 10 minutes per hive with 2 observers working simultaneously on different hives. The low number of bees departing (<c.20 per minute, see Fig 1a) meant that counts were accurate and could exclude any obviously non-forging bees

such as drones and bees leaving the hive to defecate (observers did not count bees visibly defecating as foragers, only bees who left the observers field of view).

Nectar concentration and returning bee success

Nectar quality was determined by using a refractometer (Kruss HR25/800, 21°C) measuring the percentage of sugar in the crop contents of bees returning to the entrance tube of each hive. Hive entrances were blocked and ten returning bees (returning workers with visible pollen were excluded) from each hive were captured three times daily at 0900, 1200 and 1500. Nectar was expelled from a bee's crop by applying gentle pressure to the abdomen with a small piece of polystyrene foam. The droplet emerging from the mouth was analysed. Bees were unharmed and were released to resume foraging. Success rate was defined as the percentage of these returning bees with a measurable amount of nectar in their crop.

Waggle run duration

The duration of the waggle run encodes the distance from the hive to the foraging site. We decoded waggle dances to determine foraging distances. Hives were filmed during three periods each day (0900-1000, 1200-1300 and 1500-1600) using CCTV cameras (Sony Super HAD 27x VHR30) to record the waggle dances made by returning foragers. Footage was then uploaded to an iMac computer.

On playback, the duration of the waggle phase was recorded to the nearest frame (1/25 second) using the timestamp in the video analysis software (MPEG streamclip v1.9.2). Videos were made August 23-3 September 2010, 7-13 October 2010, and 7-14 June 2011. Days where bees did not forage due to bad weather were excluded from the analysis.

We decoded the duration of the waggle run as this is more accurate than using the whole dance circuit given that the return phase of the dance circuit can vary in length due to factors such as resource quality (Seeley, 2000), crowding and whether or not food is given to other bees. Dances were decoded according to the protocols in Chapter 2 (Couvillon, Riddell Pearce, *et al* 2012), with only the cameras and video playback software differing. We also recorded the number of dances performed by each colony.

Results

Departing bees per minute

Figure 1a shows the actual mean number of bees departing the hive entrances for the 6 study colonies in the 3 trial periods before and after moving. Figure 1b shows that in all three trials the relative departure rates in the moved colonies dropped from their rates on the two previous days on the first foraging day at the new location (80.1, 32.3 & 37.1% decreases in August, October & June respectively). However, the rates for resident colonies also decreased (3.95, 16.96 & 51.5%). There is no significant difference between the moved and resident colonies' departing worker rates on foraging day 1 (first day after the move) in August (Kruskal –Wallis, H=3.61, p=0.57), October (Kruskal –Wallis, H=0.44, p=0.507), or June (Kruskal –Wallis, H=O.38, p=0.535). There is no significant difference in post-move actual departure rates on all study days combined between moved and resident hives in any trial (One-way ANOVA: August, f=0.07, p=0.788; October, f=3.64, p=0.059; June f=3.37, p=0.096) (see Figure 1a). There was also no significant difference in the number of bees departing per minute on any day after the move other than day 6 of the August trial (Kruskal –Wallis, H=4.26, p=0.039) and day 4 for the June trial (Kruskal –Wallis, H=10.39, p=0.001).
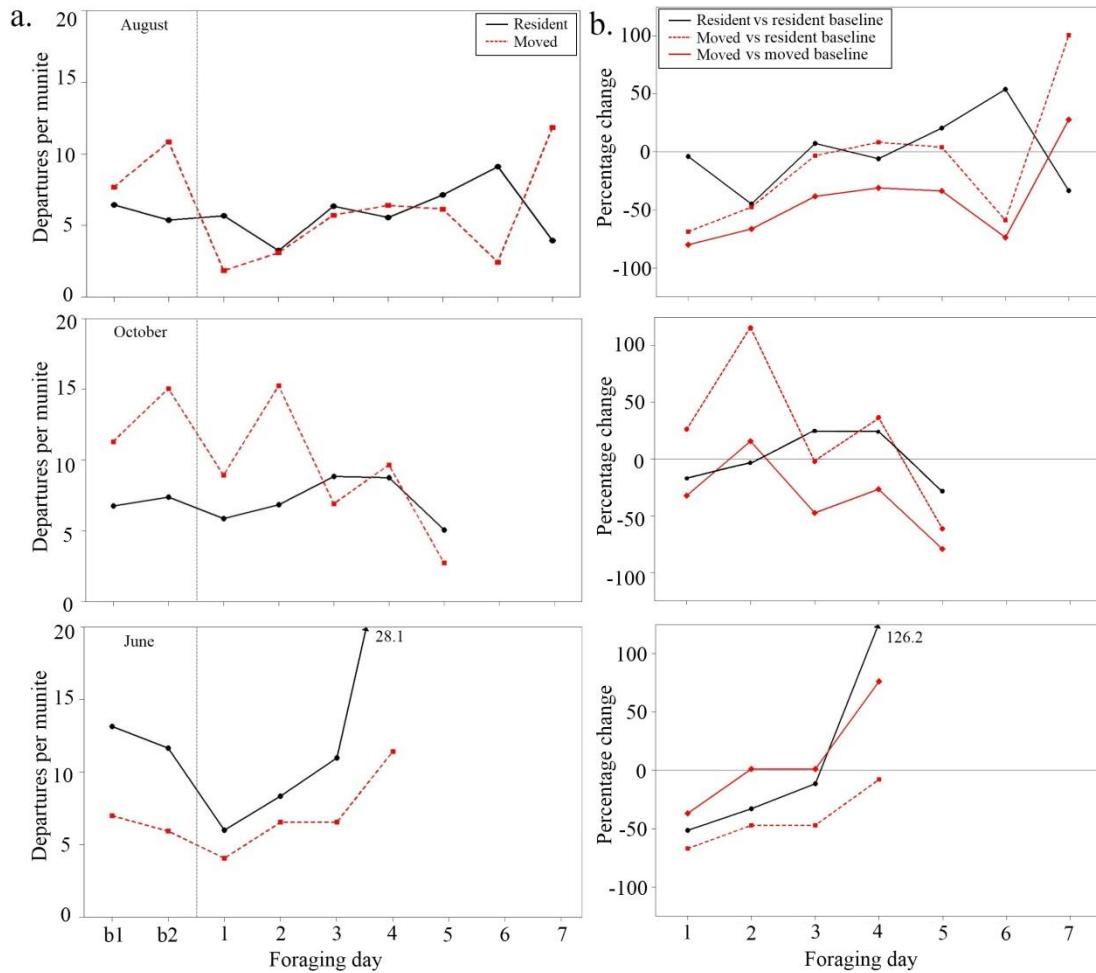
Figure 1. a) Mean actual worker bee departures per minute during the August 2010, October 2011 and June 2011 trials. Days b1 and b2 are the two days immediately before the "moved" hives were moved, (in the evening after foragers had returned to the hive) to the same location as the resident hives. b) Relative changes in departure rate from a baseline. The baseline for the resident hives (black line) is the mean departure rate for days b1 and b2 (i.e., the same hives at the same location). For the moved hives, there are two baselines: departure rate for days b1 and b2 for the resident hives (broken red line) (i.e. different hives, same location) and for the moved hives (solid red line) (i.e., same hives, different location).

Returning worker success

Overall returning workers success was low (<50%) and showed large fluctuations. Success decreased after the move for both resident and moved colonies in August (mean of days b1 & b2= 5%, mean of days 1 & 2= 4.45% for resident; mean of b1 & b2=0.55%, mean of days 1 & 2 =0% for moved colonies); increased for both resident and moved in October trial (resident, 29.3% to 31.9%; moved, 12.2% to 39.1%); and increased for resident colonies and fell for moved colonies in June trials (resident, 10.6% to 31.1%; moved 35.6% to 22.2%). Despite these fluctuations in foraging success, the data lines for resident and moved hives overlap in all three trial periods showing that resident colonies were not consistently or significantly more successful in any trial, on any day during the three trial periods other than day 1 in August (One-way ANOVA, f=9.14, p=0.039).
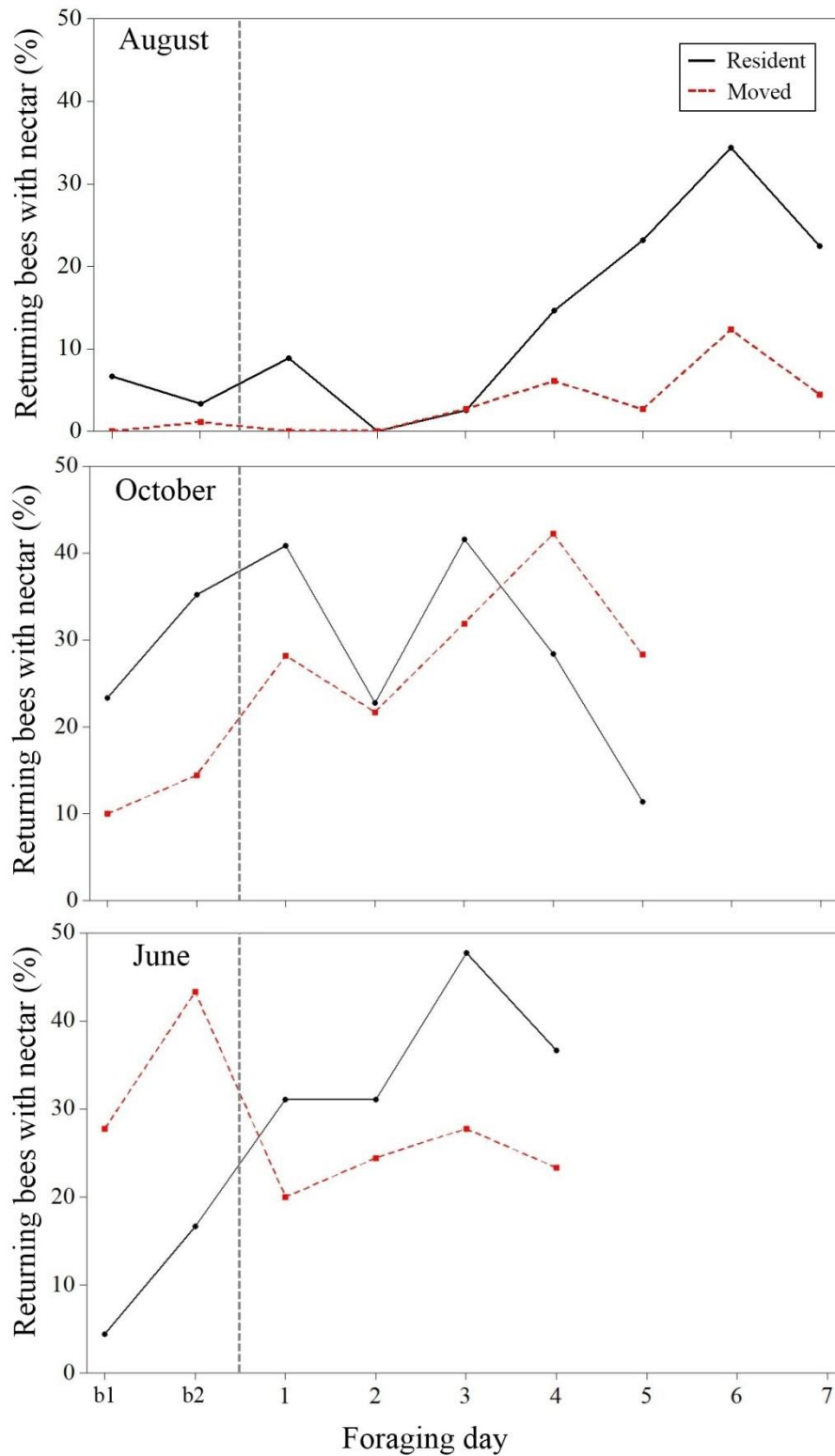
Figure 2. Proportion of returning worker bees with detectable nectar amounts in their crop in the August 2010, October 2011 and June 2011 trials before (b1 & b2) and after (days 1-7) the "moved" colonies are relocated to LASI. The vertical grey dashed line indicated the time of the relocation of hives. To the left of this line resident colonies (black line) are at LASI but

moved colonies (red line) are at Wakehurst Place. Data on the right og the vertical line are for all colonies at LASI (i.e after the move).

Waggle run duration

Figure 3 shows that on the first foraging day after the move, moved and resident hives foraged at similar distances, as indicated by similar waggle run durations, in the August and June trials (One-way ANOVA: August, f=1.54, p=0.22; June f=0.24, p=0.631). However, in the October trial, the resident hives performed no dances on day 1 while the moved hives danced normally. In August the resident and moved colonies foraged over similar distances on all days other than days 5 and 6 when the moved colonies foraged at greater distances than the resident colonies (One-way ANOVA: f=4.55, p=0.037; f=12.38, p= 0.001 for days 5 and 6 respectively) and over a greater range of distances (mean waggle phase duration, resident =1.76s, moved =2.27s; range, resident =3.75s, moved =7.86s).

During October the foraging distances of resident and moved colonies were different before the move but not significantly different on any day after the move (f=021, p=0.646), (other than foraging day 1, see above). In June, resident colonies foraged at greater distances on days 2, 3 and 4 (mean, resident, 1.9s; moved, 1.3s) than moved hives (One-way ANOVA: f= 18.56, p<0.005; f= 6.73, p=0.011; f= 8.12, p=0.005 on days 2, 3 & 4 respectively).
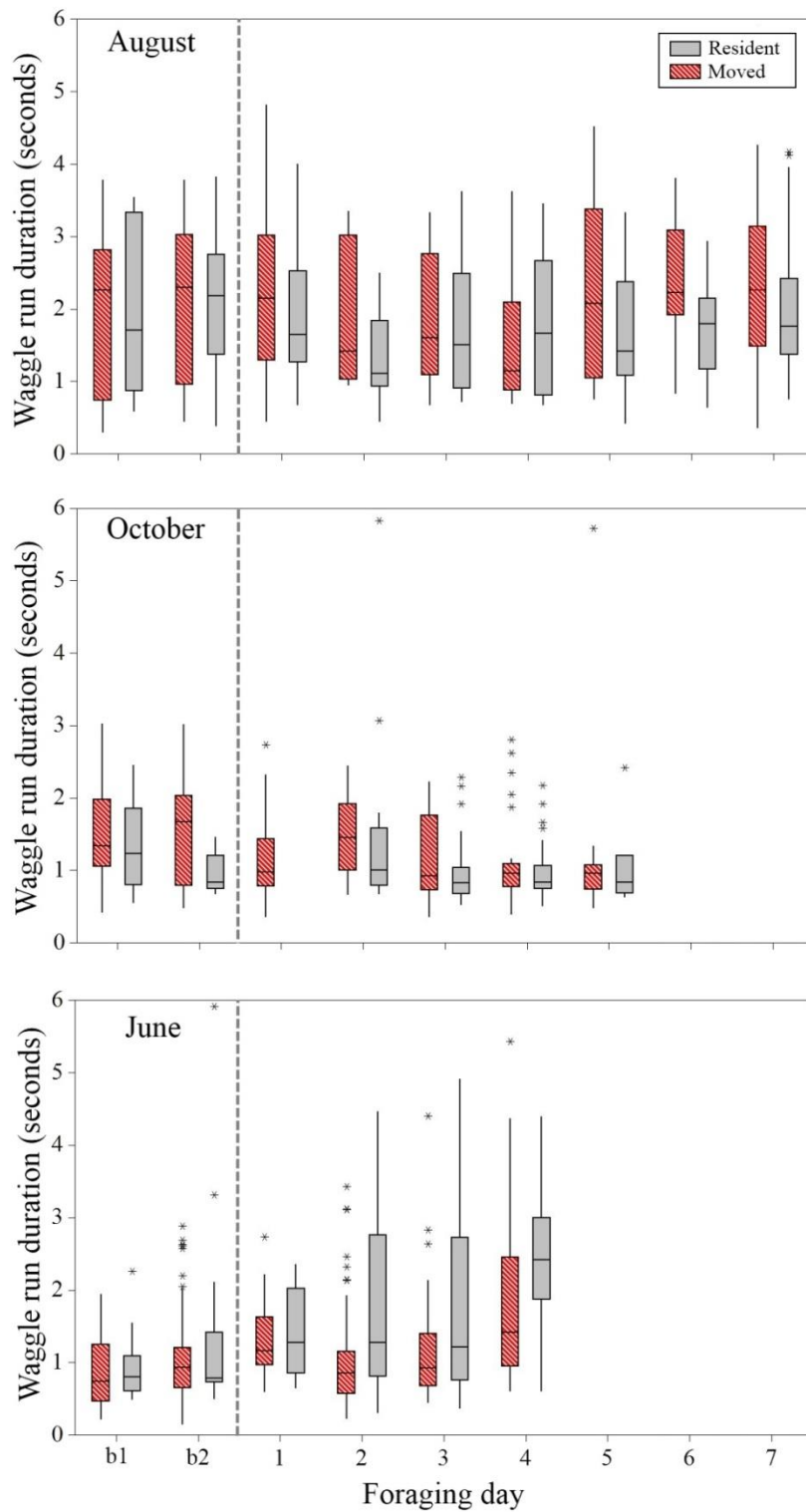
Figure 3. Durations of waggle runs of dancing bees in August, October and June trials. Longer waggle runs indicate greater foraging distance. Days b1 and b2 are the two days immediately before the "moved" hives were moved, in the evening after foraging had ended, to the same location as the resident hives. The vertical grey dashed line shows the time the

moved hives were relocated to LASI. Boxes show the interquartile range and the middle bar shows the median.

Nectar concentration

Moved hives failed to return with measurable nectar until day 3 after the move in August. The resident hives collected nectar with a mean concentration of 18.2% and 18.6% on days b1 and 1 but failed to collect nectar on days b2 and 2. There were fluctuations in the concentration of nectar collected between hives and trial periods but moved and resident hives brought back similar concentrations of nectar after the move with the exception of day 4 in August where moved hives found better quality nectar (56.8% vs. 45.1%; One-way ANOVA: f=15.29, p=0.001) and day 3 in October where the resident hives brought back higher quality nectar (32.2 vs. 28; One-way ANOVA: f=4.45, p=0.037). In October, moved hives found poorer quality nectar before the move than resident colonies (One-way ANOVA: b1, f=31.18, p<0.000; b2, f=21.66, p<0.000), however, on the first day after the move there was no significant difference between the nectar concentration found by moved and resident colonies (One-way ANOVA: f=0.13, p=0.721).
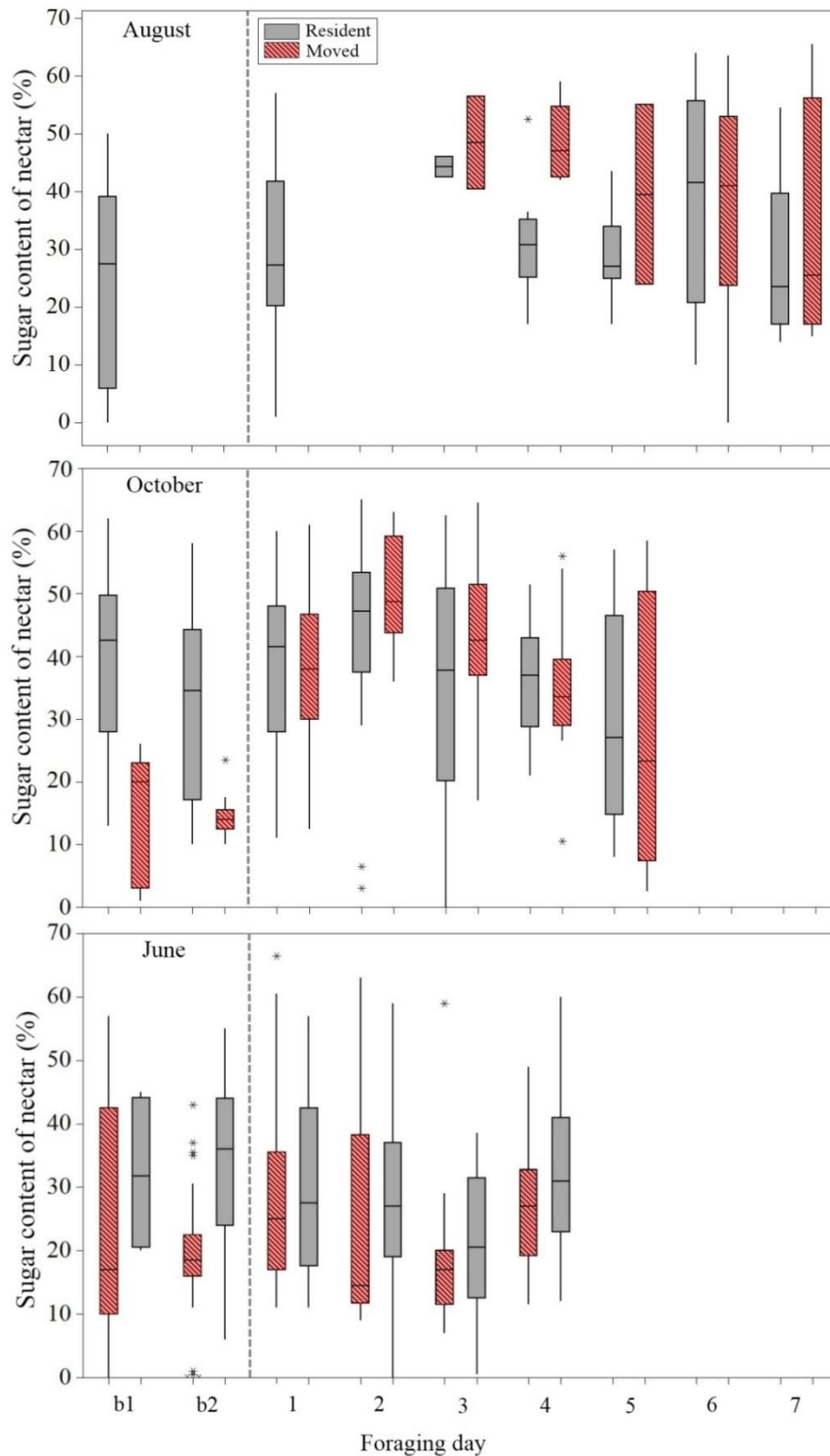
Figure 4. Sugar concentration of nectar in a sample of returning worker bees in the three study periods, August 2010, October 2011 and June 2011. Days b1 and b2 are the two days immediately before the "moved" hives were moved, in the evening after foraging had ended, to the same location as the resident hives. The vertical grey dashed line shows the time the

moved hives were relocated to LASI. Boxes show the interquartile range and the middle bar shows the median.

Mean number of dances performed

During the August trial moved and resident colonies performed similar numbers of dances both before and after the move (mean dances per day before, 25.5 & 26.5; after, 21 & 25.6). However, during the October trial moved hives performed more dances than the resident hives before (moved, 37 per day; resident, 14.5) and after (moved, 36.7 per day; resident, 19.2) the move. In the June trial moved hives performed more dances than the resident hives before the move (68.5 vs. 19) but fewer after the move (41.8 vs. 58.3).
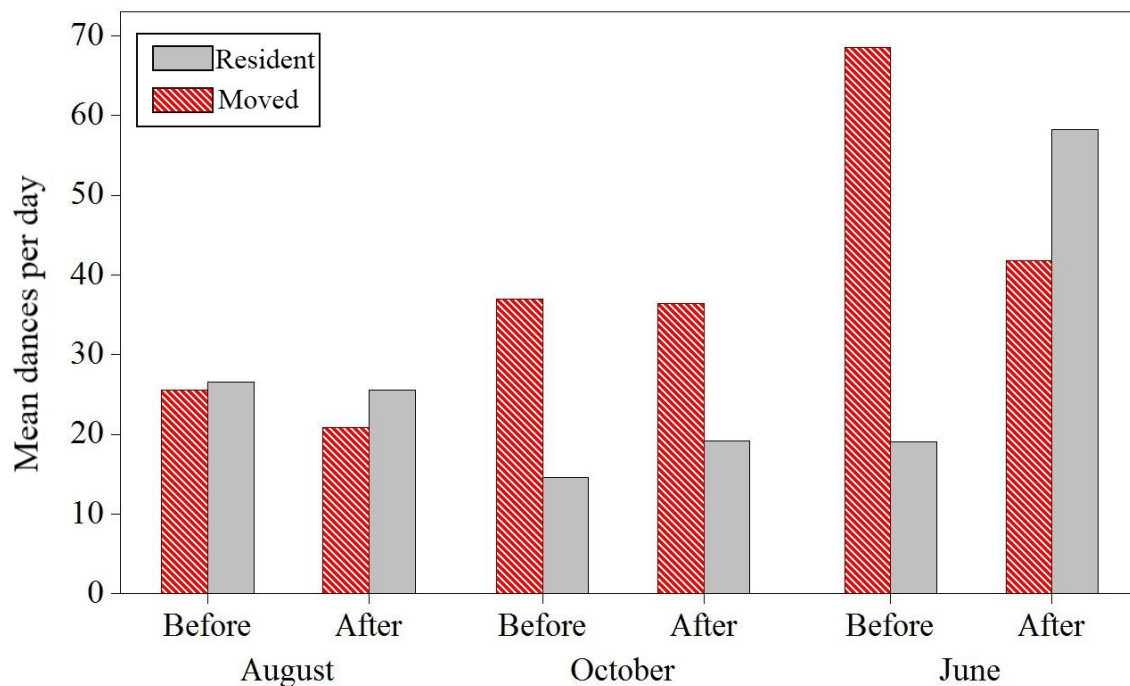

Figure 5. Mean number of dances per day (from all 3 hours of video footage per hive per day) before and after the move in the August 2010, October 2011 and June 2011 trials.

Discussion

Our results show no consistent differences in the four measures of foraging performance for resident versus colonies moved into the same location. There were differences in foraging performance before the move, such as lower mean nectar concentration for moved hives in October (14.5% vs 35%) and lower mean departures per minute for the moved hives than the resident hives in June (12.4 vs 6.5), presumably due to the difference in location and food availability. Overall, climatic conditions and seasonal resource availability were more likely an influence on the foraging performance of both moved and resident colonies than the relocation to a novel environment. Bees were able to rapidly find new and high quality sources of food after the move with moved hives bringing back similar quality nectar to resident colonies on the first day after the move in October and June (for August, see below). If foraging performance was poor it was poor for both resident and moved colonies.

In the case of departure rates there was no consistent trend for the moved hives to have lower rates with the exception of day 1 in the August trial (figure 1a). Although departure rates of moved colonies dropped immediately after the move, they also dropped for the resident colonies suggesting that weather conditions were more likely to be the cause with 0.2mm & 0.6mm of rain on b1 & b2 in August and 0mm on days 1-7.We found a great deal of overlap in the changes in departure rates of the colonies in all three trials, indicating that moved and resident colonies were changing their departure rates by similar proportions (figure 1b). There was no clear or consistent trend in the success of returning workers other than the fact the success was surprisingly low, with less than 50% of returning workers having nectar in their crop. In August workers experienced the lowest success and less than 10% of both resident and moved returning workers had measurable nectar, with both groups having 0% success on day 3. Cool damp weather reduces nectar availability to insects (Peat & Goulson, 2005) and was almost certainly an important factor in poor foraging performance in the August trial in addition to August being the most challenging month of the year for bees to find food as bees are forced to travel much greater distances (Beekman & Ratnieks 2000; Couvillon *et al*, in prep).

 In the October and June trials, during which the weather was drier than in the August trial (1mm of rain fell on day 1 and 0.2mm on day2 in June but this was overnight and did not interrupt foraging), success was greater, but never more than 50%. The nectar collected on successful foraging days after the moves was of similar concentrations for resident and moved hives on all days other than day 6 in August and day 4 in June. The nectar collected by the moved hives before the move was of lower concentration than that located after the

move by both groups and before the move by resident colonies. However, on the first day after the move both groups had located similar quality nectar. Variation in plant availability at the two sites can account for this difference. Greater coverage of ivy (*Hedera helix*), which is a major food source for honeybees from early September to early November was observed at LASI than at Wakehurst place.

In the case of foraging distance, as shown by dance decoding, foragers from both moved and resident colonies foraged at similar distances during most foraging days. On the days where the resident and moved hives did forage at different distances, the moved hives foraged at greater distances than resident hives in August (days 5 & 6) but the reverse was the case in June (days 2, 3 & 4). In October, the resident hives performed no dances on day 1 while the moved hives danced normally, one possible explanation is that the resident hives did not dance as they knew where flowers were but they were not sufficiently exciting to elicit dancing, whereas the threshold for the moved hives was lower so they did dance. Sherman and Visscher (2002) found that the waggle dance was more important in southern California under winter foraging conditions, with colonies prevented from performing oriented waggle dances losing more weight. We found that colonies performed more dances per day in June, and there was no significant difference in the mean number of dances per day for either resident or moved hives before or after the move in August or October. A possible explanation for this is that there was more dancing in June because there was more available food whereas although dancing may be more important in August, there were fewer sources available worth advertising.

A colony of bees has many foragers in the field at once, up to 25% of the colony's workers (Seeley, 1995). These foragers collect information on food availability over an area surrounding the hive of up to 100km^2 (Beekman and Ratnieks 2000) and share this information with their nestmates via the waggle dance. Seeley (1987) showed that if a food patch (100m^2) is within 1000m of the hive entrance there is a 70% chance of the colony locating it. This chance drops to 50% for a patch located 2000m from the hive entrance. Once a resource has been located by a scout, the number of visiting foragers increases rapidly as recruits are informed via the waggle dance (Seeley & Visscher 1988). Seeley and Visscher (1988) also showed that the waggle dance allows colonies to locate better quality food and that scout bees can discover a flower patch 610m away within 200 minutes of it being placed (sooner for nearer resources). Large numbers of recruit bees, presumably directed by the scout's waggle dances, then arrived within 50 minutes of its discovery by the scout. This shows that honey bee colonies have considerable ability to rapidly track both spatial and

temporal changes in food availability. If food is available in the landscape it is likely a honey bee colony will locate it. The location of floral resources varies with season, but also from day to day, and even at different times of day as some plants only produce nectar at certain times of day (Irwin *et al*, 2004). The fact that honey bee colonies have evolved mechanisms to track these changes may mean that a colony moved to a novel environment is not at a great disadvantage. In addition, honey bee colonies naturally change their location when a swarm establishes a new nest. In European A. mellifera, the new nest is within a few kilometres of the natal nest (Seeley 2010, Honey bee democracy). However, swarms of African *A. mellifera* (Ratnieks, 1990) and Asian *Apis dorsata* (Paar *et al* 2000) migrate longer distances. The Asian giant honeybee *Apis dorsata* forms massive single-comb colonies which usually hang from a tree branch or the eaves of buildings. Although colonies regularly migrate over many kilometres, we find that they often return to their original nest site — even after an absence of up to two years. How the bees do this is unknown, as workers live for only a few weeks.

This study involved moving bees to a novel location, but which was similar in terms of climate and available forage. It is possible that moving bees over much larger distances into different climatic conditions and resource availability may have a much greater impact on foraging efficiency. It is also possible that it may take longer to adjust to a new foraging location if the plant species are different from the known location as odour memory plays an important role in foraging and location of food sources (Hammer & Menzel, 1995). Free (1959) showed that after a move, foragers tended to forage on species they had previously visited if they were available. However, our study has produced some encouraging results. With many studies focusing on factors which can potentially harm bees, such as pesticides, pest and pathogens, and lack of forage (Henry et al, 2012; Cox-Foster *et al*, 2007; Goulson, 2003) and much of the media focused on the decline in honeybees and other pollinators (Oldroyd, 2007; Aizen & Harder, 2009; CCD steering committee, 2009; Potts et al, 2010; Ratnieks & Carreck 2010, & Carrington, 2012) it is reassuring to find a factor which seems not be detrimental to honey bee colonies.

Alternative methods

After the completion of this study there are aspects of the experiment which I now feel could be carried out more effectively. The use of observation hives was very useful to allow observation of the dances of returning foragers but they contain far fewer bees than a standard hive. Using larger hives may give a more natural picture of the foraging efforts.

Weighing our hives could provide important information on foraging success of colonies. We did weigh our hives during this study, but artificial feeding was required to keep the small colonies from starving during bad weather; as such the weight of hives fluctuated greatly and was of little value in determining foraging success. Using large, self-sufficient hives that do not require food supplements would allow a comparison on the mass of resident and moved colonies.

Measuring the volume of nectar brought into the hive by returning foragers, as well as its sugar concentration, would provide more information on the quality and availability of resources located. However, this would require more accurate methods of extracting the nectar from the crop of the bee or accurate measurement of individual bee mass on leaving and returning to the hive. Including the mass and quality of pollen brought into the hive would also add further information on how each colony is foraging, but keeping the area of brood in each colony would need to be closely monitored to allow for fair comparison.

In addition to investigating the foraging durations based on bees waggle dances, the efficiency of a foraging trip could be examined by recorded the period of time a forager spent out on a foraging trip using RFID tags. This information, coupled with the quality and volume of nectar brought back, would be an extremely useful tool in investigating forager efficiency.

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