Personality and Cognition in Honey Bees (*Apis mellifera*) at the Individual and Colony Level

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The goal of this dissertation was to investigate cognition and personality in honey bees by studying both the individual behavior of nectar foragers (Chapters 1-3) and the collective behavior of honey bee colonies (Chapters 4-5). The first two chapters investigate the extent to which foraging honey bees understand the location information in the waggle dances they use to communicate about the location of high-quality resources. Chapter 1 demonstrates that—contrary to prior claims—honey bees following waggle dances do not use cognitive maps to reject dances for "implausible" locations that are unlikely to yield food. Chapter 2 reports that experienced foragers are more likely to pay more attention to the location information in waggle dances when the cost of incorrectly returning to a familiar food source is greater. However, not all bees responded to waggle dances in the same way; while some of them paid attention to the location information conveyed by the waggle dance, others ignored it. Chapter 3 demonstrates that individual honey bee foragers differ in their personalities, and that bees' activity levels and restlessness are consistent over time and across testing situations. Chapter 4 extends this study of personality differences to the colony level, by quantifying the extent to which colonies differ in their collective behavior and assessing the relationship between these traits and colony fitness.

We show that honey bee colonies differ consistently on five collective personality measures, and that colonies' foraging activity levels and aggressiveness are correlated with productivity and winter survival. Chapter 5 continues this study of colony-level behavior by asking whether reproductive swarms of honey bees display differences in their collective decision-making behaviors. We find that swarms searching for a new nest site differ consistently in their decision-making behaviors, with some colonies performing more waggle dances and shaking signals and making more scouting trips than others. However, these behavioral differences do not seem to lead to consistent differences in decision-making speed, which we interpret as evidence that chance discoveries of high-quality nest sites have a large influence on the speed with which honey bee swarms are able to choose a new home.

Biographical Sketch

Maggie Wray was born on March 5, 1984 in Atlanta, Georgia. She spent her childhood in Atlanta, Alexandria, VA and Boca Raton, FL. She started riding horses when she was 9, and worked at stables from age 13 until the end of high school in order to pay for riding lessons. Maggie attended Boca Raton Community High School, where she received 5's in all of her A.P. courses and graduated 3rd out of 497 students. As an undergraduate, Maggie attended Princeton University. Having enjoyed the introductory physics classes she took in her freshman year, she spent the following three summers doing research in the astrophysics department. While she enjoyed the small class sizes and numerous research opportunities that the astrophysics department offered, she also took a number of biology classes and developed a strong interest in astrobiology and the evolution of intelligent life in the universe. During her time at Princeton, she pursued these interests by working on the Optical SETI project and completing a senior thesis project that used the Drake Equation to estimate the number of habitable planets in the galaxy. In her senior year she became especially interested in the evolution of intelligence, and decided that the best way to understand how and why intelligent behavior evolves would be to study the behavior of other intelligent species here on Earth. So, after graduating *cum laude* from Princeton in the spring of 2006, Maggie entered the Ph.D. program in Cornell's department of Neurobiology and Behavior, where she has spent the past five years studying the fascinating and intelligent individual and collective behaviors of honey bees.

This thesis is dedicated to my husband, James Wray,

my mother, Lisa Reid, my father, Dep Kirkland, and my sister Emily Kirkland,

for believing in me and supporting me in everything I choose to do.

You are what makes it all worthwhile.

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I also want to thank Heather Mattila, who was a postdoc in Tom's lab during my first two years at Cornell. Heather was like a second advisor to me, who showed me how to work with bee colonies and taught me much of what I know about statistics. I am still inspired by her organization and productivity, and her remarkable ability to stay calm and upbeat in the middle of a chaotic field season. Thanks, Heather, for being a great friend, collaborator, and role model.

Thanks also to Barrett Klein, who was one of Tom's graduate students at UT Austin and collaborated with me on my first two research projects. Working with Barrett made those summers at Cranberry Lake infinitely more fun and enjoyable. His enthusiasm and excitement about insects are remarkable, and his sense of humor managed to make the longest and most difficult days in the field seem fun.

I also want to thank all of the field assistants who helped to make this work possible. For help with the feeders at CLBS, thanks to Michael Cornell, Matthew Myers, Heather Doviak, Casey Nelson, Amy Schmitte, Noel Fitzgerald, Leslie Teagarden, Alex Coll, Jessica Donohue, and Andrew Schneider. Sean Griffin assisted with the behavioral testing and colony weight measurements described in Chapter 4, and Yohan Cho performed observations of swarms and helped to measure foraging rates as described in Chapter 5. Finally, for assistance with video coding, thanks to Amanda Armstrong, Yohan Cho, Rachel Kery, Jane Park, Carolyn Phillips, Annie Smith, Michael Smith, and April Yeaney.

The staff at the Cranberry Lake Biological station were instrumental in enabling me to complete the work described in the first three chapters of this dissertation. Alex Weir provided us with space at the CLBS to perform these experiments and allowed students at the field station to spend time working at our feeders as part of their independent research projects. Larry Rathman was also very helpful with the boats at the field station.

I want to express my appreciation for all of the staff members in Cornell's Department of Neurobiology and Behavior, who were unfailingly friendly and pleasant to be around. You have a remarkable ability to make even complicated administrative tasks seem easy! I especially want to thank Lori Miller for all her help with BioNB 2210. Lori was an incredible course assistant during the three years I was head TA for the course, and I will be eternally grateful for her help with the class; I could not have done it without her. The staff at the Cornell Statistical consulting unit, especially Françoise Vermeylen, also provided valuable assistance with various aspects of the data analysis described here.

Finally, I want to thank the other Neurobiology and Behavior graduate students, including Jessie Barker, Caitlin Stern, Becky Cramer, Julian Kapoor, Karl Berg, Jude Scarl, Chris Wilson, Juliana Rangel, Kevin Loope, Julie Miller, Biz Turnell, Glenn Stamps, Paul Shamble, Gil Menda, Lynn Fletcher, and Sheng-Feng Shen. I really enjoyed all the time we spent together, and I feel very fortunate to have shared my time at Cornell with such a great group of people!

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Chapter 1

Honeybees do not reject dances for ‘implausible’ locations:
Reconsidering the evidence for cognitive maps in insects [1]

1.1 Abstract

Results from a previous study, known as the “Lake Experiment” (Gould & Gould 1982) suggest that honeybee foragers may assess the locations advertised by waggle dances that they follow, and reject dances for “implausible” locations that are unlikely to yield food. However, alternative explanations for these results—which do not require bees to use cognitive maps or to evaluate the “plausibility” of potential food sources—have also been proposed. To address this ambiguity, we repeated the study and used video analysis of dance followers in the hive to determine whether they refused to respond to implausible dances for a feeder on a lake. We found that bees following lake dances were just as likely to leave the hive as bees following control dances for a feeder on land. Bees also took the same amount of time to leave the hive after following dances for both locations, suggesting that their motivation to leave did not depend on the plausibility of the dance they had followed. Contrary to prior findings, our results provide no evidence that honeybees assess the plausibility of information contained in waggle dances or reject dances for locations that are unlikely to yield food. Thus, we conclude that the original Lake Experiment should no longer be cited as evidence that honey bees possess cognitive maps, “insight,” or “imagination.”

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[1] This work was originally published by Wray, M.K., Klein, B.K., and Seeley, T.D. (2008), Honeybees do not reject dances for ‘implausible’ locations: reconsidering the evidence for cognitive maps in insects, *Animal Behaviour, 76*, 261–269, doi: 10.1016/j.anbehav.2008.04.005.

1.2 Introduction

The honeybee waggle dance is a complex and versatile form of symbolic communication, and the flexibility with which bees utilize it suggests that they may possess relatively sophisticated cognitive abilities (Gould & Gould 1988; Seeley 2003). Laboratory experiments have demonstrated that honey bees are capable of complex forms of learning, such as the ability to learn same-difference relationships (Giurfa et al. 2001), which were previously believed to exist only in vertebrates (Giurfa 2003). However, there have been fewer tests of the cognitive abilities that honey bees utilize under natural conditions. In particular, the degree to which honeybees "understand" or "interpret" the messages in their dances—as opposed to producing and reacting to them automatically—is still largely unknown.

Yet there is one study that claims to provide evidence that bees assess the "plausibility" of dances they follow before responding to them: the "Lake Experiment" described by Gould & Gould (1982). In this study, honeybee foragers were trained to visit a feeder on a boat in the middle of a lake. When the trained foragers performed dances for this location, no recruits arrived at the lake feeder during five out of six days of training, despite the fact that dances for an equidistant feeder on land elicited heavy recruitment (Gould & Gould 1982). Similarly low recruitment levels to feeders on a lake were also observed in a more recent study by Tautz et al. (2004). The most frequently cited explanation for these results is that "while still in the hive, the [dance followers] used the direction and distance information in the dance to position the advertised site on their mental map, determined that it was in the lake and therefore implausibly located, and refused to respond" (Gould 1990). Followers of dances for sites on the shore, however, "would 'picture' a more believable location and act on the information" (Gould & Gould 1988).

Because the Lake Experiment suggests that honeybees use a cognitive map to assess the "plausibility" of dances, this study has frequently been discussed as potential evidence for cognitive maps in bees (e.g. Gould 1986; Shettleworth 1998). The question of whether bees possess a cognitive map, i.e. an internal representation of the spatial relationship of objects in their environment (Wehner & Menzel 1990), is a longstanding debate which has recently been renewed in the literature (see Menzel et al. 2005).

Beyond its role in the cognitive map debate, the Lake Experiment has also been cited in papers on animal communication and deception (e.g. Fitch & Hauser 2002; Crist 2004) because of its implication that bees can interpret and assess the veracity of messages they receive. Furthermore, some authors have proposed that if bees are actively evaluating the "plausibility" of information they receive from dances and comparing that information with their own knowledge of the surroundings, then perhaps honeybees have more than just a cognitive map—this suggests that they may possess "insight" (Gould & Gould 1988), "imagination" (Gould 1990), or some form of consciousness (Griffin 2001).

Despite widespread interest in the Lake Experiment, most authors acknowledge that the data "are not sufficient to provide a fully convincing case" (Griffin 2001), and "can be interpreted in more than one way" (Shettleworth 1998). Indeed, there are a number of alternative explanations for the observed lack of recruits at lake feeders which do not involve bees rejecting dances for "implausible" locations. Dyer & Seeley (1989) point out that recruits in the original Lake Experiment may have in fact left the hive, but failed to arrive at the feeder because bees flying over a lake surface may become disoriented, lose altitude, and drown (von Frisch 1967). Tautz et al. (2004) offer three additional explanations for why recruits in their experiment might have left the hive but failed to arrive at the lake feeder: (1) the indication of distance in the dance directions may have been less precise for lake locations, making them harder for recruits to locate, (2) experienced foragers did not seem to help recruits find the lake feeder, which could have made it more difficult for them to locate it, or (3) recruits may have flown over the lake at a different altitude than the trained foragers who provided the dance instructions, causing recruits to miscalculate the distance to the feeder.

Knowing only that few recruits arrive at lake feeders, it is impossible to determine whether bees following lake dances are refusing to leave the hive, or are leaving the hive but failing to arrive at the lake feeder for reasons such as those mentioned above.

Data on the behavior of dance followers inside the hive—which were not collected during the original Lake Experiment (Dyer & Seeley 1989; Gould & Towne 1989) or by Tautz et al. (2004)—are required in order to discriminate between these possibilities. If bees do not leave the hive after following dances for the lake feeder, this would provide strong support for the hypothesis that they are refusing to respond to dances for "implausible" locations. However, if as many bees leave the hive in response to lake dances as to land dances, then this hypothesis can be rejected. Our study tested this hypothesis by repeating the original Lake Experiment and videotaping the behavior of both dancers and dance followers in the hive in addition to recording the number of recruits at lake and land feeders.

1.3 Methods

1.3.1 Study Site and Subjects

We performed this experiment at the Cranberry Lake Biological Station (CLBS) in the Adirondack State Park, Saint Lawrence County, New York State (44°09'N, 74°48'W). This study site is surrounded by more than 20 km of forest, and offers very few natural food sources for honeybees. These conditions made it relatively easy to elicit dancing for artificial food sources, and guaranteed that nearly all of the dances performed in the hive were for our artificial feeders. In addition, the lack of other honeybee colonies in the surrounding area ensured that every unmarked bee we observed at our feeders was from one of our colonies.

We conducted two trials of the experiment; the methods for both were the same unless otherwise noted. We performed Trial 1 on 15-16 August 2006 with a colony of approximately 3000 Italian (*Apis mellifera ligustica*) honeybees (queens from C. F. Koehnen and Sons, Inc., Glenn, CA) which had been brought to the CLBS from the Liddell Field Station in Ithaca, NY one week earlier. During the week leading up to the experiment the bees were permitted to forage freely, both in the surrounding area and at a feeder which was available twice a day for two to three hours. The colony was housed in a two-frame observation hive which was kept in a small wooden hut to prevent exposure to the elements (see Seeley 1995, Figures 4.2, 4.4).
We used a second colony of approximately 3000 New World Carniolan (*Apis mellifera carnica*) honeybees (queens from C. F. Koehnen and Sons, Inc., Glenn, CA) for Trial 2, which took place on 17-18 August 2006. This colony was moved from Liddell Field Station to the CLBS on 15 July, and for the following month foraged exclusively on wild sources. This observation hive was housed in a classroom approximately 20 m away from the wooden hut used in Trial 1.

1.3.2 Experimental Layout

We trained one group of 20-25 bees to visit a feeder in a 5 m long boat anchored in Cranberry Lake (hereafter, the “lake feeder”) and trained a separate group of bees from the same colony to an equidistant feeder on land (the “land feeder”) (Figure 1.1). All trained bees were labeled with shellac-based paint marks; the color of each bee's thorax indicated the feeder to which she had been trained, and the colors on her abdomen allowed her to be individually identified.

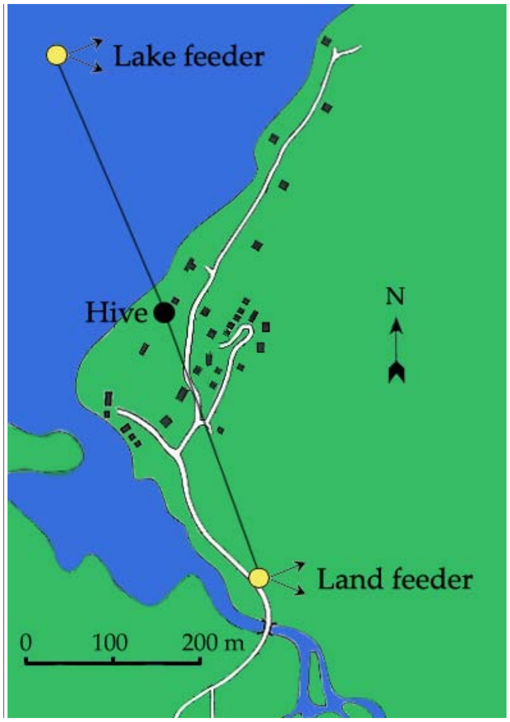

Figure 1.1 – Location of the hive and the land and lake feeders at the Cranberry Lake Biological Station. Both the land and the lake feeder are 300 m from the observation hive. The lake feeder is 260 m offshore. Arrows emanating from the two feeders indicate the wind direction in the two trials: from the WSW (Trial 1) and the WNW (Trial 2).

Both feeders provided bees with sucrose solution (0.5-2.5 M). To facilitate training, and more closely approximate the bees' experience of foraging at natural food sources, each feeder had a distinctive scent which was present both in the sugar solution and in a reservoir on top of which the jar of solution rested. In trial 1, anise scent was used at the lake feeder and orange scent was used at the land feeder. These scents were switched in Trial 2 to control for any effects the scents might have on the bees' behavior. The presence of distinct scents also tends to increase recruitment levels by enabling recruits to find feeders more easily (Tautz & Sandeman 2003). So, using scents allowed us to better test the hypothesis that bees would not arrive at the lake feeder by increasing the likelihood that bees who left the hive in response to lake dances would then be able to locate the lake feeder.

Both the land and lake feeders were located 300 m from the hive. At this distance (260 m from the nearest shore), the lake feeder was clearly distinguishable from any location on land, both visually (Figure 1.2) and—presumably—via dance instructions obtained from a trained forager. This distance was chosen to maximize the likelihood that bees assessing the location's plausibility would interpret it as an implausible place to find food. At this distance, our feeder should seem even more implausible to bees than the feeder used in the original Lake Experiment, which was located only 165 m from the hive (Gould & Gould 1982).

Figure 1.2 – 360 degree view from the Lake feeder at 300 m from hive (260 m offshore). The location of the observation hive is indicated with an arrow.

The angle between our land and lake feeders was approximately 180 degrees, with the result that dances for the two sites pointed in nearly opposite directions (Figure 1.1). It was thus highly unlikely that recruits following instructions from a dance for one site would accidentally encounter the other feeder. It was also improbable that bees following dances for the land feeder (hereafter, "land followers") would mistake those dances as pointing to a location on the water, or that bees following dances for the lake feeder (hereafter, “lake followers”) would interpret that dance as pointing to a location on land. Our design differed slightly from that of Gould & Gould (1982), who placed the land feeder on the shore of the lake, creating a 90 degree angle between the two feeders. The 180 degree angle we used should have made it even easier for dance followers to distinguish dances pointing to the lake from those pointing towards land.

1.3.3 Performing the Experiment

From 0900 hours on the first day of each trial (15 Aug. for Trial 1, 17 Aug. for Trial 2) until approximately 1400 hours on the following day, bees were trained to visit the land and lake feeders using techniques described by von Frisch (1967). The testing period began at 1500 hours on the second day, at which point the concentration of the sugar solution was raised from 0.5 M to 2.5 M to encourage bees to perform dances for the feeders. During the following two to three hours (Trial 1: two hours, Trial 2: three hours), all visits to each feeder by both trained bees and new recruits (unmarked bees) were recorded. Recruits were caught in Ziploc bags to avoid overcrowding at the feeder and to prevent them from returning to the hive to dance.

For two hours (from 1500 to 1700), we videotaped (Panasonic AGDVC 30) the area of the observation hive in which bees were performing and following dances (the “dance floor”). These tapes enabled us to analyze the behavior of both dancers and dance followers, and allowed us to see when followers entered the tunnel leading out of the hive (which we used as an indication that they were leaving the hive). To ensure that all dancing bees could be accurately identified in the videos, throughout each two-hour test period an observer at the hive pointed to each bee that performed a dance and read her identifying paint marks aloud; this information was included on the audio track of the tapes.

1.3.4 Video Analysis

In defining a “dance”, we attempted to minimize our chances of counting bees as having ‘rejected’ a dance who had not obtained sufficient information from the dance they were following to be able to leave the hive. Because most dance followers that are able to locate a food source indicated by a dance have followed the dance for an average of approximately 8 waggle runs (Judd 1995), and followers often do not begin to follow a dance until after the dancer has already performed one or two waggle runs, we defined a “dance” as a sequence of 10 or more consecutive waggle runs performed by a bee in a particular region on the comb.

We defined a “dance follower” as a bee that was no more than one bee-width away from a dancing bee, was facing the dancer, and followed her movements for at least two complete waggle runs (a.k.a. “dance circuits”). This allowed us to avoid counting as “dance followers” bees who simply happened to be walking past the dancer (we ignored bees that followed only one circuit), and prevented us from biasing our analysis against any bees that recognized quickly that the dance indicated an ‘implausible’ location and rejected it after only following a small number of waggle runs (we counted any bee that had followed at least two runs as a “dance follower”).

Across both trials, 228 land and 201 lake dances—performed by 33 land and 37 lake bees—satisfied the criteria described above. For each of these dances, we noted the dancer’s identity and feeder. For a randomly selected subset of these dances (50 land and 51 lake dances, performed by 23 land and 22 lake bees), we counted the number of waggle runs performed during that dance (a measure of the quality of the food source; Seeley & Towne 1992) and the mean duration of the waggle run (a measure of the distance to the food source; averaged over five dance circuits). Then, for the first two followers of each of these dances, we noted the number of dance circuits they followed, the next action they performed (whether they left the hive, followed another dance, or moved off-screen), and the time that elapsed before the next action. If the bee's next action was to follow another dance, the identity and feeder of the dancer were noted. All video analyses were performed using Final Cut Pro version 4 (Apple Inc.).

1.3.5 Statistical Tests

All statistical tests except power analyses were performed using SAS version 8.02 (SAS Institute 1999). For analyses of dance data (i.e. number of circuits/dance), multiple dances performed by the same dancer were treated as subsamples of that dancer (experimental unit) to avoid pseudo-replication. From the perspective of a dance follower, however, each dance represented a discrete and unique set of information, so the dance, rather than the dancer, was treated as the experimental unit. Thus, for analyses of dance followers (i.e. number of dance circuits followed), followers of different dances were treated as independent whereas followers of the same dance were treated as subsamples of that dance.

When numerical data met normality assumptions, two-way ANOVAs (trial and feeder effects) were performed (Proc GLM). Where effects were significant, means were separated with a Tukey Standardized Range test. Data that did not initially meet normality assumptions were log transformed or averaged for each bee to improve normality, or a non-parametric Wilcoxon test was used. The reduced power of the non-parametric tests made it necessary to pool data across trials and ignore subsampling effects. Chi-square tests were performed for categorical data (Proc FREQ). All *P*-values are for two-tailed tests; alpha values for all tests were set at 0.05. Means are reported as LSmean ± standard error. To avoid Type II errors, and maximize our chance of detecting significant differences between the land and lake followers, we did not apply Bonferroni corrections for multiple tests performed on the same data set.

Power analyses were performed using G*Power version 3 (Faul et al. in press). Reported values are from post-hoc power tests based on our total sample size and degrees of freedom. Estimates of small, medium, and large effect sizes for ANOVA tests (small: f=0.1, medium: f=0.25, large: f=0.4) and Chi-square tests (small: w=0.1, medium: w=0.3, large: w=0.5) were based on Cohen's (1988) recommendations.

To ensure that followers could be accurately classified as leaving the hive in response to the dance they had followed (as opposed to leaving the hive to scout for new food sources, or to return to a previously-visited food source), our analysis only included bees that left the hive within 130 s after they had stopped following the dance (this eliminated only the largest 3.5% of values). Similarly, bees that followed a second dance were only analyzed as such if they began following that dance within 130 s after they had stopped following the first dancer.

1.4 Results

1.4.1 Behavior of Dance Followers in the Hive

Bees following dances for the lake site were just as likely to leave the hive as bees following dances for the land site (Table 1.1; Chi-square test). Our power for this test (n=178) was 0.27 for a small effect size, 0.98 for a medium effect size, and 1.0 for a large effect size. We also found no difference between land and lake bees in the amount of time it took for the bees that left the hive after following a dance to exit the hive (Figure 1.3; two-way ANOVA with subsampling and log transformation; Feeder effect: $F_{1,75}$ = 0.0, P=0.95; Trial effect: $F_{1,75}$=0.01, *P*=0.93; Trial × Feeder interaction: $F_{1, 75}$=3.11, *P*=0.08; Subsampling effect: $F_{75, 32}$=1.28, *P*=0.22). Our power for this test (n=111) was 0.18 for a small effect size, 0.74 for medium effect size, and 0.99 for large effect size.

Table 1.1 – Number of land and lake followers that left the hive.

All data are for bees that left the hive within 130 s after they stopped following the dance.

Dance	Left Hive	Trial 1	Trial 2	Both Trials
Land	Yes	31	27	58
	No	15	17	32
Lake	Yes	32	21	53
	No	15	20	35
$\chi^2_{df=1}$		0.005	0.89	0.34
$P_{\text{Chi-square}}$		0.94	0.35	0.56

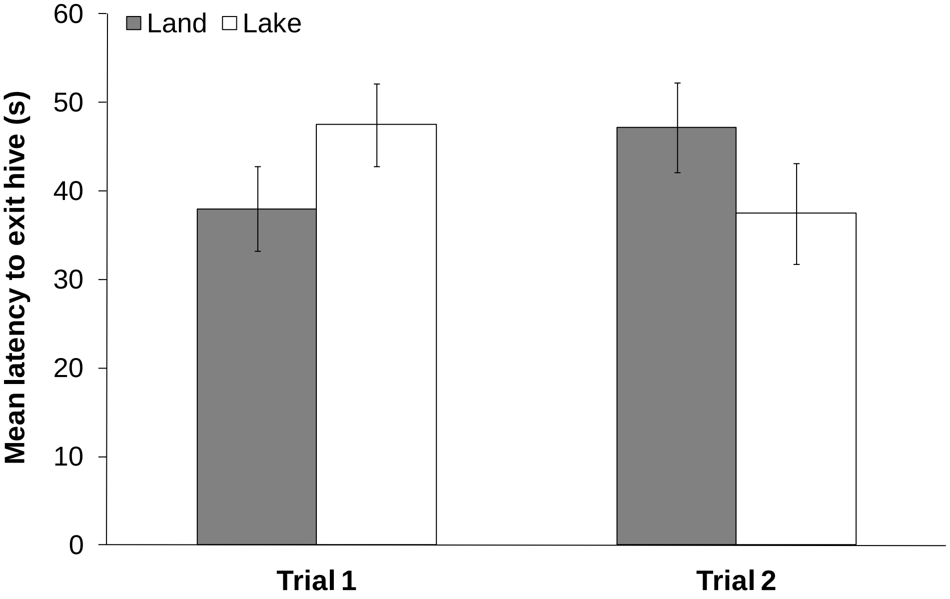

Figure 1.3 – Bees following land and lake dances did not differ in their latency to leave the hive (*P* = 0.95). Latency to leave was the mean number of seconds (± SE; before log transformation) between the time when a follower stopped following a dance and when she exited the hive.

Bees following land dances tended to follow slightly more dance circuits (7.9±0.37 circuits/follower) than bees following lake dances (6.8±0.37 circuits/follower) (two-way ANOVA with subsampling; Feeder effect: $F_{1,97}$ =4.1, P=0.047; Trial effect: $F_{1,97}$=0.8, P=0.38; Trial×Feeder interaction: $F_{1,97}$=1.4, P=0.25; Subsampling effect: $F_{97,98}$=1.0, P=0.47). Regardless of the type of dance they were following, dance followers that subsequently left the hive followed more dance circuits on average (8.1±0.35 circuits/follower) than dance followers that did not leave the hive (6.1±0.45 circuits/follower) (Wilcoxon test; Z=-3.13, P=0.002).

For those bees that followed a second dance instead of leaving the hive, the location (land or lake) of the first dance they had followed made no difference in the amount of time it took them to begin following a second dance (Wilcoxon test; Z=-0.74, P=0.46). However, the second dance they followed was significantly more likely to be for the same feeder as the first dance they had followed than would be expected by chance (Table 1.2; Chi-square test).

Table 1.2 – Location advertised by first and second dances followed.

Data represent bees that did not leave the hive after following the first dance, but instead followed a second dance within 130 s after they had stopped following the first dance. Data are presented for both trials combined.

1^{st} dance	2^{nd} dance	Number of followers
Land	Land	22
	Lake	5
Lake	Land	6
	Lake	19
$\chi^2_{df=1}$		17.26
$P_{\text{Chi-square}}$		<0.0001

1.4.2 Dance Information

Dancers for the lake feeder and for the land feeder performed the same average number of circuits per dance (Figure 1.4; two-way ANOVA with subsampling and log transformation; Feeder effect: $F_{1,39}$=0.002, P=0.96; Trial effect: $F_{1,40}$= 0.41, P=0.52; Subsampling effect: $F_{40,58}$=1.39, P=0.12; Trial × Feeder interaction: $F_{1,40}$=1.42 P=0.24). Our power for this test was 0.17 for a small effect size, 0.70 for a medium effect size, and 0.98 for a large effect size.

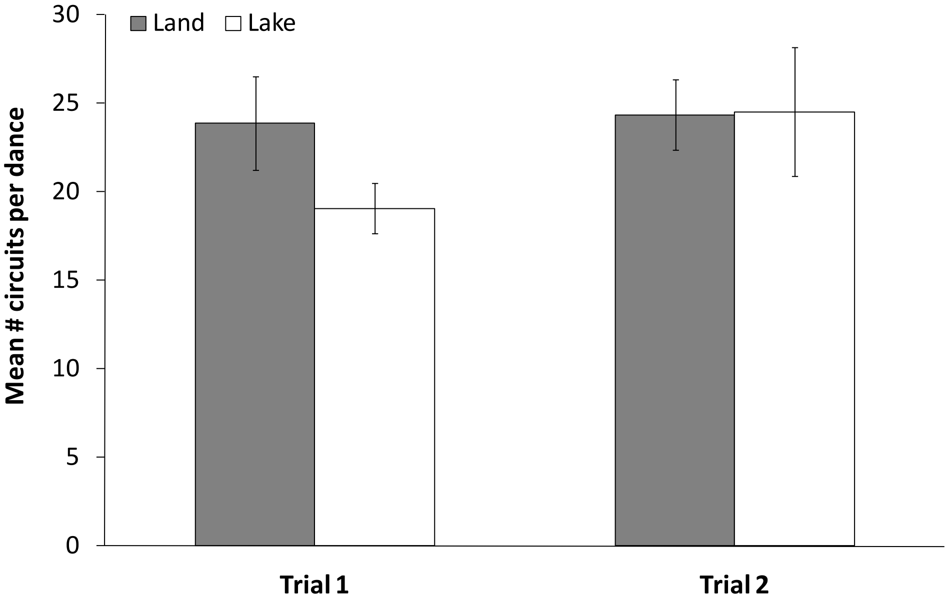

Figure 1.4 – Mean circuits per dance for bees trained to visit land and lake feeders in Trials 1 and 2 (± SE). Dances did not differ between land and lake bees (P=0.96).

The average duration of waggle runs was significantly greater for bees performing dances for the lake feeder (0.96 ± 0.02 s) than it was for bees performing dances for the land feeder (0.72 ± 0.02 s) (Figure 1.5; two-way ANOVA, averaged across dances for each bee, no transformations; Feeder effect: $F_{1,40}$=51.51, P<0.0001). In addition, the average waggle run duration was slightly greater for bees from Trial 2 (0.88 ± 0.02 s) than it was for bees from Trial 1 (0.80 ± 0.02 s) (Figure 1.5; Trial effect: $F_{1,40}$=5.48, P=0.02). [The Trial × Feeder interaction was not significant; $F_{1,40}$=1.99, P=0.17.]

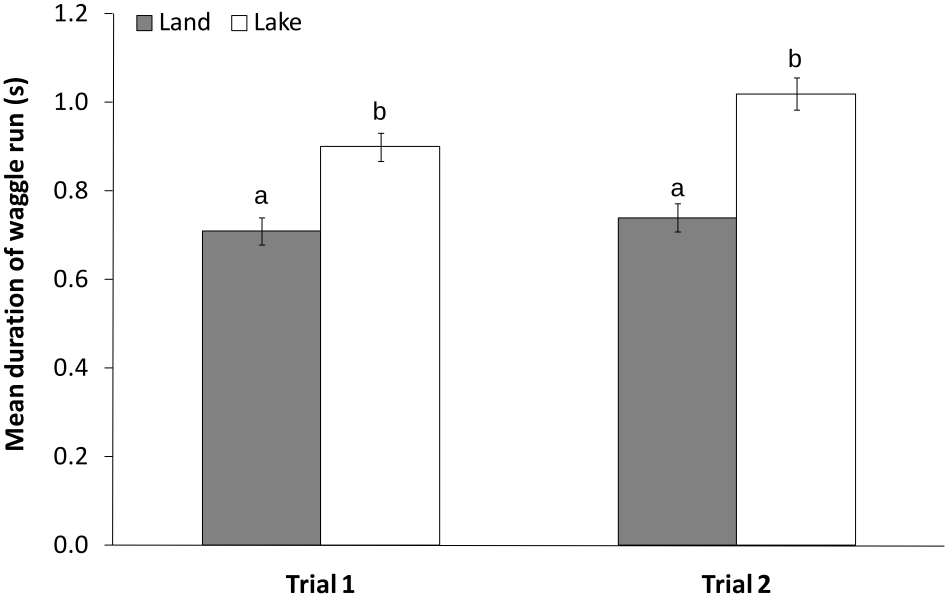

Figure 1.5 – Mean duration (± SE) of dancing bees' waggle runs in Trials 1 and 2. Dances for the lake site were significantly longer than dances for the land site (P<0.0001); significant differences are indicated by the letters above the bars.

1.4.3 Recruitment to Land and Lake Feeders

We captured new, unmarked recruits at the lake feeder in both experimental trials (Figure 1.6). If we pool our data across both trials, there was no significant difference in the number of recruits that arrived at the land feeders as compared to the lake feeders (87 vs. 99; Chi-square: χ^2_1=0.77, P=0.38).

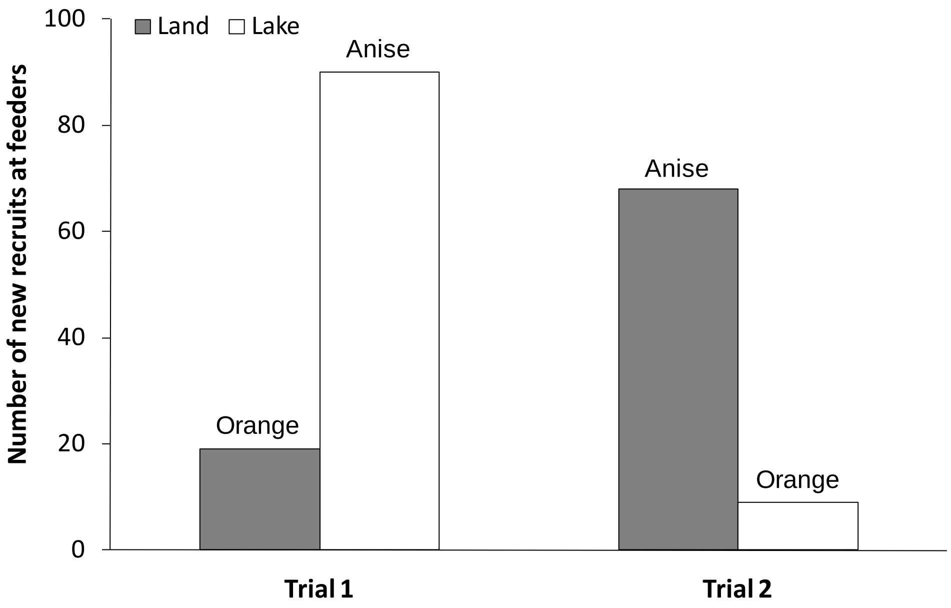

Figure 1.6 – Number of new recruits that were caught at the land and lake feeders in Trial 1 (2 hours) and Trial 2 (3 hours). The scent of the sucrose solution that was used at the feeder is indicated above each bar. There was no difference between the number of recruits that arrived at land and lake feeders (*P*=0.379). Significantly more bees arrived at feeders with anise scent (*P*<0.001).

However, there was also a strong interaction between trial and feeder associated with the change in scents from Trial 1 to Trial 2 (Figure 1.6). In Trial 1, many more recruits arrived at the lake feeder (90 recruits; anise scent) than the land feeder (19 recruits; orange scent), whereas in Trial 2 far more new recruits arrived at the land feeder (68 recruits; anise scent) than at the lake feeder (9 recruits; orange scent). Across the two trials, there was a significant difference in the number of recruits that arrived at anise-scented feeders as compared to orange-scented feeders (158 vs. 28; Chi-square: $\chi^2_1=90.86$, $P<0.001$).

In Trial 1, the wind was blowing from the WSW at 12.9 to 20.9 km/h, and in Trial 2, the wind was coming from the WNW at 0 to 4.8 km/h. In neither trial was the wind blowing scents towards the hive from either of the feeders. On the contrary, in both cases the wind was blowing the scent almost perpendicular to the direction to the hive (Figure 1.1).

1.5 Discussion

1.5.1 Behavior of Followers in the Hive

The critical prediction of the hypothesis that bees reject dances for lake sites is that bees following “implausible” (lake) dances should leave the hive in response to these dances less often than bees following dances for “plausible” (land) locations. Instead, our data clearly show that bees following “implausible” lake dances left the hive just as frequently as bees following “plausible” land dances. If any difference between the land and lake followers existed, we should have detected it because even for a medium effect size our power was 98%.

Although bees did ultimately leave the hive in response to dances for “implausible” (lake) sources, if these followers recognized the directions as unusual or potentially misleading then we might expect them to have been less motivated to leave the hive—and thus have taken longer to leave—than followers of land dances. However, for dance followers who left the hive, followers of “implausible” dances took no longer to leave the hive than followers of “plausible” dances. Thus, it seems that following “implausible” dances did not cause bees to appear “confused” or “uncertain.” Instead, they appeared just as motivated to leave the hive as followers of dances advertising a “plausible” location, which suggests that they did not interpret the lake location as being “implausible” at all.

If not all bees were equally able to distinguish between “plausible” and “implausible” dances, and the lake followers that remained in the hive were the only ones that recognized the location as nonsensical, then we might expect to see a behavioral difference between the land and lake followers that remained in the hive. In particular, bees that had followed lake dances might begin following a second dance more quickly, since their reason for not responding to the first dance was its “implausibility”, not a lack of motivation to forage. However, lake followers took no less time to follow a second dance than did land followers. Thus, we have no evidence to suggest that the lake followers that remained in the hive did so because they interpreted the dance they had followed as “implausible.”

Regardless of the “plausibility” of the first dance they followed, there was a strong tendency for bees that followed second dances to follow a dance for the same location as the first dance they had followed. This effect could potentially result from dances being distributed in such a way that a bee randomly following the next dance she encountered would tend to follow multiple dances for the same site. However, dances did not seem to be clumped together on the dance floor according to the site that was being advertised. Nor did bees seem to simply follow the next dance that they encountered; instead, they often travelled long distances across the dance floor before following a second dance, sometimes walking directly past one dance to follow a more distant one. An alternative explanation for this result is that, although bees did not seem to assess the “plausibility” of the locations indicated by dances they followed, they were capable of discriminating between dances for different locations and were actively seeking out multiple dances for the same source.

1.5.2 Dance Information

The fact that we found no difference in the average number of circuits per dance for land and lake feeders suggests that dancers for the land feeder were not advertising their site more enthusiastically than dancers for the lake feeder (or vice versa). Thus, if followers are responding to dances based on the advertised quality of the food sources (as indicated by the number of waggle runs per dance), followers' responses should not be biased towards one feeder or the other.

Honeybees' "odometers" have been shown to run at slower speeds over water than over land (Tautz et al. 2004) due to the lower optic flow they experience when flying over water, so we expected that the distance to the feeder (indicated by the duration of the bees' waggle runs) would be shorter for bees trained to the lake feeder. However, we found the opposite: waggle runs of bees trained to the lake feeder were significantly longer than those of bees trained to the land feeder. There are a number of possible reasons for the discrepancy between our results and those of Tautz et al. (2004). There might be differences in the relative amounts of visual contrast (and, therefore, optic flow) at our two study sites. This difference could result, for example, from there being more waves on our lake (greater visual contrast) or fewer shadows on the path along which our land bees flew (less visual contrast). Alternatively, the difference between our findings and those of Tautz et al. (2004) could result from the bees in the two studies flying at different relative altitudes, which could provide them with different amounts of optic flow.

1.5.3 Recruitment to Land and Lake Feeders

We found that while there is quite a strong effect of scent on recruitment levels (discussed below), when data were pooled across trials there was no significant difference between the number of recruits arriving at the land and the lake stations. This suggests that the location of the feeder (on the lake or on land) did not have a significant effect on recruitment levels. Moreover, in both trials we found that recruits were able to successfully locate the feeder on the water. This finding contrasts with the results of both Gould & Gould (1982) and Tautz et al. (2004), who reported either no recruits or very low recruitment levels to feeders on the water as compared to those on land.

The fact that our recruitment results do not match those of Tautz et al. (2004) may be due to scent differences, especially because our feeders' scents seemed to have a greater impact on recruitment levels than their locations (see discussion below). Since they were studying the behavior of trained foragers, rather than recruits, Tautz et al. (2004) used unscented feeders, which is likely to have resulted in an overall decrease in recruitment levels. If there were few recruits to begin with, then if bees had slightly more difficulty locating a feeder on the water than they did locating a feeder on a lake, this could have led Tatutz et al. (2004) to observe no recruits at all at the lake feeder.

It is difficult to interpret the relationship between our recruitment results and those of the original Lake Experiment, since we cannot be positive that scent was used in that experiment, and data on the exact number of bees recruited to land as compared to lake feeders do not exist (Fred Dyer, pers. comm.). However, it is clear that our results do not match reports that "no recruits came" to a feeder on a lake (Gould 1984). On the contrary, recruits arrived at the lake feeder in both trials of our experiment, and in trial 1 over 90 recruits arrived over the course of two hours. Thus, while it is difficult to determine the extent to which our results disagree with the existing data from the original Lake Experiment, we found no evidence to support the claim that very few bees arrive at lake feeders as compared to equidistant feeders on land.

1.5.4 Effects of Feeders' Scents on Recruitment Levels

We chose to use scents in this experiment, as opposed to using unscented feeders, for a number of reasons. The first of these is that training bees is greatly facilitated by the use of scents (von Frisch 1967, p. 17), and is specifically recommended when training bees over water (von Frisch 1967, p. 111). The second reason is that under natural conditions, bees rarely pollinate unscented food sources (von Frisch 1967, p. 48), and thus the behavior of bees being recruited to unscented feeders has been found to be rather different from their behavior when flying to scented feeders (Tautz & Sandeman 2003). Under ordinary conditions, bees that are recruited to unknown food sources tend to rely on both dance information (to locate the general vicinity of the food source) and scent (to pinpoint the food source's exact location) (Dyer 2002). When no scent is present, recruits are known to have a much more difficult time locating feeders, resulting in very low recruitment levels (Tautz & Sandeman 2003; Riley et al. 2005).

Our use of scents also makes sense in the context of previous studies. Tautz et al. (2004) did not need to use scents because they were not studying the behavior of recruits—they were studying the behavior of trained foragers, who have already visited the feeder and thus do not need scents in order to locate it . However, Fred Dyer, who performed the original Lake Experiment described by Gould & Gould (1982), is fairly certain that scents were used during the experiment (pers. comm.). In our study, the aim of collecting recruitment data was to test the hypothesis that recruits would not arrive at the lake feeder, and using scents gave us the best chance of falsifying this hypothesis by providing us with higher recruitment levels overall.

It turned out that the scent of the feeders (anise vs. orange) had a more dramatic effect on recruitment levels than the location of the feeders (land vs. lake); across both trials, significantly more bees arrived at the anise-scented feeder than at the orange-scented feeder. The difference in recruitment levels was not the product of dance followers in the hive responding preferentially to dancers that smelled of anise, because in both trials bees following dancers with either scent were equally likely to subsequently leave the hive. Instead, the higher recruitment levels to anise-scented feeders are probably the result of recruits being better able to locate anise-scented feeders after leaving the hive. This is consistent with the fact that bees recruited by a dance to visit an unknown food site are known to use scent primarily to pinpoint the precise location of the food source once they arrive in the vicinity (Dyer 2002).

Similarly different recruitment levels to feeders with different scents were obtained in a past study where equal numbers of trained bees performed dances simultaneously for two equidistant feeders with 2.5 M sugar solution, and approximately 4-12 times more recruits arrived at the anise-scented feeder than at a peppermint-scented feeder (Seeley, unpublished data). Although we used orange instead of peppermint scent, our recruitment results (4.7 to 7.5 times more recruits to anise in Trials 1 and 2 respectively) are consistent with these earlier findings. Our observation that substantially different recruitment levels can occur at otherwise equally desirable feeders with different scents may be important to consider in future studies involving honeybee recruitment.

1.5.5 Conclusions

Based on our initial hypothesis that bees reject dances for “implausible” locations, we made the following three predictions: (1) bees following lake dances should be less likely to leave the hive than bees following land dances, (2) followers of lake dances should take longer to leave the hive than followers of land dances, and (3) very few (if any) recruits should be observed at the lake feeder. None of these predictions were supported by our data. Thus, we can confidently reject the hypothesis that honeybee foragers are rejecting dances for “implausible” locations that are unlikely to yield food.

There are a number of hypotheses that could explain why bees might not reject dances advertising a location on a lake. One possibility is that bees do not have a cognitive map, and thus do not recognize the dances as pointing to a location on a lake. A second possibility is that bees have a cognitive map, but they either only use it for navigation outside of the hive, or it is overridden by other cues, such as odour, when they follow a dancing bee. A third explanation is that bees can use a cognitive map to determine that the dances indicate a location on the lake, but they do not interpret lakes as being “implausible” places to find food—either because they are not assessing the “plausibility” of dances at all, or because they do sometimes find food on bodies of water (in the form of flowering water plants, for instance). Lastly, it is possible that dance followers recognize the lake as being an unusual—even “implausible”—place for a dance to indicate, but because “there is no evidence of lying by dancers, and no advantage to dissimulation in a colony of non-reproductive sisters” (Gould 1990), they respond to the dances because they have no reason to believe the signal is in error.

Our data do not allow us to discriminate among these alternative hypotheses, so we cannot currently determine why dance followers did not reject dances for “implausible” lake sources. Consequently, while our study offers no evidence that bees possess cognitive maps, it also does not allow us to reject that hypothesis. However, if our bees did have cognitive maps, they clearly were not using them to reject dances indicating food sources that were “implausibly” located in the middle of a lake. On the contrary, we found no evidence that the bees—either before or after they left the hive—had any hesitation about responding to dances for locations on the lake. Thus, we conclude that the original “Lake Experiment” (Gould & Gould 1982), upon closer examination, does not provide convincing evidence that honeybees assess the “plausibility” of information contained in waggle dances and use this information to reject dances for implausible locations.

1.6 References

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Hillsdale, NJ: Lawrence Erlbaum Associates.

Crist, E. 2004. Can an insect speak? The case of the honeybee dance language. *Social Studies of Science*, 34, 7-43.

Dyer, F. C. 2002. The biology of the dance language. *Annual Review of Entomology*, 47, 917-949.

Dyer, F. C. & Seeley, T. D. 1989. On the evolution of the dance language. *The American Naturalist*, 133, 580-590.

Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. in press. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*.

Fitch, W. T. & Hauser, M. D. 2002. Unpacking "honesty": vertebrate vocal production and the evolution of acoustic signals. In: *Acoustic Communication* (Ed. by Simmons, A. M., Fay, R. R. & Popper, A. N.), pp. 65-137. New York: Springer.

Giurfa, M. 2003. The amazing mini-brain: lessons from a honey bee. *Bee World*, 84, 5-18.

Giurfa, M., Zhang, S., Jenett, A., Menzel, R. & Srinivasan, M. V. 2001. The concepts of 'sameness' and 'difference' in an insect. *Nature*, 410, 930-933.

Gould, J. L. 1984. The natural history of honey bee learning. In: *The Biology of Learning. Dahlem Workshop on the Biology of Learning, Berlin 1983, October 23-28* (Ed. by Marler, P. & Terrace, H. S.), pp. 149-180. Berlin: Springer-Verlag.

Gould, J. L. 1986. The locale maps of honey bees: do insects have cognitive maps? *Science*, 232, 861-863.

Gould, J. L. 1990. Honey bee cognition. *Cognition*, 37, 83-103.

Gould, J. L. & Gould, C. G. 1982. The insect mind: Physics or metaphysics? In: *Animal Mind-Human Mind* (Ed. by Griffin, D. R.), pp. 269-298. Berlin: Springer-Verlag.

Gould, J. L. & Gould, C. G. 1988. *The Honey Bee*. New York: W. H. Freeman.

Gould, J. L. & Towne, W. F. 1989. On the evolution of the dance language: response to Dyer and Seeley. *The American Naturalist*, 134, 156-159.

Griffin, D. R. 2001. *Animal Minds: Beyond Cognition to Consciousness*. Chicago: University of Chicago Press.

Judd, T. M. 1995. The waggle dance of the honey bee: Which bees following a dancer successfully acquire the information? *Journal of Insect Behavior*, 8, 343-354.

Menzel, R., Greggers, U., Smith, A., Berger, S., Brandt, R., Brunke, S., Bundrock, G., Hulse, S., Plumpe, T., Schaupp, F., Schuttler, E., Stach, S., Stindt, J., Stollhoff, N. & Watzl, S. 2005. Honey bees navigate according to a map-like spatial memory. *Proceedings of the National Academy of Sciences*, 102, 3040-3045.

Riley, J. R., Greggers, U., Smith, A. D., Reynolds, D. R. & Menzel, R. 2005. The flight paths of honeybees recruited by the waggle dance. *Nature*, 435, 205-207.

SAS Institute. 1999. *SAS/STAT User's Guide, Version 8*. Cary, NC.

Seeley, T. D. 1995. *The Wisdom of the Hive*. Cambridge, MA: Harvard University Press.

Seeley, T. D. 2003. What studies of communication have revealed about the minds of worker honey bees. In: *Genes, Behavior, and Evolution in Social Insects* (Ed. by Kikuchi, T.), pp. 22-33. Sapporo: University of Hokkaido Press.

Seeley, T. D. & Towne, W. F. 1992. Tactics of dance choice in honey bees: do foragers compare dances? *Behavioral Ecology and Sociobiology*, 30, 59-69.

Shettleworth. 1998. *Cognition, Evolution, and Behavior*. New York: Oxford University Press.

Tautz, J. & Sandeman, D. C. 2003. Recruitment of honeybees to non-scented food sources. *Journal of Comparative Physiology A*, 189, 293-3000.

Tautz, J., Zhang, S., Spaethe, J., Brockmann, A., Si, A. & Srinivasan, M. 2004. Honeybee odometry: performance in varying natural terrain. *PLoS Biology*, 2, 915-923.

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

Wehner, R. & Menzel, R. 1990. Do insects have cognitive maps. *Annual review of neuroscience*, 13, 403-414.

Chapter 2

Honey bees use social information in waggle dances more fully when foraging errors are more costly[2]

2.1 Abstract

Social animals can obtain valuable information from group members, but can also experience conflicts between personal information obtained through their own experience and social information obtained from other individuals. The waggle dance is a valuable source of social information for honey bees (*Apis mellifera*), and many dance followers are experienced foragers who also have personal information about familiar food sources. Following waggle dances can "reactivate" these experienced bees to return to their familiar food source, however it is unclear whether experienced foragers make full use of the social information provided by waggle dancers or whether they rely primarily on their own personal information. Based on studies of social information use in other organisms, we hypothesized that foragers would pay more attention to the social information in waggle dances when foraging errors that can arise from ignoring social information are more costly. We manipulated the cost of reactivation errors by training bees to visit feeders that were either close (100 m) or far (1000 m) from the hive, and found that temporarily unemployed foragers who had been trained to forage at more distant feeders were more likely to pay attention to social information about food-source location.

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2.2 Introduction

Accurate information can increase an organism's fitness by enabling it to respond more effectively to challenges and opportunities. An animal's own experiences and trial-and-error interactions with the environment can provide it with "personal information" about its surroundings (Danchin et al. 2004; Dall et al. 2005), and group-living animals can also obtain "social information" by interacting with and observing other group members (Wagner and Danchin 2010). However, sometimes animals can face a situation in which the social information they obtained from group members conflicts with their own personal information (e.g. Kendal et al. 2004; van Bergen et al. 2004) and they must choose which one to use (reviewed by Kendal et al. 2005; Rieucau and Giraldeau 2011). In such situations, individuals tend to rely more on social information when it is especially persuasive (Rieucau and Giraldeau 2009), or when personal information is difficult to acquire (Boyd and Richerson 1988), unreliable or outdated (van Bergen et al. 2004), or costly to use (Kendal et al. 2004).

Social insects such as honey bees (*Apis mellifera*) live in large, integrated groups of relatives in which social information is constantly being exchanged, through the use of both inadvertent social cues and intentional signals (Seeley 1998). The most sophisticated of these signals is the waggle dance—perhaps the most complex form of communication in non-human animals—which provides worker bees with valuable social information about high-quality resources. Interestingly, while some of the bees who follow waggle dances are naïve foragers with no personal information about nearby food sources, nearly 80% of dance followers are experienced foragers whose visits to a rich food source were interrupted by nightfall or bad weather (Biesmeijer and de Vries 2001; Biesmeijer and Seeley 2005). These bees already possess personal information about the location, scent, and availability of their familiar food source (Reinhard et al. 2004; Beekman 2005; Reinhard et al. 2006), and by following waggle dances they can obtain social information about the scent and location of profitable food sources. However, if the social information provided by waggle dancers does not match their personal information, foragers will be forced to choose between these two sources of information. Paying attention to social information about the scent and location of the food sources dancers are advertising could reduce a dance follower's chance of mistakenly making a risky and energetically costly trip back to their familiar food source when it is still unrewarding. But following dances takes time, which may not be worthwhile if personal information is cheap and reliable. Thus, the extent to which experienced foragers rely on social information from waggle dances, as opposed to their personal information, is likely to depend on the relative costs, benefits, and reliability of these two types of information (Grüter and Farina 2009b; Brockmann and Sen Sarma 2009; Grüter and Farina 2009a).

Previous studies suggest that temporarily unemployed foragers often rely on social information about the scent and/or location of food sources that they obtain from waggle dancers. Multiple studies have shown that contact with a dancer carrying a familiar scent can prompt temporarily unemployed foragers to return to their familiar food source (von Frisch 1967 p. 32-33; Johnson 1967; von Frisch 1968), whereas contact with a dancer carrying an unfamiliar scent generally does not have the same effect (von Frisch 1967 p. 33, 43). And Karl von Frisch (1967) found that temporarily unemployed foragers trained to unscented feeders are less likely to respond to waggle dances advertising feeders in unfamiliar locations. In one such test, 16 of the 19 bees following dances for a feeder in an unfamiliar direction did not respond to the dances, and two bees were observed to

> *"turn decisively away from the dancer—one of them not until she had run along after her for a few circuits—as if after the sudden realization: 'That is none of my affair.' Thereafter they remained at rest on the comb. They had understood and heeded the indication of direction." (p. 153).*

von Frisch's observations suggest that experienced bees who are familiar with a food source's location can distinguish between dances advertising familiar and unfamiliar locations after following only a few dance circuits. And while dance followers may be able to obtain scent information from dancers even more rapidly than they can decipher location information (Biesmeijer and Seeley 2005), the location a dancer advertises will tend to be a more reliable source of information than the scent a dancer carries if food sources in multiple locations share the same scent.

These findings suggest that experienced bees frequently use social information from waggle dancers, although they may use different information (scent or location) depending on the circumstances. However, under some conditions, dance followers appear to ignore social information completely. In a study by Grüter et al. (2008), temporarily unemployed foragers almost always ignored social information provided by waggle dancers, and instead simply used their personal information to return to their familiar food source. The extent to which bees rely on social information from waggle dances, as opposed to their personal information about the location and availability of familiar food resources, is likely to be affected by a number of factors such as the quality and reliability of personal and social information and the costliness of errors that could result from using inaccurate or out-of-date personal information.

Grüter and Ratnieks (2011) recently showed that honey bee foragers who experience unrewarding conditions at their familiar feeder are more likely to rely on location information from waggle dances, which suggests that bees pay more attention to social information when their personal information is out of date. Our study asks whether the costliness of errors that can arise from relying solely on personal information also affects an unemployed forager's tendency to rely on social information about food resources. We hypothesized that temporarily unemployed foragers should follow dances more carefully and rely more fully on location information from waggle dances when reactivation errors are more costly. To test this hypothesis, we trained bees to visit one of two identically scented feeders located in opposite directions from the hive, shut off both feeders for several hours, and then monitored the responses of these temporarily unemployed foragers who followed waggle dances advertising just one of the feeders. The bees who had foraging experience at the non-advertised feeder faced a choice between relying on their personal information about food-source location and the social information about food-source location provided by the waggle dancers. Bees who relied on their personal information, or who paid attention only to the scent information from waggle dancers, should tend to return to their familiar food source after following a dance. But bees who paid attention to the location information in the waggle dances should tend to either remain in the hive (waiting for news that their familiar food source is again rewarding), or fly out to the location indicated by the dancer. We predicted that bees trained to more distant feeders (1000 m vs. 100 m) would use the social information in the waggle dance more fully and therefore would be less likely to make errors when deciding where and when to resume foraging.

2.3 Methods

2.3.1 *Study site and colonies*

Tests were performed with two colonies of honey bees (*Apis mellifera*) housed in 2-frame observation hives (Seeley 1995; Figure 4.2) that we transported to the Cranberry Lake Biological Station (CLBS) in the Adirondack State Park (44°09'N, 74°48'W) on July 7, 2007. The CLBS is surrounded by forests that contain few flowers, so there are no local bee colonies and it is relatively easy to train bees to forage at artificial feeders. During each test, one colony was housed in a small wooden hut at the CLBS (Seeley 1995; Figure 4.4) while the other was kept in reserve at a marina approximately 6.5 km away.

2.3.2 *Training*

We began each experimental trial by training groups of 30-60 bees to visit each of two artificial feeders located 100 m (Trials 1 and 3) or 1000 m (Trials 2 and 4) north and south of the hive using techniques described by von Frisch (1967). Trained foragers were individually marked using shellac-based paints. The paint mark on each bee's thorax indicated the feeder she was trained to visit, and 1-3 marks on her abdomen indicated her identity. Any bee who switched feeders during the training process was captured in a freezer bag (Seeley 1995 p. 78).

To train bees to 100 m, we placed two feeders next to the hive and then gradually moved each feeder and its group of foragers farther away, until one was 100 m to the north and the other was 100 m to the south of the hive. To train bees to 1000 m, we began with the feeders at 100 m and then moved them gradually to 1000 m. To obtain a new group of bees for the 1000 m trials, we allowed the foragers that had been trained to 100 m feeders to recruit a new group of bees to the feeders as they were moved farther away from the hive, and then captured the bees from the 100 m test.

On the day after feeders reached their final destinations, trained bees were allowed to forage freely from 09:00-16:00. Throughout the day, the sucrose concentrations were adjusted so that trained bees kept returning to the feeder but did not perform dances to recruit additional bees to the feeder. Sucrose concentrations ranged from 0.5 M to 2.5 M and were always equal at both feeders. During training and testing periods, both feeders were scented using anise extract, which was present in the sugar solution (50 μl/L) and in a reservoir on top of which the jar of solution rested (Seeley 1995 Figure 4.5, 4.6). The scent levels approximated what bees encounter at natural food sources (Seeley 1995) and ensured that dance followers were exposed to both external and internal fragrances, as is typical for bees foraging on flowers (von Frisch 1967 p. 224-227).

2.3.3 Testing

On testing days (Jul. 12, 17, 31; Aug. 9, 2007), we filled both feeders with sucrose solution and allowed bees to forage freely from approximately 09:00-12:00. We based our initial sucrose concentrations on the levels that had been necessary to keep bees visiting the feeder during the previous training day (0.5 M in Trials 1 & 2, 1.0 in Trial 3, 2.0 in Trial 4) and adjusted them if necessary to keep trained bees visiting without attracting additional recruits. After the morning feeding period, we removed the feeders with sucrose solution and replaced them with clean, empty feeders. Trained bees periodically returned to inspect these empty feeders, but the frequency of their visits soon decreased. We monitored each empty feeder and recorded the number and identity of inspecting bees until there was an average of 1 inspector arriving at the feeders every 15 minutes. At that time (~14:00), we removed the empty feeder from one feeding station ('food' location) and replaced it with a feeder containing concentrated sugar solution (2.5 M); the other feeder ('no food' location) remained empty. The location with food alternated between trials (Trials 1&4: south feeder; Trials 2&3: north feeder). The testing period began when the first marked bee to return to the food location flew back to the hive and performed a waggle dance for her feeder.

During the testing period, an observer at each feeder recorded the arrival time and identity of each marked bee who arrived and also captured every bee, with the exception of 2-3 bees at the 'food' location who were designated as dancers. The anise scent of the waggle dancers was familiar to all of the temporarily unemployed trained foragers in the hive, regardless of the feeder they had been trained to visit. However, the location information in the waggle dances was only familiar to bees who had been trained to visit the advertised feeder.

Throughout each testing period, we videotaped (AGDVC 30; Panasonic) the area of the hive in which bees were performing and following dances (the "dance floor"; von Frisch 1967 p. 36-37). An observer at the hive (M.K.W.) pointed at and announced the identity of each marked bee in the vicinity of a dancer. Testing continued until no more marked bees were observed following dances in the hive (~2-3 hours). All captured bees were released at the end of each testing period.

2.3.4 Video coding and analysis

After the experiment, one of us (M.K.W.) reviewed the video recordings from Trials 1 and 2 to quantify the behavior of marked bees inside the hive. Technical difficulties prevented us from obtaining video data from our recordings of Trials 3 and 4, so for these trials we report only the feeder data (where and when trained bees were captured). The behavior of each marked bee who was pointed out on the video recordings was monitored throughout the testing period, and the times (if any) when the bee entered or exited the hive, performed trophallaxis, or followed a dance were noted. Each bee who was less than one bee length from a dancer and faced the dancer for at least one complete dance circuit (waggle run + return phase) was classified as a dance follower. We counted the number of dance circuits a bee followed as the number of circuits during which she was no more than one bee-width from a dancer, was facing the dancer, and was actively following the dancer's movements by running alongside or behind her. The end of a following bout occurred when the dancer stopped dancing, the follower turned ≥90° away from the dancer and walked away from her, or the follower was pushed away from the dancer and did not resume following her for two or more dance circuits (Girard et al. 2011). All trained bees from Trials 1 & 2 were classified according to the definitions in Table 2.1.

We excluded from our analysis any bees who did not visit a feeder on the day before and on the morning of the test day because they were likely to have died or switched to visiting a different food source. However, we did not exclude bees who visited a feeder on the day before testing but not on the morning of the test day (39 out of 229 bees). Even after an entire day without food rewards, approximately 85% of bees remember the location and scent of a feeder they have regularly visited (Beekman 2005), so bees who visited a feeder on the day before testing presumably still remembered its location on the test day.

Table 2.1 – Categories that were used to classify marked bees from Trials 1 and 2 based on their behavior during testing periods. Temporarily unemployed foragers who returned to a familiar feeder after following a dance were classified as reactivated bees, whereas bees who followed a dance for an unfamiliar feeder and arrived at that feeder were classified as recruits (Gil and Farina 2002; Fernandez, Gil et al. 2003). Definitions of reactivated bees, recruits, inspectors and scouts are based on Biesmeijer & de Vries (2001).

Behavioral category	Definition
Reactivated bee	A bee who returned to her familiar feeder in response to external information (from a waggle dance)
Recruit	A bee who followed a dance for an unfamiliar feeder and subsequently arrived at that feeder
Unresponsive follower	A bee who followed a dance, but did not subsequently leave the hive
Uncaptured follower	A bee who followed a dance and left the hive, but was not captured at either feeder
Inspector	A bee who arrived at her familiar feeder during the testing period without obtaining new external information (from waggle dances)
Scout	A bee who arrived at the unfamiliar feeder during the testing period without obtaining new external information (from following dances)
Inactive bee	A bee who was observed in the hive, but never followed a dance
Unobserved bee	A bee who was not observed in the hive or at the feeder during the testing period

2.3.5 Statistical Methods

Statistical tests were performed using SAS v9.2. Categorical data were tested using Chi-square tests (proc FREQ), comparisons among group means were performed using 2-tailed t-tests for two groups (Proc t-test) and two-way ANOVA tests for multiple groups (Proc GLM), and means for significant effects were separated using a Tukey-Kramer post-hoc test. Dance-following data were log transformed before performing Tukey tests to meet homogeneity of variance assumptions. All reported *P*-values are for two-tailed tests ($\alpha=0.05$), and average values are reported as LS_{mean} ± standard error.

2.4 Results

2.4.1 Did bees pay attention to the location information in waggle dances?

Among the bees for whom the dances indicated an unfamiliar location, 65% of them (51 out of 78) returned to their familiar food source, while 35% of them (27 out of 78 bees) went to the unfamiliar location advertised by the dancers (Figure 2.1). In contrast, nearly all of the bees (79 of 80 bees; 99%) for whom dances indicated their familiar feeder returned to that location, with the exception of one scout bee who arrived at the empty, unfamiliar 100 m feeder without having followed any dances in the hive. Bees from both colonies and at both distances were more likely to return to their familiar feeder when the dances in the hive were advertising their familiar feeder (Chi-square: Colony 1: $X^2_{df=1}=14.4$, $P=0.0001$; Colony 2: $X^2_{df=1}=15.3$, $P<0.0001$; 100m: $X^2_{df=1}=19.9$, $P<0.0001$; 1000m: $X^2_{df=1}=9.22$, $P=0.0024$).

Waggle dances advertising unfamiliar locations were less effective at stimulating bees to return to their familiar feeder. Bees who followed dances advertising an unfamiliar location were less likely to be reactivated to their familiar site (36%, 19/53) than bees who followed dances advertising a familiar location (100%, 23/23; Chi-square: $X^2_{df=1}=12.2$, $P=0.0005$). A quarter (13/53; 25%) of the bees who followed dances for an unfamiliar feeder used the location information in the waggle dance and arrived at the advertised feeder before the end of the testing period. The remaining 40% of bees who followed dances for unfamiliar locations but did not arrive at a feeder either did not leave the hive in response to these dances (6/53; 11%) or left the hive but did not arrive at a feeder (15/53; 28%). Since bees who followed dances advertising their familiar feeder always returned to that feeder, the 64% of bees who did not return to their familiar feeder after following dances for an unfamiliar location presumably paid attention to the location information and recognized that these dances were not advertising their familiar food source.

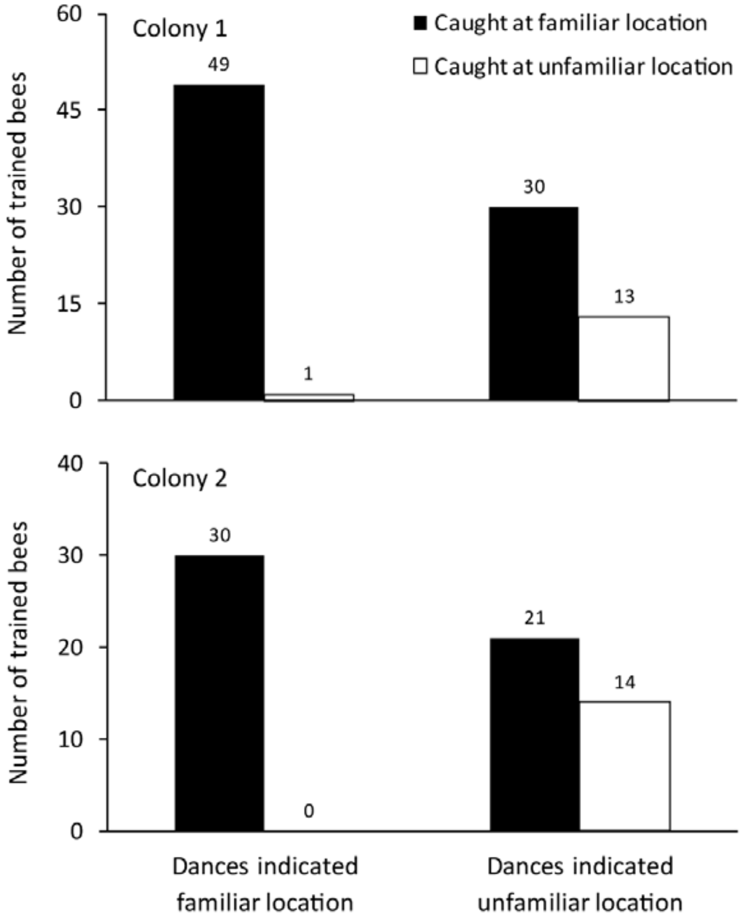

Figure 2.1 – Locations where trained bees from colony 1 and colony 2 were captured during testing periods. Nearly all bees whose familiar feeder was advertised during the testing period were caught at that location. In contrast, some of the bees who were unfamiliar with the advertised location returned to their familiar feeder whereas others flew to the unfamiliar feeder advertised by the dancers.

2.4.2 Effects of distance

Among the bees who followed dances for unfamiliar locations (Trial 1:100 m, 30 bees; Trial 2: 1000m, 23 bees), the fraction who did not leave the hive in response to these dances was higher at 1000m than at 100m (22% vs. 3.3%; Chi-square $X^2_{df=1}$=4.39, P=0.036; Figure 2.2), and the fraction who arrived at either of the two feeders was lower (43% at 1000m vs. 73% at 100m; Chi-square: $X^2_{df=1}$= 4.85, P=0.028). If we assume that bees who followed dances for unfamiliar locations but did not return to their familiar feeder were paying attention to the location information in the dances—and therefore never left the hive, left the hive but did not arrive at a feeder, or flew to the advertised feeder—then we can conclude that 57% of these bees (17 out of 30) attended to location information at 100 m (Figure 2.2), whereas 74% (17 out of 23) attended to location information at 1000 m.

In comparison, distance had no effect on the behavior of bees who followed dances advertising familiar locations (Figure 2.2). All of these bees responded to dances by leaving the hive, and all of them were caught at their familiar feeder, both at 100m (10 bees) and 1000m (13 bees).

2.4.3 Dance-following behavior

Bees who were recruited to unfamiliar feeders spent more time in the hive following dances when feeders were farther away from the hive (100 m: 20±5 min; 1000 m: 48±7 min; 2-tailed t-test: t_{11}=-3.11, P=0.01). They also followed dancers for slightly more following bouts (100 m: 6.4 ± 3.8 bouts, n=9; 1000m: 8.8 ± 3.0 bouts, n=4) and more dance circuits (100m: 15.3 ± 3.0 circuits, n=9; 1000 m: 16.0 ± 3.8 circuits, n=4), but these differences were not statistically significant (2-tailed t-tests: P>0.05).

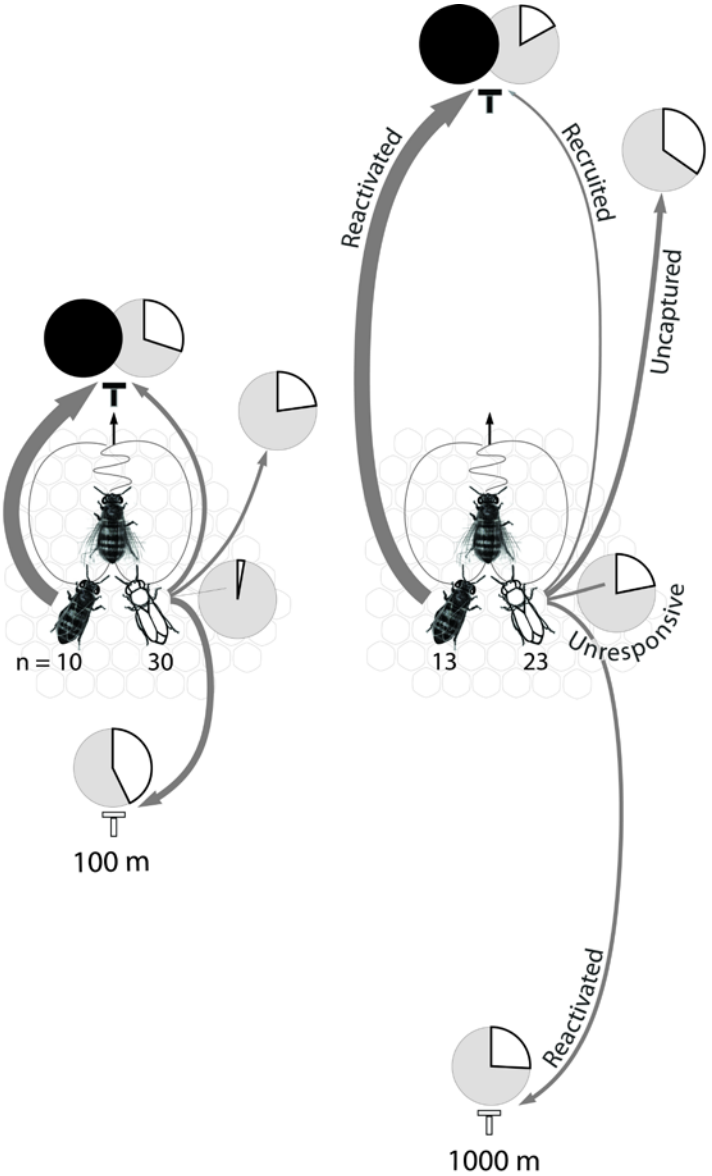

Figure 2.2 – Responses of bees from Trials 1 and 2 who followed dances for feeders located either 100m (on left) or 1000m (on right) from the hive. In each trial, there were two types of dance followers: bees who were familiar with the feeder that the dancers were advertising (in black), and bees who had been trained to visit the other feeder and were thus unfamiliar with the advertised location (in white). Pie symbols and arrow thicknesses represent the fraction of bees in each group who returned to their familiar feeder ("reactivated"), flew to an unfamiliar feeder ("recruited"), left the hive but were not caught ("uncaptured") and followed a dance but did not leave the hive ("unresponsive"). The number beneath each type of dance follower indicates the size of that group of bees. During testing periods, a reservoir of anise scent was present at both feeders, but the advertised feeder (indicated in black) contained concentrated 2.5 M sucrose solution whereas the non-advertised feeder (indicated in white) did not.

Waggle dance followers who paid attention to the location information in the dance and were recruited to an unfamiliar feeder actively followed dances for an average of 15.5 ± 2.3 dance circuits (Figure 2.3), left the hive an average of 2.4 ± 0.3 times, and took 73.7 ± 39.6 minutes from the start of the testing period to successfully locate the advertised feeder (n=13). In contrast, bees who relied only on scent or personal information and were reactivated to their familiar, but unprofitable, feeder followed dancers for an average of 3.9 ± 1.2 dance circuits (Figure 2.3), left the hive 1.5 ± 0.2 times, and arrived at their familiar feeder 49 ± 5.6 minutes after the start of the testing period (n=20). Among bees who followed dances for an unfamiliar location, those who were recruited to an unfamiliar feeder spent more time following dances than those who were reactivated to a familiar feeder (11.6±5.0 vs. 5.7±1.3 minutes; t_{53}=4.58, *P*<.0001) and followed dancers for more dance circuits (15.5 ± 2.3 vs. 3.7 ± 0.7 circuits; Tukey post-hoc test: *P*=0.004).

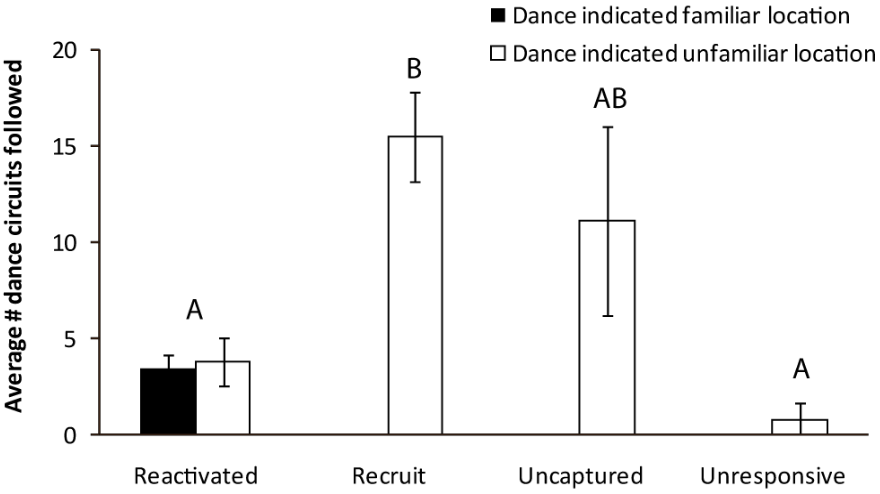

Figure 2.3 – Average number of dance circuits followed by different categories of bees as defined in Table 2.1. Groups with different letters were significantly different based on a post-hoc Tukey tests (Reactivated bees vs. recruits: P=0.004; Recruits vs. unresponsive followers: P=0.0004).

2.5 Discussion

There are two main results of this study. First, we found that temporarily unemployed foragers were more likely to be reactivated to their feeder if the dances performed in the hive indicated a familiar location rather than an unfamiliar location. This finding shows that many of these bees must have paid attention to location information in the dances. Second, we found that temporarily unemployed foragers who followed dances indicating an unfamiliar location were less likely to leave the hive and less likely to return to their familiar feeder when the feeders were farther from the hive. This finding shows that these bees must have paid more attention to the location information in the dances when the distance to their familiar food source—and, thus, the cost of making a reactivation error—was greater. In contrast, the temporarily unemployed foragers who followed dances indicating a familiar location always left the hive and always returned to their familiar feeder, regardless of the feeder's distance from the hive. The strong reactivation of these bees probably reflects the fact that for these bees there was no conflict between personal information and social information; both stimulated them to return to their familiar feeder.

These results support previous studies showing that individuals are likely to use social information more fully when relying on personal information alone could lead to costly errors. For example, Kendal et al. (2004) showed that guppies with experience feeding from one of two feeders typically preferred to continue feeding at that location even after observing group members feeding elsewhere. But when visiting their familiar feeder became costly because it required them to go behind a barrier and lose sight of the rest of their shoal, individuals were more likely to pay attention to social information and visit the feeder where they had observed group members feeding. Our findings suggest that—like guppies—honey bees can evaluate the relative cost of using personal as opposed to social information, and can increase their reliance on social information when using personal information becomes more costly. These results add to our understanding of social learning in insects (reviewed by Leadbeater and Chittka 2007) by demonstrating that honey bee foragers are able to flexibly alter their reliance on social as opposed to personal information under different conditions.

Interestingly, even when the feeders in our study were close to the hive, so the cost of making a reactivation error (i.e., returning to their familiar feeder when it remained empty) was low, some bees still paid attention to the socially-provided location information in the waggle dance and were not reactivated by dances that advertised an unfamiliar location. Over 40% of the bees who followed dances for an unfamiliar feeder 100 m from the hive arrived at the location advertised by the dancers, and 25% of them either did not leave the hive in response to dances or left the hive but never arrived at either feeder. Bees who followed dances for familiar locations always arrived at their familiar feeder, so these uncaptured bees presumably had paid attention to the location information waggle dancers advertised and were searching for the advertised feeder. These findings suggest that even when the errors that could arise from relying solely on personal information were not particularly costly, many bees (about 70%) preferred to rely on social information from waggle dancers when deciding where and when to resume foraging.

Our results differ markedly from those of Grüter et al. (2008), who found that most temporarily unemployed foragers relied only on personal information after following waggle dances, and returned to their familiar feeder regardless of the scent or location advertised by the dancers. One possible reason for this difference is that our study was performed in an environment with fewer alternative food sources, and foragers in such locations may place greater emphasis on socially-acquired information from waggle dances if personal information is consequently less reliable (Grüter et al. 2008) or more difficult to obtain. The difference in the lengths of our testing periods could also account for some of the difference in our results. Previous studies suggest that it can take hours for bees following waggle dances to find the locations that dancers are advertising (Seeley 1983), and the bees in our study who were recruited to an unfamiliar feeder took an average of 74 minutes to locate it. Indeed, several bees in our study who had left the hive but had not yet arrived at either feeder were probably still searching for the advertised feeder when we ended the testing period. Since Grüter et al. (2008) used 40-minute testing periods, it is possible that some of the dance followers in their study who did not arrive at either feeder were searching for the advertised feeder but were unable to find it by the end of the testing period.

Previous studies of temporarily unemployed foragers have had conflicting results. Numerous researchers have found that bees pay attention to social information provided by waggle dancers (odor information: Johnson 1967; Biesmeijer and Seeley 2005; location information: von Frisch 1967), but others have found that bees can be reactivated equally well by any waggle dance, regardless of its odor or location information (Grüter et al. 2008). Our results, and those of Grüter and Ratnieks (2011), suggest that experienced dance followers are able to flexibly adjust the degree to which they rely on social as opposed to personal information, and presumably also the type of social information they use (scent or location information), in response to the costs and benefits associated with these strategies. This may explain why previous studies of dance-following behavior have produced such different results, and why "even small changes in experimental design can lead to very different recruitment patterns" (Grüter and Farina 2009b).

Interestingly, even under the same experimental conditions, we observed a significant amount of variability among bees in their responses to the waggle dances they followed. While some dance followers returned to their familiar feeder regardless of the location dancers were advertising, other bees paid attention to the socially-provided location information in the dance and flew out in search of the advertised feeder. Because we used different bees in each testing trial, we do not know if these differences in workers' dance-following behavior are consistent over time. Recent studies have shown that some honey bees are more impulsive than others (Burns and Dyer 2008) and tend to make faster but less accurate foraging decisions. If these decision-making strategies are consistent across contexts, then more impulsive foragers might also tend adopt the faster, but potentially less accurate, strategy of ignoring social information about scent and location when following waggle dances as temporarily unemployed foragers. Kurvers et al. (2010) recently demonstrated that personality and social information use are correlated in barnacle geese, and investigating whether this is also true of honey bees could be a promising area for future studies.

In conclusion, our results show that, at least under some conditions, many temporarily unemployed foragers do pay attention to the social information in waggle dances. Moreover, our results show that temporarily unemployed foragers pay more attention to the social information in waggle dances when their familiar food sources are farther away. Previous studies have shown that honey bee foragers are sophisticated in how they produce waggle dances, being highly sensitive to environmental factors such as the distance to a food source and the risk of predation, and varying the duration and tempo of their dances based on their estimate of a food source's overall profitability (Seeley 1994; Seeley et al. 2000; Abbott and Dukas 2009). Our findings suggest that experienced honey bee foragers display a similar level of sophistication in how they follow waggle dances, paying more attention to the social information in waggle dances when the cost of making a reactivation error is higher.

2.6 References

Abbott, K., & Dukas, R. 2009. Honeybees consider flower danger in their waggle dance. *Animal Behaviour*, 78, 633-635.

Beekman, M. 2005. How long will honey bees (*Apis mellifera L.*) be stimulated by scent to revisit past-profitable forage sites? *Journal of Comparative Physiology A*, 191, 1115-1120.

Biesmeijer, J., & Seeley, T. 2005. The use of waggle dance information by honey bees throughout their foraging careers. *Behavioral Ecology and Sociobiology*, 59, 133-142.

Biesmeijer, J. C., & de Vries, H. 2001. Exploration and exploitation of food sources by social insect colonies: a revision of the scout-recruit concept. *Behavioral Ecology and Sociobiology*, 49, 89-99.

Boyd, R., & Richerson, P. J. 1988. An evolutionary model of social learning: the effects of spatial and temporal variation. In: *Social learning: psychological and biological perspectives*, (Ed. by T. R. Zentall & B. G. Galef), Psychology Press.

Brockmann, A., & Sen Sarma, M. 2009. Honeybee dance language: is it overrated? *Trends in Ecology & Evolution*, 24, 583-583.

Burns, J. G., & Dyer, A. G. 2008. Diversity of speed-accuracy strategies benefits social insects. *Current Biology*, 18, R953-R954.

Dall, S., Giraldeau, L., Olsson, O., McNamara, J., & Stephens, D. 2005. Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, 20, 187-193.

Danchin, É., Giraldeau, L.-A., Valone, T. J., & Wagner, R. H. 2004. Public Information: From Nosy Neighbors to Cultural Evolution. *Science*, 305, 487 -491.

Girard, M., Mattila, H., & Seeley, T. 2011. Recruitment-dance signals draw larger audiences when honey bee colonies have multiple patrilines. *Insectes Sociaux*, 58, 77-86.

Grüter, C., Balbuena, M. S., & Farina, W. M. 2008. Informational conflicts created by the waggle dance. *Proceedings of the Royal Society B*, 275, 1321-1327.

Grüter, C., & Farina, W. M. 2009a. Why do honeybee foragers follow waggle dances? *Trends in Ecology & Evolution*, 24, 584-585.

Grüter, C., & Farina, W. M. 2009b. The honeybee waggle dance: can we follow the steps? *Trends in Ecology & Evolution*, 24, 242-247.

Grüter, C., & Ratnieks, F. L. W. 2011. Honeybee foragers increase the use of waggle dance information when private information becomes unrewarding. *Animal Behaviour*, 81, 949-954.

Johnson, D. L. 1967. Communication among honey bees with field experience. *Animal Behaviour*, 15, 487-492.

Kendal, R. L., Coolen, I., van Bergen, Y., & Laland, K. N. 2005. Trade-offs in the adaptive use of social and asocial learning. *Advances in the Study of Behavior*, 35, 333-379.

Kendal, R., Coolen, I., & Laland, K. 2004. The role of conformity in foraging when personal and social information conflict. *Behavioral Ecology*, 15, 269-277.

Kurvers, R. H. J. M., Van Oers, K., Nolet, B. A., Jonker, R. M., Van Wieren, S. E., Prins, H. H. T., & Ydenberg, R. C. 2010. Personality predicts the use of social information. *Ecology Letters*, 13, 829-837.

Leadbeater, E., & Chittka, L. 2007. Social learning in insects - From miniature brains to consensus building. *Current Biology*, 17, R703-R713.

Reinhard, J., Srinivasan, M. V., Guez, D., & Zhang, S. W. 2004. Floral scents induce recall of navigational and visual memories in honeybees. *Journal of Experimental Biology*, 207, 4371-4381.

Reinhard, J., Srinivasan, M. V., & Zhang, S. W. 2006. Complex memories in honeybees: can there be more than two? *Journal of Comparative Physiology A*, 192, 409-416.

Rieucau, G., & Giraldeau, L.-A. 2009. Persuasive companions can be wrong: the use of misleading social information in nutmeg mannikins. *Behavioral Ecology*, 20, 1217 -1222.

Rieucau, G., & Giraldeau, L.-A. 2011. Exploring the costs and benefits of social information use: an appraisal of current experimental evidence. *Philosophical Transactions of the Royal Society B*, 366, 949 -957.

Seeley, T. D. 1983. Division of labor between scouts and recruits in honeybee foraging. *Behavioral Ecology and Sociobiology*, 12, 253-259.

Seeley, T. D. 1995. *The wisdom of the hive: the social physiology of honey bee colonies.* Harvard University Press.

Seeley, T. D., Mikheyev, A. S., & Pagano, G. J. 2000. Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *Journal of Comparative Physiology A*, 186, 813-819.

Seeley, T. D. 1994. Honey Bee Foragers as Sensory Units of Their Colonies. *Behavioral Ecology and Sociobiology*, 34, 51-62.

Seeley, T. D. 1998. Thoughts on information and integration in honey bee colonies. *Apidologie*, 29, 14.

van Bergen, Y., Coolen, I., & Laland, K. N. 2004. Nine-spined sticklebacks exploit the most reliable source when public and private information conflict. *Proceedings of the Royal Society B*, 271, 957-962.

von Frisch, K. 1967. *The Dance Language and Orientation of Bees.* First Edition edn. Belknap Press.

von Frisch, K. 1968. The role of dances in recruiting bees to familiar sites. *Animal Behaviour*, 16, 531-533.

Wagner, R. H., & Danchin, É. 2010. A taxonomy of biological information. *Oikos*, 119, 203-209.

Chapter 3

Personality in honey bee foragers:
Consistent individual differences in activity levels and restlessness[3]

3.1 Abstract

Consistent individual differences in behavior, or “personalities”, are present in a wide variety of animal species. In European honey bees (*Apis mellifera*), individual workers differ in the probability that they will perform different tasks, and may also exhibit characteristic differences in the way they carry out the same task. Recent studies have shown that workers differ in the problem-solving strategies they use to choose among available resources and have suggested that diversity in workers’ foraging behaviors may improve their colony’s ability to effectively acquire resources. Our study investigates whether individual honey bees foraging at the same food source display behavioral differences similar to the differences in “personality” or “temperament” that have been observed in other animal species. We trained groups of worker bees to visit an artificial feeder and measured how individuals responded to a series of behavioral tests. We found that individuals differed consistently in how active and restless they were during their visits to the feeding station. These behavioral differences were consistent over time and across different testing situations, and can therefore be described as personality differences. We suggest that individual differences in workers’ activity and restlessness could improve colony productivity and survival, and might provide an additional explanation for why genetic diversity has been shown to improve colony fitness in honey bees.

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3.2 Introduction

There is abundant evidence that non-human animals differ in their personalities—i.e. they display individual differences in their behavior that are consistent over time, across contexts, or in different situations (Sih & Bell 2008). While researchers once believed that behavioral variation among individual organisms within the same population represented non-adaptive fluctuations around an adaptive mean behavior (Wilson 1998), many personality differences are now believed to represent adaptive alternative frequency-dependent behavioral strategies that have important evolutionary consequences (Dall et al. 2004) and may be related to life-history tradeoffs (Biro & Stamps 2008).

Personality differences have been documented in a wide range of species, including aquatic invertebrates (octopus: Mather & Anderson 1993; and squid: Sinn & Moltschaniwskyj 2005; crayfish: Vainikka et al. 2010), spiders (Johnson & Sih 2007; Pruitt & Riechert 2011), and insects (water striders: Sih & Jason V. Watters 2005; crickets: Kortet & Hedrick 2007; Wilson et al. 2010)**.** Studies of personality in social insects are less common, but there is abundant evidence that behavioral differences among individuals are important within insect colonies as well. In honey bees, genetic diversity increases colony productivity and fitness (Mattila & Seeley 2007), thermoregulation ability (Jones et al. 2004), disease resistance (Seeley & Tarpy 2007), and foraging effectiveness (Mattila et al. 2008; Mattila & Seeley 2010a; Girard et al. 2011), presumably at least in part because of the behavioral diversity that genetic diversity produces among a colony's workers. The "response threshold" model hypothesizes that differences among workers in their tendency to perform certain tasks (i.e. their response thresholds) result in more effective division of labor (Bonabeau et al. 1996), and behavioral differences among workers from different patrilines within a colony are thought to be one of the main mechanisms by which genetic diversity results in increased colony success (reviewed by Oldroyd & Fewell 2007). Although few formal tests of this hypothesis have been performed (for an exception, see Mattila & Seeley 2010a), honey bees of different genotypes are known to differ in their tendency to perform tasks such as guarding and defending the hive (Breed et al. 1990), undertaking (Trumbo et al. 1997), and performing waggle dances and shaking signals (Arnold et al. 2002; Schneider & Duong 2008). Bees from different patrilines also vary in their responsiveness to sucrose, which affects the type of resource they prefer to collect (nectar, pollen, or water; Pankiw & Page Jr 2000),

While much of the behavioral variation that has been observed among workers in honey bee colonies involves differences in their tendency to perform a given task, recent studies suggest that workers can also differ in how they perform the same task. In bumble bees, individual workers have been shown to vary in the probability they will respond to elevated temperatures and CO_2 levels and in the duration of their fanning response (Weidenmüller 2004). Studies of bumble bees and honey bees have also shown that individual foragers differ in their decision-making strategies, with some bees consistently opting for a slow and accurate strategy and others tending to make faster, less accurate decisions (Chittka et al. 2003; Burns & Dyer 2008). Furthermore, individual honey bees have been found to differ in their foraging strategies when choosing among resources with different levels of cost and reward, with some bees basing their choice of flower on reward, some on effort, and some on flower color (Cakmak et al. 2009).

Our study extends the study of behavioral differences among individual worker honey bees by asking whether bees foraging at the same resource display consistent differences in their behavior similar to the personality traits that have been observed in other animal species. To answer this question, we trained groups of individually-marked bees to an artificial feeder and monitored the bees' behaviors during three different tests [of activity, boldness and exploration] over a two-day period. We then assessed the consistency of bees' behavior and asked whether individual bees exhibited consistent differences in their behavior during these three tests.

3.3 Methods

3.3.1 Setup and study subjects

Two colonies of honey bees (*Apis mellifera*) were used in this study. The colonies' queens were super-sisters (i.e. sisters who share the same father and thus ¾ of their genes), each of whom was inseminated with sperm from 10 different drones. These queens were obtained from Glenn Apiaries (Fallbrook, CA) in May, 2008. Before testing, we transferred the queen and ~4000 workers from each colony into a 2-frame observation hive (Seeley 1995; Fig. 4.2). Both colonies were transported to Cranberry Lake, NY (in the Adirondack State Park) on June 24, 2008. Colony 1 was taken by boat (~6.5 km) to the Cranberry Lake Biological Station (CLBS) (44°09'N, 74°48'W) and installed in a small wooden hut (Colony 1: Seeley 1995; Fig. 4.4), while Colony 2 was remained at the marina. On July 10, after testing with Colony 1 was complete, we moved Colony 1 to the marina and set up Colony 2 at the CLBS. The CLBS contains few natural nectar sources for honey bees, which enabled us to train large groups of bees to regularly return to artificial feeding stations over the course of several days. In addition, as there are no local bee colonies in the area we could be confident that all of the bees we saw at our feeding station belonged to the colony we were studying. Behavioral tests (described below) were performed on one group of bees at a time. We tested three different groups of bees from each of the two colonies. Tests with Colony 1 (groups 1-3) were performed from July 1-9, and tests with Colony 2 (groups 4-6) were conducted from July 14-23.

3.3.2 Testing

On the first day of each testing trial, a group of 50-60 bees was trained to visit an artificial feeder 350 m from the hive using techniques described by Karl von Frisch (1967), and all trained bees were individually labelled with shellac-based paints. During the following two days, the feeder was supplied with sucrose solution from 8:30 am to 5:30 pm (described by Seeley 1995; p.77-79, Fig. 4.5-4.7), and trained bees were allowed to travel freely between the hive and the feeding station. The concentration of the sucrose solution at the feeder ranged from 0.2 M to 2.5 M, and was adjusted throughout the day based on weather conditions and bees' visitation rates to ensure that trained bees returned to the feeding station but did not perform waggle dances to recruit additional bees. Any unmarked recruits that landed at the feeder were captured with an aspirator. Throughout the day, we conducted a series of behavioral tests to test for the presence of consistent behavioral differences between individual nectar foragers on 3 of the 5 temperament traits reviewed by Réale et al. (2007): activity, boldness, and exploration (described below). For each group of bees, tests were repeated 2-3 times on each of the two test days, for a total of 4-6 test repetitions. Bees' behavior during all tests was videotaped (Sony Handycam DCR-HC90) for subsequent analysis.

Activity test

"Activity" is defined by Réale et al. (2007) as "the general level of activity of an individual", as measured in situations that are not risky or novel. We measured bees' activity during a 10-minute time period during which conditions at the feeding station remained stable and bees were allowed to travel undisturbed between the feeding station and the hive. We collected activity data during three time periods per day (morning, mid-day, and afternoon), each of which began at least 5-10 min after the conclusion of the previous behavioral test.

A video player with variable speed playback (Sony digital videocassette recorder DSR-30) was used to review the video tapes and record each time a bee landed on or lifted off from the feeder during the activity tests. We counted the number of trips each bee made to the feeding station during each test period (=”trips to the feeder per test”). If a bee made multiple trips to the feeding station, we subtracted the time when she left the feeding station from the time when she returned to obtain a measure of the time it took her to fly back to the hive, unload the sugar solution she was carrying, and return to the feeding station (=“duration of round trip to the hive”). For each trip a bee made to the feeding station, we also calculated the time that she spent off the feeder during temporary interruptions in feeding (=”interruption time”), starting from the time she first touched down at the feeding station. This time was subtracted from the total duration of her visit (last departure – first arrival) to obtain the amount of time she spent on the feeder during each trip (=”time on feeder”). Trips to the feeding station that were separated by ~1 minute or more were treated as multiple visits.

Boldness test

Boldness is defined as: “an individual’s reaction to any risky situation, but not new situations” (Réale et al. 2007). Boldness is frequently studied in the context of anti-predator behavior or responses to humans (e.g. ‘docility’). Studies in numerous species have found evidence that boldness is consistent across contexts (e.g. in fishing spiders: Johnson & Sih 2007), and individual animals’ level of boldness has been shown to affect their survival and reproduction (Smith & Blumstein 2008).

We tested the boldness of the bees at our feeding station by poking each bee on the tip of her abdomen with a paintbrush three times (1 poke/second) or until she flew up from the feeder. Each trained forager had been marked with paint on the previous day, so being poked with a paintbrush was not a new experience for these bees. However, being disturbed from behind by a large foreign object was a potentially risky situation, as it could represent an attack by a predator. A similar experimental protocol was used by Johnson and Sih (2007) to study boldness in fishing spiders; in this study, each spider was “firmly poked from behind with a pencil” and the duration of its response (time spent underwater) was recorded. In our test, only one bee was disturbed at a time. In most cases, disturbances occurred within 30-60 s after a bee landed at the feeding station and began drinking from the feeder. Each bee was disturbed only once during each testing period (~1 hour, or until all bees visiting the feeding station had been disturbed). The boldness test was repeated three times each day (mid-morning, early afternoon, and late afternoon).

Behavioral coding for the boldness test was performed with The Observer version 5.0 (Noldus et al. 2000). For each bee who was disturbed, we recorded the amount of time between when she first touched down at the feeding station and when she began drinking from the feeder (=”latency to begin feeding”), the time between when she first touched down at the feeding station and when she was first disturbed (“landing to disturbance”), and the amount of time she spent drinking before being disturbed (“drinking before disturbance”). Also, we calculated the time between when she was first poked and when she began responding (=”response latency”), and we recorded her “response magnitude” (no response=0, moved=1, walked=2, flew onscreen=3, flew offscreen=4). We recorded the end of her response as the time when she settled down again at the feeder and resumed drinking for at least 5 s. Some bees responded to each poke with the paintbrush but briefly resumed drinking between pokes; in these cases, we subtracted any time a bee spent drinking from the feeder between the start and stop of her response from her reaction time to obtain the time she spent actively responding to the disturbance (=”response duration”). We gave bees who did not react at all to our disturbance a response magnitude and duration of 0, and did not include them in response latency calculations. We expected that bolder bees would display less extreme responses to our disturbances (longer response latencies, smaller response magnitudes, and shorter response durations).

Exploration test

“Exploration” is defined by Réale et al. (2007) as an individual’s reaction to a situation that is new, but not necessarily risky. Exploration is one of the most commonly studied animal personality traits (novel object tests, open field tests, etc.), and has been shown to be heritable in some species (e.g. Dingemanse et al. 2002). We measured bees’ exploration during a 15-minute time period during which a second feeder on a differently colored plate was placed on one corner of the feeding station (see Figure 3.8). The location (front right, front left, back right, back left) and color (blue or yellow) of the novel feeder were alternated in each test period. The familiar feeder was always white, and was always located in the center of the feeding station. This test was performed twice each day (mid-morning and mid-afternoon).

Behavioral coding of videos from the exploration test was performed with The Observer version 5.0 (Noldus et al. 2000). For each trip a bee made to the feeding station, we recorded the identity (familiar or novel) of the feeder on which she first landed. The fraction of trips during which a bee landed on the novel feeder was taken as a measure of her willingness to explore the novel feeder (=”fraction of landings on novel feeder”). To assess whether some bees were more hesitant to land after arriving at the feeding station, we also recorded the amount of time from when each bee first flew into the camera’s field of view and when she landed on one of the feeders (=”landing latency”). Once a bee had begun feeding, we recorded the number of times, if any, when she switched to the other feeder or lifted off of her feeder and landed again; together, these were taken as a measure of the total number of times a bee’s feeding was interrupted (=”number of interruptions”).

3.3.3 Statistical methods

Statistical analyses described below were performed using SAS version 9.2 (© 2008 SAS Institute Inc). For activity and exploration tests, data for any bee who made multiple trips to the feeding station during a given testing period were averaged across trips to obtain a mean measure of the bee’s performance during that testing period. For boldness tests, bees were typically only disturbed once in each testing period; if any bee was accidentally disturbed a second time, only her first response was included in our analysis. For statistical tests that required a single data point for each bee (correlations and factor analysis), calculations were performed using each bee’s averaged score across all test repetitions. Before analysis, quantitative variables that did not initially meet normality assumptions ($P < 0.05$ in Shapiro-Wilk W test) were transformed to improve normality. All reported P values are for 2-tailed tests, with $\alpha=0.05$. Average values are reported as mean ± SE.

Consistency

Consistency of bees’ behavior over time across repeated measurements of the same test was assessed using Kendall’s coefficient of concordance (Briffa et al. 2008), which was calculated using the MAGREE macro (http://support.sas.com/kb/25/006.html). As this test does not permit missing data, only bees who had participated in all the rounds of a given test were included in consistency analyses.

Mixed models

To test for differences among bees, we used the likelihood ratio test (LRT) to compare the fit of general linear mixed models (proc HPMIXED) that did and did not include ‘Bee ID’ as a random factor. Significant results indicate that bees’ identities accounted for a significant portion of the variance in their responses to the test (Martin & Réale 2008). Preliminary analysis revealed that random interaction terms (e.g. Bee ID*Day, Bee ID*Test_period) were not significant, so these terms are not included in the models we report here.

All bees who participated in more than one test repetition were included in these analyses. Colony, group (nested within colony), day (1-2), testing period (1-3), hour of day, and sugar concentration were included as fixed factors in all mixed models. For the boldness test, the number of times each bee was poked and bees’ “landing to disturbance” and “drinking before disturbance” times were also included as fixed factors. In the exploration test, the color and location of the novel feeder were included as fixed factors. For significant categorical variables, LS means tests were used to test for differences between levels of the variable. Testing period within a day was included in our models as a continuous factor, but in cases where this factor was significant we re-ran our models including it as a categorical variable to test for significant differences between time periods (1 vs. 2, 2 vs. 3, 1 vs. 3).

For the exploration test, we also fit a separate model using one line for each trip a bee made to the feeding station (rather than averaging across all the trips a bee made within each testing period) in order to determine whether bees' approach duration, approach direction, or the interaction between their approach direction and the novel feeder's location affected the likelihood that bees would land on the novel feeder. As this was a 1/0 response, this model was fit using a generalized linear model with a binary distribution (proc GLIMMIX; dist=Binary). Bee ID was not included in this model because it was not significant in our analysis of bees' average behavior (fraction of a bee's landings that were on the novel feeder).

Correlations and factor analysis

For correlations between individual pairs of variables (proc CORR), we report Pearson's correlation coefficient *r* for variables that were normally distributed, and Spearman's correlation coefficient ρ for variables with significant deviations from normality. Multiple correlations performed using the same data set were corrected for multiple comparisons using the Step-down Bonferroni correction (Holm 1979).

We also performed a factor analysis (Proc factor; method=ml; priors=smc) to test for the presence of latent factors that may have influenced the bees' performances across our different behavioral tests. Factor analysis was performed using each bee's average behavior across all testing trials. All of the variables on which bees had been found to differ consistently from one another were included in the analysis. Following the Kaiser-Guttman rule, we extracted all principal components with eigenvalues greater than 1 (Kaiser 1960), and rotated them using a varimax rotation.

3.4 Results

3.4.1 *Differences among bees*

Activity test

Across all colonies (*n*=2), groups within a colony (*n*=3), days (*n*=2) and testing periods within a day (*n*=3), bees made an average of 1.85 ± 0.02 trips to the feeding station per 10-minute testing period, spent 81.85 ± 0.82 s on the feeder per trip, and took an average of 3.90 ± 0.03 min to fly back and forth to the feeding station between trips (*n*=1462). The average amount of time a bee spent on the feeder during any given testing period ranged from under 30 to over 240 s (Figure 3.1a) and the average amount of time it took them to make a round trip to and from the hive ranged from 1.05 to 8.68 min (Figure 3.1b).

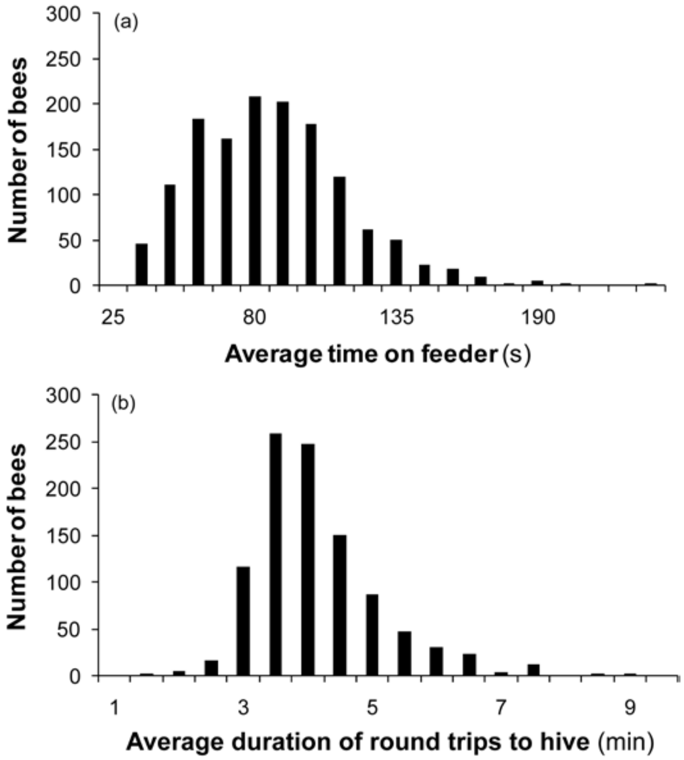

Figure 3.1 – Distribution of bees' behavioral scores during the activity test. Both the average amount of time bees spent on the feeder during each trip (a) and the duration of their round trips to the hive (b) spanned a large range of values.

Individual bees differed consistently in the number of trips they made to the feeding station, the average time they spent on the feeder during each trip (Figure 3.2), the average duration of their round trips to the hive, and the average duration of any "interruptions" during which when they temporarily flew up from the feeder (Table 3.1). These variables were all consistent over time (Kendall's coefficient of concordance: *P*<0.0001 for all variables except interruption duration: *P*= 0.002).

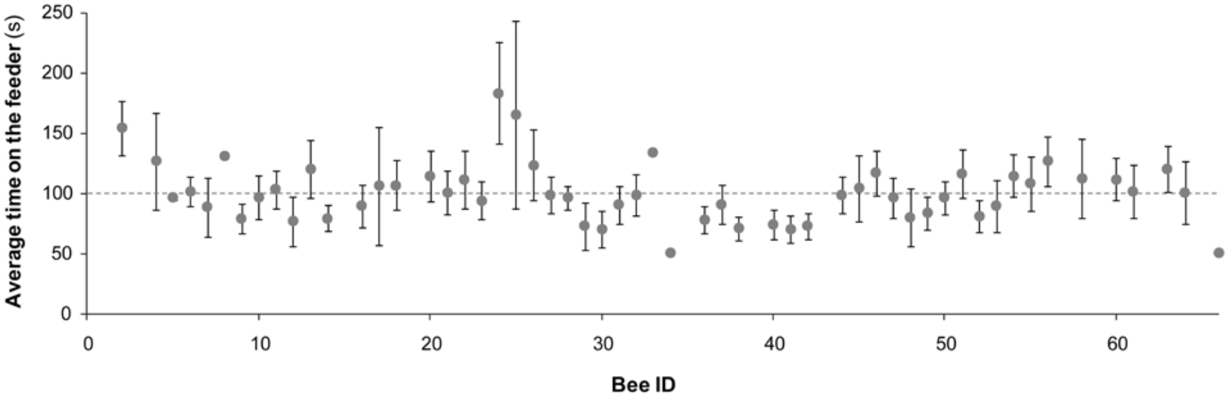

Figure 3.2 – Average amount of time that bees from colony 1, group1 spent on the feeder during the activity test. Circles represent each bee's mean score (± SE) from across all of the testing periods (3 tests on each of 2 days) during which she visited the feeder. The dashed line represents the average score across all of the bees in this group.

Bees who spent more time on the feeder during each trip to the feeder also took longer to make round trips to the hive (Pearson's correlation: *r*=0.29, *P*<0.0001; Figure 3.3), and consequently made significantly fewer trips to the feeding station during each testing period (Pearson's correlation: *r* =-0.43, *P*<0.0001).

Table 3.1 – Differences among individual bees in tests of activity, boldness, and exploration. For each variable, general linear mixed models were fit to the distribution of bees' behaviors during each testing period, and a likelihood-ratio test (LRT) was used to compare the fit of models with and without random Bee ID factor. Significant values, in bold, indicate that bees' identities accounted for a significant portion of the variability.

	LRT (X^2)	*df*	*P*
Activity test			
Number of trips to the feeding station per test	11.80	1	**0.0006**
Average time on feeder	11.00	1	**0.0009**
Average duration of round trips to hive	13.68	1	**0.0002**
Average interruption time	10.49	1	**0.001**
Boldness test			
Latency to begin feeding	28.75	1	**<0.0001**
Response latency	3.23	1	0.07
Response magnitude	1.55	1	0.21
Response duration	0.00	1	1
Exploration test			
Number of trips to the feeding station per test	27.07	1	**<0.0001**
Latency to land on a feeder	31.53	1	**<0.0001**
Fraction of landings on novel feeder	0.00	1	1
Average number of interruptions	3.88	1	**0.0483**

Boldness test

The time it took for bees to begin feeding after they had landed at the feeding station ("feeding latency") ranged from 0.03 to 66.23 s. Once per testing trial, each bee was disturbed with a paintbrush shortly after she had begun drinking from the feeder. 82% of bees reacted to these disturbances, and 71% of them flew up from the feeder. The average response latency was

0.60 ± 0.15 s (n=1519) after the start of the disturbance. The response duration, from the time a bee first reacted until she resumed drinking from the feeder, ranged from 0 to 74.86 s, and lasted an average of 3.85 ± 0.15 s (n= 1515).

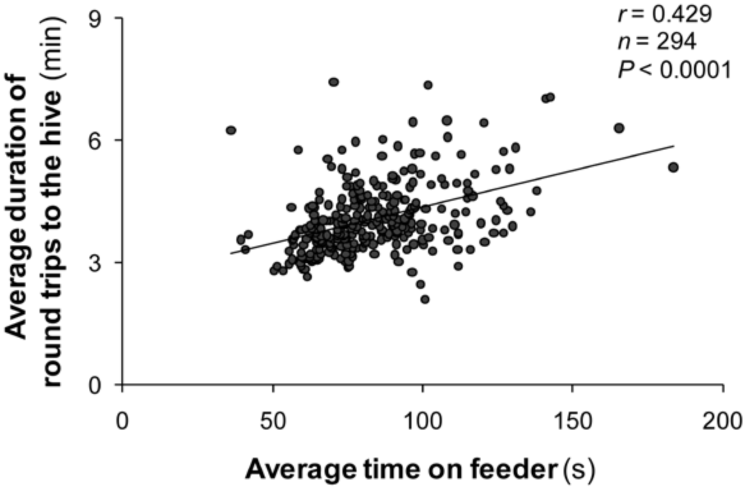

Figure 3.3 – Correlation between time on feeder and round trip duration during activity tests. Bees who spent more time on the feeder during the activity test also took more time to make round trips to the hive between visits to the feeding station.

Bees differed significantly from one another in the amount of time it took them to begin feeding after they first landed at the feeding station (LRT: $X^2_{df=1}$=28.75, P< 0.0001), and individual bees' feeding latencies were consistent across test repetitions (Kendall's coefficient of concordance: P<0.0001). However, individual bees did not differ consistently in their response latency, response magnitude, or response duration during individual testing periods (Table 3.1).

Exploration test

On average, bees made 2.71 ± 0.03 trips to the feeding station during each round of the exploration test. Bees spent an average of 1.49 ± 0.03 s approaching the feeding station, from when they were first seen on the video screen to when they touched down at one of the feeders. Interruptions, during which the bee flew up from a feeder, occurred during an average of 12 ±

1% of bees' trips to the feeding station. Bees switched to the other feeder during 64% of these interruptions; during the remaining 36% the bee lifted off and landed again on the same feeder.

Bees differed in the number of trips they made to the feeding station during each round of the exploration test, the amount of time they took to approach and land on one of the feeders, and the number of times they lifted off of a feeder while drinking (number of interruptions) (Table 3.1). Bees' behaviors on these three measures were consistent over time (Kendall's coefficient of concordance: $P<0.0001$; P<0.0001; $P=0.0028$). The bees' average scores from the two testing periods within a day and their average scores from within each of the two days of testing were positively correlated (Figure 3.4).

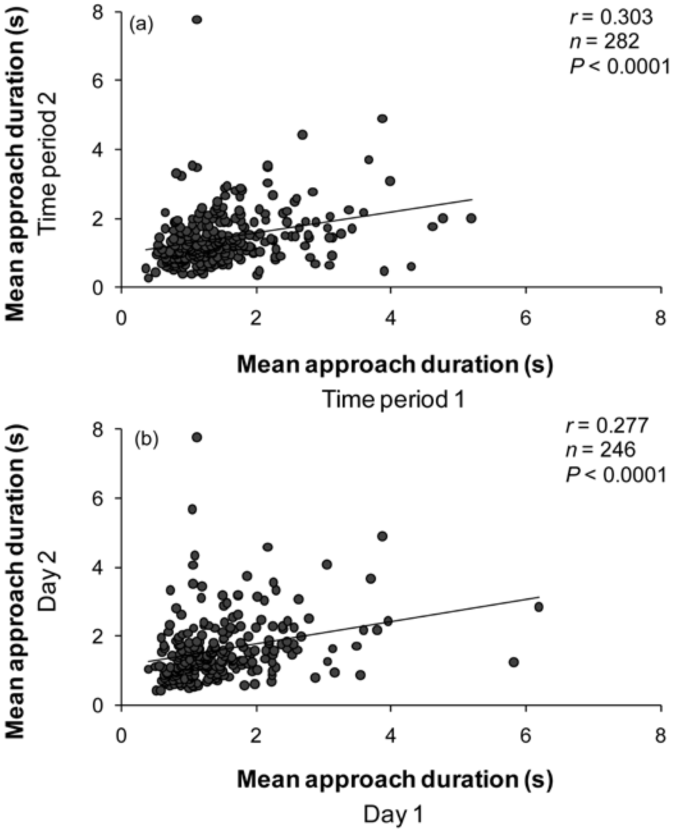

Figure 3.4 – Average time bees took to approach and land on a feeder in the exploration test. Tests were performed during two time periods each day. Average scores from the two time periods (a) and from the two days (b) were both positively correlated.

Bees did not differ significantly from one another in their tendency to land on the novel feeder. They did treat the novel feeder differently than the familiar feeder; across all testing periods, only 25% of bees' initial landings were on the novel feeder (674/2698; see supplementary Table 3.3), and bees were twice as likely to switch feeders during a given trip to the feeding station if they had landed on the novel feeder ($F_{1,2680}$=15.03, P<0.0001). However, within each testing period, bees did not differ from one another in the fraction of times they landed on the novel feeder.

3.4.2 Personality traits

Performing a factor analysis of bees' scores on all of the variables on which bees differed significantly from one another (Table 3.1), we found that bees' behavior was best described by two main factors (Table 3.2).

Table 3.2 – Factor analysis of variables for which bees exhibited consistent behavioral differences. The two factors with eigenvalues >1 were retained. Factor loadings >0.4 are bold and flagged by an '*'

		Factor 1 "Activity"	**Factor 2** "Restlessness"
Activity	Total trips to the feeding station	**0.90***	-0.14
	Average time on feeder	**-0.54***	0.16
	Average duration of round trips to the hive	**-0.48***	0.04
	Average interruption time	0.12	**0.45***
Boldness	Tests during which bee visited feeder	**0.63***	-0.33
	Average latency to begin feeding	-0.19	**0.56***
Exploration	Total trips to the feeding station	**0.86***	0.18
	Average latency to land on a feeder	0.05	-0.04
	Average number of interruptions while feeding	-0.17	**0.42***

The first factor represented the bees' activity level. Bees with high activity levels made more trips to the feeding station (Figure 3.5), spent less time on the feeder during each visit, and took less time to make round trips to the hive between feeder visits.

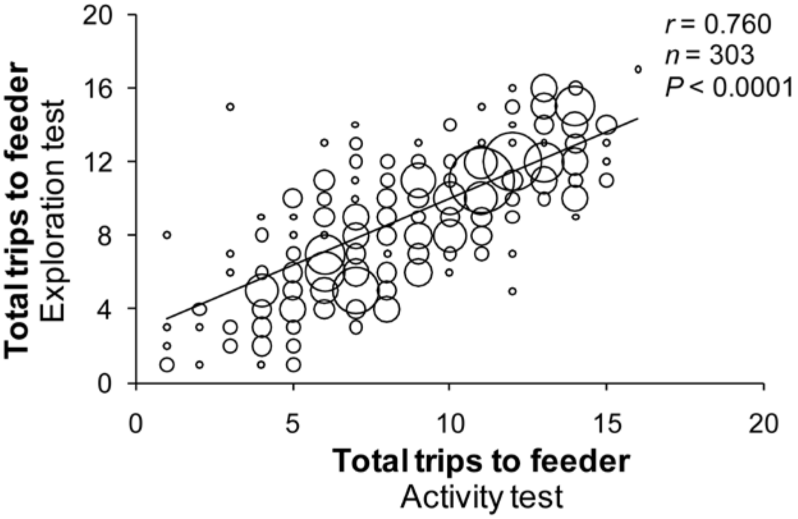

Figure 3.5 – Relationship between the total number of trips bees made to the feeding station during the activity test and the exploration test, summed over all testing trials. The size of each circle represents the number of bees who had that same number of trips in each test.

The second factor represented bees' restlessness while at the feeding station. Bees with high scores on Factor 2 had more frequent and longer "interruptions" in the activity and exploration tests during which they temporarily flew up from the feeder (Figure 3.6), and they took longer to settle down and begin feeding after arriving at the feeding station during the boldness test. Bees' activity scores (Factor 1) and restlessness scores (Factor 2) were not correlated (Pearson's correlation: *NS*).

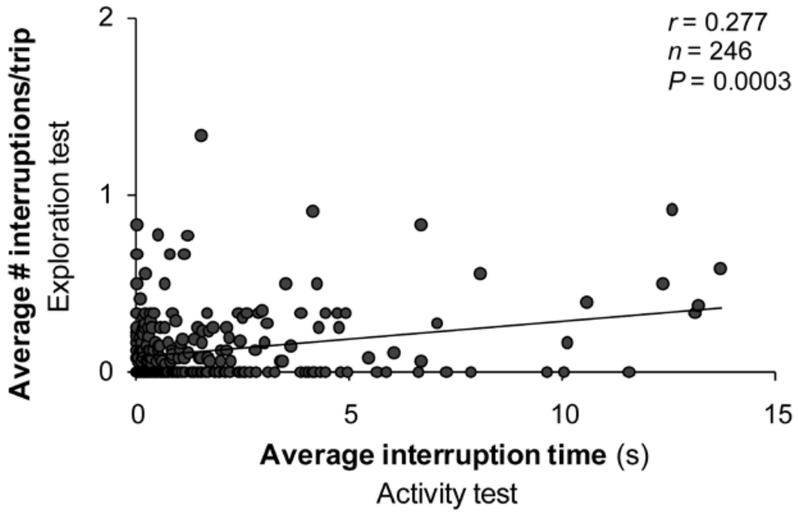

Figure 3.6 – Correlation between bees' average interruption time in the activity test and the average number of times they flew up from the feeder (i.e. number of "interruptions") during the exploration test.

3.4.3 Effects of fixed factors

Test and landing order

In later test repetitions within a day, bees tended to make more trips to the feeding station (Activity test, $F_{1,1422}$=4.24, P= 0.0237 ; Exploration test, $F_{1,965}$=29.62 , P<0.0001) and spend less time on the feeder per trip ($F_{1,1363}$=26.16, P < 0.0001; Figure 3.7). Bees in later testing periods also took less time to approach and land on one of the two feeders in the exploration test ($F_{1,965}$=12.46, P =0.0004). However, these effects did not carry over across days; bees did not spent any less time on the feeder in Day 2 than they had in Day 1 (Figure 3.7), and there was no difference in bees' approach durations on Day 2 of testing as compared to Day 1 ($F_{1,965}$=0.18, P=0.673).

During the exploration test, bees who returned to the feeder more than once during a testing period tended to have shorter approach durations in later trips ($F_{1,2654}$=6.81,

P=0.009), and were more likely to land on the novel feeder in subsequent landings ($F_{1,2653}$=21.36, P< 0.0001).

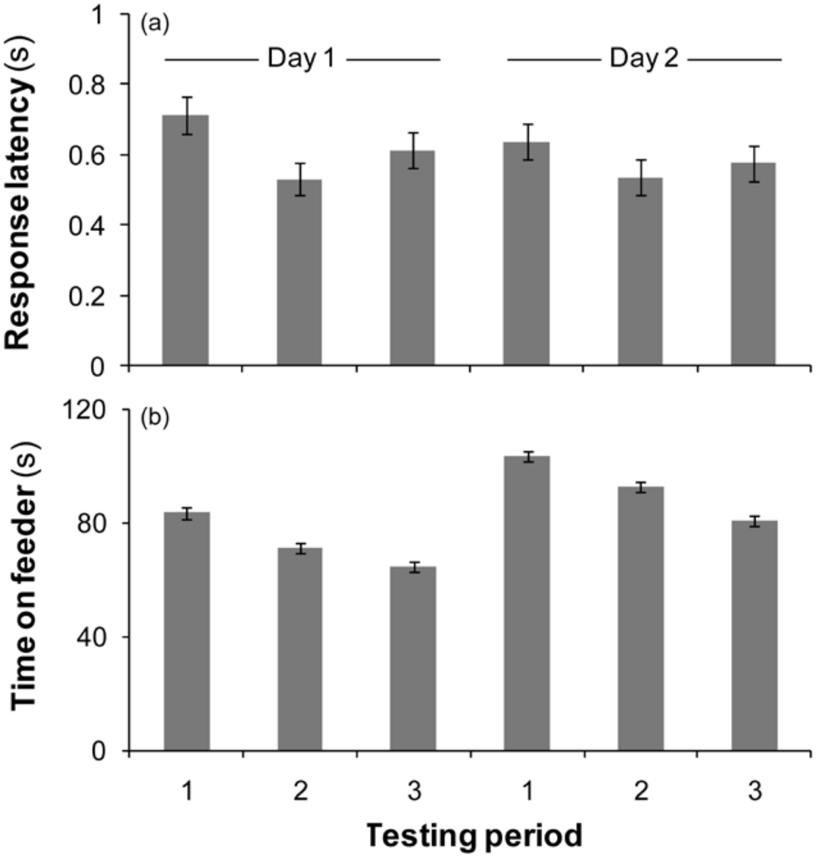

Figure 3.7– Bees' average response latencies (a) and average time spent on the feeder (b) across testing periods during the two days of testing. There were no significant effects of day or time period on bees' response latencies. In contrast, the amount of time bees spent on the feeder decreased during the course of each day, but did not differ significantly across days.

Sugar concentration

When the sugar solution in the feeder was more concentrated, bees tended to spend more time on the feeder per trip ($F_{1,1363}$=172.36, P <0.0001) and took less time to begin drinking after landing at the feeder during the boldness test ($F_{1,1434}$=4.30, P=0.038).

However, sugar concentration did not significantly affect the number of visits bees made to the feeding station or the duration of their round trips to the hive.

The sugar concentration did not affect bees' average number of interruptions during the exploration test ($F_{1,965}$=0.85, P=0.3567), but the total duration of interruptions during the activity test was shorter when sugar solution was more concentrated ($F_{1,1363}$=34.38, P<0.0001).

Novel feeder color and location

During the exploration test, a greater fraction of bees' landings were on the novel feeder ($F_{1,965}$ =4.01, P<0.0456) during trials when the novel feeder was blue, rather than yellow. The probability that a bee would land on the novel, rather than the familiar, feeder during a given trip to the feeding station was also strongly affected by the interaction between the direction in which the bee approached the feeding station and the location of the novel feeder ($F_{21,2650}$=12.54, P<0.0001). Bees were more likely to land on the novel feeder than the familiar feeder when it was the first feeder they encountered as they approached the feeding station, but if they approached the feeding station from any other direction they were more likely to land on the familiar feeder (Figure 3.8; Supplementary Table 3.3).

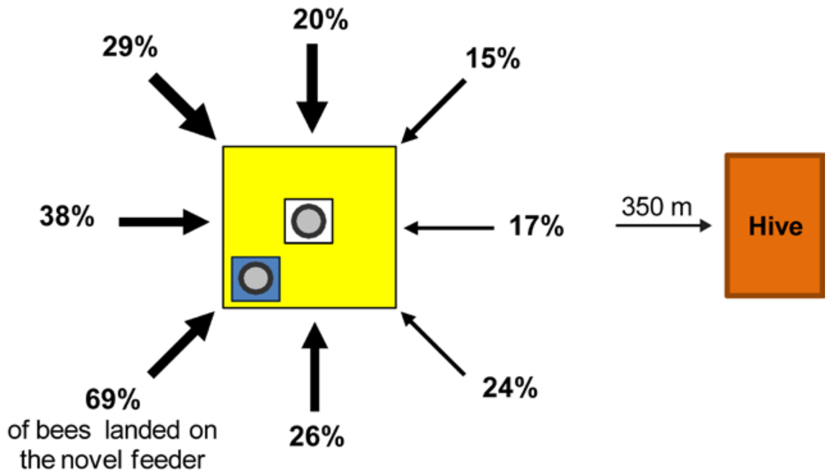

Figure 3.8 – Likelihood that bees would land on the novel feeder in the exploration test. Each arrow's thickness indicates the fraction of bees who approached the feeding station (large yellow square) from that direction, and its percentage represents the probability that a bee approaching from that direction would land on the novel feeder (small blue square). For most approach directions, less than half of the bees landed on the novel feeder. However, bees who approached the feeding station from the direction closest to the novel feeder (bottom left) were more likely to land on the novel feeder (69% of bees) than the familiar feeder. In this figure, the novel feeder is in the back left corner of the feeding station; complete results are presented in Supplementary Table 3.3.

3.5 Discussion

We found that individual honey bee foragers consistently differed in how active and restless they were during their visits to an artificial feeder. Individual bees differed significantly in their activity levels, with some bees consistently spending less time on the feeder during each visit and taking less time to make round trips to the hive between visits. Bees also differed in their restlessness; more restless bees took longer to settle down and begin drinking after they arrived at the feeding station, and interrupted their feeding by flying up from the feeder more frequently and for longer time periods. These behavioral differences among bees were consistent over time and across different testing situations.

Our observation that individual bees display consistent differences in their activity levels is consistent with previous studies in other species. Personality differences in activity levels have been described in a large number of animal species (Gosling 2001), and activity is one of five major behavioral traits that Réale et al. (2007) proposed as part of a conceptual framework for researchers studying animal personality and temperament. This finding also fits with previous studies in honey bees, which have reported that some bees were observed at feeders more often than others during experiments (Johnson & Wenner 1966) and have found that bees from colonies with different genotypes differ in the number of foraging trips they make from the hive (Guzmán-Novoa & Gary 1993). Several studies in ants have also suggested that individual workers differ in their activity rates (Oster & Wilson 1979; Cole 1992), but few of them measured the consistency of individuals' activity levels over time or how their activity levels are related to other behaviors. Interestingly, consistent differences in foraging activity levels have also been found among equally-sized colonies of honey bees (Wray et al. 2011), although it is not yet clear whether differences in colonies' activity levels result from differences in the average foraging rates of the workers within each colony or in the proportion of each colony's workforce that is engaged in foraging tasks. However, within a given colony, it seems that changes in foraging rate over time are regulated by varying the number of foragers that leave the hive rather than altering the level of activity of these foragers (Thom et al. 2000), which fits with our finding that individual foragers' activity levels are consistent over time.

To our knowledge, the differences we discovered in bees' restlessness have not previously been described in the literature, but they fit with previous observations that bees appear to vary in their responsiveness to changes in sucrose concentration and their "skittishness" when artificial feeders are moved or disturbed (authors; personal observation). We can think of two potential proximate mechanisms for the differences in restlessness we observed among bees. One could be that the restlessness is a startle response, and the variability we saw in bees' restlessness represents differences in bees' levels of anti-predator, or vigilance, behavior. Alternatively, restlessness could be related to bees' responsiveness to sucrose, and bees with high levels of restlessness could be bees who have high sucrose response thresholds and are less satisfied with the food provided at the feeder.

We found that bees' activity levels and restlessness were consistent both within and across days. In general, the time period within a day during which a test took place affected bees' behavior more than the testing day (significant fixed factor in 7 vs. 4 out of 12 mixed models). This suggests that changes over time in bees' mean behavior were probably due to temperature fluctuations rather than learning or habituation. We found that behavioral differences among our bees persisted across time periods and thus were not affected by changing temperature; similar effects of temperature on personality were recently described in a study of subsocial spiders (*Anelosimus studiosus*: Pruitt et al. 2011). The consistency in our bees' behavior across days differs from recent studies of bumble bees (Muller et al. 2010) and gloomy octopuses (Pronk et al. 2010) in which individuals' behaviors were observed to be much more consistent within a day than across days. We did not find this with our bees; in general, the correlations between their behaviors within a day were not stronger than the correlations between their behaviors across days. Also, in contrast to findings by Pronk et al. (2010) that octopus' behavioral correlations change from day to day, we found that the same behavioral correlations were present in both Day 1 and Day 2 and thus seem to be more permanent than the episodic personalities found in the gloomy octopus.

Interestingly, we found that bees did not differ consistently from one another in their response to disturbances, as indicated by their response latency, duration, and magnitude in our boldness tests. This negative result is unlikely to have been due to insufficient power given that our sample size (approximately 300 individuals) was considerably greater than the average for studies measuring the repeatability of behavior (average n=39; Bell et al. 2009) and we did observe significant differences among bees in other behavioral tests with the same sample size. This lack of consistent differences among bees is somewhat surprising because boldness, or response to risky situations, (Réale et al. 2007) is a behavioral trait that has been observed in many species—including several invertebrates such as fiddler crabs (Reaney & Backwell 2007) and house crickets (Wilson et al. 2010). Indeed, fishing spiders differed consistently in their boldness in response to a very similar behavioral test on which our own test was based (Johnson & Sih 2007). Why might we have failed to observe consistent differences in boldness among the bees in our study? It is possible that even though our testing procedure was similar to the methodology from a previous study that did reveal individual differences in boldness, the bees in our study did not view our disturbance as a particularly risky or threatening, in which case our assay would not have provided a good measure of bees' boldness. Future studies could focus more specifically on testing for the presence of boldness differences among bees by exposing bees to actual predators, more realistic simulations of predators (i.e. pinching), or alarm pheromone. Alternatively, it is possible that individual bees do not differ consistently from one another in their boldness because there is no selective pressure for them to do so. Boldness is an important component of individual variation that affects survival and reproductive success in a number of animal species. But in honey bee colonies, natural selection operates primarily between, rather than within, colonies and selects for traits that improve individual workers' indirect, rather than their direct, fitness. This can result in very different behavioral outcomes; for instance, bold behaviors that would be very detrimental to an individual animal’s fitness [e.g. fatal stinging of intruders] can actually benefit the inclusive fitness of worker honey bees if their death permits the queen to produce more reproductive offspring (queens and drones).

We also found that bees did not differ from one another in their tendency to land on the novel feeder during the exploration test. In general, bees were less likely to land on the novel feeder than on the familiar feeder, although they were more willing to land on the novel feeder if it was the first feeder they encountered as they approached the feeding station, and bees that returned to the feeder more than once within a testing period were more likely to land on the novel feeder in subsequent visits. This suggests that bees recognized the new feeder as a novel object and preferred to avoid it. But we found no evidence that individual bees differed consistently from one another in their willingness to explore the novel feeder. Why might we have failed to see differences among bees in their exploratory behavior? It is possible that while bees did appear to recognize the new feeder as novel, this situation was not different enough from baseline conditions to elicit true exploratory behavior and provide a reliable measure of the bees’ willingness to explore new environments. Alternatively, it might be that the fraction of times bees landed on the novel feeder was not the best measure of their response to novelty. A recent study with bumble bees showed that most of the variation in bees’ latency to feed from artificial flowers with novel colors was captured by their “investigation time”, or the time they spent hovering near the flower before landing (Muller et al. 2010). So, it is possible our bees did differ in their exploratory behavior but that these differences were reflected in bees’ landing latencies rather than the fraction of times they landed on the novel feeder. The bees in our study did differ significantly in their landing latencies during the exploration test, and bees that returned to the feeding station multiple times within a testing period had shorter landing latencies in later trips, which suggests that landing latency may indeed represent their reaction to the novel feeder. Furthermore, landing latencies were not correlated with bees' activity levels or restlessness, so it is possible that this variable represented a separate 'exploration' trait, with bees who took less time to approach and land on one of the feeders during the exploration test being more exploratory than bees who took longer to land. We cannot be certain that the consistent differences in our bees' landing latencies during this test represented differences in their exploratory behavior because we do not have a measure of bees' baseline landing latencies prior to the introduction of the novel feeder, and we do not have any other measures of exploration with which to correlate bees' landing latencies in this test. However, future studies that include more tests of bees' exploratory behavior should be able to determine whether a bee's latency to land at a novel feeding station is related to other measures of her willingness to explore novel objects or environments.

Although we only observed bees' behavior while they were at the feeding station, we found that the duration of their round trips to the hive—which included time spent in the hive unloading food—was related to the time they spent on the feeder. This suggests that the differences we saw in bees' activity levels at the feeding station may also carry over to their behaviors in the hive. Future studies could perform observations of bees both inside and outside the hive to determine whether bees that are more active and restless during foraging trips also display high levels of activity and restlessness within the hive. More focused observations over longer timescales could also be used to determine whether these personality traits are consistent over the course of bees' development or change over time (e.g. Johnson & Sih 2007). The development of personality is an under-studied but intriguing topic (Stamps & Groothuis 2010) that would be especially fascinating to study in honey bees because of their age-based polyethism (Huang & Robinson 1996). Furthermore, these same studies could investigate the mechanistic basis for the differences we observed in bees’ level of activity and restlessness and determine whether they result primarily from differences in bees’ genotype, age, rearing temperature (Becher et al. 2009), or previous experiences (e.g. Frost et al. 2007).

Previous research has shown that genetically diverse honey bee colonies are better able to capitalize on available resources (Mattila & Seeley 2007; Mattila et al. 2008; Mattila & Seeley 2010b), and variations in workers’ foraging strategies (Burns & Dyer 2008) are hypothesized to enhance a colony’s foraging efficiency. The consistent differences we observed in the activity and restlessness of nectar foragers may also provide benefits to honey bee colonies. In individual animals, differences in activity rates have been linked to variation in life-history strategies (Biro & Stamps 2008; Réale et al. 2010), with more active individuals tending to have shorter lives. For a colony of honey bees, having workers with a variety of activity levels might allow highly active workers to be very productive foragers, but potentially wear out or suffer predation at higher rates, while less active bees would be less productive but have longer lifespans. These less active workers could be of particular importance during times such as colony swarming events when the queen temporarily stops laying eggs and the colony’s workforce is not constantly being replaced by new workers. Variation in restlessness among workers within a colony could also serve a useful function. If restlessness is an anti-predator response, it may be useful to have some foragers who are highly vigilant and likely to avoid predation, while others are willing to forage even in risky environments. Alternatively, if differences in restlessness are due to variation in bees’ satisfaction with the same quality of nectar, colonies may benefit from having some bees who abandon low-quality food sources in order to search for exceptional ones, as well as other less selective bees who are willing to forage on any available nectar.

Finally, future studies of personality in honey bees could investigate whether activity or restlessness are correlated with other consistent behaviors, either within or across contexts, as part of a behavioral syndrome. Behavioral syndromes are suites of behaviors that are correlated across situations, and can involve correlations between the same behavior in different contexts (e.g. activity in the context of foraging or brood-care) or different behaviors within the same context (e.g. activity and boldness in the context of foraging) (Sih et al. 2004; Sih & Bell 2008). Studies that test bees' activity and restlessness as well as their 'impulsiveness' (Burns & Dyer 2008) and problem-solving behaviors (Cakmak et al. 2009) could determine whether bees that are more restless or more active also tend to be more "impulsive". Future studies could also investigate whether bees' activity levels in a foraging context are related to their willingness to dance for a food source or the number of waggle runs they perform for the same food source (Seeley 1994).

Our results demonstrate that individual honey bee foragers visiting the same food source display differences in their activity levels and restlessness that are consistent over time and across testing situations. The behavioral differences among the honey bees in our study were similar to the personality differences observed in previous studies in other organisms, demonstrating that the framework of personality research can be applied successfully to social insect species. Differences in personality traits such as activity and restlessness may serve to increase the behavioral diversity of workers within a honey bee colony and consequently improve colony survival and reproduction.

3.6 References

Arnold, G., Quenet, B., Papin, C., Masson, C., & Kirchner, W. H. 2002. Intra-colonial variability in the dance communication in honeybees (*Apis mellifera*). *Ethology,* 108, 751-761.

Becher, M. A., Scharpenberg, H., & Moritz, R. F. A. 2009. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera L.*). *Journal of Comparative Physiology A*, 195, 673-679.

Bell, A. M., Hankison, S. J., & Laskowski, K. L. 2009. The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, 77, 771–783.

Biro, P., & Stamps, J. 2008. Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, 23, 361-368.

Bonabeau, E., Theraulaz, G., & Deneubourg, J. L. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society B,* 263, 1565–1569.

Breed, M., Robinson, G., & Page, R. 1990. Division of labor during honey bee colony defense. *Behavioral Ecology and Sociobiology,* 27, 395-401.

Briffa, M., Rundle, S. D., & Fryer, A. 2008. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society B: Biological Sciences,* 275, 1305-1311.

Burns, J. G., & Dyer, A. G. 2008. Diversity of speed-accuracy strategies benefits social insects. *Current Biology*, 18, R953-R954.

Cakmak, I., Sanderson, C., Blocker, T. D., Lisa Pham, L., Checotah, S., Norman, A. A., Harader-Pate, B. K., Tyler Reidenbaugh, R., Nenchev, P., Barthell, J. F., & Wells, H. 2009. Different solutions by bees to a foraging problem. *Animal Behaviour,* 77, 1273-1280.

Chittka, L., Dyer, A. G., Bock, F., & Dornhaus, A. 2003. Psychophysics: Bees trade off foraging speed for accuracy. *Nature,* 424, 388.

Cole, B. J. 1992. Short-term activity cycles in ants: Age-related changes in tempo and colony synchrony. *Behavioral Ecology and Sociobiology,* 31, 181-187.

Dall, S. R. X., Houston, A. I., & McNamara, J. M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters*, 7, 734-739.

Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K., & van Noordwijk, A. J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, 64, 929-938.

Frost, A., Winrow-Giffen, A., Ashley, P., & Sneddon, L. 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society B*, 274, 333-339.

Girard, M., Mattila, H., & Seeley, T. 2011. Recruitment-dance signals draw larger audiences when honey bee colonies have multiple patrilines. *Insectes Sociaux*, 58, 77-86.

Gosling, S. 2001. From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, 127, 45-86.

Guzmán-Novoa, E., & Gary, N. E. 1993. Genotypic Variability of Components of Foraging Behavior in Honey Bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 86, 715-721.

Holm, S. 1979. A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics*, 6, 65-70.

Huang, Z.-Y., & Robinson, G. E. 1996. Regulation of honey bee division of labor by colony age demography. *Behavioral Ecology and Sociobiology*, 39, 147-158.

Johnson, D. L., & Wenner, A. M. 1966. A relationship between conditioning and communication in honey bees. *Animal Behaviour*, 14, 261-265.

Johnson, J. C., & Sih, A. 2007. Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, *Dolomedes triton*. *Animal Behaviour*, 74, 1131-1138.

Jones, J. C., Myerscough, M. R., Graham, S., & Oldroyd, B. P. 2004. Honey bee nest thermoregulation: Diversity promotes stability. *Science*, 305, 402 -404.

Kaiser, H. F. 1960. The Application of Electronic Computers to Factor Analysis. *Educational and Psychological Measurement*, 20, 141 -151.

Kortet, R., & Hedrick, A. N. N. 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biological Journal of the Linnean Society*, 91, 475–482.

Martin, J. G. A., & Réale, D. 2008. Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, 75, 309–318.

Mather, J. A., & Anderson, R. C. 1993. Personalities of octopuses (*Octopus rubescens*). *Journal of Comparative Psychology*, 107, 336.

Mattila, H. R., & Seeley, T. D. 2010a. Promiscuous honeybee queens generate colonies with a critical minority of waggle-dancing foragers. *Behavioral Ecology and Sociobiology*, 64, 875–889.

Mattila, H. R., & Seeley, T. D. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, 317, 362-364.

Mattila, H. R., Burke, K. M., & Seeley, T. D. 2008. Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. *Proceedings of the Royal Society B: Biological Sciences*, 275, 809-816.

Mattila, H. R., & Seeley, T. D. 2010b. Promiscuous honeybee queens generate colonies with a critical minority of waggle-dancing foragers. *Behavioral Ecology and Sociobiology*, 64, 875-889.

Muller, H., Grossmann, H., & Chittka, L. 2010. `Personality' in bumblebees: individual consistency in responses to novel colours? *Animal Behaviour*, 80, 1065-1074.

Noldus, L. P. J. J., Trienes, R. J. H., Hendriksen, A. H. M., Jansen, H., & Jansen, R. G. 2000. The Observer Video-Pro: New software for the collection, management, and presentation of time-structured data from videotapes and digital media files. *Behavior Research Methods, Instruments, & Computers*, 32, 197-206.

Oldroyd, B. P., & Fewell, J. H. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology & Evolution*, 22, 408-413.

Oster, G. F., & Wilson, E. O. 1979. *Caste and Ecology in the Social Insects.* Princeton University Press.

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

Pronk, R., Wilson, D. R., & Harcourt, R. 2010. Video playback demonstrates episodic personality in the gloomy octopus. *J Exp Biol*, 213, 1035-1041.

Pruitt, J., Demes, K., & Dittrich-Reed, D. 2011. Temperature Mediates Shifts in Individual Aggressiveness, Activity Level, and Social Behavior in a Spider. *Ethology*, 117, 318-325.

Pruitt, J., & Riechert, S. 2011. How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proceedings of the Royal Society B*, 278, 1209-1215.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291–318.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B*, 365, 4051 -4063.

Reaney, L. T., & Backwell, P. R. Y. 2007. Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behavioral Ecology*, 18, 521 -525.

Schneider, S. S., & Duong, N. 2008. Intra-patriline variability in the performance of the vibration signal and waggle dance in the honey bee, *Apis mellifera*. *Ethology*, 114, 646-655.

Seeley, T. D. 1995. *The wisdom of the hive: the social physiology of honey bee colonies.* Harvard University Press.

Seeley, T. D., & Tarpy, D. R. 2007. Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society B: Biological Sciences*, 274, 67.

Seeley, T. D. 1994. Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, 34, 51-62.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *Quarterly Review of Biology*, 241–277.

Sih, A., & Bell, A. M. 2008. Insights for Behavioral Ecology from Behavioral Syndromes. *Advances in the Study of Behavior*, 38, 227-281.

Sih, A., & Jason V. Watters. 2005. The mix matters: Behavioural types and group dynamics in water striders. *Behaviour*, 142, 1417-1431.

Sinn, D. L., & Moltschaniwskyj, N. A. 2005. Personality traits in dumpling squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. *Journal of Comparative Psychology*, 119, 99–110.

Smith, B. R., & Blumstein, D. T. 2008. Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, 19, 448-455.

Stamps, J., & Groothuis, T. G. G. 2010. The development of animal personality: relevance, concepts and perspectives. *Biological Reviews*, 85, 301-325.

Thom, C., Seeley, T. D., & Tautz, J. 2000. A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. *Apidologie*, 31, 2.

Trumbo, S., Huang, Z., & Robinson, G. 1997. Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. *Behavioral Ecology and Sociobiology*, 41, 151-163.

Vainikka, A., Rantala, M. J., Niemelä, P., Hirvonen, H., & Kortet, R. 2010. Boldness as a consistent personality trait in the noble crayfish, *Astacus astacus*. *acta ethologica*, 14, 17-25.

von Frisch, K. 1967. *The Dance Language and Orientation of Bees.* First Edition edn. Belknap Press.

Weidenmüller, A. 2004. The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, 15, 120-128.

Wilson, A. D. M., Whattam, E. M., Bennett, R., Visanuvimol, L., Lauzon, C., & Bertram, S. M. 2010. Behavioral correlations across activity, mating, exploration, aggression, and antipredator contexts in the European house cricket, *Acheta domesticus*. *Behavioral Ecology and Sociobiology*, 64, 703–715.

Wilson, D. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London: Series B*, 353, 199-205.

Wray, M. K., Mattila, H. R., & Seeley, T. D. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Animal Behaviour*, 81, 559-568.

3.7 Supplementary information

Table 3.3 – Fraction of bees that landed on the novel feeder in the Exploration test for each combination of approach direction and novel feeder location. The rows in which more than 50% of bees landed on the novel feeder are indicated with an asterisk *. The rows for which bees' approach direction matched the location of the novel feeder are shaded in green.

Novel feeder location	**Bees' approach direction**	**Total landings**	**Landings on novel**	**% landings novel**
Back left	Bottom left	103	71	69% *
Back left	Bottom middle	69	18	26%
Back left	Bottom right	38	9	24%
Back left	Left	79	30	38%
Back left	Right	84	14	17%
Back left	Top left	65	19	29%
Back left	Top middle	123	25	20%
Back left	Top right	72	11	15%
Back right	Bottom left	100	4	4%
Back right	Bottom middle	100	13	13%
Back right	Bottom right	72	49	68% *
Back right	Left	106	10	9%
Back right	Right	20	11	55% *
Back right	Top left	109	12	11%
Back right	Top middle	79	9	11%
Back right	Top right	37	15	41%
Front left	Bottom left	89	14	16%
Front left	Bottom middle	75	9	12%
Front left	Bottom right	21	4	19%
Front left	Left	85	36	42%
Front left	Right	40	8	20%
Front left	Top left	182	96	53% *
Front left	Top middle	120	19	16%
Front left	Top right	53	19	36%
Front right	Bottom left	105	12	11%
Front right	Bottom middle	134	12	9%
Front right	Bottom right	35	16	46%
Front right	Left	117	9	8%
Front right	Right	26	16	62% *
Front right	Top left	137	20	15%
Front right	Top middle	158	36	23%
Front right	Top right	63	26	41% *

Chapter 4

Collective personalities in honey bee colonies are linked to colony fitness [4]

4.1 Abstract

Personality differences (i.e. consistent between-individual differences in behavior) play an important role in the lives of humans and other animals, influencing both their day-to-day actions and their long-term reproductive success. For organisms living in highly-structured groups of related individuals—such as colonies of social insects—personalities could also emerge at the group level. However, while numerous recent studies have investigated individual-level personality, the phenomenon of collective personality in animal groups has received little attention. In this paper, we apply the concept of collective personality to colonies of honey bees (*Apis mellifera*). We document the presence of consistent differences among colonies across a wide range of collective behaviors and demonstrate a link between colony-level personality traits and fitness. The colonies in our study exhibited consistent behavioral differences in traits such as defensive response, foraging activity, and undertaking, and several of these traits were correlated as part of a behavioral syndrome. Furthermore, some of these traits were strongly tied to colony productivity and winter survival. Our results show that the concept of collective personality is applicable to colonies of social insects, and that personality differences among colonies can have important consequences for their long-term survival and reproduction. Applying the concept of personality to close-knit animal groups can provide important insights into the structure of

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behavioral variability in animal populations and the role that consistent between-group behavioral differences play in the evolution of behavior.

4.2 Introduction

Personality differences can have profound effects on the behaviors and long-term fitness of individual organisms. Human psychologists have long recognized that people consistently differ in their responses to different situations (Allport 1937; Mischel & Shoda 1995), and that personality traits can affect reproductive success (Eaves et al. 1990). More recently, studies have shown that non-human animals also display consistent, heritable differences in personality traits such as boldness, tendency to explore, and aggressiveness (Wilson 1998; Gosling 2001; Bell et al. 2009) and that these traits can be linked to survival and reproductive success (Dingemanse & Reale 2005; Smith & Blumstein 2008).

The word “personality” is typically used to refer to consistent behavioral differences among *individual* organisms. However, to the extent that cooperative *groups* differ from one another in their collective behaviors, these groups can also be thought of as having “collective personalities” (Stewart 2003). In humans, for example, studies of collective personality have shown that some groups tend to be more communicative or more aggressive than others (Hofmann & Jones 2005), just as some individuals are more extroverted or less agreeable than others. These collective personality differences can have important effects on a group’s ability to survive and function. In human organizations and teams, for instance, collective personality can affect a group’s performance (Stewart 2003; Hofmann & Jones 2005) and its ability to attract additional members (Anderson et al. 2010).

The concept of collective personality has been applied primarily to human social groups, but this idea is also relevant to many animal groups, especially cooperative groups of related individuals whose genetic interests are aligned. In a social insect colony, for example, workers' actions are so well coordinated that the colony behaves as a single "superorganism" (Hölldobler & Wilson 2008) and nearly all reproduction occurs at the colony level—either during colony fissioning or when males and queens leave to found new colonies. In such groups, any fitness consequences of collective personality should be especially apparent because natural selection operates primarily on differences among colonies (between-group selection) rather than among individuals within a colony (within-group selection) (Korb & Heinze 2004; Bergmüller et al. 2007).

Previous work suggests that social insect colonies often differ in their collective behavior. Beekeepers claim that "every colony of honey bees has an individual character" (Weiss 1983), and breeders of honey bees (*Apis mellifera*) report marked differences in the productivity and temperament of their colonies (Laidlaw 1979; Laidlaw & Page 1997). Numerous studies have shown that honey bee colonies differ in traits such as defensive response (e.g. Collins et al. 1982; Breed 1991; Hunt et al. 1998; Guzmán-Novoa et al. 2002), hygienic behavior (e.g. Rothenbuhler 1964; Arathi & Spivak 2001) and pollen hoarding (e.g. Hellmich et al. 1985; Pesante et al. 1987). Several of these differences in colony-level behavior are consistent across multiple rounds of testing (e.g. Giray et al. 2000; Guzmán-Novoa et al. 2003), and some studies have found correlations between one or more of these behaviors (e.g. Page et al. 1995a; Giray et al. 2000; Guzman-Novoa et al. 2002).

Taken together, these findings suggest that the concept of collective personality is highly relevant to the study of colony-level behavior in social insects. However, relatively few studies have quantified the range of behavioral variation that characterizes colonies of European honey bees with naturally mated queens; most previous studies involved both Africanized and European bees or used colonies from artificially selected genetic lines (although see Breed & Rogers 1991). Furthermore, we know very little about the relationship between these collective behavioral traits and colony-level fitness.

Our study expands on prior results by applying the concept of collective personality to social insects and exploring the link between colony-level personality and fitness. We established 25 equally sized honey bee colonies in empty hives, assessed the consistency of their responses to a variety of colony-level behavioral tests, and monitored their subsequent growth and survival to determine whether any of these behavioral traits were linked to colony fitness. Observing our colonies from the colony-founding stage allowed us to equalize their size, weight, and physiological state, and provided them with a demanding test of fitness. In temperate climates, only 24% of newly founded colonies survive their first winter (Seeley 1978), so forcing colonies to found new nests encouraged high levels of productivity and emphasized performance differences among colonies. Furthermore, by testing the same colonies on a variety of behavioral tests, we were able to determine whether these colony-level traits formed suites of correlated behaviors, or “behavioral syndromes” (Sih et al. 2004a; Sih et al. 2004b). In individual animals, correlations among personality traits like activity, aggression, and boldness vary across populations according to ecological factors like the level of predation (Bell 2005; Dingemanse et al. 2007), and are thus likely to be adaptive.

4.3 Methods

4.3.1 Study Colonies

On 20 May 2009, we created 25 artificial swarms from genetically unrelated colonies of European honey bees with naturally mated queens. We created each swarm by transferring 1 kg of workers (~7500 bees) and their mother queen from a single colony to a screened wooden swarm cage using standard methods (Seeley & Visscher 1985). Caged bees were fed 1:1 sucrose solution ad libitum for three days to induce the physiological state of bees in a natural swarm. On the evening of 23 May, we installed each swarm in an 8-frame full-depth Langstroth hive body with alternating frames containing full and partial sheets of wax foundation. Colonies were housed in the same apiary, at the Liddell Field Station of Cornell University in Ithaca, New York (42°26′N, 76°30′W). Hives were arranged in four rows, with adjacent hives at least 2 m apart to minimize drifting of bees.

During the course of the study, some colonies constructed enough comb to nearly fill their hives. An additional hive body was added to any colony that had built comb filling approximately 90% of its hive.

All colonies received standard treatments against Varroa mites (Apistan®) in April and against American Foulbrood (Terra Pro, Walter T. Kelley Co., Clarkson, KY) in late June.

4.3.2 Behavioral Tests

We performed behavioral tests to assess each colony’s level of defensive response and foraging activity, the diversity of pollen its foragers collected, and its workers’ tendency to repair damaged comb (“comb repair”), run across comb (“runniness”), and remove dead bees from the hive (“undertaking”).

Testing schedule

To ensure that behavioral differences among colonies were not due to variations in colony weight or population size, all tests were performed during the first five weeks of the study, before colonies had reared new foragers or gained significant weight. Each colony was also checked periodically to confirm that its queen was healthy and laying well.

To assess the consistency of each colony’s response, we tested colonies six times at approximately weekly intervals: on 25–26 May, 31 May–1 June, 6–7 June, 13–14 June, 17 and 19 June (it rained on 18 June), and 26–27 June. Each round of tests lasted two days. On day one, we measured each colony’s level of foraging activity, collected pollen foragers to assess pollen diversity, and added 100 dead bees to each hive in the evening. On day two, we assessed the colonies’ undertaking speeds and measured colonies’ runniness and defensive response. The schedule for comb repair measurements is described below.

Foraging activity

On the first day of each round of tests, we measured colonies’ foraging activity at three times: morning (08:30-10:00), mid-day (13:00-14:30), and evening (18:00-19:30). At each time, observers counted the total number of bees entering the hive and the number entering with pollen during four 1-minute intervals. The average of these twelve measurements provided a measure of each colony’s daily foraging activity. We used the average total number of returning bees as our measure of foraging activity, as this was strongly correlated with the number of returning pollen foragers (r=0.896, P<0.0001). During each 1-minute interval, two colonies were monitored simultaneously by two different observers, following a randomized order that was determined using a random number generator (http://www.randomizer.org/).

Pollen diversity

We measured each colony's pollen diversity by capturing approximately 30 returning pollen foragers from each colony and counting how many different colors of pollen they carried. We collected pollen foragers on the first day of testing, immediately following the morning measure of foraging activity (10:30–12:15).

We collected pollen foragers from five colonies at a time by screening off each hive's entrance and transferring returning pollen foragers to a screened cage (7 × 6 × 6.5 cm) until 30 foragers had been collected or 45 minutes had passed. Collected foragers were anesthetized with CO_2 and frozen. Later that day, one person (M.K.W.) separated pollen foragers into groups according to pollen color, and a second person (H.R.M.) reviewed these groupings. In the event of disagreement, we combined foragers into a single color group. Once groupings had been finalized, we counted the color groups in each colony and the bees within each group and computed pollen diversity using the Shannon diversity index (Magurran 1988).

Undertaking speed

Our undertaking test challenged colonies to see how quickly they would remove dead workers from the hive. At the end of the first day of testing (19:30-20:45), we placed 100 paint-marked dead bees on the floor at the back of each hive (farthest from the entrance). Dead bees were added to one row of colonies at a time, and the order of the rows was randomized across test repetitions. Between 09:20 and 10:45 on the second day of testing, we counted the number of paint-marked bees that remained in each hive.

Dead bees were obtained by removing approximately 0.3 kg of bees from a colony in a nearby apiary two days before each round of testing, anesthetizing them with CO_2 and freezing them for 24 hours. The following day, we removed the dead bees from the freezer, marked each bee's thorax with paint, placed 100 bees into each of 25 plastic bags, and returned them to the freezer until the time of testing.

Runniness

We video recorded a frame of bees from each colony to measure the speed with which the bees in our colonies ran across their comb—a trait that beekeepers call "runniness" or "nervousness" (Hunt et al. 1998). Runniness was evaluated on the second day of testing, after data for the undertaking test were collected (10:30–12:30). Tests were performed in a barn next to the apiary; the barn doors were open so bees were sheltered from the wind but still exposed to natural light. Colonies were tested one at a time in a randomized order.

To estimate runniness, we slowly removed a single frame from the center of a hive, using no smoke to minimize disturbance to the bees. In the barn, the frame was placed onto a support that held it vertically in front of a video camera. We recorded the bees in a 10x10 cm^2 area in the center of the frame for two minutes. After the first minute, the bees were disturbed by dropping a brick (~ 2 kg) 20 cm onto the top of the frame support.

The video recordings were scored as follows. First, we covered the video monitor with a transparent sheet of Mylar containing 10 randomly located dots, and selected the 10 workers closest to those dots as focal bees. Workers inside cells, engaged in trophallaxis, or carrying pollen were not selected. Each focal bee's path was traced onto the Mylar sheet for 5 seconds before the disturbance and her path length was measured (cm) using a digital-plan measure

(Scale Master Classic; Calculated Industries, Carson City, NV). Using the same procedure, we measured the path lengths of a second set of 10 focal bees for a 5-second interval that began 1 second after the disturbance. We converted path lengths to speeds (cm/sec) and calculated the average speed of the 10 pre- and post-disturbance bees for each colony. Colonies' pre-disturbance and post-disturbance speeds were significantly correlated (r=0.524, P=0.007), so we treated the average pre-and post-disturbance speeds as our measure of each colony's overall level of runniness.

Defensive response

Following the runniness tests (14:00–15:30), we measured each colony's reaction to a potential threat using a standard assay of colony-level defensive behavior (Collins & Kubasek 1982). We exposed each colony to 0.05 ml of 98% isopentyl acetate (Sigma-Aldrich #112674; St. Louis, MO) on a piece of filter paper placed just outside the hive entrance and then we disturbed the colony by dropping a brick (~ 2 kg) 20 cm onto the hive cover three times: at 5 seconds, 10 seconds, and 15 seconds after presenting the isopentyl acetate (e.g. Collins et al. 1994; Gervan et al. 2005).

We used a video camera to record the number of bees outside the hive from 30 seconds before the start of the disturbance until one minute after the brick was dropped for the final time. The number of bees was greatest 20 to 30 seconds after exposure to the pheromone (i.e. 5 to 15 seconds after the final time the brick was dropped onto the hive cover). A colony's defensive response was quantified as the number of bees outside the hive during this time period (average of the 20, 25, and 30-second measurements), minus the number of bees outside the hive during the pre-disturbance period.

Comb repair

We measured a colony's ability to repair damaged areas of comb by cutting out a 5 × 5 cm^2 area of comb from the middle of a central brood frame and then measuring the area that remained unrepaired (to the nearest half cm^2) 24 hours and 3 days later. The same area of comb was excised in each round of testing. We report the average of 1-day and 3-day comb repair measurements, with the exception of the first two rounds of tests, for which only 1-day measurements were made. Because colonies did not have comb at the time of our first round of testing (24 May), we performed comb repair tests on 30 May, 5 June, 13 June, 21 June, 12 July, and 27 July.

4.3.3 Fitness Measures

To measure colonies' productivity and fitness, we monitored their weight, the amount of comb they constructed, and the number of bees they reared from the time of colony founding until the end of the summer.

We recorded colony weights every 2–4 days from 24 May–3 August, and for every 6–10 days thereafter. Weights were measured by placing each hive on a platform scale (Detecto model 4510; precision ±0.05 kg). Each hive's empty weight was subtracted from its current weight to obtain the weight of bees, comb, and food in the hive.

Each colony (swarm) was initially installed in a hive without comb, so colonies were required to construct comb in which to store food and raise young bees. The area of worker and drone comb constructed was measured to the nearest half cm^2 every 4–7 days from 26 May until 24 August, and every other week thereafter until 21 September.

Workers and drones take approximately 12 and 14 days to undergo metamorphosis and emerge from their sealed brood cells as adults (Winston 1987), so we determined the number of individuals that each colony produced by recording the total area of sealed worker and drone brood at approximately bi-weekly intervals (every 11–14 days) throughout the summer (5 June–27 September). Areas of sealed worker and drone brood were estimated using the same methods as comb construction measurements and then multiplied by 4.3 workers/cm^2 and 2.6 drones/cm^2 (Seeley & Visscher 1985) to estimate the number of individuals that were reared.

Throughout the winter, we checked colonies for signs of life every time they were weighed. Colonies still alive at the beginning of March were considered to have survived the winter.

Although we did not measure colony fitness directly by recording swarming events, the traits that we measured—nest size (comb construction), food stores and population size (colony weight), population growth (amount of sealed brood), and survival—are often used as estimates of fitness in studies of honey bees (Seeley 1985) so we assumed that they provided a good estimate of colonies' actual fitness.

4.3.4 Statistical Methods

Statistical analyses were performed using SAS version 9.2 and JMP version 9.2. Data that did not initially meet normality assumptions ($p \leq 0.05$ in Shapiro-Wilk W-test, JMP v.8.0) were transformed using a log or square-root transformation to improve normality. All reported *P*-values are for two-tailed tests. Alpha values for all tests were set at 0.05. For statistical tests that required a single data point for each colony, calculations were performed using each colony's averaged score across all repetitions of the tests. Average values are reported as mean ± standard error (s.e.m.).

Differences among colonies were assessed by fitting a restricted maximum likelihood linear mixed model to the colonies' distribution of scores on each of our behavioral and fitness tests (proc MIXED, SAS v. 9.2) that included 'ColonyID' as a random factor. The likelihood ratio test was then used to compare the fit of these models to models without a 'Colony ID' factor (Martin & Réale 2008). This allowed us to determine whether the colonies' identities accounted for a significant portion of the variance in their scores, and hence whether there were significant differences among them. Test repetition, weight at time of testing (for behavioral tests), and row in the apiary were included in mixed models as fixed factors. Effects of apiary row were never significant and are not reported.

We assessed the consistency of colonies' performances across repeated measurements of the same trait using three different measures: Cronbach's alpha, which measures reliability of repeated tests (Budaev 1997; Burns 2008); the intra-class correlation coefficient, which is a standard measure of reliability (Bell et al. 2009); and Kendall's coefficient of concordance, which tests for consistency of behavioral differences between individuals over time (e.g. Durr & Smith 1997; Briffa et al. 2008). All repeatability tests were performed using SAS v. 9.2. Cronbach's α was computed using Proc CORR, Kendall's coefficient of concordance (W) was calculated using the MAGREE macro (http://support.sas.com/kb/25/006.html), and Intra-class correlation coefficients were computed using the INTRACC macro (http://support.sas.com/kb/25/031.html).

We performed a principal components analysis using JMP v. 8 to test for the presence of behavioral syndromes in the form of correlations between our collective personality variables.

All collective personality variables were included in the analysis, and only the two components with eigenvalues greater than 1 were extracted. PCA components were rotated using a Varimax rotation.

For correlations between individual pairs of variables, we report Pearson's correlation coefficient (proc CORR). Two-sample t-tests (proc TTEST) were used to make comparisons between groups of colonies (e.g. survivors and non-survivors).

Because there was variability in the 25 colonies' average behavior across test repetitions, standardized scores are used instead of raw scores in figures representing the degree of behavioral variation among colonies. Test scores were standardized by subtracting a colony's test score from the mean score across all colonies in that round of testing, and dividing the resulting number by the standard deviation of all 25 colonies' scores for that testing round (Hair et al. 1998). The resulting standardized scores, or "z-scores", have no units and thus can be used to compare scores across behavioral tests that are measured using different variables (Svartberg et al. 2005; Uher et al. 2008).

4.4 Results

4.4.1 Differences among colonies

There was considerable variation among colonies in their responses to our behavioral tests. Colonies differed significantly in their defensive response, comb repair, foraging activity, runniness, and undertaking (LRT: $X^2 \geq 16.2$, df=1, $P < 0.0001$ for each test). Some of these differences were quite dramatic; for example, in our test of defensive response (Figure 4.1A) the average number of bees responding was nearly forty times greater for the most defensive colony

(67.7 ±12.6 bees) compared to the least defensive colony (1.7 ± 2.7 bees). Similarly striking differences were present in all other behavioral tests (Figure 4.1B–Figure 4.1E) with the exception of pollen diversity (LRT: X^2=2.6, *df*=1, *P*=0.11; Figure 4.1F).

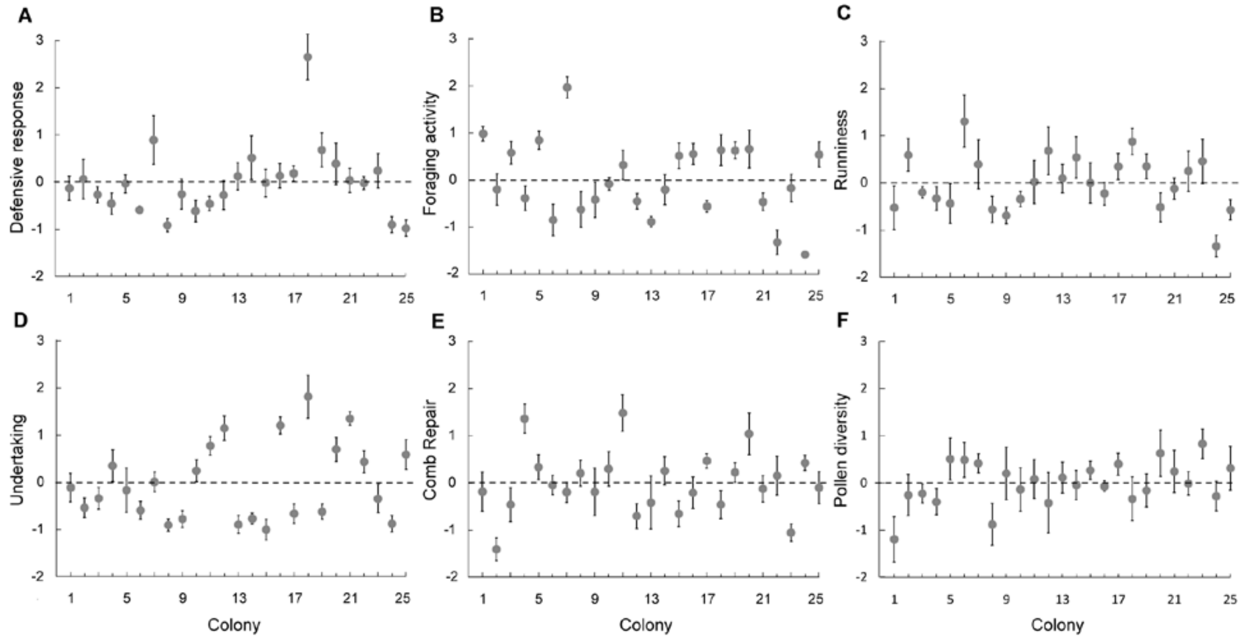

Figure 4.1 – Colonies differed significantly from one another in their average scores on all behavioral tests, with the exception of pollen diversity. The overall average of the 25 colonies' scores on each test is indicated with a dashed line. Behavioral responses are plotted using standardized scores to account for test-to-test variation in average scores across all 25 colonies.

Colonies also differed dramatically in their growth and productivity (Figure 4.2). Colonies differed significantly in all measures of productivity: weight gain over time (LRT: X^2=664.3, *df*=1, *P*<0.0001), amount of comb constructed (LRT: X^2=341.4, *df*=1, *P*<0.0001), and number of individuals reared (LRT: X^2=27.6, *df*=1, *P*<0.0001). Colony sizes were equal at the start of the study, but by the end of the summer the most successful colony was more than seven times heavier and had constructed over four and a half times more comb than the least successful colony (Figure 4.2). Colonies also differed in their ability to survive the winter; a quarter (6 out of 25) were dead by the beginning of February, and only about half (11 out of 25) survived to the beginning of March.

4.4.2 Consistency over time

The behavioral differences among colonies were consistent over time for all traits except pollen diversity (Table 4.1), according to three statistical tests that are commonly used to evaluate behavioral consistency. Because colonies were neither behaviorally consistent nor significantly different from one another in the diversity of pollen they collected, this test was excluded from further analysis.

While the colonies' scores relative to one another on most behavioral tests were consistent across rounds of testing, a colony's absolute test scores varied over time (Categorical 'test repetition' factor: $P<0.0001$ for all behavioral tests). Similarly, each colony's absolute scores on our fitness measures changed over time as the summer progressed and the colony grew (Continuous 'time' factor: $P<0.0001$ for all fitness measures), but the colonies' performances relative to one another remained highly consistent throughout the study (Table 4.1).

4.4.3 Behavioral syndromes

We found evidence that some colony-level behavioral traits were correlated with one another. There was a significant correlation between colonies' foraging activity and defensive response ($r=0.45$, $P=0.024$), and between their runniness and defensive response ($r=0.47$, $P=0.017$). However, we found no correlation between colonies' foraging activity and runniness ($r=-0.006$, $P=0.97$).

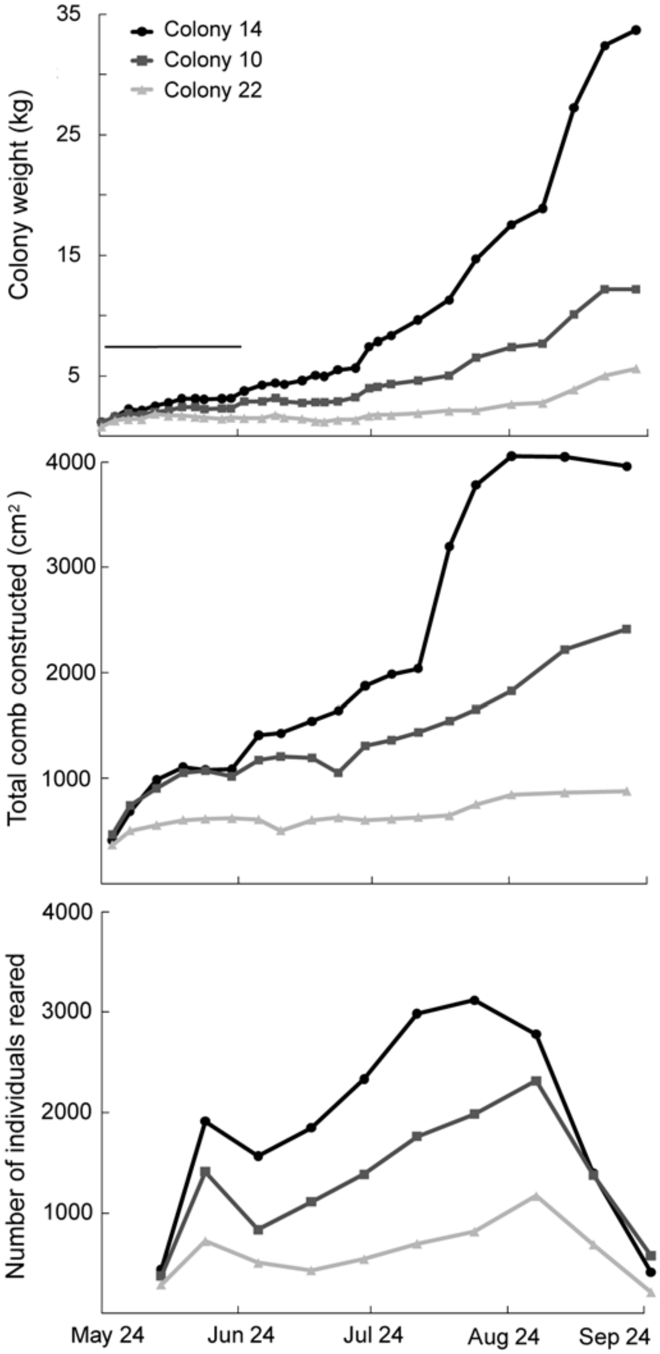

Figure 4.2 – Performance of the largest, smallest, and median colonies over the course of the summer. Significant differences among colonies were observed on all of our performance measures: weight gain, amount of comb constructed, and number of individuals reared. Disparities between the colonies became more pronounced over time. The horizontal line indicates when behavioral tests were conducted.

Table 4.1 – Consistency of behavioral tests and fitness measures.
For behavioral differences among colonies to be characterized as "collective personality" differences, they must be consistent over time. Colonies' relative scores were consistent over time on all behavioral tests and fitness measures except pollen diversity. Consistency was measured using tests of reliability, including Cronbach's α, the Intra-class correlation coefficient, and Kendall's coefficient of concordance. All three tests yielded similar results. Cronbach's α test values above 0.6 are regarded as acceptably reliable (Nunnally 1967).

	Cronbach's alpha (α)	**Reliability (ICC)**	**Kendall's coefficient (W)**
Behavioral tests			
Defensive response	0.81	0.41 $P < 0.001$	0.43 $P < 0.0001$
Comb repair	0.75	0.34 $P < 0.001$	0.44 $P < 0.0001$
Foraging activity	0.88	0.57 $P < 0.001$	0.61 $P < 0.0001$
Runniness	0.60	0.22 $P < 0.001$	0.33 $P = 0.0007$
Undertaking	0.90	0.57 $P < 0.001$	0.67 $P < 0.0001$
Pollen diversity	0.38	0.09 $P=0.049$	0.19 $P=0.25$
Fitness measures			
Weight/growth	0.96	0.80 $P < 0.001$	0.81 $P < 0.0001$
Comb construction	0.96	0.80 $P < 0.001$	0.81 $P < 0.0001$
Brood production	0.91	0.45 $P < 0.001$	0.65 $P < 0.0001$

Principal components analysis, which is often used to test for the presence of behavioral syndromes (Dingemanse et al. 2010), suggests that approximately 66% of the variance in our colonies' five collective personality scores can be explained by a behavioral syndrome consisting of two distinct components. Runniness, defensive response and amount of comb left unrepaired loaded strongly on the first principal component (Table 4.2), suggesting that runny colonies were also more defensive and tended not to repair areas of comb that had been excised from their hive. Foraging, defensive response, and undertaking loaded strongly onto the second component (Table 4.2), suggesting that colonies with high foraging activity also tended to be more defensive and removed dead workers from the colony more rapidly. Defensive response was strongly related to both of these principal components.

Table 4.2 – Component loadings from PCA analysis.
The two principal components extracted from our personality variables account for 66% of the variation in our personality scores (PC1=37.4%, PC2=28.2%). Significant factor loadings are indicated in bold.

	Factor 1	Factor 2
Defensive response	**0.601**	**0.613**
Comb repair	**-0.773**	0.234
Foraging activity	0.042	**0.791**
Runniness	**0.823**	0.120
Undertaking	-0.117	**0.746**

4.4.4 Correlations between collective behavior and fitness

Colony weight is a good proxy of fitness because it measures a colony's population size and food reserves. Colonies' average weights from the time of colony founding until the end of the summer were significantly correlated with two collective behaviors: foraging activity and defensive response (Figure 4.3). Colonies that were more active foragers and colonies with greater defensive responses at the start of the summer went on to gain more weight. Colonies' foraging activity at the start of summer was also correlated to the amount of comb they constructed (r=0.70, P<0.0001) and the total number of individuals they reared (r=0.74, P<0.0001).

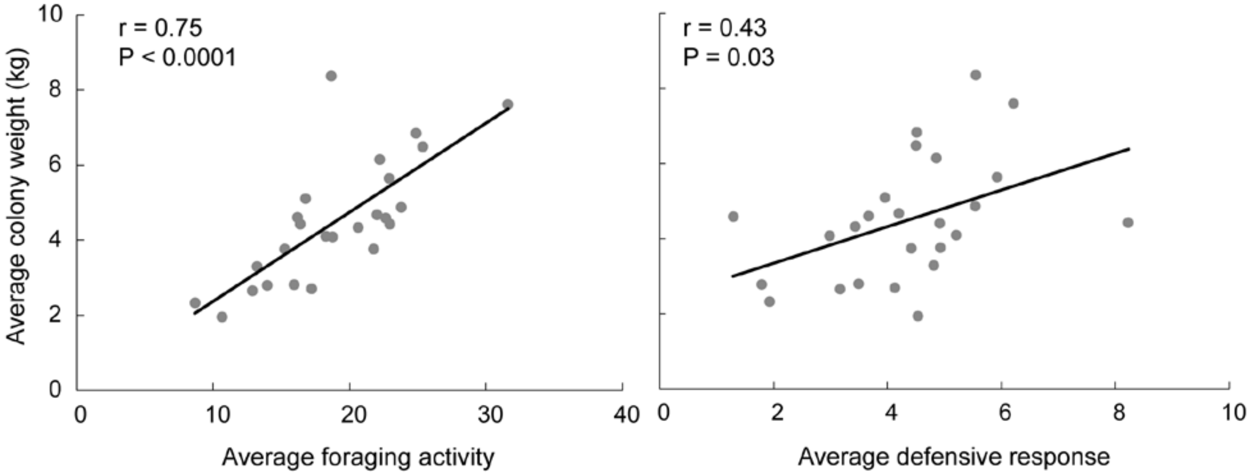

Figure 4.3 – Foraging activity and defensive response were correlated with average weight during the summer months. Colonies' summer weights were measured every 2–6 days from the time of colony founding (24 May) through the end of the summer (21 September). A colony's total weight included the weight of the bees themselves, the weight of the food they collected, and the weight of any comb they constructed.

Another proxy of fitness is a colony's ability to survive the winter. We found that colonies that survived through the beginning of March were significantly more defensive (t_{22}=4.06, P=0.0005) and had higher levels of foraging activity (t_{22} = 2.35, P=0.028) than colonies that died (Figure 4.4).

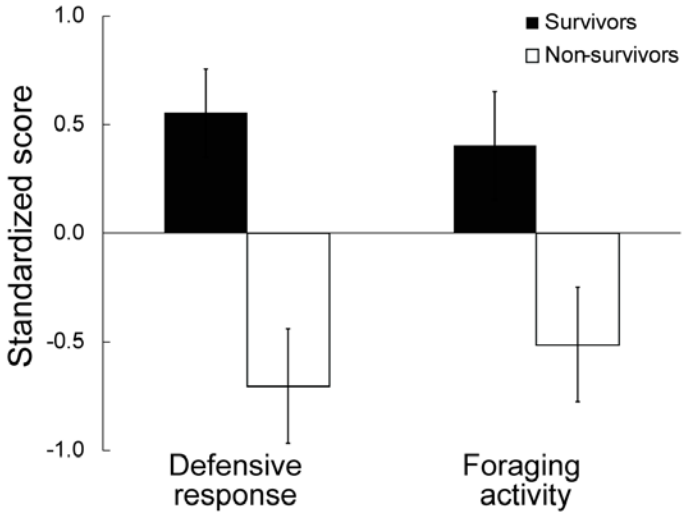

Figure 4.4 – Average levels of defensive response and foraging activity (± s.e.m.) were significantly higher for the group of colonies that survived the winter (n=14) than for the colonies that died earlier in the season (n=11). Defensive response and foraging activity are plotted using standardized scores so that colonies' performances on these two behavioral tests can be directly compared.

4.5 Discussion

4.5.1 Personality differences among colonies

Our colonies differed significantly from one another in their collective behavior. These differences could not have been due to differences in population size, environment, or available resources because colonies started the study with similar numbers of bees installed in empty hives in the same apiary, and their behavior was tested at the beginning of the summer before they had gained significant weight. Furthermore, their behavioral differences were significant even after accounting for colonies' weights at the time of testing. Our queens were unrelated and were naturally mated, so behavioral differences among colonies could have been due to genetic differences or gene by environment interactions (Dingemanse et al. 2010). Alternatively, this behavioral variability could have arisen from very small, random differences among colonies that were enhanced by competition with nearby colonies for the same ecological or social niche (Bergmüller & Taborsky 2010). Exactly how these types of consistent behavioral differences among colonies are generated will be a promising area for future studies.

The behavioral differences we observed are in line with those reported in previous studies of behavioral traits such as defensive response (e.g. Collins et al. 1988) and undertaking (Page et al. 1995b). Interestingly, the lack of consistent differences among colonies in the diversity of pollen they collected is also consistent with prior studies. While the diversity of pollen collected by honey bee colonies is often used to estimate the diversity of pollen sources in the surrounding area (Barth & Da Luz 1998), previous studies report no differences among colonies in the number of pollen sources they visit (Dimou et al. 2006), and no effect of genetic diversity on the diversity of pollen collected (Woyciechowski & Warakomska 1994). In combination with these findings, our results suggest that the variety of pollen sources collected by a honey bee colony's foragers is a reflection of the availability of pollen in the environment, rather than an inherent behavioral trait of the colony.

4.5.2 Consistency over time

We also found that our colonies' relative scores on the behavioral tests were consistent over time for all behavioral traits and fitness measures except pollen diversity. These results agree with previous studies showing that colony-level behavioral traits such as defensive response and flight activity are consistent across multiple rounds of testing (e.g. Giray et al. 2000; Guzmán-Novoa et al. 2003).

While the colonies' rankings relative to one another were consistent, their average scores did vary across test repetitions. For our fitness measures, this variability was largely a result of the growth of the colonies over the course of the summer. Thus, while their absolute scores on our fitness measures (colony weight, amount of comb constructed, and number of bees reared) increased over time, colonies' performances relative to one another remained highly consistent. For our behavioral tests, which were conducted over a much shorter time span, the variations in colonies' average scores across test repetitions were probably due to differences in weather conditions. Meteorological variables including temperature, solar radiation, humidity, and wind velocity are known to affect colony-level behaviors such as defensive response (Southwick & Moritz 1987) and flight activity (Burrill & Dietz 1981). Weather conditions were essentially constant during any given behavioral test (lasting 1–2 hours), but differences in temperature, cloud cover, and wind speed could have altered the bees' behavior across rounds of testing (conducted 6–7 days apart)

4.5.3 Behavioral syndromes

Results from a principal components analysis suggest that all five colony-level behaviors form a behavioral syndrome with two components. The first component (PC1) is composed of defensive response and runniness, which are strongly correlated, and the amount of comb unrepaired. PC1 could reflect differences among colonies in their sensitivity to disturbance, with more sensitive colonies being more likely to respond to threats, more nervous and excitable (“runny”) when beekeepers or predators disturb them, and more wary of—and thus less willing to repair—areas of their hive that have been disrupted. The second component (PC2) is composed of defensive response and foraging activity, which are strongly correlated, and undertaking. PC2 might reflect differences among colonies in their ability to respond flexibly to changes in their environment, with more flexible colonies being better able to mobilize their workforce in response to new challenges (a predator attacking the colony, or a disease killing numerous workers) or opportunities (the presence of available forage). Alternatively, PC2 might represent differences among colonies in their tendency to engage in risky behaviors, or behaviors that involve leaving the hive.

Interestingly, our test of defensive response was strongly related to both principal components. Previous studies have shown that colony defensive response results from at least two distinct behaviors, guarding and stinging (reviewed by Breed et al. 2004). So, it could be that colonies’ guarding behavior was related to one of these principal components (PC2) and their stinging behavior was related to the other (PC1). Colonies with high levels of either guarding or stinging behavior would both have scored high on our test of defensive response, which measured the number of bees that exited the hive in response to a disturbance, even though the underlying mechanisms for their strong response would be different. This possibility could be tested by measuring both guarding and stinging behavior in a group of colonies and determining how those two measures correlated with PC1 and PC2.

A number of the correlations between behavioral traits that we found in our study have also been reported by previous researchers. For instance, the tendency of bees to run on combs is often thought to be associated with defensive response, and Guzmán-Novoa et al. (2002) found positive genetic correlations between runniness and multiple aspects of defensive response, including number of stings and tendency to fly up and hang from combs. The relationship between defensive response and undertaking in our principal components analysis was also reported by Page et al. (1995b), who found a positive correlation between colony-level defensive response and the number of dead bees removed from hives. On the other hand, the positive correlation we observed between defensive response and foraging activity differs from findings from Page et al. (1995b), who found no correlation between colony defensive response and number of returning foragers, and Giray et al. (2000), who reported a negative correlation between colony defensive response and flight activity. However, the trade-off between foraging and defensive response observed by Giray et al. (2000) may have resulted from studying single-cohort colonies in which all workers were the same age, whereas our colonies contained workers of different ages.

4.5.4 Relationship between colony-level personality and fitness

We found that differences in our colonies' collective behaviors (defensive response and foraging activity) were related to differences in their subsequent fitness (productivity and survival). Colony productivity was more strongly correlated with foraging activity, whereas defensive response was a better predictor of winter survival.

The relationship between foraging activity and colony productivity is not surprising as colonies that are more active foragers tend to bring in more resources, facilitating comb building, brood rearing, and food storage, and consequently decreasing the probability of starvation during the following winter. However, the reasons why defensive response is linked to colony survival and productivity are less clear. Larger colonies are generally more defensive (reviewed by Winston 1987), but when colony sizes are approximately equal colony defensiveness and population size are unrelated (e.g. Page et al. 1995b; Giray et al. 2000). What our results suggest is that, among colonies with approximately equal population sizes at the time of testing, the more defensive colonies will subsequently go on to be more productive, grow larger, and have a higher likelihood of winter survival. Interestingly, there is some evidence for a correlation between aggressiveness and colony fitness across races of bees, with Africanized honey bee colonies behaving more aggressively and also tending to outcompete colonies of European honey bees (Fletcher 1991). However, ours is the first study to show a positive correlation between aggressive behavior and subsequent growth and survival among colonies of European honey bees. Exploring the causes for this relationship should be a promising area for future studies.

4.5.5 Conclusions

We found that honey bee colonies differ in their collective behavior across a diverse array of behavioral traits, that these behavioral differences are consistent over time, that these traits form a behavioral syndrome, and that some of these behavioral traits are related to colony-level fitness. Thus, it appears that colonies of honey bees differ in their "collective personalities" in a way that ultimately affects their fitness.

How do these collective personality differences arise? In individual animals, personality differences are likely to result from differences in their physiological, behavioral, or morphological state (Dall et al. 2004; McElreath & Strimling 2006; Stamps 2007). Collective personality differences may also arise from differences in state-dependent variables such as population size or resource availability (e.g. larger honey bee colonies being more defensive; Winston 1987), but differences in the collective personality of a group could also arise from the behavioral responses of individual group members. Previous work with social insects has clearly demonstrated that individual workers consistently differ in their behavior on a wide range of traits, including their responsiveness to sucrose (Scheiner et al. 2004) and their tendency to perform various different tasks such as guarding (Breed et al. 1990), undertaking (Trumbo et al. 1997), and fanning (Weidenmüller 2004). Consistency in worker-level behavior should be favoured by selection if task specialization increases colony efficiency (Bergmüller et al. 2010). However, the relationship between the behavioral types of individual workers and the behavioral phenotype of the colony as a whole is not necessarily straightforward because while some individual-level behaviors will have a direct additive effect on the colony's behavior, others will have a more indirect, non-additive effect on the colony-level response (Robinson & Page 1988). For example, hygienic behavior in honey bee colonies is an additive function of the performance of individual workers (Arathi & Spivak 2001; Paleolog 2009), but colony-level defensive behavior is not equal to the sum of the workers' phenotypes because the presence of just a few highly defensive individuals in a hive can incite less defensive colony members to join in an attack (Guzmán-Novoa et al. 2004; Paleolog 2009).

Some of the collective personality traits we observed here may be related to personality traits that have been studied in individual animals; for example, "defensive response" could be analogous to "aggression", and "foraging activity" could be related to general activity level, exploration, or feeding behavior. Likewise, "runniness" might be comparable to activity level, reactivity, or boldness. Other traits, however (e.g. comb repair, undertaking), may be unique to honey bees or other social insects. The behavioral correlations we observed may also be related to suites of correlated personality traits—or "behavioral syndromes"—that have been observed in individual animals. For instance, the correlation between defensive response and runniness in our colonies may be analogous to correlations between aggressiveness and general activity level that have been observed in other animals (e.g. more aggressive field crickets are more active in general; Kortet & Hedrick 2007). Similarly, the relationship we observed between defensive response and activity could potentially be related to the positive correlation between aggression and boldness that has been observed in a number of animal species, including sticklebacks (Huntingford 1976), great tits (Verbeek et al. 1996), and kangaroo rats (Dochtermann & Jenkins 2007). Previous studies have shown that the presence and structure of behavioral syndromes in individual animals can vary between populations (Bell 2005) and can be altered by environmental conditions such as predation pressure (Bell & Sih 2007). If colony-level behavioral correlations show similar levels of variability, then we might expect them to differ among populations of social insect colonies that live in different habitats. For example, colonies of Africanized honey bees (*A. m. scutellata*) tend to have more predators than European colonies (Winston 1987) so these two races of bees might be expected to differ in the structure of their colony-level behavioral syndromes.

Our finding that some collective personality traits in honey bees were related to colony-level fitness is also in line with prior findings from studies of individual animals. Previous studies of personality and fitness in various animal species have found correlations between personality differences and differences in survival and reproductive success (Dingemanse & Reale 2005), particularly when selection pressures are frequency-dependent (Wilson 1998), vary over time (Dingemanse et al. 2004), or result in trade-offs between survival and reproduction (Boon et al. 2008).

Our study is the first to apply the concept of collective personality to groups of non-human animals and to investigate the relationship between collective personality and group-level fitness. Together with previous findings, our results demonstrate that animal personalities and behavioral syndromes can emerge at the level of the collective, and that between-colony differences in personality can be related to colony-level fitness in social insects. Just as studies of personality in individual organisms have increased our understanding of the inter-individual behavioral differences among animals in a population, future studies of collective personality should improve our understanding of the consistent behavioral differences we observe among groups of social animals. To the extent that collective personality differences result in consistent differences in the competitive abilities of animal groups, they will also affect the inclusive fitness of the individuals in those groups. Thus, we anticipate that future studies documenting collective personality in animal groups will improve our understanding of the role that consistent behavioral differences—both between individuals and between groups—play in the evolution of behavior by natural selection.

4.6 References

Allport, G. W. 1937. *Personality: A psychological interpretation.* New York: Hold, Rinehart & Winston.

Anderson, M., Haar, J., & Gibb, J. 2010. Personality trait inferences about organizations and organizational attraction: An organizational-level analysis based on a multi-cultural sample. *Journal of Management and Organization*, 16, 140.

Arathi, H., & Spivak, M. 2001. Influence of colony genotypic composition on the performance of hygienic behaviour in the honeybee, *Apis mellifera L.*. *Animal Behaviour*, 62, 57-66.

Barth, O., & Da Luz, C. 1998. Melissopalynological data obtained from a mangrove area near to Rio de Janeiro, Brazil. *Journal of Apicultural Research*, 37, 155-163.

Bell, A. M., Hankison, S. J., & Laskowski, K. L. 2009. The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, 77, 771–783.

Bell, A. M., & Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, 10, 828–834.

Bell, A. 2005. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology*, 18, 464-473.

Bergmüller, R., Russell, A. F., Johnstone, R. A., & Bshary, R. 2007. On the further integration of cooperative breeding and cooperation theory. *Behavioural Processes*, 76, 170-181.

Bergmüller, R., Schürch, R., & Hamilton, I. M. 2010. Evolutionary causes and consequences of consistent individual variation in cooperative behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 2751 -2764.

Bergmüller, R., & Taborsky, M. 2010. Animal personality due to social niche specialisation. *Trends in Ecology & Evolution*, 25, 504-511.

Boon, A. K., Réale, D., & Boutin, S. 2008. Personality, habitat use, and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus*. *Oikos*, 117, 1321–1328.

Breed, M., Robinson, G., & Page, R. 1990. Division of labor during honey bee colony defense. *Behavioral Ecology and Sociobiology*, 27, 395-401.

Breed, M. D. 1991. Defensive behavior. In: *The "African" Honey Bee*, (Ed. by M. Spivak, D. J. C. Fletcher, & M. D. Breed), Westview Press.

Breed, M. D., Guzman-Novoa, E., & Hunt, G. J. 2004. Defensive Behavior of Honey Bees: Organization, Genetics, and Comparisons with Other Bees. *Annual Review of Entomology*, 49, 271-298.

Breed, M. D., & Rogers, K. B. 1991. The behavioral genetics of colony defense in honeybees: Genetic variability for guarding behavior. *Behavior Genetics*, 21, 295-303.

Briffa, M., Rundle, S. D., & Fryer, A. 2008. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1305.

Budaev, S. V. 1997. " Personality" in the guppy (*Poecilia reticulata*): A correlational study of exploratory behavior and social tendency. *Journal of Comparative Psychology*, 111, 399–411.

Burns, J. 2008. The Validity of Three Tests of Temperament in Guppies (*Poecilia reticulata*). *Journal of Comparative Psychology*, 122, 344-356.

Burrill, R. M., & Dietz, A. 1981. The response of honey bees to variations in solar radiation and temperature. *Apidologie*, 12, 319–328.

Collins, A., & Kubasek, K. 1982. Field Test of Honey Bee (*Hymenoptera, Apidae*) Colony Defense Behavior. *Annals of the Entomological Society of America*, 75, 383-387.

Collins, A. M., Daly, H., Rinderer, T., Harbo, J. B., & Hoelmer, K. 1994. Correlations Between Morphology and Colony Defense in *Apis mellifera L.*. *Journal of Apicultural Research*, 33, 3-10.

Collins, A. M., Rinderer, T. E., Harbo, J., & Bolten, A. 1982. Colony defense by Africanized and European honey bees. *Science*, 218, 72-74.

Collins, A. M., Rinderer, T. E., & Tucker, K. W. 1988. Colony defence of two honeybee types and their hybrid 1. Naturally mated queens. *Journal of Apicultural Research*, 27, 137-140.

Dall, S. R., Houston, A. I., & McNamara, J. M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters*, 7, 734–739.

Dimou, M., Thrasyvoulou, A., & Tsirakoglou, V. 2006. Efficient use of pollen traps to determine the pollen flora used by honey bees. *Journal of Apicultural Research*, 45, 42-46.

Dingemanse, N. J., Dochtermann, N., & Wright, J. 2010. A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Animal Behaviour*, 79, 439–450.

Dingemanse, N. J., & Reale, D. 2005. Natural selection and animal personality. *Behaviour*, 142, 1159–1184.

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. 2004. Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society B: Biological Sciences*, 271, 847-852.

Dingemanse, N. J., Kazem, A. J., Réale, D., & Wright, J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, 25, 81-89.

Dingemanse, N. J., Wright, J., Kazem, A. J. N., Thomas, D. K., Hickling, R., & Dawnay, N. 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology*, 76, 1128-1138.

Dochtermann, N. A., & Jenkins, S. H. 2007. Behavioural syndromes in Merriam's kangaroo rats (*Dipodomys merriami*): a test of competing hypotheses. *Proceedings of the Royal Society B: Biological Sciences*, 274, 2343-2349.

Durr, R., & Smith, C. 1997. Individual differences and their relation to social structure in domestic cats. *Journal of Comparative Psychology*, 111, 412–418.

Eaves, L. J., Martin, N. G., Heath, A. C., Hewitt, J. K., & Neale, M. C. 1990. Personality and reproductive fitness. *Behavior Genetics*, 20, 563-568.

Fletcher, D. J. 1991. Interdependence of Genetics and Ecology in a Solution to the African Bee Problem. In: *The "African" Honey Bee*, Westview Studies in Insect Biology (Ed. by M. K. Spivak, D. J. Fletcher, & M. D. Breed), Westview Press.

Gervan, N., Winston, M., Higo, H., & Hoover, S. 2005. The effects of honey bee (*Apis mellifera*) queen mandibular pheromone on colony defensive behaviour. *Journal of Apicultural Research*, 44, 175-179.

Giray, T., Guzmán-Novoa, E., Aron, C. W., Zelinsky, B., Fahrbach, S. E., & Robinson, G. E. 2000. Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera* . *Behavioral Ecology*, 11, 44-55.

Gosling, S. 2001. From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, 127, 45-86.

Guzman-Novoa, E., Hunt, G. J., Page Jr, R. E., & Fondrk, M. K. 2002. Genetic correlations among honey bee (*Hymenoptera: Apidae*) behavioral characteristics and wing length. *Annals of the Entomological Society of America*, 95, 402–406.

Guzmán-Novoa, E., Hunt, G. J., Uribe, J. L., Smith, C., & Arechavaleta-Velasco, M. E. 2002. Confirmation of QTL effects and evidence of genetic dominance of honeybee defensive behavior: Results of colony and individual behavioral assays. *Behavior Genetics*, 32, 95–102.

Guzmán-Novoa, E., Hunt, G. J., Uribe-Rubio, J. L., & Prieto-Merlos, D. 2004. Genotypic effects of honey bee (*Apis mellifera*) defensive behavior at the individual and colony levels: the relationship of guarding, pursuing and stinging. *Apidologie*, 35, 15-24.

Guzmán-Novoa, E., Prieto-Merlos, D., Uribe-Rubio, J., & Hunt, G. 2003. Relative reliability of four field assays to test defensive behaviour of honey bees (*Apis mellifera*). *Journal of Apicultural Research*, 42, 42-46.

Hair, J. F., Tatham, R. L., Anderson, R. E., & Black, W. 1998. *Multivariate Data Analysis*. 5th edn. Upper Saddle River, New Jersey: Prentice Hall.

Hellmich, R. L., Kulincevic, J. M., & Rothenbuhler, W. C. 1985. Selection for high and low pollen-hoarding honey bees. *Journal of Heredity*, 76, 155-158.

Hofmann, D. A., & Jones, L. M. 2005. Leadership, collective personality, and performance. *Journal of Applied Psychology*, 90, 509–522.

Hölldobler, B., & Wilson, E. O. 2008. *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies*. 1st edn. W. W. Norton & Company.

Hunt, G. J., Guzman-Novoa, E., Fondrk, M. K., & Page, R. E. 1998. Quantitative trait loci for honey bee stinging behavior and body size. *Genetics*, 148, 1203-1213.

Huntingford, F. A. 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour*, 24, 245–260.

Korb, J., & Heinze, J. 2004. Multilevel selection and social evolution of insect societies. *Die Naturwissenschaften*, 91, 291-304.

Kortet, R., & Hedrick, A. N. N. 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biological Journal of the Linnean Society*, 91, 475–482.

Laidlaw, H. H. 1979. *Contemporary queen rearing.* Hamilton, Illinois: Dadant & Sons.

Laidlaw, H. H., & Page, R. E. 1997. *Queen Rearing and Bee Breeding.* 1st edn. Cheshire, Connecticut: Wicwas Pr.

Magurran, A. 1988. *Ecological diversity and its measurement.* Princeton, NJ: Princeton University Press.

Martin, J. G., & Réale, D. 2008. Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, 75, 309–318.

McElreath, R., & Strimling, P. 2006. How noisy information and individual asymmetries can make 'personality' an adaptation: a simple model. *Animal Behaviour*, 72, 1135–1139.

Mischel, W., & Shoda, Y. 1995. A cognitive-affective system-theory of personality: Reconceptualizing situations, dispositions, dynamics, and invariance in personality structure. *Psychological Review*, 102, 246-268.

Nunnally, J. C. 1967. *Psychometric Theory*. New York: McGraw Hill.

Page, R. E., Waddington, K. D., Hunt, G. J., & Fondrk, M. K. 1995a. Genetic determinants of honey bee foraging behaviour. *Animal Behaviour*, 50, 1617–1625.

Page, R. E., Robinson, G. E., & Nasr, M. E. 1995b. Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera L.*). *Behavioral Ecology and Sociobiology*, 36, 387-396.

Paleolog, J. 2009. Behavioural characteristics of honey bee (*Apis mellifera*) colonies containing mix of workers of divergent behavioural traits. *Animal Science Papers and Reports*, 27, 237-248.

Pesante, D., Rinderer, T. E., & Collins, A. M. 1987. Differential pollen collection by Africanized and European honeybees in Venezuela. *Journal of Apicultural Research*, 26, 4-29.

Robinson, G. E., & Page, R. E. 1988. Genetic determination of guarding and undertaking in honeybee colonies. *Nature*, 333, 356-358.

Rothenbuhler, W. C. 1964. Behaviour genetics of nest cleaning in honey bees 1. Responses of 4 inbred lines to disease-killed brood. *Animal Behaviour*, 12, 578-583.

Scheiner, R., Page, R. E., & Erber, J. 2004. Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie*, 35, 133-142.

Seeley, T. D., & Visscher, P. K. 1985. Survival of honeybees in cold climates: The critical timing of colony growth and reproduction. *Ecological Entomology*, 10, 81-88.

Seeley, T. D. 1978. Life-history strategy of the honey bee, *Apis mellifera*. *Oecologia*, 32, 109-118.

Seeley, T. D. 1985. *Honeybee Ecology: A Study of Adaptation in Social Life.* Princeton, NJ: Princeton University Press.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. 2004a. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology*, 79, 241-277.

Sih, A., Bell, A., & Johnson, J. C. 2004b. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, 19, 372-378.

Smith, B. R., & Blumstein, D. T. 2008. Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, 19, 448-455.

Southwick, E., & Moritz, R. 1987. Effects of meteorological factors on defensive behavior of honey bees. *International Journal of Biometeorology*, 31, 259-265.

Stamps, J. A. 2007. Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters*, 10, 355–363.

Stewart, G. L. 2003. Toward an understanding of the multilevel role of personality in teams. In: *Personality and work: reconsidering the role of personality in organizations*, (Ed. by M. R. Barrick & A. M. Ryan), pp. 183-204. John Wiley and Sons.

Svartberg, K., Tapper, I., Temrin, H., Radesäter, T., & Thorman, S. 2005. Consistency of personality traits in dogs. *Animal Behaviour*, 69, 283–291.

Trumbo, S., Huang, Z., & Robinson, G. 1997. Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. *Behavioral Ecology and Sociobiology*, 41, 151-163.

Uher, J., Asendorpf, J. B., & Call, J. 2008. Personality in the behaviour of Great Apes: Temporal stability, cross-situational consistency and coherence in response. *Animal Behaviour*, 75, 99–112.

Verbeek, M., Boon, A., & Drent, P. 1996. Exploration, aggressive behavior and dominance in pair-wise confrontations of juvenile male great tits. *Behaviour*, 133, 945-963.

Weidenmüller, A. 2004. The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, 15, 120-128.

Weiss, K. 1983. The influence of rearing condition on queen development. In: *Queen rearing: Biological basis and technical instruction*, (Ed. by F. Ruttner), pp. 133. Bucharest: Apimondia Publishing House.

Wilson, D. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London: Series B*, 353, 199-205.

Winston, M. L. 1987. *The Biology of the Honey Bee.* Cambridge, Massachusetts: Harvard University Press.

Woyciechowski, M., & Warakomska, Z. 1994. Worker's genetic diversity has no relation to pollen diversity in a honey bee colony (*Apis mellifera L.). Journal of Ethology*, 12, 163-167.

Chapter 5

Consistent personality differences in house-hunting behavior but not decision speed in swarms of honey bees (*Apis mellifera*) [5]

5.1 Abstract

Speed-accuracy tradeoffs are a common feature of decision-making processes, both in individual animals and in groups of animals working together to reach a single collective decision. Individual organisms display consistent differences in their "impulsivity", and vary in their tendency to make rapid, impulsive choices as opposed to slower, more accurate decisions. However, we do not yet know whether groups of animals might consistently differ in their tendency to prioritize decision speed over accuracy. We challenged 17 swarms of honey bees (*Apis mellifera*) to simultaneously choose a new nest site in each of three locations, and measured their decision speeds in each trial. We found that swarms displayed consistent personality differences in the number of waggle dances and shaking signals they performed and in how actively they scouted for new nest sites. However, swarms did not consistently differ in how long they took to choose a nest site. We suggest that house-hunting *A. mellifera* swarms may place an especially high emphasis on decision accuracy when choosing a nest-site, and that chance events—such as the time when each swarm discovers a sufficiently high-quality nest site—may consequently play a greater role in determining a swarm's decision speed than intrinsic characteristics such as a swarm's "impulsivity".

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5.2 Introduction

For most decision-making tasks in humans and non-human animals, there is a tradeoff between speed and accuracy such that increasing decision speed will tend to decrease accuracy (reviewed by Chittka et al. 2009). Conversely, increasing decision difficulty (Dyer and Chittka 2004), decreasing urgency (Reddi and Carpenter 2000; Rival et al. 2003) or increasing the cost of errors (Chittka et al. 2003) will tend to reduce decision speed while increasing accuracy.

These same tradeoffs also apply to groups of organisms working together to make collective decisions. Some of the best examples of collective decision-making behavior are exhibited by social insects, in which the hundreds or thousands of individuals within each colony effectively behave as a single “superorganism” (Hölldobler and Wilson 2008). These insect societies possess a “collective intelligence” (Franks 1989) with decision-making processes and abilities that are strikingly similar to those of vertebrate brains (Passino et al. 2008; Marshall and Franks 2009; Seeley 2010). Colonies of insects are subject to the same tradeoff between speed and accuracy as individual animals (Passino and Seeley 2006; Marshall et al. 2006; Sumpter and Pratt 2009), and in some cases will flexibly alter the degree to which they emphasize decision speed versus accuracy based on factors such as environmental conditions (e.g. Franks et al. 2003), decision urgency (Dornhaus et al. 2004), and colony size (Franks et al. 2006).

At the level of the individual, research on personality—also known as “behavioral syndromes”, or “temperaments”—has demonstrated that many organisms display differences in their behavior that are consistent over time and across situations (reviewed by Gosling 2001; Sih et al. 2004; Réale et al. 2007). In addition to varying in personality traits like aggression and boldness, both humans (Phillips and Rabbitt 1995) and non-human animals (Burns and Rodd

2008) are often consistent in their tendency to favor speed as opposed to accuracy when making decisions, with “cautious” individuals consistently opting for slower, more accurate decisions and more “impulsive” individuals making faster but less accurate choices. Consistent differences in decision-making strategies have also been demonstrated in individual social insects, including bumble bees (Chittka et al. 2003; Burns 2005) and honey bees (Burns and Dyer 2008).

At the group level, colonies of honey bees (*Apis mellifera*) differ from one another in personality traits such as aggression and foraging activity (Wray et al. 2011). However, previous studies have not investigated whether colonies also display consistent differences in their decision-making strategies, such as their tendency to emphasize decision speed as opposed to accuracy.

One of the most difficult decisions a colony of insects ever faces is choosing a new nest site (Seeley and Buhrman 1999; Franks et al. 2002). Nevertheless, many social insects are able to select the highest quality site with impressively high levels of accuracy (Seeley and Buhrman 2001; Franks et al. 2006). Research with rock ants (*Temnothorax albipennis*) has demonstrated that colonies can select the best nest site even when it is over 9 times farther away than a poorer quality site (Franks et al. 2008). In honey bees *(A. mellifera)*, researchers have shown that most swarms are able to correctly choose the best of five potential nest sites (Seeley and Buhrman 2001), and models of the house-hunting process predict that swarms will generally be 90-95% accurate at selecting the best available site (Passino and Seeley 2006).

Decision speed should also be important to colonies, especially in species that leave their former nest before choosing a new home (e.g. *A. mellifera*) and are therefore exposed to the elements and to potential predators during the house-hunting process. However, previous accounts of the decision-making process suggest that colonies can vary widely in the time it takes to choose a nest site. In ants, decision speed varies with the size of the colony (Franks et al. 2006), the urgency of their decision (Franks et al. 2003; Dornhaus et al. 2004; Healey and Pratt 2008), the previous experiences of colony members (Langridge et al. 2008), and the colony's familiarity with the surrounding environment (Franks et al. 2007; Stroeymeyt et al. 2010). In Western honey bees (*A. mellifera*), most swarms take 2-3 days to choose a new nest site but there is a great deal of variability in decision speed; some swarms will make their decision in a single afternoon (Seeley and Visscher 2004) whereas other swarms will take as long as 1-2 weeks to decide (Lindauer 1955, 1961). In one extreme case, a swarm that had been house-hunting for two weeks gave up its search and began building comb on the tree branch where it was clustered—a location in which it had no chance of surviving the winter (ibid). Our study extends the investigation of consistent differences in decision-making strategies from the individual to the colony level, by assessing whether swarms of Western honey bees (*A. mellifera*) vary in the speed with which they select a new nest site. We directly compared the decision-making behavior of 17 swarms of bees by testing them simultaneously, under the same environmental conditions, and measuring the amount of time they took to choose a nest site. This decision-making process was repeated in three different locations to determine whether the differences in swarms' behaviors and decision speeds were consistent over time.

5.3 Materials & Methods

5.3.1 Swarm preparation

On 25 May 2010, we created 18 artificial swarms of Western honey bees *(Apis mellifera*). Each swarm was prepared by transferring 1 kg of workers (~7500 bees; Mitchell 1970) and their mother queen from a source colony to a screened wooden swarm cage (15 x 25 x 35 cm) using standard methods (Seeley and Visscher 1985). Source colonies were located in two nearby apiaries. Queens were genetically unrelated and had been naturally mated. Caged bees were fed 1:1 sucrose solution ad libitum for three days until they began producing wax scales, indicating that they were in a physiological state similar to that of bees in a natural swarm.

5.3.2 Decision-making tests

Study dates and locations

Decision-making tests were performed in Ithaca, New York on 28-29 May (Trial 1), 1-2 June (Trial 2), and 7-10 June (Trial 3). To present bees with a new problem in each round of testing, the three trials were conducted in different locations, all of which were surrounded by large tracts of forest. Moving the swarms to a new location in each trial prevented bees from simply returning to the nest sites they had discovered in earlier testing trials, which would have eliminated the independence of the results in the three trials. Trial 1 was located in an open field (42°27’22”N, 76°26’49”W) 0.5 km away from the Liddell Field Station of Cornell University (42°27’39”N, 76°26’42”W). Trial 2 was located in a field (42°29’48”N, 76°25’47”W) 4.7 km away from the Trial 1 site, and Trial 3 was located in a hilltop field (42°27’49”N, 76°22’21”W)

6 km away from the sites for Trials 1 and 2. After the final trial, swarms were installed in hives in an apiary at the Liddell Field Station.

Swarm setup

Shortly before each round of testing began, wooden swarm stands were set up ≥30 m apart from one another in the testing location. In a previous study, positioning swarms 20 m apart was sufficient to prevent bees from different swarms from drifting between the two stands (Rangel et al. 2010). Each swarm stand consisted of a 40 x 40 cm board mounted on a 130 cm wooden stake. A strip of wood was nailed to the front of the board to support the queen cage. Each swarm stand was anchored in place by attaching it to a steel rod pounded securely into the ground. The top of each stand was painted a distinctive color for easy identification by the bees, and a colorful empty hive was placed next to the swarm stand. Hive entrances were closed to prevent bees from entering. A random number generator (http://www.randomizer.org/) was used to assign swarms to stands, and a removable label was attached to each stand to indicate the number of the swarm assigned to it.

Swarms were set up in the afternoon on the first day of testing (13:00-14:30, Trials 1 and 3; 17:30-18:50, Trial 2) by attaching each swarm’s caged queen to the swarm stand and shaking the worker bees onto a hive cover on the ground in front of the swarm stand. Bees crawled up the stand and formed a cluster around the queen cage within 30-60 minutes. To ensure that the swarms remained well-fed, and thus all dancing bees and bees landing on the swarm were nest site scouts rather than nectar foragers, an inverted quart-sized jar of sucrose solution was attached to each swarm stand. Bees could obtain sugar solution through a series of small holes in the jar's cap. The level of sugar solution in these jars was monitored, and jars were refilled whenever necessary.

Measurements during decision-making

After swarms were set up, three observers monitored them between the hours of 06:45–19:00 and counted each swarm's rate of waggle dances (observer 1; MKW), shaking and piping signals (observer 2; TDS), and scouting activity (observer 3; YC). Swarm stands were set up in a circular pattern so that observers could systematically collect data by moving around the circle. Each observer collected data from every swarm 3 times per hour.

In *A. mellifera* swarms, waggle dances serve to inform scout bees on the swarm about the distance and direction to a potential nest site; these dances are performed by scouts who have visited the site and judged it to be of suitable quality (Lindauer 1955, 1961). The rate of waggle dances on our swarms was measured by scanning each swarm's surface for 30 s and counting the number of bees simultaneously performing waggle dances. If different counts were obtained throughout this 30-s interval, we recorded the maximum observed number of dances.

The "shaking signal", also called the "vibration signal" (Schneider and Lewis 2004), stimulates bees to increase their activity levels (Nieh 1998; Seeley et al. 1998). These signals are typically observed throughout the nest-site selection process, and appear to be performed by a specialized group of workers (Visscher et al. 1999). The rate of shaking signals was measured by scanning a swarm's surface for 15 s and counting the number of bees performing shaking signals. The surface of the swarm was scanned only once during each 15-s period to avoid double counting the same bees.

During the house-hunting process in A. *mellifera*, the vast majority of bees in the swarm are inactive; only about 5% of the workers act as nest-site scouts, searching for and visiting potential nest sites (Seeley et al. 1979). We estimated the level of scouting activity on a swarm by counting how many flying bees landed on a swarm during two 15-s counts and taking the average of these counts.

Piping signals and swarm takedown

Any swarm that took off to fly to its chosen nest site would ultimately return to the swarm stand to cluster around its caged queen. However, if multiple swarms were to take off at once, bees from different swarms might mix in the air. So, we prevented swarms from taking off by monitoring the level of piping signals and taking each swarm down once it had reached a threshold of 5 or more pipes within a 15 s interval. Piping signals in *A. mellifera* swarms are produced by scout bees who have sensed a quorum of scouts at the chosen nest site (Visscher and Seeley 2007); these signals stimulate the quiescent bees in the swarm cluster to warm up their flight muscles in preparation for takeoff (Seeley and Tautz 2001). Piping typically begins about an hour before takeoff and crescendos immediately before departure (Tautz & Seeley 2001), so it reliably indicates when a swarm has chosen a nest site and is preparing to take off. We measured the rate of piping signals by putting one ear close to the swarm and counting the number of piping signals heard during a single 15-s interval. Once the threshold level of piping (5+ pipes/15 s) was reached, the swarm was shaken from its swarm stand into the nearby hive and the hive entrance was opened to allow bees to freely travel in and out. Each hive contained two frames of honey and three empty frames, and provided a temporary home for the bees throughout the remainder of the trial.

Each swarm's decision speed was calculated as the number of hours between when it was set up and when it was taken down, minus any time during which swarms were unable to fly to a new nest site—namely, during the evening and early morning (18:00-8:00), or during rainy weather (9 June). The swarm's decision rank was the speed of its decision relative to the other swarms, with the fastest swarm receiving rank 1. Swarms that were taken down within ½ hour of each other received equal decision ranks.

Once all swarms had been taken down, they were left in their hives overnight and then transferred back to their swarm cages early in the morning before the bees were active. After each decision-making trial, swarms were weighed again, and if necessary a small number of additional bees from the original source colony was added to any underweight swarms. The source colonies did not yet have new queens, so these additional workers were sisters to those in the swarm. Swarm 4 had been created from a small source colony that would have been endangered by removing additional workers from the colony, so this swarm was dropped from the study after the first two trials of testing.

Statistical methods

All reported *P*-values are for two-tailed tests, with $\alpha=0.05$. Average values are reported as mean ± standard error (s.e.m.). Data that did not initially meet normality assumptions ($p \leq 0.05$ in Shapiro-Wilk W-test, JMP v.8.0) were transformed using a log or square-root transformation to improve normality. All statistical analyses were performed using SAS version 9.2. Correlations between two variables were calculated using Pearson's correlation coefficient (proc CORR). We assessed the consistency of swarms' performances (decision time, dances, etc.) across the three test trials using Kendall's coefficient of concordance (W), which tests for consistency of behavioral differences between individuals over time (Durr and Smith 1997; Briffa et al. 2008) and was computed using the MAGREE macro (*http://support.sas.com/kb/25/006.html*).

To test for differences among swarms, we fitted a restricted maximum likelihood linear mixed model (proc MIXED, SAS v. 9.2) to swarms' average scores during each hour of testing (rate of dancing, shaking, and scouting activity) or to swarms' overall scores on each test trial (decision time and decision rank). We used the likelihood ratio test to compare the fit of models with and without a random 'Colony ID' factor (Martin and Réale 2008) to determine whether swarms' identities accounted for a significant portion of the variance in their scores, and hence whether there were significant differences among them. We included as fixed factors the number of hours until takedown, maximum hourly temperature (°C) and hourly solar radiation ($W \cdot h/m^2$), none of which were significantly correlated ($r \leq 0.3$, $P \geq 0.07$). Additional weather variables (hour of day, hours after setup, minimum temperature, average and maximum wind speed, and soil temperature) were strongly correlated with other fixed factors and had unacceptably high variance inflation factors (VIF>10), so they were excluded from the analysis to avoid multicollinearity.

5.4 Results

5.4.1 Decision-making behavior

Decision speed

Across the three trials, there was a great deal of variation in the swarms' decision speeds (Table 5.1). The fastest decision time in any single trial was 2.05 hours (Swarm 13, Trial 2),

Table 5.1– Decision times for the 17 swarms that completed all three testing trials. Average values are mean ±SE.

Swarm	Trial 1	Trial 2	Trial 3	Average
1	4.90	9.40	8.92	7.7 ± 1.4
2	4.72	3.35	7.28	5.1 ± 1.2
3	5.95	4.72	6.20	5.6 ± 0.5
5	7.32	6.47	15.28	9.7 ± 2.8
6	5.92	3.18	7.35	5.5 ± 1.2
7	6.73	3.17	16.05	8.7 ± 3.8
8	3.43	2.78	9.05	5.1 ± 2.0
9	5.82	4.73	16.25	8.9 ± 3.7
10	6.87	7.75	7.35	7.3 ± 0.3
11	4.92	3.27	13.27	7.2 ± 3.1
12	5.33	4.52	14.58	8.1 ± 3.2
13	5.97	2.05	14.42	7.5 ± 3.6
14	4.20	9.00	7.30	6.8 ± 1.4
15	6.02	4.78	8.10	6.3 ± 1.0
16	4.50	3.27	6.30	4.7 ± 0.9
17	6.45	3.97	7.57	6.0 ± 1.1
18	6.82	8.03	16.12	10.3 ± 2.9

whereas the slowest was 16.25 hours (Swarm 9, Trial 3) (Table 5.1). Within each trial, there were large differences among swarms in the time they took to reach a decision. The difference in decision times between the fastest and slowest swarm ranged from 3.88 hours in Trial 1 to 10.05 hours in Trial 3. When swarms' decision times were averaged across all three trials, they ranged from 4.7 ± 0.9 hours (Swarm 16) to 10.3 ± 2.9 hours (Swarm 18). However, swarms' decision times and ranks were not consistent across the three trials (Kendall's coefficient: Decision Time: $F_{15,31}$=1.68, P=0.109; Decision Rank: $F_{15,31}$=1.85, P=0.073), and swarms did not differ from one another in their decision times or ranks (Table 5.2).

Table 5.2 – Differences among swarms, as determined by the likelihood ratio test (LRT). Significant *P*-values are in bold.

	LRT (X^2)	***df***	***P***
Decision time (hours)	0.36	1	0.5499
Decision rank (1-18)	2.17	1	0.1406
Waggle dances/30 sec	10.89	1	**0.001**
Returning bees/15 sec	41.64	1	**<.0001**
Shaking signals/15 sec	13.4	1	**0.0003**

Waggle dances, scouting activity and shaking signals

The average rate of waggle dances performed during a given testing trial ranged from 0.3–4.0 dances/30 s. Swarms differed from one another in their dancing rates (Table 5.2; Figure 5.1a), and the rate of dancing on each swarm was the most consistent decision-making variable across the three testing trials (Kendall's coefficient: $F_{15,31}$=2.21, P=0.030). For example, swarm 14 consistently stood out as the swarm with the most dances in each of the three trials (Table 5.3).

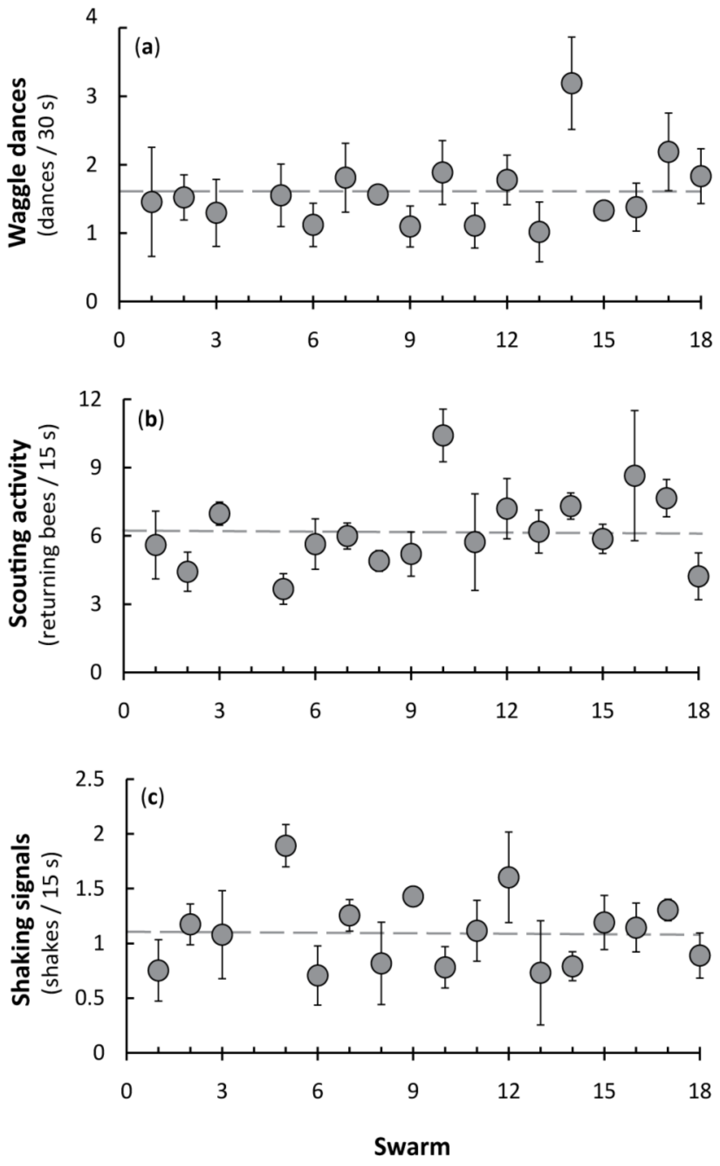

Figure 5.1 – Average rate of waggle dances (a), scouting activity (b), and shaking signals (c) per unit time for each swarm across the three decision-making trials. Circles represent each swarm's mean score across the three trials, and error bars represent ± SE. Dashed lines indicate the average of all 17 swarms' behavioral scores. Swarm 4 was excluded from the study and is not included above (see methods section).

Within each trial, there was also variation in level of scouting activity (1.7– 13.6 returning bees/15 sec.) and rate of shaking signals (0–2.3 shakes/15 sec.) (Table 5.3). Across all three trials, swarms differed significantly from one another in their levels of scouting activity and their rates of shaking signals (Table 5.2; Figure 5.1b-c). While the swarms' levels of scouting activity were consistent across testing trials (Kendall's coefficient: $F_{15,31}$=2.03, *P*=0.047), their rates of shaking signals were not (Kendall's coefficient: $F_{15,31}$=1.80, *P*=0.081).

Table 5.3 – Swarms' minimum, maximum, and average scores on decision-making variables. Superscript numbers indicate which swarm had the minimum and maximum score in each individual trial and across trials 1-3. The average score for each trial represents the mean performance of all of the swarms in that trial. Trials with the same superscript letter (a, b) did not differ significantly from one another (Tukey test, *P*>0.05).

	Trial 1	**Trial 2**	**Trial 3**	**Trials 1-3 Average**
Decision time (Hours)				
Minimum	3.4^{8}	2.1^{13}	6.2^{3}	4.7 ± 0.9^{16}
Maximum	7.3^{5}	9.4^{1}	16.3^{9}	10.3 ± 2.9^{18}
Average score	5.6 ± 0.3 *a*	5.0 ± 0.6 *a*	10.7 ± 1.0 *b*	
# Waggle dances / 30 sec				
Minimum	1.5^{16}	0.3^{13}	0.3^{1}	1.0 ± 0.4^{13}
Maximum	4.0^{14}	3.7^{14}	1.9^{14}	3.2 ± 0.7^{14}
Average score	2.2 ± 0.2 *a*	1.6 ± 0.2 *b*	1.0 ± 0.1 *b*	
# Returning bees / 15 sec				
Minimum	2.9^{2}	1.7^{11}	2.2^{18}	3.7 ± 0.7^{5}
Maximum	13.6^{16}	8.2^{10}	10.8^{10}	10.4 ± 1.2^{10}
Average score	7.1 ± 0.6 *a*	5.3 ± 0.4 *a*	6.2 ± 0.6 *a*	
# Shaking signals / 15 sec				
Minimum	0.7^{14}	0.0^{13}	0.45^{10}	0.71 ± 0.3^{6}
Maximum	2.3^{5}	1.9^{12}	1.7^{5}	1.89 ± 0.2^{5}
Average score	1.3 ± 0.1 *a*	0.84 ± 0.1 *b*	1.1 ± 0.1 *a,b*	

In Trial 3, swarms that decided faster than others had higher levels of scouting activity (r=-0.69, *P*=0.002), but this trend was not present in Trials 1 and 2. Swarms' average rates of dancing and shaking were not related to their decision times or decision ranks in any of the three testing trials.

Differences among trials

Swarms' decision speeds were significantly slower in Trial 3 than they had been in Trials 1 and 2 (Tukey test, *P*<0.0001; Table 5.3; Figure 5.2a), whereas rates of dancing and shaking were higher in Trial 1 than in Trials 2 and 3 (Table 5.3).

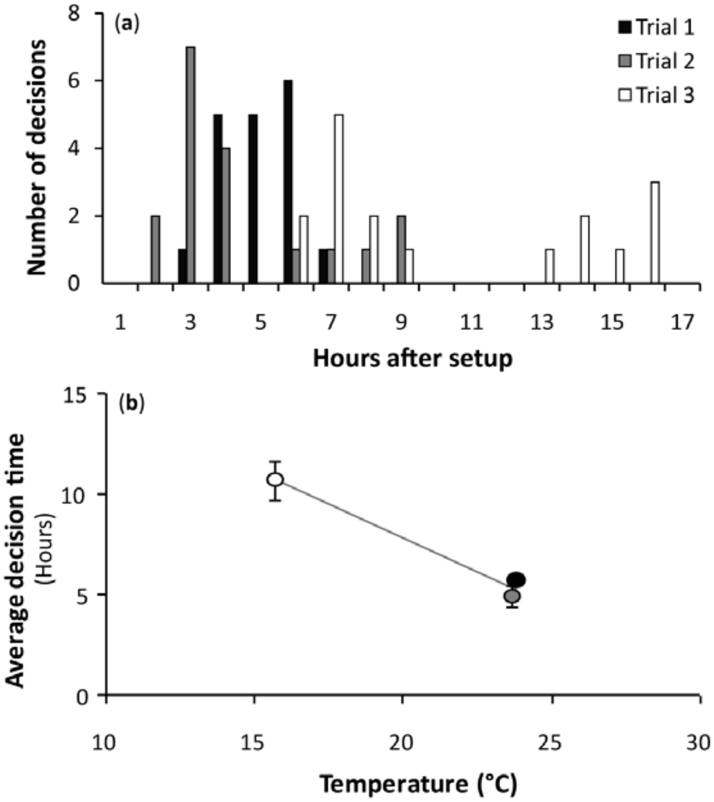

Figure 5.2 – (a) Histogram of swarms' decision times in the three testing trials. This included the time it took swarms to locate potential nest sites, reach a consensus decision, and prepare for takeoff. Swarms in Trial 3 took longer to make their decisions, on average, than swarms in Trials 1 and 2. (b) Relationship between average daytime temperatures and swarms' mean decision times during testing trials 1-3.

Weather conditions also differed across the three trials (Table 5.4). The daytime maximum and minimum hourly temperatures were significantly lower in Trial 3 than in Trials 1 and 2 (Tukey test, $P<0.0001$), and the daytime maximum and average hourly wind speeds were higher in Trial 3 than in Trial 1 (both $P<0.0001$) or Trial 2 ($P=0.011$, $P=0.003$). Across the three testing trials, warmer average daytime temperatures were associated with shorter decision times (Pearson's correlation coefficient: $r=-0.62$, $P=0.0008$; Figure 5.2b).

Table 5.4 – Weather conditions from 6 am – 8 pm during testing trials 1-3. Values represent mean ± SE. Trials with the same superscript letter (a, b) are not significantly different from one another (Tukey test; $P>0.05$). There was no precipitation during trials 1 and 2.

	Trial 1	**Trial 2**	**Trial 3**
Mean maximum hourly temperature (°C)	23.8 ± 0.8^{a}	23.7 ± 1.3^{a}	15.7 ± 0.5^{b}
Highest hourly temperature (°C)	27	28	21
Mean minimum hourly temperature (°C)	22.6 ± 0.8^{a}	22.3 ± 1.5^{a}	14.9 ± 0.6^{b}
Lowest hourly temperature (°C)	18	11	6
Mean precipitation (cm)	—	—	0.03 ± 0.02
Mean maximum wind speed (Kph)	13.6 ± 1.0^{a}	19.4 ± 1.8^{a}	25.3 ± 1.1^{b}
Mean average wind speed (Kph)	7.3 ± 0.5^{a}	10.7 ± 0.8^{a}	14.8 ± 0.7^{b}
Mean solar radiation ($W \cdot h/m^2$)	420 ± 76^{a}	505 ± 70^{a}	367 ± 46^{a}

5.5 Discussion

5.5.1 Decision time and signaling

The swarms in our study took 1-3 days to locate and select a nest site, which is similar to the range of decision times observed in previous studies (Lindauer 1955, 1961; Camazine et al. 1999; Seeley and Buhrman 1999). The longest decision time in our study (16.25 hours) also precisely matched the longest decision time (16 h) predicted by models of honey bee nest site selection (Passino et al. 2008).

Swarms consistently differed from one another in the rate at which they performed waggle dances during the three testing trials. However, waggle dance rate was unrelated to the time it took swarms to reach a decision. This fits with data from a previous study in which the number of dances performed per minute was not correlated with swarms' decision times (Seeley and Visscher 2004). Why would swarms with more dances not select a nest site more rapidly? If all of the dances on a swarm were for the same site, this would indeed tend to lead to a rapid decision. However, if these dances were all for different locations, the swarm would have more potential options to consider, making their decision more difficult and increasing the amount of time it should take them to reach a decision. Given the complex relationship between waggle dance rate and decision time, it is not surprising that we saw no direct relationship between these variables.

Swarms also differed in the rate at which they performed shaking, or "vibration", signals but here, too, the signal production rate was unrelated to the time it took swarms to reach a decision. This is in line with previous results showing that removing shaking bees from a swarm slows down the takeoff process but does not increase the time it takes swarms to choose a nest site and begin preparing for takeoff (Donahoe et al. 2003). Why might this be? Apparently, swarm bees that receive shaking signals tend to increase their activity levels and are more likely to fly from the swarm, but they are no more likely to follow dances than bees that are not shaken (Lewis and Schneider 2000). So, shaking signals might increase the chance that the recipient bee will leave the swarm to actively search for a new, undiscovered site. This increase in exploration for new sites could speed up the decision-making process, if it enabled the swarm to discover a high-quality site more rapidly. But if the swarm had already discovered a good site, it could slow down the process of quorum formation by reducing the number of scout bees on the swarm who could be recruited to already-existing sites, and thereby reducing the buildup of bees at the highest-quality site. Our results demonstrate that although the rate of shaking signals does differ consistently among swarms, increased shaking rates do not necessarily lead to faster decisions.

The swarms in our study also consistently differed from one another in their level of scouting activity. This suggests that our swarms differed either in the percentage of bees on each swarm that were acting as nest site scouts or in each scout's average level of flight activity. In the third trial, these differences were related to decision time, with swarms that had higher levels of scouting activity reaching a decision more rapidly. A similar relationship between scouting activity and decision speed has been observed in ant colonies; larger colonies, which deploy more scouts, tend to locate the best sites more rapidly and complete their moves in less time (Franks et al. 2006). Our study suggests that consistent differences in scouting activity can also exist among honey bee colonies of the same size.

5.5.2 The role of chance in the nest-site selection process

While our swarms displayed consistent personality-like differences in their decision-making behavior during testing trials, their decision speeds were inconsistent and varied considerably across testing trials. This lack of consistency suggests that differences in swarms' decision speeds in any given testing trial may reflect chance differences in the time at which each swarm locates a sufficiently high-quality site, rather than differences that are intrinsic to the swarms. Depending on the number of high-quality sites a swarm locates, the amount of time it takes to locate the first high-quality site, and the similarity of the sites it locates—all of which are likely due to chance—the nest site selection process can differ dramatically in its complexity (Seeley and Buhrman 2001; Passino and Seeley 2006; Passino et al. 2008). For instance, if a swarm happens to discover a single high-quality site very early in the process, it will reach a decision quickly. But if it happens to find multiple sites of similar quality, or if it experiences a long delay before finding a high-quality site, then the decision-making process will tend to be much slower (Lindauer 1955, 1961; Passino et al. 2008; reviewed in Seeley 2010). It is possible that swarms may have some ability to influence the number of candidate nest sites they locate and the speed with which they are found, if they differ in characteristics such as their level of scouting activity or the quality threshold their scouts utilize. However, the *quality* of the sites that a swarm discovers will be almost entirely due to chance, which may explain the large variability we observed in swarms' overall decision times and ranks.

Chance is likely to play a larger role in the nest-site selection process when decision accuracy is more critical, because as swarms place a greater emphasis on decision accuracy as opposed to speed, their threshold for the minimum-quality site they are willing to accept will increase, and the number of acceptable sites (i.e. sites above their minimum threshold quality) will decrease. As these sites become rarer, the amount of time it takes a swarm to successfully locate one or more of these acceptable sites by searching randomly in the environment will become more variable, and luck will therefore play a greater role in determining which swarms in a given group locate sites most rapidly and consequently make the fastest decisions. The fact that we observed such high levels of variability across decision-making trials in our study suggests that our swarms may have been emphasizing accuracy over speed in their decision-making process.

Previous research suggests that accuracy is a critical element of the nest-site selection process in Western honey bees (*A. mellifera*). Swarms generally display high levels of accuracy when choosing among an array of potential nest sites (Seeley and Buhrman 2001), and key features of their decision-making system, such as quorum size and dance decay rate, are set at values that generally lead to very high accuracy (>90-95%) but not necessarily high speed (Seeley 2003; Seeley and Visscher 2004; Passino and Seeley 2006). Moreover, from a fitness perspective, it seems likely that natural selection should favor high accuracy, rather than high speed, in *A. mellifera* swarms. For open-nesting bee species such as red dwarf honey bees (*Apis florea*), the number of candidate nest sites is virtually unlimited and there is very little variance in the quality of available sites (Oldroyd et al. 2008; Makinson et al. 2011). In contrast, Western honey bees (*A. mellifera*) nest in cavities, which are relatively rare and highly variable in quality. For a colony, of *A. mellifera*, the cavity it occupies will influence its maximum size, its ability to defend itself against predators, the cleanliness and temperature of its nest, and the amount of energy that is required for nest-site construction (Seeley and Morse 1978). Indeed, choosing a suitable nest site can literally make the difference between life and death, as a colony needs a spacious and protective nest cavity in order to survive the winter (Seeley 2010). Furthermore, each colony has only one chance to make a correct decision; unlike *A. florea* (Oldroyd et al. 2008) and many species of ants (Dornhaus et al. 2004) which will move to a new nest site if their initial choice proves unsatisfactory, *A. mellifera* colonies that choose poorly are generally stuck with this decision. The comb, food, and brood in these large colonies are energetically costly to produce and cannot be moved to a new location, so the expense of moving to a new nest site virtually always outweighs the benefit (Seeley 1985).

In general, increasing the costliness of errors increases the emphasis that decision-makers place on accuracy as opposed to speed, a relationship that has been demonstrated in individual honey bee and bumble bee foragers (Avarguès-Weber et al. 2010; Chittka et al. 2003). Given the severe fitness consequences of an incorrect decision in the context of nest-site selection, it is perhaps unsurprising that swarms of Western honey bees (*A. mellifera*) seem to place a high emphasis on accuracy as opposed to speed in their decision-making process. In contrast, prior studies have demonstrated that swarms of red dwarf honey bees (*A. florea*)—for whom the cost of choosing a poor-quality nest site is relatively low—tend to make faster, less accurate decisions than swarms of Western honey bees, and often simply choose a general direction in which to travel rather than a specific nest site to occupy (Oldroyd et al. 2008; Makinson et al. 2011).

Interestingly, in contrast to rock ant colonies that make faster, less accurate decisions when environmental conditions are harsh (Franks et al. 2003; Scholes and Suarez 2009), our honey bee swarms took significantly longer to make decisions in the third trial of the study, when weather conditions were harshest. Could this indicate that the tradeoff between speed and accuracy in honey bee swarms is insensitive to external factors like weather conditions? Perhaps, but the relationship between "harsh" conditions and decision speed in honey bees is confounded by the fact that flight activity dies off when it rains or becomes cold, cloudy and windy, so swarms' decision-making effectively shuts down in poor weather and prevents them from speeding up their decision-making. However, future studies could alter the urgency of the decision-making process by manipulating swarms' food reserves, thereby varying the amount of time swarms could spend searching for a nest site before running out of energy. If honey bee swarms are found to emphasize accuracy even when conditions are urgent, this would strongly support the idea that bees are relatively inflexible in their tendency to place a high premium on accuracy in their decision-making process.

Speed-accuracy tradeoffs are an important part of many decision-making processes in both individual animals and animal groups. There is increasing evidence in individual bees that the speed-accuracy model is important for decision-making (Dyer et al. 2007), and that individuals differ consistently in their tendency to make quick, as opposed to accurate, decisions (Chittka et al. 2003; Burns 2005; Burns and Dyer 2008) However, our results suggest that for house-hunting swarms of Western honey bees (*A. mellifera*), chance plays a greater role in determining swarms' decision speeds than intrinsic characteristics of the swarms themselves. Making an accurate nest-site decision is crucial for *A. mellifera* swarms, and this necessarily high emphasis on accuracy may explain why chance plays such a large role in the decision-making process, and therefore why swarms did not differ consistently in their "impulsivity".

5.6 References

Avarguès-Weber, A., de Brito Sanchez, M. G., Giurfa, M., & Dyer, A. G. 2010. Aversive Reinforcement Improves Visual Discrimination Learning in Free-Flying Honeybees. *PLoS One*, 5, e15370.

Briffa, M., Rundle, S. D., & Fryer, A. 2008. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1305.

Burns, J. G. 2005. Impulsive bees forage better: the advantage of quick, sometimes inaccurate foraging decisions. *Animal Behaviour,* 70, e1–e5.

Burns, J. G., & Dyer, A. G. 2008. Diversity of speed-accuracy strategies benefits social insects. *Current Biology*, 18, R953-R954.

Burns, J. G., & Rodd, F. H. 2008. Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Animal Behaviour*, 76, 911-922.

Camazine, S., Visscher, P., Finley, J., & Vetter, R. 1999. House-hunting by honey bee swarms: collective decisions and individual behaviors. *Insectes Sociaux*, 46, 348-360.

Chittka, L., Skorupski, P., & Raine, N. E. 2009. Speed-accuracy tradeoffs in animal decision making. *Trends in Ecology & Evolution*, 24, 400-407.

Chittka, L., Dyer, A. G., Bock, F., & Dornhaus, A. 2003. Psychophysics: Bees trade off foraging speed for accuracy. *Nature*, 424, 388-388.

Donahoe, K., Lewis, L. A., & Schneider, S. S. 2003. The role of the vibration signal in the house-hunting process of honey bee (*Apis mellifera*) swarms. *Behavioral Ecology and Sociobiology*, 54, 593-600.

Dornhaus, A., Franks, N. R., Hawkins, R. M., & Shere, H. N. S. 2004. Ants move to improve: colonies of *Leptothorax albipennis* emigrate whenever they find a superior nest site. *Animal Behaviour*, 67, 959-963.

Durr, R., & Smith, C. 1997. Individual differences and their relation to social structure in domestic cats. *Journal of Comparative Psychology*, 111, 412–418.

Dyer, A. G., & Chittka, L. 2004. Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A*, 190, 759-763.

Dyer, A. G., Whitney, H. M., Arnold, S. E. J., Glover, B. J., & Chittka, L. 2007. Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of Antirrhinum majus flower colour. *Arthropod-Plant Interactions*, 1, 45–55.

Franks, N. R., Dornhaus, A., Best, C. S., & Jones, E. L. 2006. Decision making by small and large house-hunting ant colonies: one size fits all. *Animal Behaviour*, 72, 611-616.

Franks, N. R., Dornhaus, A., Fitzsimmons, J. P., & Stevens, M. 2003. Speed versus accuracy in collective decision making. *Proceedings of the Royal Society B*, 270, 2457-2463.

Franks, N. R., Hardcastle, K. A., Collins, S., Smith, F. D., Sullivan, K. M. E., Robinson, E. J. H., & Sendova-Franks, A. B. 2008. Can ant colonies choose a far-and-away better nest over an in-the-way poor one? *Animal Behaviour*, 76, 323-334.

Franks, N. R. 1989. Army Ants: A Collective Intelligence. *American Scientist*, 77, 138-145.

Franks, N. R., Hooper, J. W., Dornhaus, A., Aukett, P. J., Hayward, A. L., & Berghoff, S. M. 2007. Reconnaissance and latent learning in ants. *Proceedings of the Royal Society B*, 274, 1505-1509.

Gosling, S. 2001. From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, 127, 45-86.

Healey, C. I. M., & Pratt, S. C. 2008. The effect of prior experience on nest site evaluation by the ant *Temnothorax curvispinosus*. *Animal Behaviour*, 76, 893-899.

Hölldobler, B., & Wilson, E. O. 2008. *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies.* 1st edn. W. W. Norton & Company.

Langridge, E. A., Sendova-Franks, A. B., & Franks, N. R. 2008. How experienced individuals contribute to an improvement in collective performance in ants. *Behavioral Ecology and Sociobiology*, 62, 447-456.

Lewis, L. A., & Schneider, S. S. 2000. The modulation of worker behavior by the vibration signal during house hunting in swarms of the honeybee, *Apis mellifera*. *Behavioral Ecology and Sociobiology*, 48, 154-164.

Lindauer, M. 1955. Schwarmbienen auf Wohnungssuche. *Zeitschrift für vergleichende Physiologie*, 37, 263-324.

Lindauer, M. 1961. *Communication among Social Bees: Revised Edition.* Harvard University Press.

Makinson, J. C., Oldroyd, B. P., Schaerf, T. M., Wattanachaiyingcharoen, W., & Beekman, M. 2010. Moving home: nest-site selection in the Red Dwarf honeybee (Apis florea). *Behavioral Ecology and Sociobiology*, 65, 945-958.

Marshall, J. A. R., Dornhaus, A., Franks, N. R., & Kovacs, T. 2006. Noise, cost and speed-accuracy trade-offs: decision-making in a decentralized system. *Journal of the Royal Society Interface*, 3, 243-254.

Marshall, J. A. R., & Franks, N. R. 2009. Colony-level cognition. *Current Biology*, 19, R395-396.

Martin, J. G. A., & Réale, D. 2008. Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, 75, 309–318.

Mitchell, C. 1970. Weights of workers and drones. *American Bee Journal*, 110, 468-469.

Nieh, J. C. 1998. The honey bee shaking signal: function and design of a modulatory communication signal. *Behavioral Ecology and Sociobiology*, 42, 23-36.

Oldroyd, B. P., Gloag, R. S., Even, N., Wattanachaiyingcharoen, W., & Beekman, M. 2008. Nest site selection in the open-nesting honeybee Apis florea. *Behavioral Ecology and Sociobiology*, 62, 1643-1653.

Passino, K. M., & Seeley, T. D. 2006. Modeling and analysis of nest-site selection by honeybee swarms: the speed and accuracy trade-off. *Behavioral Ecology and Sociobiology*, 59, 427-442.

Passino, K. M., Seeley, T. D., & Visscher, P. K. 2008. Swarm cognition in honey bees. *Behavioral Ecology and Sociobiology*, 62, 401-414.

Phillips, L. H., & Rabbitt, P. M. A. 1995. Impulsivity and speed-accuracy strategies in intelligence test performance. *Intelligence*, 21, 13-29.

Rangel, J., Griffin, S. R., & Seeley, T. D. 2010. Nest-site defense by competing honey bee swarms during house-hunting. *Ethology*, 116, 608-618.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291–318.

Reddi, B. A. J., & Carpenter, R. H. S. 2000. The influence of urgency on decision time. *Nature Neuroscience*, 3, 827-830.

Rival, C., Olivier, I., & Ceyte, H. 2003. Effects of temporal and/or spatial instructions on the speed-accuracy trade-off of pointing movements in children. *Neuroscience Letters*, 336, 65-69.

Schneider, S. S., & Lewis, L. A. 2004. The vibration signal, modulatory communication and the organization of labor in honey bees, *Apis mellifera*. *Apidologie*, 35, 117-131.

Scholes, D. R., & Suarez, A. V. 2009. Speed-versus-accuracy trade-offs during nest relocation in Argentine ants (*Linepithema humile*) and odorous house ants (*Tapinoma sessile*). *Insectes Sociaux*, 56, 413-418.

Seeley, T. D., & Visscher, P. K. 1985. Survival of honeybees in cold climates: The critical timing of colony growth and reproduction. *Ecological Entomology*, 10, 81-88.

Seeley, T. D. 1985. *Honeybee Ecology: A Study of Adaptation in Social Life.* Princeton University Press.

Seeley, T. D. 2003. Consensus building during nest-site selection in honey bee swarms: the expiration of dissent. *Behavioral Ecology and Sociobiology*, 53, 417-424.

Seeley, T. D. 2010. *Honeybee Democracy*. Princeton University Press.

Seeley, T. D., & Buhrman, S. C. 1999. Group decision making in swarms of honey bees. *Behavioral Ecology and Sociobiology*, 45, 19-31.

Seeley, T. D., & Buhrman, S. C. 2001. Nest-site selection in honey bees: how well do swarms implement the “best-of-N” decision rule? *Behavioral Ecology and Sociobiology*, 49, 416-427.

Seeley, T. D., & Morse, R. A. 1978. Nest site selection by the honey bee, *Apis mellifera*. *Insectes Sociaux*, 25, 323-337.

Seeley, T. D., Morse, R. A., & Visscher, P. K. 1979. The Natural History of the Flight of Honey Bee Swarms. *Psyche*, 86, 103-113.

Seeley, T. D., & Tautz, J. 2001. Worker piping in honey bee swarms and its role in preparing for liftoff. *Journal of Comparative Physiology A*, 187, 667-676.

Seeley, T. D., & Visscher, P. K. 2004. Quorum sensing during nest-site selection by honeybee swarms. *Behavioral Ecology and Sociobiology*, 56, 594-601.

Seeley, T. D., Weidenmuller, A., & Kuhnholz, S. 1998. The shaking signal of the honey bee informs workers to prepare for greater activity. *Ethology*, 104, 10-26.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology*, 79, 241-277.

Stroeymeyt, N., Giurfa, M., & Franks, N. R. 2010. Improving decision speed, accuracy and group cohesion through early information gathering in house-hunting ants. *PLoS ONE*, 5, e13059.

Sumpter, D. J. T., & Pratt, S. C. 2009. Quorum responses and consensus decision making. *Philosophical Transactions of the Royal Society B*, 364, 743-753.

Visscher, P. K., & Seeley, T. D. 2007. Coordinating a group departure: who produces the piping signals on honeybee swarms? *Behavioral Ecology and Sociobiology*, 61, 1615-1621.

Visscher, P. K., Shepardson, J., McCart, L., & Camazine, S. 1999. Vibration signal modulates the behavior of house-hunting honey bees (*Apis mellifera*). *Ethology*, 105, 759-769.

Wray, M. K., Mattila, H. R., & Seeley, T. D. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Animal Behaviour*, 81, 559-568.