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Honey Bee Foraging: Persistence to Non-Rewarding Feeding Locations and Waggle Dance Communication

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A thesis submitted for the degree of Master of Philosophy

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Declaration

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 another degree.

Hasan Al Toufailia

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Summary

The honey bee, *Apis mellifera*, is important in agriculture and also as a model species in scientific research. This Master's thesis is focused on honey bee foraging behaviour. It contains two independent experiments, each on a different subject within the area of foraging. Both use a behavioural ecology approach, with one investigating foraging behaviour and the other foraging communication. These form chapters 2 and 3 of the thesis, after an introductory chapter.

Chapter 2. Experiment 1: Persistence to unrewarding feeding locations by forager honey bees (*Apis mellifera*): the effects of experience, resource profitability, and season

This study shows that the persistence of honey bee foragers to unrewarding food sources, measured both in duration and number of visits, was greater to locations that previously offered sucrose solution of higher concentration (2 versus 1molar) or were closer to the hive (20 versus 450m). Persistence was also greater in bees which had longer access at the feeder before the syrup was terminated (2 versus 0.5h). These results indicate that persistence is greater for more rewarding locations. However, persistence was not higher in the season of lowest nectar availability in the environment.

Chapter 3. Experiment 2: Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour

This study shows that honey bee foragers follow fewer waggle runs as the distance to the food source, that is advertised by the dance, increases, but invest more time in following these

dances. This is because waggle run duration increases with increasing foraging distance. The number of waggle runs followed for distant food sources was further reduced by increased angular noise among waggle runs within a dance. The number of dance followers per dancing bee was affected by the time of year and varied among colonies. Both noise in the message, that is variation in the direction component, and the message itself, that is the distance of the advertised food location, affect dance following. These results indicate that dance followers pay attention to the costs and benefits associated with using dance information.

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Chapter 1: General Introduction

1.1 How it started

I graduated in 2007 from the University of Damascus in Syria with a BSc degree (1st class) in Agricultural Engineering and remained there as a teaching assistant in the Department of Plant Protection. In 2009 the University of Damascus awarded me a scholarship to carry out a full-time Masters/Doctoral degree course in the UK on my favourite subject: honey bees. I wanted to obtain broad training in honey bee biology, both basic and applied, and then to return to Syria as a lecturer in apiculture at the University of Damascus.

I then started looking for a suitable UK university. After the first click of a Google search about honey bee research in the UK it became evident that the most active laboratory was that of Professor Francis Ratnieks who had recently moved to the University of Sussex. I was also attracted to Sussex because of its good reputation in research in general.

Professor Ratnieks accepted me for a Masters/Doctoral program. I came to the University of Sussex in August 2010 to do an English course and then joined the Laboratory of Apiculture and Social Insects in October 2010. Because honey bees are not active during winter, field work can only take place from April to October in the UK. As a result, my first project had to be something I could start in October. Professor Ratnieks suggested that one suitable project would be to study the persistence of foragers at feeding locations that become unrewarding, and how the duration of their persistence is affected by the rewards they received while it was rewarding. This project was suitable as honey bees are easy to train to feeders in the autumn. I was able to complete some preliminary field work by the end of October 2010, collecting some data

and developing the necessary methods. These preliminary results were promising and allowed me to plan a project during the winter and to start in spring 2011, with data also being collected in the summer and autumn under environmental conditions of differing nectar availability.

Before field work began in the spring of 2011 I also sat in on the undergraduate lecture courses in Behavioural Ecology and Social Insects, read up on honey bee foraging behaviour, and attending workshops at the University of Sussex on topics relevant to my research (using Endnote software for making reference lists in scientific publications, using the SPSS and Minitab programs for statistics and learning about the honey bee dance language). In addition, I also attended the BBKA (British Beekeepers Association) two-day conference in Stoneleigh, Warwickshire, where I attended talks and met bee scientists and beekeepers. I also attended the IUSI meeting on social insects in London 2010.

In early 2011 I also started on a second project on honey bee foraging. This one was on the waggle dance, specifically on the behaviour of bees that follow the dance to obtain information about foraging locations. This project was a contrast to the first project and mostly involved the analysis of videos of honey bee dancing made using observation hives. It was helpful to my training to have two rather different projects, but both in the same general area of honey bee foraging. Each of these projects is presented as a chapter in the thesis and will also be submitted for publication in suitable journals. In addition to my supervisor, I also worked closely with two postdoctoral researchers at LASI who are honey bee biologists: Drs. Christoph Grüter and Margaret Couvillon.

1.2 Thesis Projects to be submitted for Publication

1. Al Toufailia H, Grüter C and Ratnieks FLW. Persistence to unrewarding feeding locations by forager honey bees (*Apis mellifera*): the effects of experience, resource profitability, and season.
2. Al Toufailia H, Couvillon M, Ratnieks FLW and Grüter C. Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour.

1.3 Scientific Background

The honey bee (*Apis mellifera*) is one of the best studied insects. Honey bees live in highly organised eusocial colonies. The honey bee is important ecologically and economically because of their role in pollination in honey production, and is also an important species for basic scientific research. The number of honey bee colonies in the UK has declined c. 75% during the last century, and 25 % in Europe since 1985 (Potts et al. 2010). Several factors contribute to this decline including loss of flowers in the environment, pests and diseases. Due to the decline in hive numbers and the increasing need for pollination, research on honey bee foraging behaviour is important. In addition, research on honey bee foraging can make an important contribution to basic biological knowledge.

1.3.1 Foraging behaviour of honey bees

Honey bees (*Apis mellifera*) forage mainly on nectar and pollen which they collect from flowers. Before starting to forage, a worker bee learns landmarks surrounding the nest location on training flights. Honey bees have an excellent memory and quickly learn the position, colour, shape, and odour of rewarding food sources while foraging (Frisch 1967; Giurfa et al. 1999; Menzel 1968; Ronacher 1998). Honey bees show a high

degree of flower constancy (Aristotle ca. 340 B.C; Darwin 1876; Ribbands 1949). That is, they use the learned information to select previously rewarding flowers while ignoring other flower species (Chittka et al. 1999; Menzel 1999).

Choosing good foraging locations is important, and in the social insects nestmates often help each other to do this. Experienced foragers can use a variety of different mechanisms to direct their nestmates to food sources, including the pheromone trails used by many ants (Hölldobler & Wilson 1990) and the waggle dance of honey bees (*Apis*) (Gould et al. 1970; Seeley & Towne 1992). In many species, however, including bumble bees and many stingless bees, foragers cannot direct nestmates to specific food locations but can recruit them to foraging or give them some information about the properties of the food source, such as scent and quality (Dornhaus & Chittka 2004; Nieh 2004).

In honey bees, foragers working rewarding patches of flowers can communicate to nestmates the location (direction and distance) of a food source by means of the waggle dance, which was discovered 1944 by von Frisch and has subsequently been studied by many other researchers (Beekman & Ratnieks 2000; Beekman et al. 2004; Frisch 1967; Seeley 1995; Seeley & Visscher 2004; Waddington et al. 1994). A successful forager returns to the nest to perform the waggle dance. During this dance, the worker bee waggles her abdomen as she walks in a straight line (waggle run) then turns either to the left or right to return to the starting point of the previous waggle run (return phase), before she repeats the waggle run (Frisch 1967). The waggle run provides the direction (encoded in the angle between the body of the dancer and the direction of gravity) and distance (encoded in the duration of the waggle run) between the nest and feeding location (Frisch 1967). At least three types of information are provided to followers by a dancing bee: the presence of a rewarding food source (Frisch

1923; Thom et al. 2007), the distance and direction to the exploited food source (vector information, the 'dance language' ; Frisch 1967; Riley et al. 2005) and the food source odour, which is brought to the nest on the forager's body and in the nectar transferred to other bees during trophallaxis (Díaz et al. 2007; Frisch 1967; Gil & Farina 2002).

Foragers of many social insects are able to modulate their recruitment effort in response to the quantity and quality of food (Breed et al. 1987; Taylor 1977). For example, recruitment in the giant tropical ant *Paraponera clavata* depends on the amount of food that is available and the distance of the food source from the nest. In *P. clavata* and in honey bees, fewer workers recruit to a resource location when the distance to the nest increases (Fewell & Winston 1992; Frisch 1967). Honey bee foragers also vary in their probability to perform a recruitment dance depending on reward, including sucrose concentration and the rate of nectar flow (Frisch 1965; Seeley 1995).

A honey bee colony forages at distances of up to c. 12km (Beekman & Ratnieks 2000) and selectively exploits the most profitable food patches (Butler 1945; Seeley 1986). Honey bee colonies flexibly adjust their preference between flower patches depending on their internal and external situation. Foragers will accept only highly concentrated sugar solutions when the nest already contains a lot of honey stores, but the colony will accept even extremely dilute even (0.125 M) sugar solutions when starvation is imminent (Lindauer 1949) or when flowers are scarce (Downs & Ratnieks 2000).

1.3.2 Memory and foraging persistence

If the conditions are suitable for foraging, foragers will revisit profitable patches for days or even weeks (Butler 1945; Ribbands 1949). Route memory information enables forager honey bees and ants to return to known food locations for weeks or even months (Collett et al. 2003; Ribbands 1949). However, formerly profitable food patches are likely to disappear over time. Foragers faced with a declining resource must decide whether to persist at that resource or abandon it and search for a new food source elsewhere. If a forager decides to persist, it is likely that the foraging conditions and the economic value of the food source experienced before the deterioration affect this decision to abandon a food source. In Chapter 2, I tested the hypotheses that the persistence in visiting an unrewarding food source depends on the number of previously successful foraging trips to the food source, sucrose concentration, distance from the hive, and season of the year.

1.3.3 Dance-follower behaviour

If a forager abandons a food source after it has become unrewarding or if the forager is new to foraging (naïve forager), she can use waggle dance information provided by her nestmates to find a new food source. This requires that a forager following a dance for several waggle runs. Little is known about how waggle dance followers are able to decode the waggle dance information in the darkness of a hive. The information might be transferred by airborne sounds or air-flows (produced by the wings of the dancers), vibrations transmitted via the comb, or other tactile cues (Dyer 2002). The body position of the dance follower relative to the dancer might be important in order to acquire the vector information of the dance (Judd 1994).

Dance followers show considerable variation in the number of waggle runs they follow, from 1 to >20, in a single dance. Following more waggle runs improves informational accuracy as it has been shown to improve the accuracy of the flight paths of recruited bees (Tanner & Visscher 2009). This might be because variation exists amongst the waggle runs (De Marco et al. 2008; Dyer 2002) with dance followers (recruits) averaging multiple waggle runs to derive a single, more precise, vector (Frisch & Jander 1957; Tanner & Visscher 2008).

Previous experiments have shown that honey bee foragers need to follow more than one waggle run to locate an advertised food source. In different studies, the number was on average 8 (Judd 1994), 17 (Grüter et al. 2008), 15.5 (Wray et al. 2012) or 20-23 waggle runs (Menzel et al. 2011). Previous works suggests that dance followers need to follow about 5 waggle runs to have good information about the advertised food location (Biesmeijer & Seeley 2005; Esch & Bastian 1970; Frisch & Jander 1957; Grüter & Farina 2009a; Mautz 1971). Biesmeijer & Seeley 2005 was the only study that studied the behaviour of dance following for natural food sources as opposed to syrup feeders. They concluded that only 12-25% of followers discovered an advertized unfamiliar location but that the other followers are reactivated to foraging. Reactivation may be much simpler as it only requires the dance follower to detect a familiar floral odour on the body of the dancing bee.

It is not possible to investigate dance follower behaviour for natural dances after the followers leave the hive due to the large distances and speed of flight. Chapter 3 investigates how the characteristics of waggle dances for natural food sources and environmental factors affect dance follower behaviour. I test the hypothesis that food source distance and the waggle run noise affect dance follower behaviour, and also

whether season of the year and overall dancing activity affect the number of dance followers per dancer and the number of waggle runs followed.

Chapter 2: Persistence to unrewarding feeding locations by forager honey bees (*Apis mellifera*): the effects of experience, resource profitability, and season

2.1 Summary

The aim of this study was to investigate how the previous experience of honey bee foragers at a feeding location affects their persistence at that location once food is no longer available. We hypothesized that persistence would be greater to locations that were more rewarding (closer to the hive, higher concentration of sucrose solution), for which a bee had greater prior experience (0.5h versus 2h training access), and at times of the year of lower nectar availability in the environment. We studied individually-marked worker bees from 4 colonies trained to sucrose-solution feeders. Our results support most of the predictions. Persistence, measured both in duration and number of visits, was greater to locations that previously offered sucrose solution of higher concentration (2 versus 1molar) or were closer to the hive (20 versus 450m). Persistence was also greater in bees which had longer access at the feeder before the syrup was terminated (2 versus 0.5h). However, contrary to our prediction persistence was not higher in the season of lowest nectar availability in the environment.

2.2 Introduction

Most animals forage in a dynamic environment in which food availability is constantly changing and is neither entirely predictable nor random in space or time (Mobus & Fisher 1999). As a result, animals will often revisit previously-rewarding locations and give up on locations that are unrewarding (Gende & Sigler 2006; van Gils et al. 2003).

The availability of nectar and pollen for a colony of honey bees, *Apis mellifera*, is constantly changing. Flower patches are usually rewarding only for short periods (Frisch 1967; Seeley 1995; Vogel 1983). At a group level, each honey bee colony faces the challenge of allocating its foragers to the best nectar and pollen sources in an area greater than 100 km² (Beekman & Ratnieks 2000; Frisch 1967; Seeley 1995; Seeley et al. 2000; Visscher & Seeley 1982). At an individual level, each forager has to decide whether to continue foraging at its patch, including whether or not to recruit additional foragers (Frisch 1967; Seeley 1995), or, if the patch is deteriorating, whether to abandon it (Seeley 1995; Townsend-Mehler & Dyer 2012; Townsend-Mehler et al. 2011). Many factors determine the quality of a nectar patch, but an important currency is energy (Frisch 1967; Seeley 1995), which depends mainly on sugar concentration, the distance of the patch from the colony, and the time taken for a forager to collect a load.

In honey bees and many other social insects decision-making concerning foraging is complex because decisions made by individual foragers are influenced not only by their own private information about a food source but also by information from nestmates. Experienced honey bee foragers can choose between foraging location information from waggle dances and their own memory (Biesmeijer & Seeley 2005; Frisch 1967; Gil & Farina 2002; Grüter et al. 2008; Grüter & Ratnieks 2011; Seeley & Towne 1992), or can scout for new locations (Seeley 1995). Foragers pay greater

attention to social information when their own food source deteriorates in quality (Grüter & Ratnieks 2011).

A dancing honey bee provides information about the direction, distance and odour of a profitable food source (Dyer 2002; Frisch 1967; Grüter & Farina 2009a; Seeley 1995). However, even in a colony with many dancing bees advertising a range of food sources (Beekman et al. 2004; Waddington et al. 1994), individual foragers normally focus on a particular patch for days or even weeks if it remains rewarding (Butler 1945; Butler et al. 1943; Ribbands 1949). The importance of this private information is further strengthened by evidence that foragers with greater experience are less interested in waggle dances (Biesmeijer & Seeley 2005), and that many foragers that follow waggle dances actually return to their old patch instead of trying to locate the advertised new patch (Grüter et al. 2008; Grüter & Ratnieks 2011; Johnson 1967; Menzel et al. 2011; Wray et al. 2012).

Given the evidence that honey bees return to familiar food locations even after these become unrewarding (Frisch 1967; Grüter & Ratnieks 2011), combined with the fact that there is ongoing change in the feeding locations used by a colony (Seeley 1995; Visscher & Seeley 1982), what factors influence the degree of persistence shown by honey bee foragers to feeding locations? Although short-term responses (≤ 60 min) have been quantified in foraging honey bees that have experienced low rewards (Townsend-Mehler & Dyer 2012; Townsend-Mehler et al. 2011), this duration is likely to be too short to measure the actual degrees of persistence shown.

The aim of this study was to investigate how previous experience and reward at a feeding location affects the persistence of honey bee foragers to this location once food is no longer available. We hypothesized that persistence would be greater at locations that were more rewarding (closer, higher concentration of sucrose solution),

for which a bee had greater prior experience, and at times of the year of lower nectar availability. We studied individually-marked worker bees from 4 colonies trained to sucrose-solution feeders. Our results support most of the predictions. Persistence was greater when higher sucrose concentration and closer feeders were used, in bees which had foraged for longer at a feeder before the syrup was terminated. However, contrary to our prediction persistence was not higher in the season of lowest nectar availability in the environment.

2.3 Materials and Methods

2.3.1 Study site and organisms

Experiments were performed from April to October 2011. We used four honey bee colonies (C1-C4) of mixed European subspecies but predominantly *Apis mellifera mellifera* from the apiary of the Laboratory of Apiculture and Social Insects, University of Sussex. Colonies were kept in Langstroth hives with 2 medium-depth 10-frame boxes and always had space to store additional pollen and honey. Colony sizes change during the season, but were approximately 10,000-30,000 bees. Each colony was monitored weekly to confirm that it had a laying queen, brood, and was otherwise thriving.

2.3.2 Experimental procedure

We used standard procedures (Frisch 1967, read pages 17-18) to train 10-30 foragers from each colony to a sucrose solution feeder located 20m from the hive, or 450m in the longer distance treatment (see below). The feeders were similar to those of von Frisch (1967, Fig. 18) and consisted of a small jar 6cm high by 4cm in diameter standing on a grooved base where worker bees could take syrup. In order to help trained bees learn the location, we placed each feeder on a sheet of blue A4-paper on a small table. All

training was done in the afternoon, usually between 1230-1530. Training started by placing the feeder at the hive entrance over stands near the entrance. When a few bees were feeding at both feeders (sometimes bees feed only at one feeder but not the other), it was then moved to the designated feeder position by carrying it slowly to prevent disturbing the feeding bees. After filling up, the bees will leave the feeder and perform circling orientation flights to learn the features of the feeder and the landmarks in the surrounding. When foragers returned to the feeder for the first time after moving, we individually caught each bee gently using a standard (commercially available) queen marking cage and marked them with plastic number tags (Opalithplättchen). Unmarked bees that arrived later (potential recruits) were also marked. During each trial we counted the number of visits to the feeder made by each marked bee during the whole access period, either 0.5 or 2h.

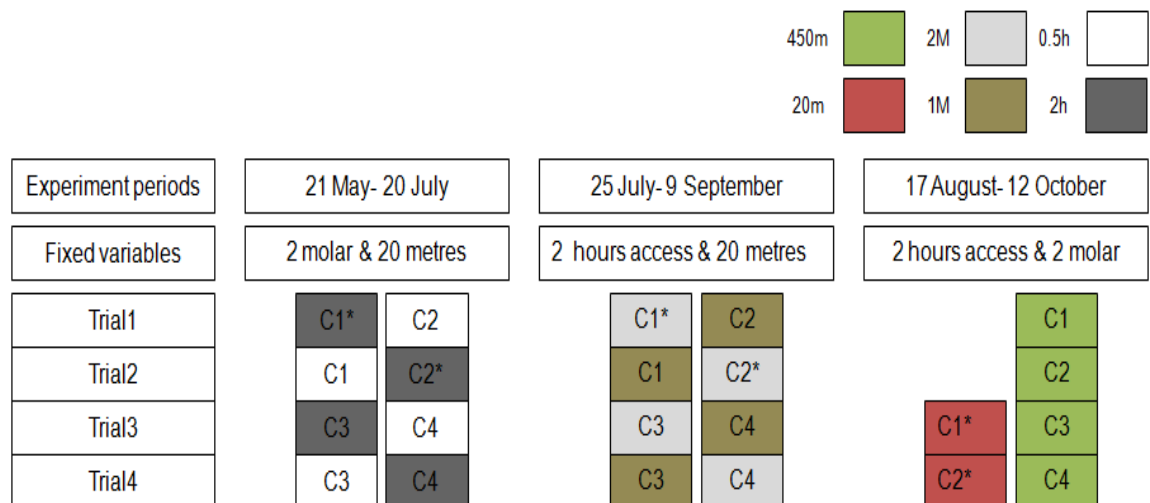


Figure 1: Sequence of the experiments, treatments and hive combinations performed between 21 May and 12 October. Rectangles containing C1-C4 represent the four study colonies. Asterisks refer to trials which were also used to compare seasonal effects. In the last experimental period, there are two boxes missing (of C3 and C4) because these two colonies were already tested in the second experimental period with 2 molar solution and 2 hours access to the feeder.

For experiments with a feeder at 450m we trained bees in two steps: first we moved a feeder with 20-30 on it to a location 350m from the hive. In order to avoid

disturbing the drinking bees during the transport, the feeder was placed inside a cardboard box. After bees were accustomed to this location and repeatedly visited the feeder, we performed the second step by moving it another 100m to its designated location by carrying it quickly without shaking the box. Both steps were performed on the same day. For each trial, we tested two colonies simultaneously (Fig.1).

We tested the effect of 4 different factors on persistence: the number and duration of rewarded visits (i.e. training visits made during the 2h or 0.5h access period), sucrose concentration (1M, 2M), distance from hive to feeder (20m, 450m), and time of the year. We used two measures of forager persistence: the total number of visits made by a marked bee after the feeder ceased to be rewarding (visit-persistence) and the maximum duration, number of days, that a marked bee returned to the empty feeder (duration-persistence).

The experiments were carried out at different times of year during the foraging season, and data were analysed over different periods. During Period 1, 21 May to 20 July (spring-early summer), we tested the effect of access duration and the number of rewarded visits. In order to create the necessary variation, we allowed foragers from one colony 2h access versus 0.5h for the other colony, with the access switched 0.5h access versus 2h. Each colony's feeder had 2M sucrose. During Period 2, 25 July to 9 September, we tested the effect of sucrose concentration. The feeders were both at 20m and both colonies had 2h access with one colony receiving 1M sucrose and the other 2M, with the trial switched 2M versus 1M. During Period 3, 17 August to 12 October, we tested the effect of distance with one hive being trained to a feeder at 20m and the other at 450m, with the trial switched 450m versus 20m. Training to the 450m feeder location was done on the same day as training to the 20m location. Both feeders had 2M sucrose and 2h access.

At the end of the training phase we emptied the feeders. Over the following days the empty feeders were recorded at their respective training location from 10.00-16.00 using video cameras (Sony HDR-XR550VE). We stopped recording at a feeder when it had not been visited by any trained bees for 2 days of good foraging weather. From the videos we were able to determine how often and for how many days the marked bees returned to the empty feeders.

2.3.3 Statistical Analyses

We analysed the data using general linear models in Minitab (version 14). We first tested whether colonies differed in our two measures of persistence, duration and visits. If not, we pooled the data from the 4 colonies to increase statistical power. If colonies did differ, we included colonies as an explanatory variable in the model. If necessary, we log or square root transformed the response variable to meet the assumptions of ANOVA (Grafan & Hails 2002). Non-significant interactions between explanatory variables ($p > 0.05$) were removed from the model. All tests are two-tailed and a significance level of $\alpha = 0.05$ was used. Descriptive statistics are given as mean \pm standard deviation.

2.4 Results

We studied 361 marked bees (Fig. 2A). Across all treatments, the average number of training visits to a feeder was 14.41 ± 10.02 (Range: 2-46). After training, the average number of visits (6h video per day) to the empty feeder (visit-persistence) was 4.29 ± 4.47 (range: 0-25) with the trained bees returning for an average of 1.89 ± 1.56 days (duration-persistence; range: 0-7). Figure 2 shows the distribution of visits and number of bees' persistence per day after training.

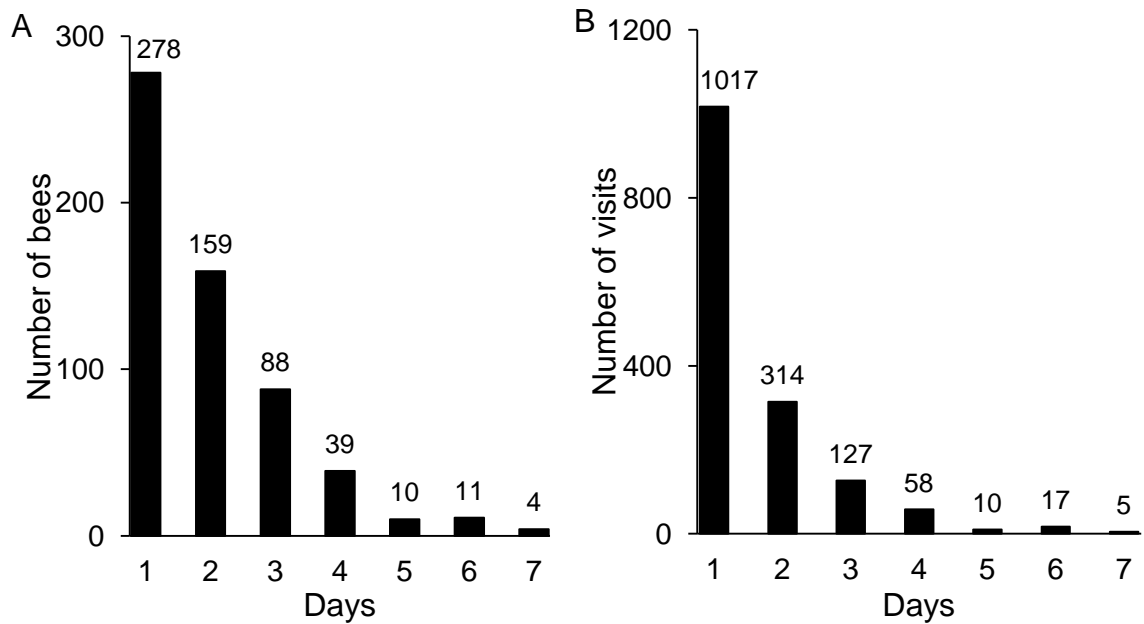


Fig. 2. The persistence on days 1-7 after training to a feeder on day 0 decreases over time. A) The data show that per day about 50% of bees give up visiting the empty feeder. B) The number of visits decreases by about 2/3 per day. Data of all treatments is shown in this figure.

In total there were 1548 visits to the unrewarding feeders. Fig. 3 shows that the bees visited the unrewarding feeder across the full 6 hours of videoing, and that the video period included the period of peak visiting activity.

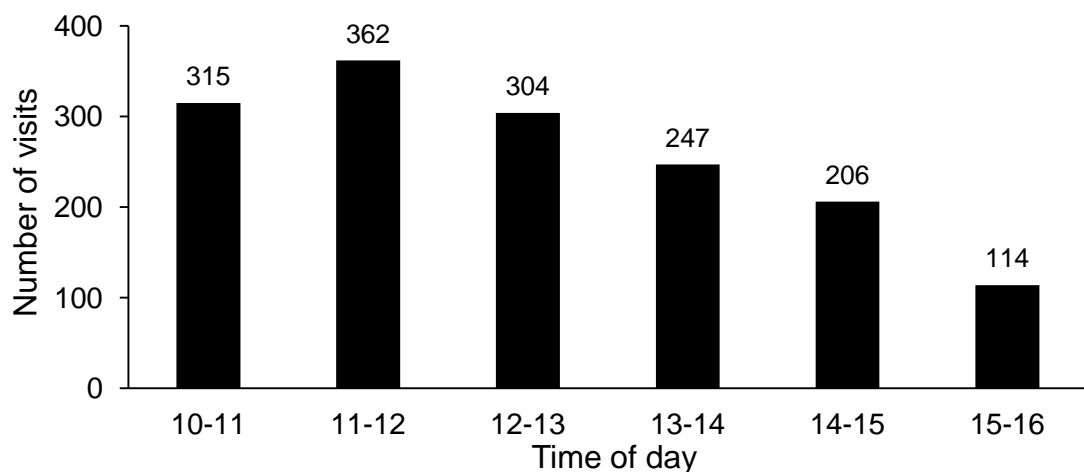


Fig. 3. The figure shows that the bees visited the unrewarding feeder across the full 6 hours of videoing. The data also show that the period of peak visiting activity (11-12) is before the training period (in the afternoon). Data across all treatments and days after training are shown.

2.4.1 Experiment 1: Effect of training access duration and number of training visits

We tested 122 bees in total, 46 with 0.5h access to the feeder (average number of training visits 4.24 ± 1.84) and 76 bees with 2h access (15.66 ± 10.87 visits). There was no difference among colonies in visit-persistence ($F_{3,118} = 2.12$, $P = 0.102$). Overall, visit-persistence was 23% greater, 1.69 versus 1.3 per bee, for 2h versus 0.5h of training access ($F_{1,120} = 3.72$, $P = 0.056$; Fig. 4A). Since bees in the 2h treatment made, on average, 3.69 times as many training visits as bees in the 0.5h treatment ($F_{1,120} = 49.75$, $P < 0.001$), we ran an additional model that included both the number of training visits and training duration as explanatory variables and found a significant interaction between these two predictor variables ($F_{1,118} = 11.11$, $P = 0.001$; Fig. 5A).

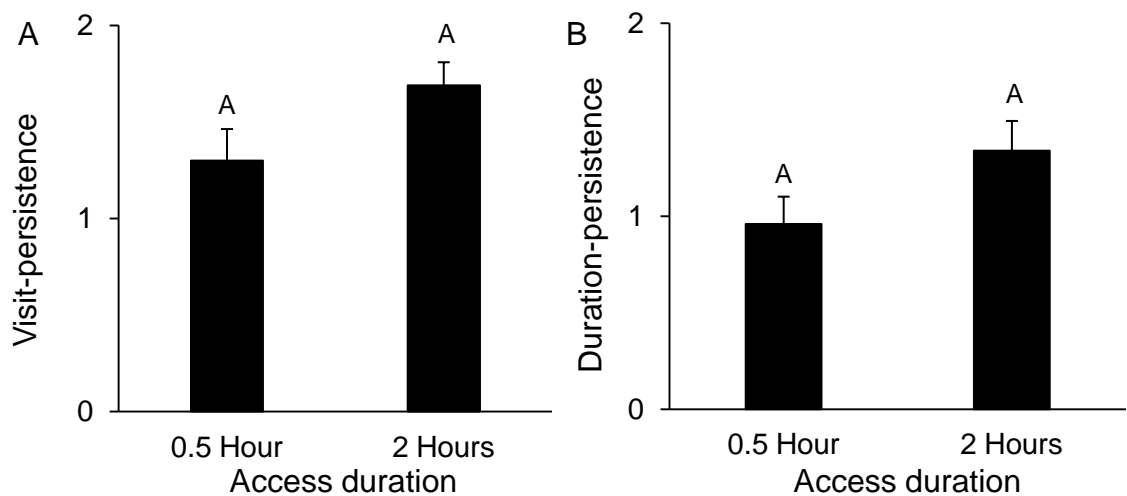


Fig.4 Both visit-persistence and duration-persistence are greater for 2h versus 0.5h of training access if the number of training visits is taken into account. Also bees return to the empty feeder for more days when they had longer access. Both feeders were at 20m distance from the hive. Figure shows means and standard-errors over the black bars of the data for 0.5h versus 2h access. (A) Above bars indicate a non statistically significant difference ($P > 0.05$) between the two access durations.

Both access duration and number of training visits significantly affected visit-persistence (training duration: $F_{1,118} = 8.74$, $P = 0.004$; Fig.4A; number of training visits: $F_{1,118} = 15.53$, $P < 0.001$; Fig. 5A). The interaction ($F_{1,118} = 11.11$, $P = 0.001$; Fig.

5A) suggests that the number of training visits has a decreasing effect as the total training duration increases.

There were significant differences between hives in duration-persistence ($F_{3,118} = 5.51$, $P = 0.001$). Hence, we included hive in the model with training duration and number of training visits. We found significant effects of hive ($F_{3,115} = 7.66$, $P < 0.001$), training duration ($F_{1,115} = 6.13$, $P = 0.015$; Fig. 4B) and the number of training visits ($F_{1,115} = 12.25$, $P = 0.001$; Fig. 5B). Additionally, we again found a significant interaction between access duration and number of training visits ($F_{1,115} = 5.86$, $P = 0.017$; Fig. 5B).

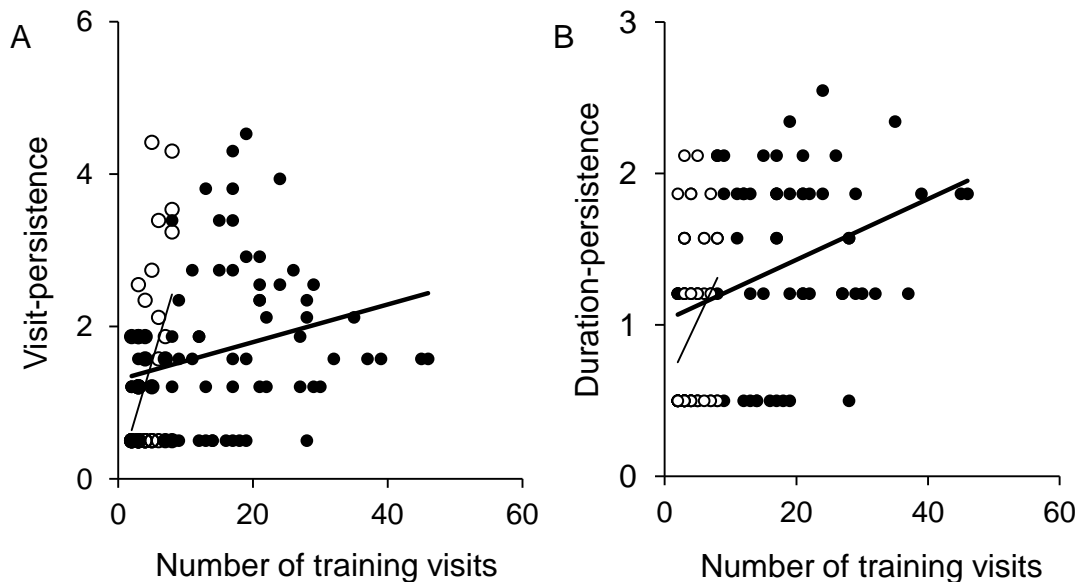


Fig. 5. The figure shows a positive effect of the number of training visit on both A) visits-persistence B) duration-persistence. A statistically significant interaction between the number of training visits and the access duration suggests that the effect of the number of training visits was weaker when the overall training duration was longer (2h). Circles show training access duration to feeder: Black circles and dark line show data of 2h access, open circles and light line show data of 0.5h access.

2.4.2 Experiment 2: Effect of sucrose concentration

We trained 86 bees with 2M (number of training visits: 19.21 ± 9.09 visits) and 60 bees with 1M sucrose (17.22 ± 10.26 visits). There was a difference between hives in visit-

persistence ($F_{3,142} = 5.10$, $P = 0.002$) and duration-persistence ($F_{3,142} = 4.83$, $P = 0.003$). There was no statistically significant effect of molarity on the number of training visits ($F_{1,144} = 1.53$, $P = 0.219$). Therefore, we ran a model with molarity and hive as explanatory variables and found that the two factors interacted ($F_{3,138} = 3.70$, $P = 0.013$). The two main effects significantly affected visit-persistence (Hives: $F_{3,138} = 5.88$, $P = 0.001$; molarities: $F_{1,138} = 12.98$, $P = 0.000$; Fig. 6A). When analysing the data on duration-persistence, we again found significant effects of hive ($F_{3,141} = 4.24$, $P = 0.007$) and molarity ($F_{1,141} = 8.08$, $P = 0.005$; Fig. 6B).

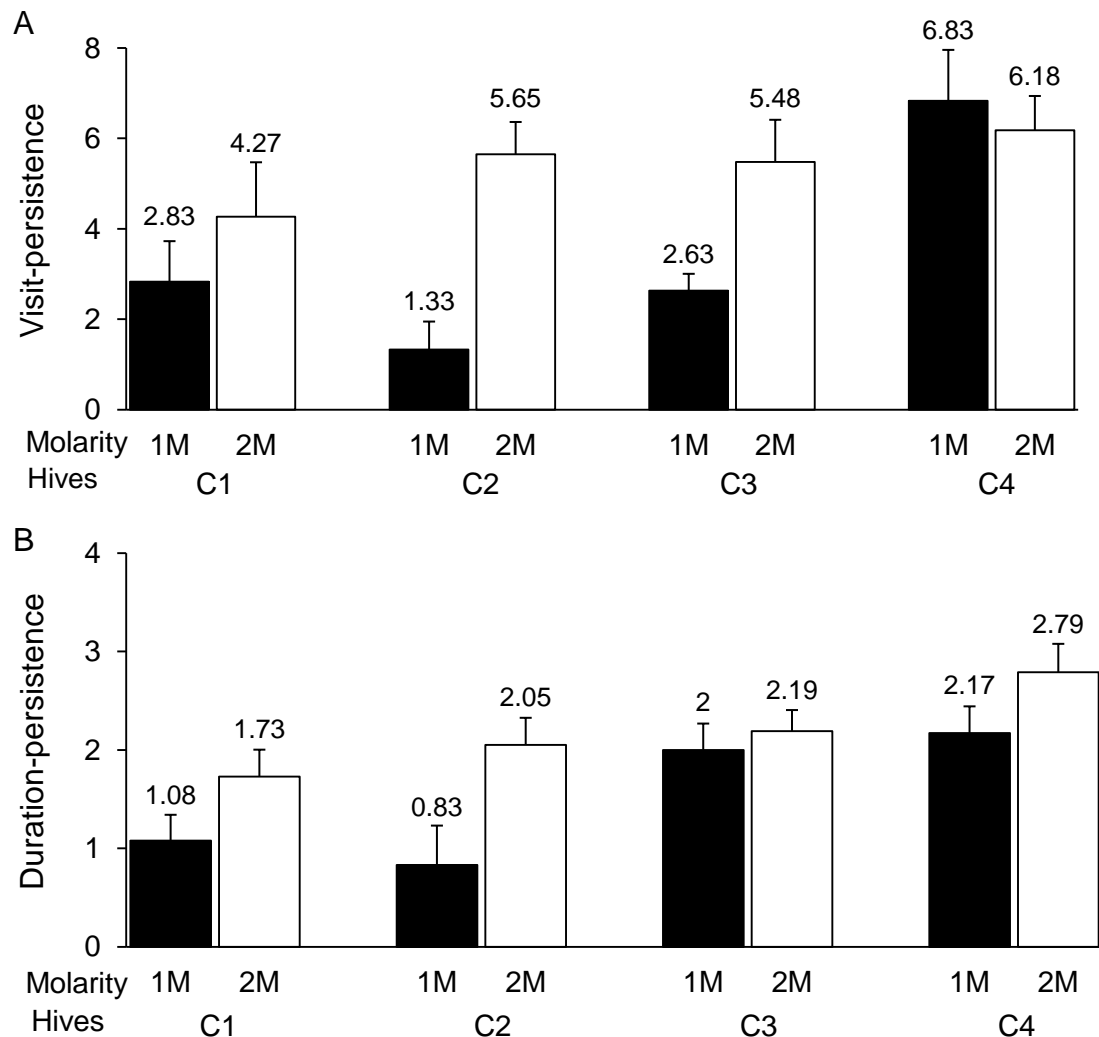


Fig. 6: A) Bees of 2 colonies (C2, C3) made significantly more visits to the empty feeder (visit-persistence) and B) bees of 2 colonies (C1, C2) visited the feeder for more days (duration-persistence) when they were trained on 2M versus 1M of the sucrose solution during the training period. Figure shows means and standard-errors over the black and white bars of the data for 1M versus 2M respectively.

2.4.3 Experiment 3: Effect of distance to feeder

We trained 82 bees at 20m (number of training visits to feeder: 18.44 ± 8.91) and 66 bees at 450m (10.14 ± 4.04 visits). As there was no difference between colonies in visit-persistence ($F_{3,144} = 1.60$, $P = 0.193$) or duration-persistence ($F_{3,144} = 1.49$, $P = 0.221$) we pooled the data across colonies. Overall, visit-persistence was 13% greater for 20m versus 450m (2.4 v 2.09 , $F_{1,146} = 4.73$, $P = 0.031$; Fig. 7A). However, since bees also made more training visits to the 20m feeder than the 450m feeder ($F_{1,146} = 49.08$, $P < 0.001$), we ran an additional model that included the number of training visits as a predictor variable. This showed that the difference in persistence is affected by the number of training visits ($F_{1,145} = 10.67$, $P = 0.001$) rather than by distance itself ($F_{1,145} = 0.09$, $P = 0.762$). When analysing duration-persistence, we again found significant differences between bees trained to feeders at 20m versus 450m. Overall, duration-persistence was 11% greater 1.68 v 1.49 for 20m versus 450m ($F_{1,146} = 5.98$, $P = 0.016$; Fig. 7B). When we again included the number of training visits in the model we found that foraging distance was significant ($F_{1,145} = 4.07$, $P = 0.046$) but that the number of training visits was not significant ($F_{1,145} = 0.03$, $P = 0.856$).

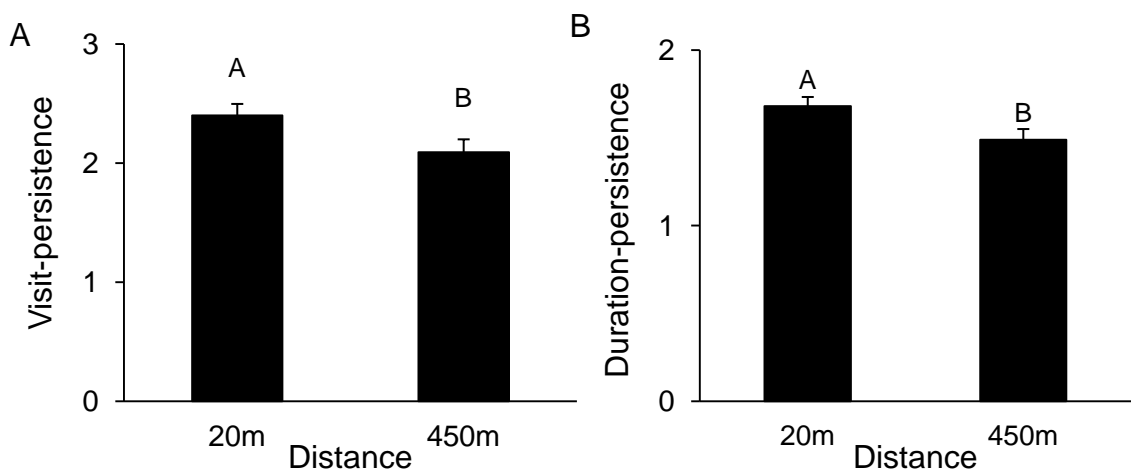


Fig. 7: The data shows that both visit-persistence and duration-persistence are significantly greater for the closer food source (20m versus 450m). Both feeders offered that same molarity (2M) and for the same duration (2h). Figure shows means and standard-errors. A & B above bars indicate a statistically significant difference ($P < 0.05$) between the two different distances.

2.4.4 Experiment 4: Effect of different seasons

Here we compared persistence only in colonies C1 and C2 (marked with asterisks in Fig.1) for which data were available at 3 different periods of the year: Season (spring) = May-June (36 bees); Season (summer) = July-August (46 bees); Season (autumn) = September- October (27 bees). For colonies C1 and C2 the average number of training visits for the 3 periods was: 12.03 ± 8.50 , 20.35 ± 10.21 , 17.11 ± 9.74 , respectively.

There were significant differences between colonies in visit-persistence ($F_{1,107} = 4.12$, $P = 0.045$). Since bees also made different numbers of training visits at different seasons, we ran a model that included colony, season, and number of training visits as explanatory variables. We found significant differences among seasons ($F_{2,102} = 3.99$, $P = 0.021$; Fig. 8A), and a significant effect of the number of training visits ($F_{1,102} = 25.16$, $P < 0.001$), but no significant effect of colonies ($F_{1,102} = 3.77$, $P = 0.055$; Fig. 8A). In addition, there was a significant interaction between season and colony ($F_{2,102} = 4.24$, $P = 0.017$, Fig 8A). A Tukey post-hoc analysis showed that the difference between summer and autumn was significant ($P = 0.038$), between spring and autumn was borderline non-significant ($P = 0.066$), and not significant between spring and summer ($P = 0.989$).

There was no difference between the two colonies in duration-persistence ($F_{1,107} = 0.20$, $P = 0.658$; Fig. 8B). Hence, we ran a model including the number of training visits and season as explanatory variables. We found significant effects of both the season ($F_{2,103} = 7.38$, $p = 0.001$) and the number of training visits ($F_{1,103} = 8.63$, $P = 0.004$). Additionally, we found a significant interaction between season and the number of visits ($F_{2,103} = 6.62$, $P = 0.002$), suggesting that the effect of experience with a particular food source on persistence depends on the season. A post-hoc test showed that the difference between seasons summer and autumn was significant ($P = 0.018$),

and borderline non-significant between spring and summer ($P= 0.062$), but not significant between spring and autumn ($P= 0.767$).

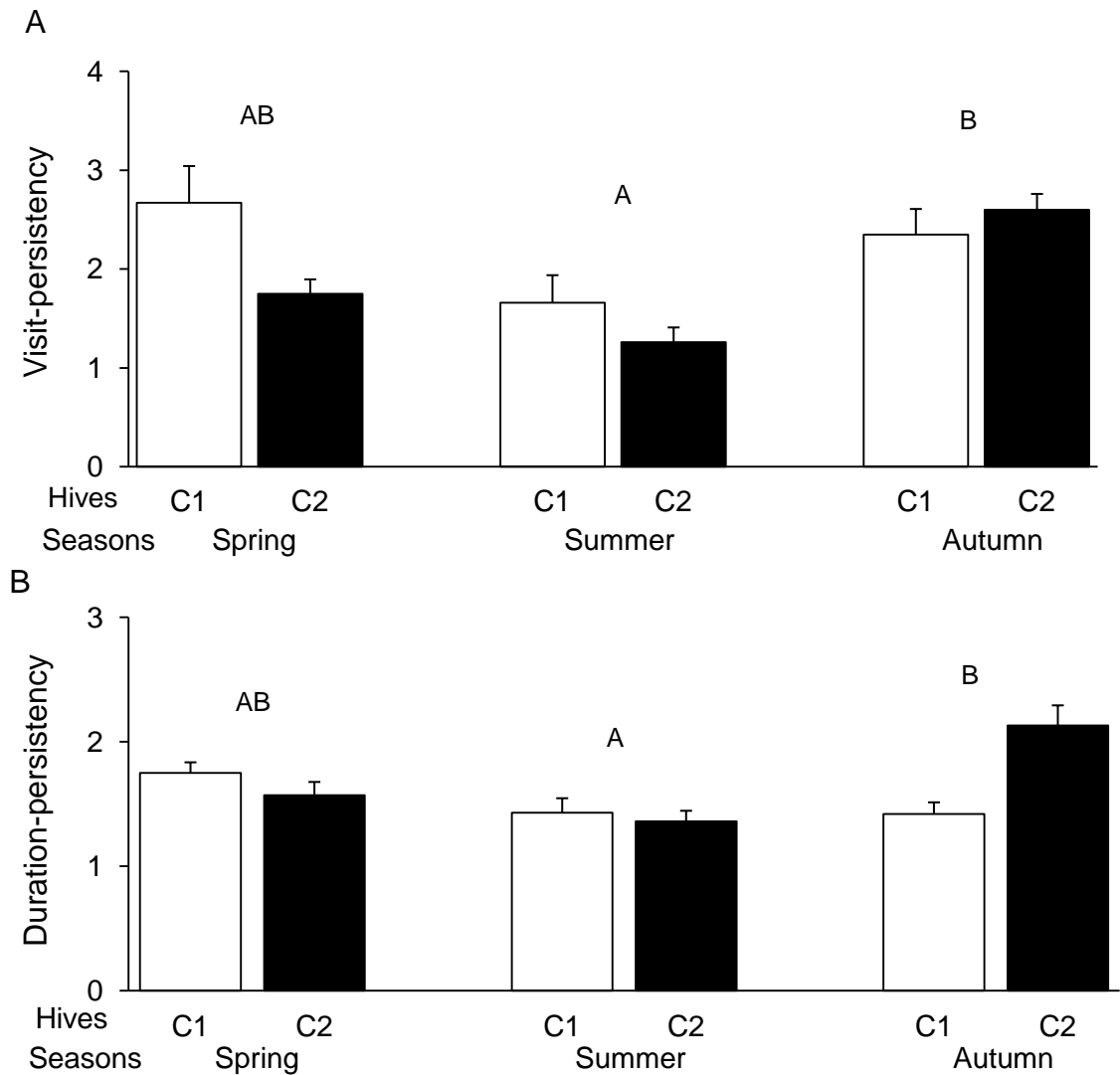


Fig.8: The data show significant differences in persistence between seasons (spring, summer and autumn). Persistence was greater in autumn both in terms of visit number and duration. The figure shows means and standard-errors for colonies C1&C2. A & B indicate a statistically significant difference ($P < 0.05$) between the different seasons.

2.5 Discussion

Our results clearly show that the persistence of honey bee foragers to an unrewarding feeding location that was previously rewarding is significantly affected by their prior experience of that location and its economic value in ways that match predictions.

Greater experience (training access of 2h v 0.5h), and greater economic value (closer to the nest, 20m v 450m; more concentrated artificial nectar, 2m v 1m sucrose) all increase persistence, both in the number of visits made and in their overall duration. Season also had an effect, with persistence being greatest in autumn.

Bees continued to visit the empty feeder for up to 7 days (1.89 ± 1.56) after only 0.5h or 2h of training access, with about 50% giving up per day (Fig.2A). In nature, a bee would likely have foraged for longer at a patch, maybe days or even a week (Butler 1945; Butler et al. 1943; Ribbands 1949). Interestingly, some bees (19 of 361 or 5.2%) interrupted visiting the empty feeder for 1 to 4 days before being recorded again at the feeder. This shows that foragers may check a location even after a long gap in visiting.

Long-term persistence was positively affected by the number of training visits made by an individual bee while the feeder offered food. Von Frisch (1923) realized that reward amount is an important factor for food quality. A larger number of training visits means more learning trials, which increases the predictability of the reward and has been shown to positively affect the retention of memory in honey bees (Menzel 1999) and short-term persistence in flower constancy (Grüter et al. 2011a).

When we trained bees to two different sucrose molarities, 3 of 4 colonies showed a positive effect of molarity on persistence (Fig. 6A) with one colony showing no effect. Previous work has shown that foragers respond to the quality of a food source during the training period by making more visits to the more concentrated feeder (Seeley 1986). However, how differently colonies respond to 1M versus 2M might depend on factors such as the availability of alternative food sources or the amount of stored honey and, therefore, varies between colonies and seasons (Lindauer 1949; Seeley 1989). Sucrose molarity has been shown to affect many other foraging-related behaviours including crop filling at the food source (Núñez 1966), memory formation

(Bitterman et al. 1983; Scheiner et al. 1999), flying speed (Frisch & Lindauer 1955), forager body temperature (Stabentheiner 1996), the probability to perform a recruitment dance (Frisch 1967; Seeley 1995), trophallactic behaviour inside the colony (Farina & Grüter 2009) and flower constancy (Grüter et al. 2011b). As a consequence of these changes, a feeder offering a highly-concentrated sucrose solution will attract many more bees than a feeder with diluted sucrose solution (Frisch 1967; Seeley 1995).

Foraging theory predicts that the time taken to collect food should play an important role in forager decisions to collect food at a particular location (Fewell et al. 1992; Schoener 1971; Stephens & Krebs 1986). For a central place forager such as a honey bee, increased foraging distance means less food is collected per unit time, and may also result in greater exposure to predators and environmental risks. In agreement with these predictions, in the giant tropical ant *Paraponera clavata* the giving-up time depends on travel time (Breed et al. 1996). In our experiments there was a significant difference in both persistence measures between the two different foraging distances (20, 450m). As expected, persistence was greater for the closer food source: visit-persistence was 13% greater for 20m versus 450m and duration-persistence was 11% greater for 20m versus 450m. Our results are to some degree ambiguous as to whether this is caused by a different number of rewarded visits during training to the closer feeder or the distance itself. To disentangle these two factors, a third treatment would have been necessary that allowed bees to make the same number of training visits at a 450m feeder as they did at the 20m feeder. The maximum distance in the experiment was only c. 0.45km, but honey bees will forage at up to c. 13km in nature. However, average foraging distances are usually much shorter (Ratnieks 2000; Steffan-Dewenter & Kuhn 2003; Visscher & Seeley 1982) and vary between months or seasons (Beekman & Ratnieks 2000; Dornhaus et al. 2006; Seeley 1997; Waddington et al. 1994).

Long-term persistence also differed between seasons, being highest in autumn and lowest in summer. These results are to some degree puzzling because we expected forager persistence to correlate negatively with natural food abundance and food abundance is higher in autumn than in summer in the study area. In autumn colonies gained more weight (personal observation measured with a hive balance) and foragers forage at shorter distances compared to summer, due to the availability of ivy, *Hedera spp.*, in autumn (Couvillon et al. 2012a; Garbuzov & Ratnieks. In preparation). On the other hand, seasonal effects were tested with only two colonies which were tested only during a few days. It is likely that day-to-day weather conditions are not always typical for a season and, therefore, can cause considerable unaccounted variation or depend on the colonies situation. Honey bee researchers are well aware of seasonal differences in acceptance thresholds of sucrose solution and daily changes of dance thresholds (Lindauer 1949; Seeley 1995). This manifests itself, for example, in the difficulty to train bees to forage from a feeder in months of food abundance (Frisch 1967, Page.18; Lindauer 1949). Furthermore, forager life-times might be longer in autumn than summer (Winston 1987) which could bias the data to suggest longer persistence in autumn.

In summary, our results are in line with previous studies showing that if the conditions are suitable for foraging, foragers will revisit favourable areas for several days (Butler 1945; Ribbands 1949). Persistence to food source locations is not only known in honey bee foragers, but also ants are known to return to food locations for weeks or even months by using their route memory (Collett et al. 2003). However, honey bees clearly take the economical value of a food source into account when deciding to abandon an unrewarding food source. This ability to abandon unrewarding food sources according

to their prior economic value illustrates how a honey bee colony is able to adaptively re-allocate its foraging force in a changing environment in economical ways.

Chapter 3: Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour

3.1 Summary

Honey bee foragers perform waggle dances to inform nestmate foragers about the presence, location and odour of profitable food sources and nest-sites. The aim of this study was to investigate how the characteristics of waggle dances for natural food sources and environmental factors affect dance follower behaviour. We hypothesised that the attractiveness of a dance, measured as the number of dance followers and their attendance, depends on the distance of the advertised food location. Additionally, we determined whether time of year and dance signal noise, quantified as the variation in waggle run direction and duration, affect dance follower behaviour. Our results show that foragers follow fewer waggle runs as the food location distance increases, but that they invest more time in following each dance. This is because waggle run duration increases with increasing foraging distance. The number of waggle runs followed for distant food sources was further reduced by increased angular noise among waggle runs. The number of dance followers per dancing bee was also affected by the time of year and varied among colonies. Our results confirm that both noise in the message, that is variation in the direction component, and the message itself, that is the distance of the advertised food location, affect dance following. These results indicate that dance followers pay attention to the costs and benefits associated with using dance information.

3.2 Introduction

Many animals make deliberate signals to conspecifics to direct them to feeding locations (Galef & Buckley 1996; Judd & Sherman 1996; Maynard-Smith & Harper 2003; Shettleworth 2010; Sumpter & Brännström 2008; Wilson 1971). These signals are especially well studied in social insects. In some cases successful foragers use signals that simply increase foraging effort (Jarau & Hrnčir 2009; Thom et al. 2007), including the vibratory signal in the honey bee whose message is “increase your work-rate” (Schneider & Lewis 2004). In other cases the signals also guide recruits to specific locations by direct guiding (Hölldobler & Wilson 1990; Jarau & Hrnčir 2009; Nieh 2004), trail pheromones (Beekman et al. 2001; Jarau & Hrnčir 2009; Nieh 2004; Wilson 1971) or the honey bee waggle dance (Frisch 1967).

Honey bees, *Apis mellifera* and other *Apis*, are well known for using the waggle dance to provide nestmates with information about the location and odour of profitable food sources (reviewed in: Couvillon 2012; Dyer 2002; Frisch 1967; Grüter & Farina 2009a; Seeley 1995). During a waggle dance, a worker bee waggles her abdomen as she walks in a straight line (waggle run) then turns either to the left or right to return to the starting point of the previous waggle run (return phase), before she repeats the waggle run. This waggle run is the information-rich part of a dance (Frisch 1967; Michelsen 2003; Seeley et al. 2000) and provides the direction and distance vector from the nest to the feeding location. A dancing bee may perform from 1 to 100 or more waggle run phases, depending on the resource quality and nectar availability (Frisch 1967; Seeley et al. 2000). Higher quality resources tend to elicit dances with more waggle runs (Frisch 1967; Seeley 1995). The dance signal is quite noisy in that waggle runs within a dance vary in direction and duration (Couvillon 2012; Couvillon et al. 2012b; De Marco et al. 2008; Dyer 2002). Dance followers reduce the effect of signal noise by averaging

several waggle runs to compute an overall vector (Frisch & Jander 1957; Tanner & Visscher 2008). Consequently, the number of waggle runs followed positively correlates with the accuracy of the flight vector (Tanner & Visscher 2009). Therefore, following more waggle runs is beneficial in terms of information accuracy, but requires more time. Previous work has reported that foragers that were successfully recruited to a food location by a waggle dance followed on average 8 (Judd 1994), 17 (Grüter et al. 2008), 15.5 (Wray et al. 2012) or 20-23 waggle runs (Menzel et al. 2011). It has been suggested that dance followers need to follow at least about 5 waggle runs to acquire precise location information (Biesmeijer & Seeley 2005; Esch & Bastian 1970; Frisch & Jander 1957; Grüter & Farina 2009a; Mautz 1971).

Dance following has mostly been studied in experimental situations with *ad libitum* nearby sucrose feeders in environments with few or zero good natural food sources (Grüter et al. 2008; Grüter & Ratnieks 2011; Menzel et al. 2011; Wray et al. 2012). This rather unnatural situation might affect how foragers use waggle dance information (Grüter & Farina 2009a). We know of only one study that investigated dance following behaviour in a naturally foraging colony (Biesmeijer & Seeley 2005). Interestingly, this study reported that dance followers followed on average only 2-4 (range 1-20+) waggle runs for different groups of bees (Biesmeijer & Seeley 2005). The authors suggested that only in 12-25% of cases did dance following lead to discovery of the advertised location. Some of the other follower bees might have resumed foraging at familiar food sources after perceiving a familiar flower odour on the dancer (Frisch 1923, 1967; Grüter et al. 2008; Reinhard et al. 2004; Ribbands 1954; Wenner & Wells 1990; Wenner et al. 1969). Reactivation does not require extensive dance following but simply the detection of a familiar odour on the dancer. This might explain why reactivated foragers follow less than 5 waggle runs on average (Grüter et al. 2008;

Grüter & Ratnieks 2011; Wray et al. 2012) although in one study reactivated bees followed more waggle runs, some >20 (Menzel et al. 2011). Biesmeijer and Seeley (2005) found that many dance followers were active foragers, which led them to hypothesize that dance following provided “confirmation” of the continued availability of a familiar food source. However, the interpretations made by Biesmeijer and Seeley (2005) about how often dances are followed for the discovery of new food patches, reactivation, or confirmation were based on assumptions about how foragers would behave after leaving the hive rather than actual observations. This is because it has not yet been possible to investigate what followers of natural dances do once they leave the hive due to the foraging distances and flight speed of foragers.

The aim of this study was to investigate how the characteristics of waggle dances for natural food sources and environmental factors affect dance follower behaviour. We tested the hypotheses that the distance of the advertised food source and the informational noise in the waggle run affect dance follower behaviour. Foragers might follow more waggle runs for distant food sources because getting lost at a greater distance from the colony is potentially more costly. On the other hand, dances for more distant food sources might arouse less interest for several reasons including the increased flight costs, the time it takes to follow the longer dance circuits, the increased chance that the food source disappeared before being located or the lack of odour molecules on the dancers body after longer flight (Frisch 1967), which function as orientation cues for followers (Díaz et al. 2007). We also tested whether the time of year and the overall dancing activity influence the number of dance followers and the number of waggle runs that a forager follows. Because signal noise might affect the attractiveness of dance to dance followers, we tested the effect of the standard deviation of waggle run duration and angle. Our results show that workers follow fewer waggle

runs as the food location distance increases, but that they invest more time in following each dance. The number of waggle runs followed for more distant food sources was further reduced by increased angular noise among waggle runs. The number of dance followers per dancing bee was also affected by the time of year and varied among colonies.

3.3 Materials and Methods

3.3.1 Study organism and hives

We used three colonies (H1-H3) of honey bees (*Apis mellifera mellifera*), each housed in an observation hive with 3 medium and 1 deep Langstroth frames. Hives were kept indoors at room temperature (c. 20°C) at the Laboratory of Apiculture and Social Insects on the campus of the University of Sussex. Each colony had a queen, brood, c. 5000 workers, and adequate honey stores but also vacant cells to store additional honey and pollen. A plastic tube 3cm in diameter and 30cm long connected each hive to the outside.

3.3.2 Dance decoding

Dances were decoded for another project, and these data were used to direct our data collection on dance following behaviour. Briefly, to decode dances, we videoed each observation hive for one hour between 0900 and 1600 on each study day using camcorders (Canon Legria HV40, HDV 1080i). Cameras were placed 1m from hives to reduce parallax and videoed an area 25cm wide × 20cm high where most dances took place. Plumb lines of nylon fishing line with heavy metal washers at the end were hung at 5cm intervals across the wall of outer glass. These appeared as thin white lines in the video and gave a vertical frame of reference.

Videos were analysed using Final Cut Express (Version 4.0). Dances were decoded frame by frame. We detected the beginning and end of each waggle run by observing, whether or not the dancer's body and wings were blurred on a single video frame. The exact beginning and end points (hour, minute, second, frame) were taken from the timer of the video software to a temporal resolution of 0.04 sec (1 frame). Dances had different numbers of waggle runs (4-32), and we averaged at least 4 waggle runs, excluding the first and last because these are significantly more variable in both duration and angle (Couvillon et al. 2012b). Averaging 4 waggle runs provides a reliable mean estimate for the entire dance (Couvillon et al. 2012b). The mean and standard deviation for both duration and angle for all waggle runs within a bout of dancing were calculated. Additionally, we recorded whether a dancer carried pollen. We analysed 602 dances (249 dances in H1; 185 in H2; 168 in H3).

3.3.3 Dance follower behaviour

We analysed dance following behaviour in 4 months (September 2009; April, May and July 2010). These months were selected because food abundance and average foraging distance varied greatly among these months (Couvillon et al. 2012a) which include spring, summer and autumn. For each decoded dance we determined the number of dance followers ten seconds after the beginning of the dance (after Grüter & Farina 2009b) and then determined the number of waggle runs these followers followed from this moment on, and also the number of other dances in the video. Waiting for 10 seconds gave surrounding bees time to identify and approach the dancer.

By analysing the movement of followers we made sure that bees which were merely standing near a dancer were not counted as followers. Followers were identified as bees facing the dancer with their heads within antennal length during the waggle run

and which followed the movement of the dancer (Grüter & Ratnieks 2011; Tanner & Visscher 2008). To quantify dance following duration we recorded how many waggle runs each dance follower then followed. A follower was considered to have stopped following a dance if she turned away from the dance and walked away. For statistical analysis, we averaged the number of waggle runs followed by all followers of a dance to obtain a mean value per dance.

3.3.4 Statistical analysis

We analysed the data using general linear models in Minitab (version 14). We first tested whether hives and pollen or non-pollen dances differed in the number of followers and the average number of waggle runs followed. If not, we pooled the data across hives or dances with and without pollen to increase statistical power. If necessary, we transformed the response variable with log or square-root transformations to meet the assumptions of ANOVA (Grafan & Hails 2002). We centered continuous explanatory variables to facilitate interpretation of interactions between them. Before including multiple continuous explanatory variables, we made sure that they correlated only weakly to avoid problems of co-linearity. Non-significant interactions between explanatory variables ($p > 0.05$) were removed from the model. All tests are two-tailed. Descriptive statistics are given as mean \pm standard deviation.

3.4 Results

We analysed the behaviour of 2405 dance followers in 602 dances. We discarded 26 dances (4.1%) either because foragers stopped dancing before the followers stopped following or because dancers left the videoed area before all dance followers finished dance following. The average number of followers was 3.98 ± 0.92 per dance (range 2-

8; Fig1A; Hive 1: 3.80 ± 0.82 , Hive 2: 4.06 ± 0.99 , Hive 3: 4.16 ± 0.91). Twenty-one percent (127) of all dances were made by foragers carrying pollen.

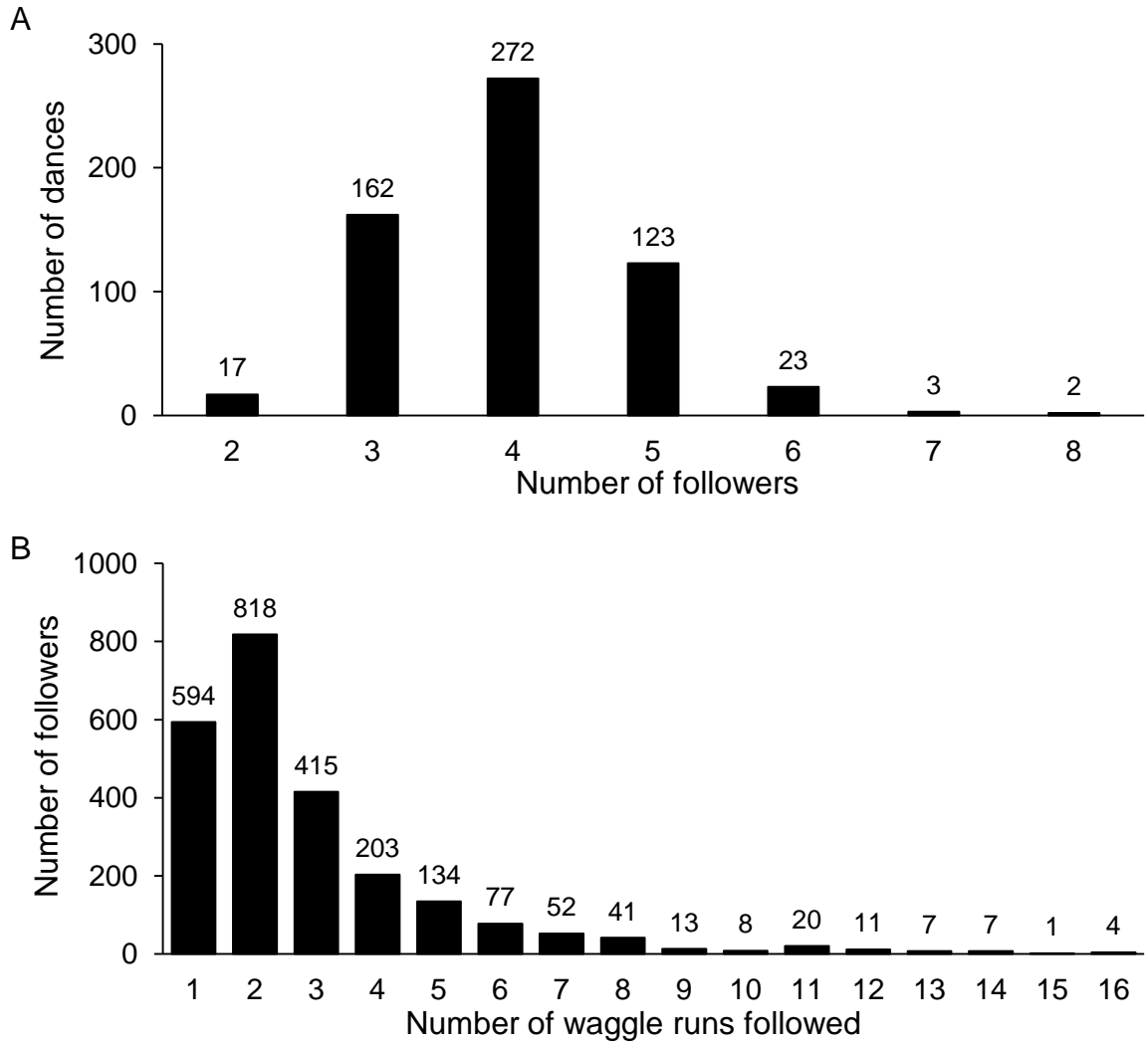


Fig.1: The data show that the maximum number of dance followers of a dance was 8, and the maximum number of waggle runs followed per dance was 16. Most followers followed dances only briefly, for 1 or 2 waggle runs. A) The distribution of the number of dance followers per dance for all 602 dances. B) The number of waggle runs followed by followers per dance.

The average duration of the waggle runs was 2.21 ± 1.27 seconds, which corresponds to a foraging distance of c. 2200 ± 1600 m (range 215-15500 m) when von Frisch's distance-waggle run duration curve is used (Frisch 1967). The number of bees dancing simultaneously in the videoed area was 1.94 ± 1.46 (range: 0-9). The average

number of waggle runs followed by each follower was 2.80 ± 1.38 (range 1-16; Fig. 1B; Hive 1: 2.75 ± 1.29 , Hive 2: 2.81 ± 1.33 , Hive 3: 2.88 ± 1.56).

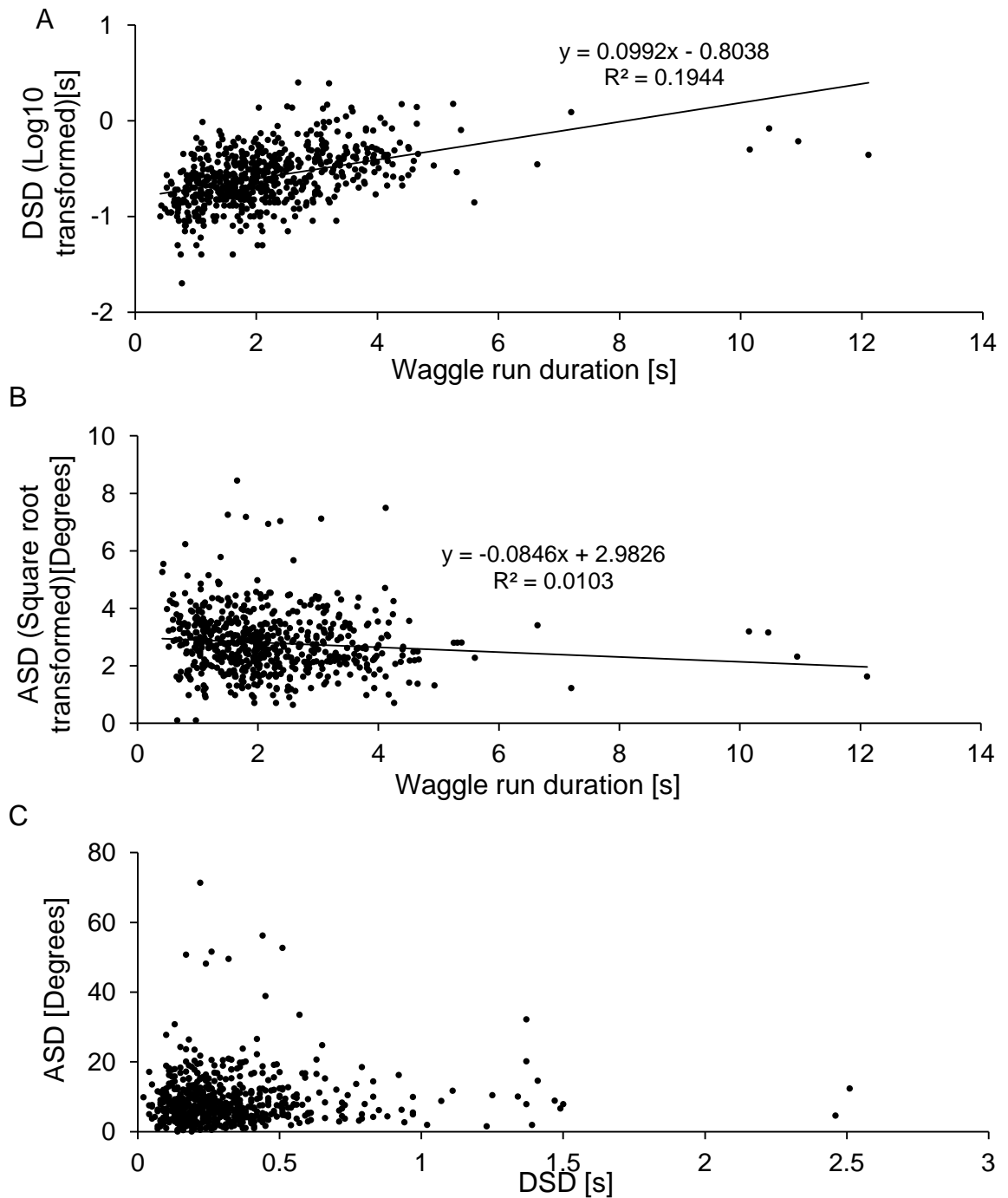


Fig.2 : A) The data show that there is a positive relationship between waggle run duration and duration standard deviation (DSD). B) There is a weak but significant negative relationship between waggle run duration and angle standard deviation (ASD). C) There is no significant relationship between these two components of noise (DSD and ASD). We used log10 and square root transformation to transform the response variable when this was necessary to meet the assumptions of ANOVA. For each data set, the best transformation was chosen.

First, we tested whether the mean waggle run duration (distance) affected the standard deviation of waggle run duration or waggle run direction within a dance. There was a significant positive effect on duration standard deviation (DSD) ($F_{1,600}=144.75$, $P<0.001$; Fig.2A), and a significant negative effect of angle standard deviation (ASD) ($F_{1,600}=6.26$, $P=0.013$; Fig.2B). Interestingly, there was no relationship between the ASD and DSD, suggesting that the two components of noise are independent from each other ($F_{1,600}=1.68$, $P=0.196$; Fig.2C).

3.4.1 Experiment 1: Factors affecting the number of waggle runs followed

We tested whether the number of waggle runs followed differed between hives and depended on whether dancers carried pollen, but found non-significant effects of hives ($F_{2,596}= 1.25$, $P=0.287$), pollen versus non-pollen ($F_{1,596}= 0.09$, $P=0.765$) and the interaction ($F_{2,596}= 2.36$, $P=0.095$). Hence, we pooled the data across dance types and hives. We ran a model including month, waggle run duration, DSD, ASD and the number of simultaneous dances as explanatory variables. We found significant effects of waggle run duration ($F_{1,592}= 3.95$, $P=0.047$; Fig. 3A), but not month ($F_{3,592}= 2.38$, $P=0.069$), DSD ($F_{1,592}= 2.72$, $P= 0.10$), ASD ($F_{1,592}= 0.02$, $P= 0.90$) or the number of simultaneous dances ($F_{1,592}= 0.35$, $P= 0.56$). Additionally, the interactions between waggle run duration and ASD ($F_{1,592}= 4.85$, $P= 0.028$), and between waggle run duration and the number of simultaneous dances ($F_{1,592}= 4.21$, $P=0.041$) were significant. This suggests that the angular noise also affected dance following but that this effect depends on the duration of the waggle run and, therefore, the distance to the food source. The second interaction suggests that the effect of waggle run duration depends on the dancing activity on the dance floor. The slope of the first interaction was positive ($y= 0.0015x - 0.642$), suggesting that the (negative) effect of the waggle run

duration increases with increasing angular noise of the dance signal. The slope of the second interaction was also positive ($y = 0.0074x - 0.642$), suggesting that the (negative) effect of the waggle run duration increases with increasing number of bees that dance at the same time.

Figure 3A shows the relationship between the indicated waggle run duration and the number of waggle runs followed. To visualize this relationship in a different way, we created 7 categories of waggle run duration: <1s, 1-2s, 2-3s, 3-4s, 4-5s, 5-6s, 6+ s) (Fig. 3B). The data shown in Fig. 3B suggest a negative relationship between the two variables.

Our findings show that followers follow fewer waggle runs for more distant food sources. However, as waggle runs and return phases take longer for further distances (Frisch 1967), followers might actually invest more time in dance following with increasing food source distance. To explore this we multiplied the average number of waggle runs followed for a particular distance category by the time it takes a dancer to make a circuit (waggle run + return phase) for the corresponding distances. Circuit duration was calculated using von Frisch's measurements (1967; Table 13). Figure 3C shows that there is indeed a remarkably strong positive relationship ($R^2 = 0.9911$) between waggle run duration per dance and the estimated time invested by dance followers to follow a dance.

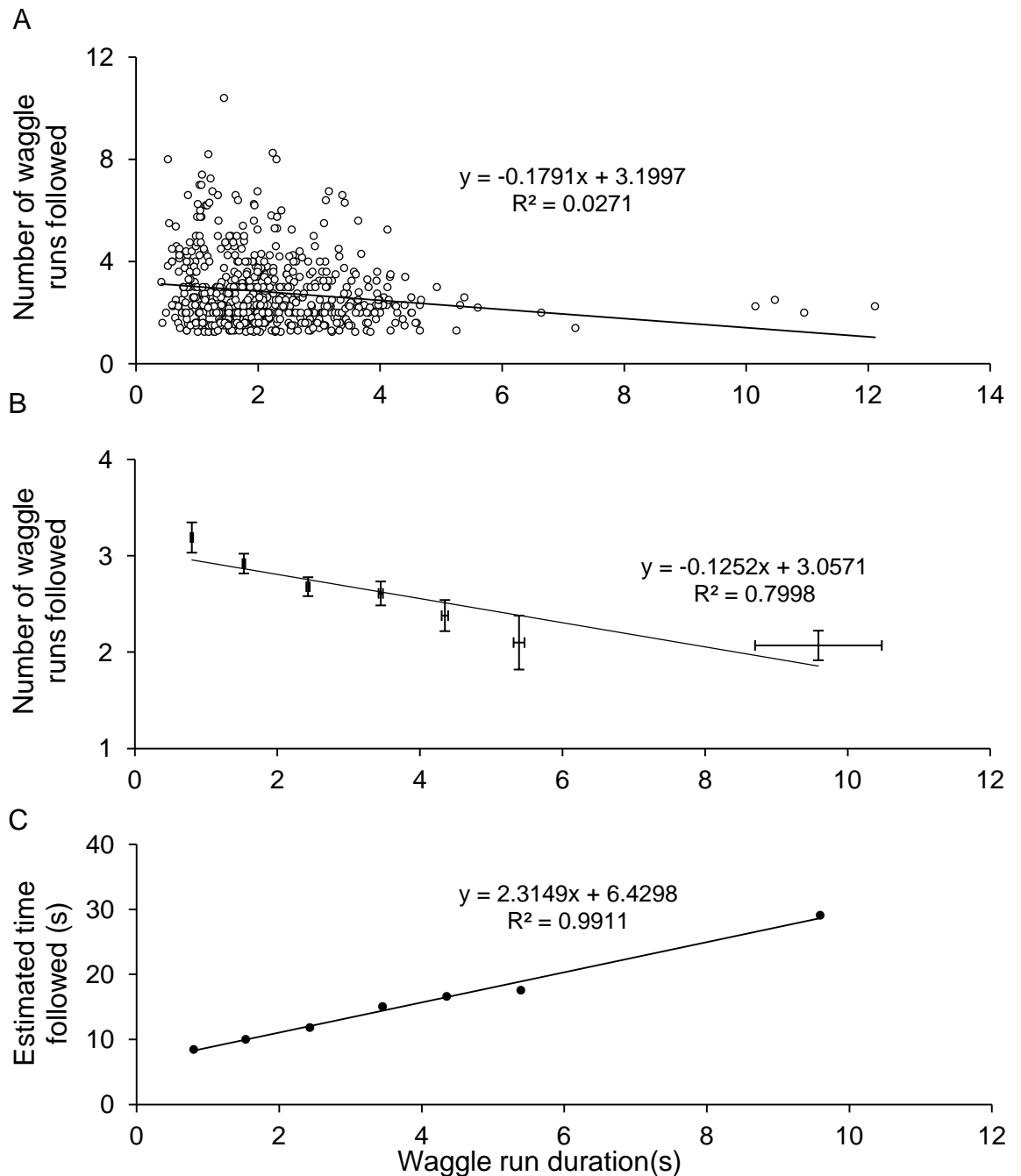


Fig.3: The data show that the number of waggles runs followed decreases with increasing waggles run duration of a dance, but with increasing waggles run duration (further distance of the food source) bees follow a dance longer in terms of time. A) The relationship between the waggles run duration and the number of waggles runs followers followed per dance. B) The same relationship but represented in a different way. We made seven categories for waggles run duration. Horizontal and vertical error bars represent standard errors of the means. C) The relationship between waggles run duration and the estimated time (sec) that followers follow the dancer. The estimated time of following was calculated using von Frisch's measurements of the total circuit duration given our waggles run durations, multiplied by the number of waggles runs followed for each category (1967; Table 13). The line represents a best-fit line of a regression analysis ($R^2 = 0.99$).

3.4.2 Experiment 2: Factors affecting the number of dance followers

First, we again tested whether the number of dance followers differed between hives and depended on whether dancers carried pollen. There was a significant effect of hives ($F_{2,596} = 4.02$, $P = 0.018$), but not type of dance ($F_{1,596} = 0.87$, $P = 0.351$) or the interaction ($F_{2,596} = 0.55$, $P = 0.579$). Hence, we analysed each hive separately but pooled pollen and non-pollen dances.

We hypothesised that the number of bees following a dance depends on the time of year (because of seasonal changes in overall foraging conditions and colony size), the number of other bees dancing (because this affects the ratio of dancers to potential followers) and waggle run duration (because this affects the area occupied by a dancing bee). Therefore, for each hive we ran a model including month, waggle run duration, and the number of simultaneous dances as explanatory variables.

In Hive 1, we found that month ($F_{3,243} = 11.08$, $P < 0.001$) and number of simultaneous dances ($F_{1,243} = 10.31$, $P = 0.002$) both had a significant effect on the number of dance followers but that waggle run duration did not ($F_{1,243} = 0.73$, $P = 0.394$). In Hive 2, we found that month was also significant ($F_{3,175} = 3.31$, $P = 0.021$), but that the other factors were not significant (waggle run duration: $F_{1,175} = 0.71$, $P = 0.402$; simultaneous dances: $F_{1,175} = 0.07$, $P = 0.799$). However, the interactions between waggle run duration and month ($F_{3,175} = 2.70$, $P = 0.047$) and waggle run duration and the number of simultaneous dances were significant ($F_{1,175} = 4.76$, $P = 0.03$). The slope of this interaction was positive ($y = 0.015x + 0.591$), which suggests that the effect of the waggle run duration increases with an increasing number of bees that dance simultaneously. In Hive 3, only month significantly affected the number of dance followers ($F_{3,162} = 9.66$, $P < 0.001$; waggle run duration: $F_{1,162} = 0.00$, $P = 0.990$; simultaneous dances: $F_{1,162} = 1.38$, $P = 0.24$). For each hive, we did a *post hoc* analysis

including a sequential Bonferroni correction for multiple testing (Sokal & Rohlf 1995) to investigate which months differ from each other in the number of followers (Table 1; Fig. 4). The results suggested that dances had most followers in September (early autumn).

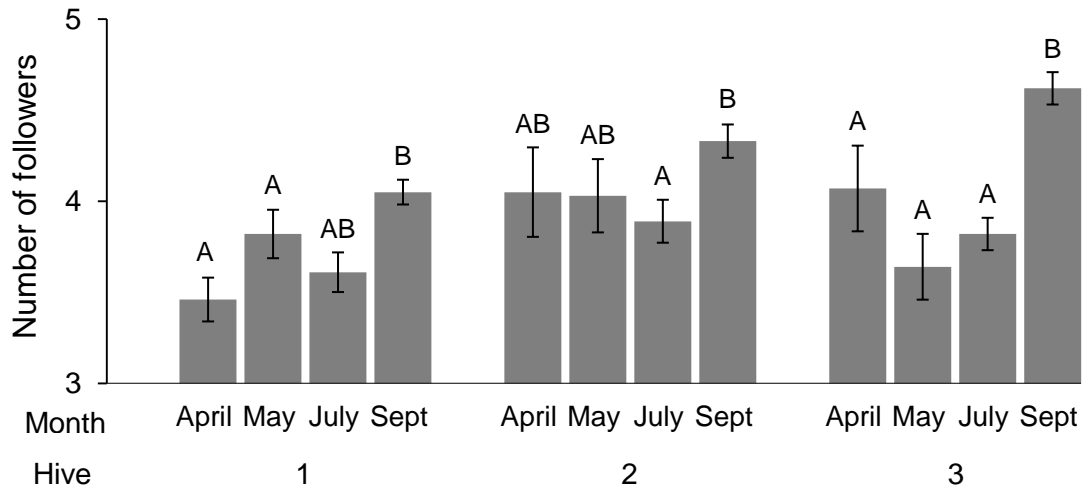


Fig.4: Dances have most followers in September compared to the other months (April, May and July). The letters above bars indicate statistically significant ($P < 0.05$) differences between months in each hive. For example, B above September in hive 2 indicates that the numbers of followers differs significantly from July (A), but that both September and July are not significantly different from May (AB). Error bars present standard errors.

Table 1: Pair-wise comparisons between months for each hive. P-values were corrected for multiple testing with sequential Bonferroni in order to avoid inflation of type-I errors.

Pairwise comparisons between months	P values		
	Hive 1	Hive 2	Hive 3
April – May	0.148	1.000	0.661
April – July	0.265	1.000	1.000
April – September	<0.001	1.000	0.029
May – July	1.000	0.948	1.000
May – September	0.014	1.000	<0.001
July – September	0.222	0.041	<0.001

3.5 Discussion

Our results show that the following of waggle dances for natural food sources is affected by the distance of the resource. As foraging distances increase bees follow

fewer waggle runs. However, since waggle run duration is longer for greater distances (Frisch 1967) followers actually invest more time in dance following with increasing food source distance (c.10s for a food source at c.1000m and c.30s for a food source at c.10,000m; Fig. 3C). Thus, although followers invest more time for distant food sources they might acquire less information because they follow fewer waggle runs. Previous research indicates that the number of waggle runs followed has a significant effect on the accuracy of flights to the advertised source (Tanner & Visscher 2009). However, it is not known whether dance followers acquire the same amount of spatial information per waggle run for nearby food sources (short circuits, short waggle runs) as for distant food sources (long circuits, long waggle runs). The waggle run provides distance and direction information, and these two vector components might differ in how easily or accurately they are decoded by dance followers. Interestingly, the two components are not equally prone to signal noise. Variation among waggle runs in the direction component depends on sensory and physical constraints that depend on the body position of the dancer (Couvillon et al. 2012a; Tanner & Visscher 2010) and this angular noise tends to decrease with increasing waggle run duration (Beekman et al. 2008; Couvillon et al. 2012b; Weidenmüller & Seeley 1999) (our Fig. 2B). On the other hand, noise in the duration component (distance) increases with increasing waggle run duration (Beekman et al. 2008; Couvillon et al. 2012b) (our Fig. 2A). Hence, while bees might need to follow fewer waggle runs to acquire accurate direction information for distant food sources, they probably need to follow more waggle runs to acquire precise information about distance. Feeder array studies (Tanner & Visscher 2009) or harmonic radar (Menzel et al. 2011) could be used in future studies to determine how dance following affects the angular and distance precision of foraging flights of varying distances.

Our results suggest that angular variation among waggle runs affects dance following, but that this effect depends on the distance of the food source. Followers actually followed fewer waggle runs for distant food sources when angular variation was greater. This suggests that followers might be less interested in following dances for distant food sources if the quality of the signal is bad. Another interesting result was that dance following for distant food sources was reduced when there were more simultaneous dances (interaction between waggle run duration and the number of simultaneous dances). A possible interpretation is that bees are quicker to abandon noisy dances for distant food sources if there are many alternative dances on the dance floor. This raises the question of how bees can estimate the overall dance activity on the dance floor. Two possibilities are vibrations perceived via the comb (Tautz 1996) and the presence of the dance pheromone (Thom et al. 2007).

In general, dance following was short (2.80 ± 1.38 , range 1-16 circuits), confirming previously reported results (Biesmeijer & Seeley 2005). While most followers followed 1-5 waggle runs (90%), only 2% followed >10. This suggests that most instances of dance following are shorter than typical for bees that successfully use the waggle dance to locate food (Grüter et al. 2008; Grüter & Farina 2009a; Menzel et al. 2011; Michelsen 2003; Wray et al. 2012). Wray et al (2012) showed that successful recruits followed on average 15.5 waggle runs and Menzel et al. (2011) showed that foragers leaving the hive in the direction of the advertised food location had followed on average more than 20 waggle runs. So why do bees following dances under natural conditions follow so few waggle runs? One explanation is that many foragers have private information about other food sources and, therefore, are not interested in following dances extensively (Biesmeijer & Seeley 2005; Grüter et al. 2008; Grüter & Farina 2009a). Since our followers were not individually marked, it is also possible that

these bees returned later to the same dances to acquire more information. However, this seems unlikely because subsequently-followed dances are likely to advertise different food sources (Biesmeijer & Seeley 2005; Fig. 4). It has also been shown that dancing attracts nectar receivers (Farina 2000). Hence, it is also possible that some of the followers were nectar receivers not interested in the food source location.

Our results showed that the number of followers differed between months and colonies. Two other factors waggle run duration and the number of simultaneous dances, had no large effects. The time of year might affect the number of dance followers for several reasons. For example, the number of followers could vary depending on colony size. This could also explain the inter colony differences in the number of dance followers. Dances had more followers in September (Fig. 4). In this month the foraging environment improved considerably compared to August due to the blooming of ivy (*Hedera helix*) and most foragers could be seen returning with the pollen from ivy (personal observation; Garbuzov & Ratnieks In preparation). It has been shown that foragers are attracted to dancers that carry familiar food odours, which in turn leads to a higher number of dance followers (Frisch 1923; Grüter et al. 2008; Grüter & Farina 2009b; Grüter & Ratnieks 2011). It is possible that dances will generally tend to have more followers when most of a colony's foragers have experience with the same food type, as was the case in September with ivy, because the nearest dancer of an unemployed forager is likely to carry a familiar and attractive odour.

In summary, our results show that dance followers respond to the characteristics of the waggle dance. However, while dancing behaviour and the factors that cause a bee to perform this signal are well understood (reviewed in: Couvillon 2012; Dyer 2002; Frisch 1967; Seeley 1995), we still have a limited understanding of how followers of

natural dances use the different informational components in their foraging decisions. More research into follower behaviour (signal receivers) and information use-strategies under natural circumstances is needed to understand, for example, why natural dance following is shorter than expected if follower bees were trying to decode the spatial information of a dance.

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