COLONY FISSIONING IN HONEY BEES: HOW IS SWARM DEPARTURE

TRIGGERED AND WHAT DETERMINES WHO LEAVES?

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SWARMING IN HONEY BEES: HOW IS A SWARM'S DEPARTURE TRIGGERED AND WHAT DETERMINES WHICH BEES LEAVE?

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Honey bees (*Apis mellifera*) live in colonies that reproduce by fissioning. When a colony divides itself, approximately two thirds of the workers along with the (old) mother queen, leave the hive as a swarm to found a new colony else where. The rest of the workers, and a (new) daughter queen, stay behind and inherit the old nest. In cold, temperate regions, this ephemeral process of colony multiplication typically occurs only once per year and takes less than 20 minutes, making it a hard-to-study phenomenon. The purpose of this dissertation, which is divided into four chapters, was to uncover the mechanisms and functional organization of the colony fissioning process in honey bees. The first chapter explored the signals used by honey bee colonies to initiate the departure of a swarm from its nest, finding that the piping signal, and the buzz-run signal, are the key signals used to initiate the swarm's departure. The second chapter searched for the identity of the individuals that performed the signals that trigger the swarm's exodus. We now know that knowledgeable nest-site scouts are the producers of the signals that trigger this sudden departure. The third chapter investigated whether honey bee swarms compete for, and defend, potential nest sites during their house-hunting process. We found that they do so, using various levels of aggression depending on the number of nest-site scouts from each swarm present at the nest site at the same time. The fourth and final chapter looked at the question of whether honey bee workers make a decision of whether to

stay in the old nest, or leave with the swarm, based on their genetic relatedness to the queen(s) that inherit the nest during colony fissioning. Our findings showed that there is no intracolonial nepotism during swarming, despite the theoretical prediction that workers should benefit from preferentially staying in the old nest based on their genetic relatedness to the daughter queen(s).   These studies have helped us better understand important aspects of the reproductive biology of honey bees, and now serve as the basis for future research regarding colony fissioning in honey bees and other social insects.

BIOGRAPHICAL SKETCH

Juliana Rangel Posada was born in Pereira, Colombia. She moved with her mother and two brothers to the United States in 1998. She attended San Diego Mesa College in San Diego, CA, where she obtained an Associate's Degree in transfer studies. In 2001, she transferred to the program of Ecology, Behavior, and Evolution at the University of California, San Diego. That same year she joined the laboratory of Dr. Daniel Feldman, where she played an important role in the visualization and analysis of the development of cortical anatomy in mice. Also in 2001, she joined the laboratory of Dr. James Nieh where, for nearly three years, she conducted multiple independent research projects exploring the mechanisms of communication of resource location in honey bees (in the United States) and stingless bees (in Brazil and Costa Rica). She graduated *cum laude* from UCSD in 2004. That same year she began her doctoral studies in the Department of Neurobiology and Behavior at Cornell University in Ithaca, NY. Working under the supervision of Dr. Thomas D. Seeley and Dr. Hudson K. Reeve, her dissertation explored the mechanisms and functional organization of reproductive swarming in the honey bee *Apis mellifera*. Juliana defended her Ph. D. dissertation in December 2009. Juliana will begin a Postdoctoral Associate Position with Dr. David R. Tarpy at North Carolina State University in January 2010. As part of a larger outreach initiative, Juliana will spearhead the comprehensive educational program "Born and Bred in North Carolina," a state-wide training initiative that will include workshops on queen rearing, and clinics on queen breeding, to the state's beekeeping community. She will also conduct field and laboratory experiments aimed at determining the mechanisms and causes of queen replacement in honey bees, or queen "supersedure," the number one problem facing beekeepers today. In her spare time, Juliana likes to play the guitar, sing folk Latin American songs, dance, and cook for friends and family.

This work is dedicated to the most important people in my life: my mother María Eugenia Posada, my brothers Pedro José Rangel and Alvaro Rangel, my dear friend Juan Diego Villegas, and the members of the Posada Family in Colombia and abroad. I would have never accomplished my career goals thus far without their love and support, both in times of bliss and in times of tribulation. I also dedicate this achievement to the memory of my late father Alvaro Rangel, who would have been very proud of seeing his children grow up to become responsible, professional adults.

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CHAPTER 1

THE SIGNALS INITIATING THE MASS EXODUS OF A HONEY BEE SWARM

FROM ITS NEST*

ABSTRACT

Animals that travel in groups must synchronize the timing of impending departures to assure group cohesion. The mechanisms used by a honey bee colony to organize the departure of a swarm from its nest remain a mystery. We examined the signals that trigger a swarm's explosive exodus from the parental nest, and we documented the concurrent changes in bee density and mobility. Using videorecordings of swarms exiting observation hives, we analyzed how bees in three swarming colonies prepared for and then performed their sudden departures. We found that over the 60 min before swarm exodus, the production of piping signals gradually increased, and ultimately peaked at the start of the swarm departure. We also found that, during swarm exodus, bee density (number of bees in 100 cm^2) dropped markedly, while the average bee velocity (mm / s), and the production of buzz-run signals spiked dramatically. Neither waggle runs nor shaking signals showed increases before or during swarm exodus. The explosive departure of a honey bee swarm from its parental nest shows how animals can use the same communication signals in different contexts; we now know that honey bees use piping and buzz-run signals to initiate both a swarm's departure from its nest and a swarm's take-off from its bivouac site. This study also demonstrates how a small minority of individuals in a social insect colony can operate as an oligarchy to make an important decision, i.e., when a swarm should leave its nest to found a new colony.

INTRODUCTION

When animals that travel about in a group move from one location to the next, they must synchronize their departures so that the group does not disintegrate. The timing of a group's departure may be decided by means that are democratic, oligarchic, or despotic (Conradt & Roper 2003, 2005). In a democracy, the decision-making process involves a group of individuals signaling their preferences about when to leave, initiating the move only when a majority of the members are in agreement. For example, in red deer (*Cervus elaphus*), a herd that has settled for the night moves the next day only when more than 60% of the adults stand up in concert (Conradt & Roper 2003). In an oligarchy, only a small number of individuals have access to the information about the conditions that must be met for their group's move, and this minority makes the decision of when to depart. This oligarchic decision-making process is exemplified by colonies of rock ants (*Temnothorax albipennis)*. During colony emigration, only a small percentage of a colony's members know when to initiate the move to the new nest site, and this minority stimulates the majority to do so using sophisticated mechanical signals (Mallon et al. 2001, Pratt et al. 2002). Finally, in a despotic situation, one individual controls when the group moves and this leader signals to the others when it is time to leave. Such is the case in the mountain baboon (*Papio ursinus*), in which a male or female leader initiates and specifies the direction of travel with exaggerated gestures of standing up (Byrne et al. 1990). Most of the studies of the mechanisms used by group-living species to initiate travel have focused on birds, cetaceans, and primates (Boinski & Garber 2000), leaving large gaps in our knowledge of group travel in invertebrates (but see Simpson et al. 2006, Buhl et al. 2006). Given their large colonies, sophisticated communication systems, and ease

of study, social insects are attractive for exploring how animals initiate a group's move from one location to the next.

Many studies of social insects have revealed how group decisions are made regarding nest building, brood rearing, food collecting, and other aspects of colony life (Camazine et al. 2001), but still little is known about how a group of social insects decides when to move to a new location (Forsyth 1981, Dyer 2000). Most of our knowledge regarding these mechanisms comes from the study of honey bee swarms. When a colony swarms, it splits into two colonies, with the mother queen and several thousand workers suddenly leaving the parental nest to found a new colony. The departing bees settle on a tree branch in a beard-like bivouac cluster, from which scout bees go searching for potential nest sites. Scout bees that find prospective home sites return to the swarm cluster and perform waggle dances to recruit other bees to their finds (Lindauer 1955). Once a sufficient number of bees has built up at one of the sites, the scout bees at this site return to the swarm and begin to produce the signals that initiate the swarm's take-off and flight to its new home (reviewed in Seeley et al. 2006, Visscher 2007).

The first signal is the high-pitched piping signal, which starts an hour or so before take-off, and primes all the bees for take-off. While piping, scouts burrow among the quiet bees in the swarm cluster, pressing their thoraces against these bees, and producing flight muscle vibrations that stimulate the quiescent bees to warm their flight muscles to at least $35^{o}C$, the temperature needed to take flight (Seeley & Tautz 2001, Seeley & Visscher 2004). Then, about 10 min prior to take-off, the same bees that were producing piping signals gradually begin to produce buzz-run signals, excitedly running about, pushing against other bees, and buzzing their wings in bursts that release the swarm's lift-off (Lindauer 1955, Rittschof & Seeley 2008). Additionally, the shaking signal (also known as the "dorso-ventral abdomen vibration

signal", or simply, the "vibration signal"), in which one bee grabs another bee and shakes her body for a second or so, seems to work as a non-specific modulatory signal (Nieh 1998) that, in the context of swarming, aids in the general activation of swarm bees before the flight to the new home (Schneider et al. 1998, Lewis & Schneider 2000, Donahoe et al. 2003, reviewed in Schneider & Lewis 2004). Thus, while much is known about how honey bee swarms organize the take-off process after they have left the parental nest, almost nothing is known about how the explosive departure of a swarm is initiated inside the parental nest (Seeley 1985, Winston 1987).

What are the signals that initiate the explosive exodus of a honey bee swarm from the parental nest? To date, only Martin (1963) has studied what happens inside a nest prior to the exodus of a swarm. Working with colonies in observation hives, Martin reported that 15 min before the exodus, worker bees appeared quiet and calm. Then a sudden agitation started, with a few workers running back and forth, sometimes buzzing their wings, performing what he called the *Schwirrlauf*, or buzz-run. He reported that the buzz-runners were silent while signaling to other bees, and that direct contact with other bees was necessary to activate them. He also reported that the mass exodus of the swarm began just a few minutes after the majority of bees in the colony had been contacted by the buzz-runners. Given that honey bees use the buzz-run to release a swarm's take-off from the bivouac site (Rittschof & Seeley 2008), it is likely that they also use the buzz-run to trigger a swarm's departure from the parental nest. Another signal that may play a role is the piping signal. Based on the studies of swarm take-off from the bivouac site, we know that bees start warming their flight muscles more than an hour before the buzz-runners appear on the swarm cluster. This increase in temperature is stimulated by individuals that perform the piping signal (Seeley & Tautz 2001). While the ambient temperature experienced by bees living inside a nest is higher than that experienced by bees hanging from a tree branch, pre-

swarm bees might also need to be stimulated to warm their flight muscles before buzz-runners can release their mass exodus from the nest.

Two other signals that might play a role in eliciting swarm exodus are the shaking signal and the waggle dance. One study found that, during the reproductive season, the queen is increasingly shaken by workers several days before swarming, presumably to activate her for flight (Allen 1959a, b). The shaking signal on workers has been found to activate quiescent swarm bees, even before they warm their flight muscles for take-off (Visscher et al. 1999). As mentioned above, scouts perform waggle dances to indicate the locations of potential home sites (Lindauer 1955, Seeley et al. 2006). If the search for a new home starts even before the swarm leaves the parental nest, perhaps bees perform the waggle dance in increasing frequency in preparation for swarm departure.

In this study, we examined three swarms originating from honey bee colonies living in observation hives, to determine how bees initiate the mass exodus of a reproductive swarm from its parental nest. We report which signals are, and which ones are not, used for this purpose.

METHODS

Study Site and Bees

Our study was conducted at the Liddell Field Station of Cornell University in Ithaca, New York State (42º26'N, 76º30'W). We created five medium-sized honey bee colonies from five larger colonies that lived in nearby bee yards, all headed by New World Carniolan queens (*Apis mellifera carnica*; Strachan Apiaries, Yuba City, California, USA). On 14 May 2007, each of the five colonies was installed with its

original queen in a three-frame observation hive (described by Seeley 1995). Bees at the entrance had access to both sides of the hive. The three frames chosen to start each colony were covered with adult bees (roughly 6 000 workers total), and were filled with sealed brood, honey, and pollen, to create the conditions present in a natural colony preparing to swarm (Winston 1987). We standardized the amount of brood, food, and workers across colonies by choosing frames that were approximately half full of sealed brood, half full of honey and pollen, and entirely covered by workers. Two weeks after establishment, the colonies were actively foraging and growing, and were more or less synchronized to start swarming in early June, which is when most natural swarms occur in the Ithaca area (Fell et al. 1977).

Video and Audio Recording

Before the observation colonies were sealed with glass covers, an electret condenser microphone (Radio Shack Model 33-3013, 70-16 000 Hz frequency response) was placed at the center of the bottom frame on one side, so we could hear the sounds made by bees during the swarming process. To detect these sounds, we connected the microphone to a voice-activated sound recorder (Sony ICD-P520), which gave us easy access to the sounds produced by each colony at any time. The observation hives were set up in a light-proof room to simulate the dark conditions inside natural honey bee nests, leaving the daylight coming in the hive entrance as a guide for the bees to find the exit. All video recordings were made in complete darkness using the night vision function of a digital video camera (Sony DCR-TRV50), which had a field of view always positioned on the same side of each hive, and consisting of a 100 mm x 133 mm area of comb located on the bottom frame, with the microphone placed at the center.

We monitored the observation hives daily to determine which signals were produced to initiate the exodus of a swarm. Knowing that bivouacked swarms are primed for departure by nest-site scouts that perform piping signals during the last hour or two before take-off, we listened in on each colony in our observation hives every 15 min from 0800 - 1500 hours, checking for piping signals. When we heard piping at a rate of at least 3 signals in 30 s, we turned on the video camera, transferred the microphone output to the camera, and started recording until after the swarm had left the hive and the remaining bees returned to the quiescent pre-swarm state. The start of swarm departure was defined as the moment at which began a rapid outflow of bees from the hive (confirmed by observing the exodus from outside the hive). The end of swarm departure was defined as the time at which the swarm had settled on a tree branch, and bees stopped flying out to join the swarm.

Video Analysis

The video recordings were sampled at intervals of 2.5 min (unless otherwise noted) and analysed at slow speed or frame-by-frame using a video editing deck (Sony DSR-30). The following measurements were taken at every time interval: (a) Bee Density = number of bees present in a 10 cm x 10 cm area; (b) Bee Mobility = number of bees crossing a vertical 3-cm line in 30 s (the line was located in the center of the camera's field of view); (c) Mobility Index = Bee Mobility / Bee Density, as an index of bee mobility with a correction for the changes in bee density over time; and (d) Average Bee Velocity = average number of mm traveled in 3 s by 10 randomly-chosen bees. To choose 10 bees at random, we placed an acetate sheet over the video screen with 10 sampling dots dispersed across the screen, and tracked the 10 bees closest to the dots. A plot of the movements made by each of the 10 randomly-chosen

bees was generated for one of the swarms, to illustrate the sudden spike in average bee velocity during the swarm exodus, and to see whether there was consistent directionality in the movement of the bees. Using the input from the microphone connected to the video camera, we measured (e) Piping Rate = number of seconds the piping signal was heard during a 30 s period. Additionally, we scanned the entire screen of the video monitor from top-left to bottom-right to measure three more signals: (f) Buzz-run Rate = number of buzz-runners observed in 30 s, identified as individuals performing the behavioral sequence described by Rittschof and Seeley (2008); (g) Waggle Run Rate = number of waggle runs observed in 30 s; and (h) Shaking Signal Rate = number of worker-worker shaking signals observed in 30 s. Finally, we measured the total number of bees observed on the video screen and recorded what proportion of those bees were producing the buzz-run before and during swarm departure. We followed buzz-running bees at slow speed from one second before to one second after each time interval to make an accurate count of all signaling bees. We did so to check whether only a small minority of individuals in the colony initiated a swarm's exodus.

Statistics

The descriptive statistics reported are the mean ± one standard error. For all statistical tests, the level of significance used was $\alpha = 0.05$. To increase the power of our tests, we used one-tailed tests when possible; we predicted a priori, based on previous studies that (1) the density of bees would drop as bees left the hive (Martin 1963), and (2) the average velocity, piping rate, and buzz-run rate would increase during the swarm departure process (Seeley & Tautz 2001, Rittschof & Seeley 2008). For each colony, we calculated the mean value for each mobility variable and each

communication signal before, during, and after swarm departure. The number of measurements per mean varied depending on the length (min) of each period in the departure process. We grouped the data from the three swarm departures and calculated one mean value for each mobility variable and communication signal using the means obtained for each swarming event. To test each measure for a difference between its mean value before and during swarm departure across the three swarms, we used paired t tests. The use of this test enabled us to keep the significance level at $\alpha = 0.05$, because for $n = 3$ (data pooled from three swarm departures), the non-parametric Wilcoxon signed-rank test would not have the power to discern differences at a significance level lower than $\alpha = 0.125$.

To test whether the direction of travel of bees before and during swarm departure was consistent, we used the drawings of distances traveled by the 10 randomly-chosen bees to measure average bee velocity (see above) to create a net travel vector from the start to the end point of each bee's travel. The angle was measured relative to straight up ($= 0^{\circ}$) and a circular histogram of vector angles was generated for each swarm. Mean vector bearing (MVB) and mean vector length (MVL) were calculated, and Rayleigh's Z test was used to test for non-random directionality of the bees' movements in each swarm (using Dr. Kirk Visscher's circular statistics software, Riverside, CA), with the null hypothesis that the circular distribution of the angles was drawn from a uniform circular distribution (as described by Gardner et. al 2008).

RESULTS

We obtained complete recordings of the departure of prime swarms from three of the five colonies. The swarm originated from colony 1 started the exodus at 12:55 p.m., the swarm from colony 2 originated at 12:15 p.m., and the swarm from colony 3

issued at 10:20 a.m. All three colonies showed a similar pattern in the production of signals and the movement of bees before, during, and after the swarm exodus, as exemplified by the signaling and mobility patterns observed in colony 1 (Fig. 1.1, Table 1.1). Over the hour or so before departure, there were no apparent changes in the behavior of bees, except for a gradual increase in the piping rate and a slight rise in the buzz-run rate when getting close to the departure. During the departure, the signals that showed strong increases were the piping and the buzz-run signals, with the production of buzz-runs surging most strongly. We did not observe any surges in the production rates of waggle runs or shaking signals. In fact, neither signal was observed in high rates for any of the three swarm phases. After the swarm left, all communication signals were barely produced.

As with the production of the piping and buzz-run signals, we observed conspicuous changes in density, mobility, mobility index, and velocity during the swarm exodus (Fig. 1.1, Table 1.1). The density of bees sharply decreased, a not surprising result as many bees were leaving the hive to join the swarm. Likewise, the mobility, and mobility index rapidly increased during the departure and then dropped to below pre-swarm levels after the swarm left. Similarly, the average velocity of 10-randomly chosen bees jumped during the exodus, and dropped dramatically after, indicating how the bees that remained in the colony went back to performing tasks that did not require much movement across the comb. For example, the distance and direction traveled by each of the 10 randomly-chosen bees in colony 1 at time (t) = 20, 60, 100, 108, 120, and 160 min from the beginning of the video recording is shown in Fig. 1.2. While at most times the bees monitored were either motionless or barely moving, there was a sudden increase in distance traveled at t = 108 min, with some of the bees moving out of the field of view before the 3 s had passed. By t = 120 min, the bees in the hive were again nearly motionless. There was no 'goal-oriented'

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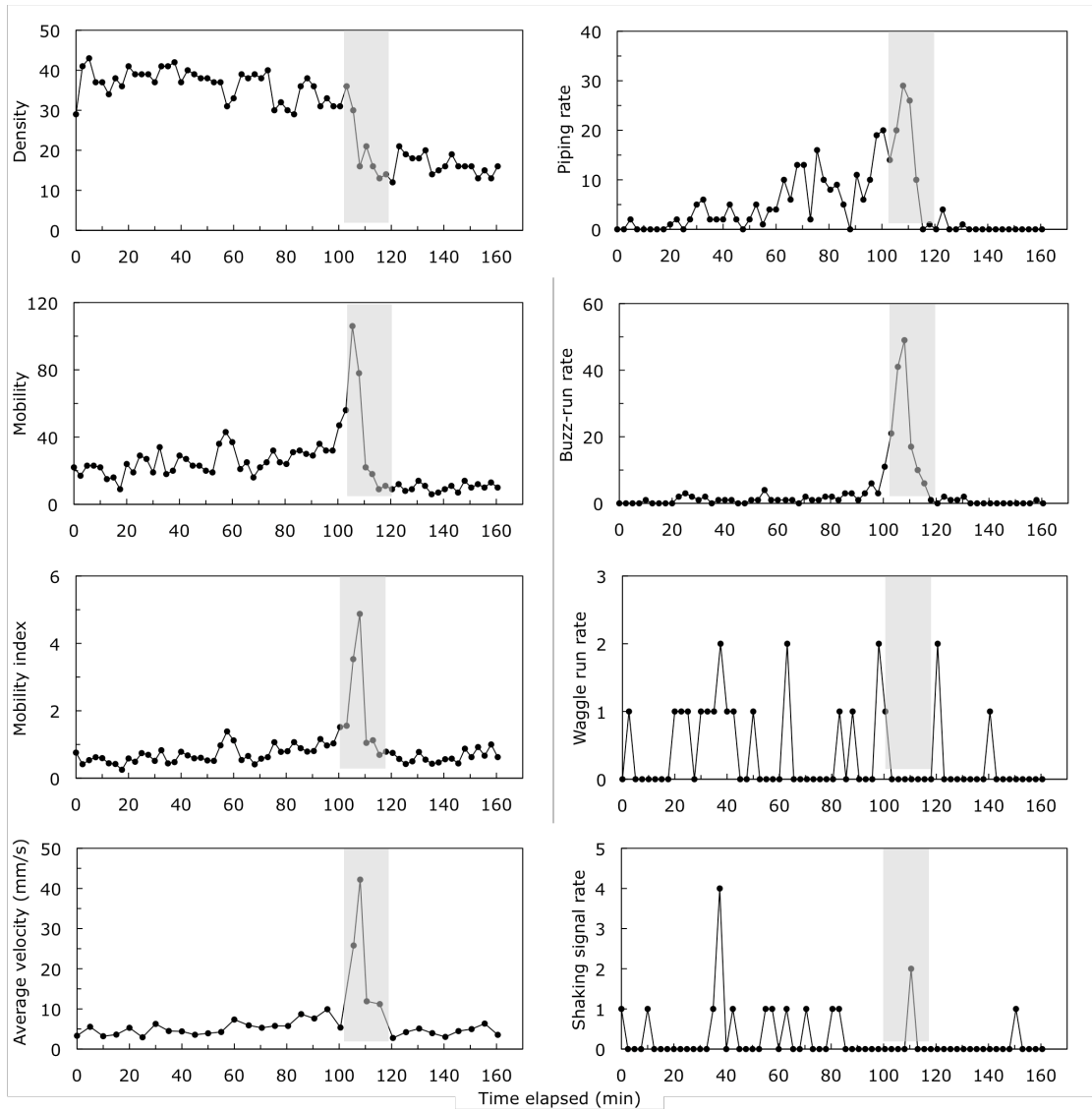
Figure 1.1. Changes in bee density, mobility and signal production inside the nest before, during (grey box) and after the swarm departure from colony 1. The time elapsed (min) was set at t = 0 when the video recording started; measurements were made every 2.5 min (except for measurements of average velocity, made every 5 min) for a total of 160.5 min. See Methods for the units of the variables plotted here.

Table 1.1. Duration of video recordings, aspects of honey bee mobility, and triggering signals produced in three honey bee colonies that swarmed in June 2007

Observation	Colony 1, 2 June 2007			Colony 2, 7 June 2007			Colony 3, 14 June 2007		
	Before	During	After	Before	During	After	Before	During	After
Video duration (min)	100.5	17.5	42.5	40.0	12.5	10.0	52.5	11.25	46.25
Piping rate	5.0 ± 0.8	14.3 ± 4.3	0.3 ± 0.2	4.1 ± 0.8	26.0 ± 1.4	0.0 ± 0.0	2.8 ± 0.8	12.8 ± 3.1	0.3 ± 0.1
Buzz-run rate	0.9 ± 0.3	45.1 ± 18.4	0.3 ± 0.1	0.2 ± 0.1	82.7 ± 14.6	0.0 ± 0.0	1.5 ± 1.1	58.0 ± 9.5	2.1 ± 0.8
Waggle run rate	0.5 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
Shaking signal rate	0.3 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.6 ± 0.2	0.6 ±0.2	1.2 ± 0.7	1.0 ± 0.3	0.6 ± 0.3	1.3 ± 0.4
Density	36.5 ± 0.6	20.9 ± 3.3	16.3 ± 0.6	40.5 ± 0.7	17.9 ± 3.8	9.2 ± 0.6	44.6 ± 0.8	28.7 ± 2.2	26.2 ± 1.2
Mobility	25.7 ± 1.2	42.9 ± 14.3	10.1 ± 0.6	32.5 ± 1.8	54.7 ± 10.6	2.9 ± 0.6	22.0 ± 3.8	98.0 ± 14.4	8.7 ± 1.7
Mobility Index	0.7 ± 0.0	1.9 ± 0.6	0.6 ± 0.0	0.8 ± 0.0	3.4 ± 0.5	0.3 ± 0.0	0.5 ± 0.1	3.4 ± 0.4	0.3 ± 0.1
Velocity (mm / s)	5.4 ± 0.4	22.8 ± 7.3	4.3 ± 0.4	6.1 ± 0.4	22.3 ± 2.8	3.8 ± 0.4	3.4 ± 0.3	20.7 ± 2.7	2.8 ± 0.3

Each recording was divided into three segments: Before, during, and after the departure of a swarm from the parental nest. Values are shown as X ± S.E.
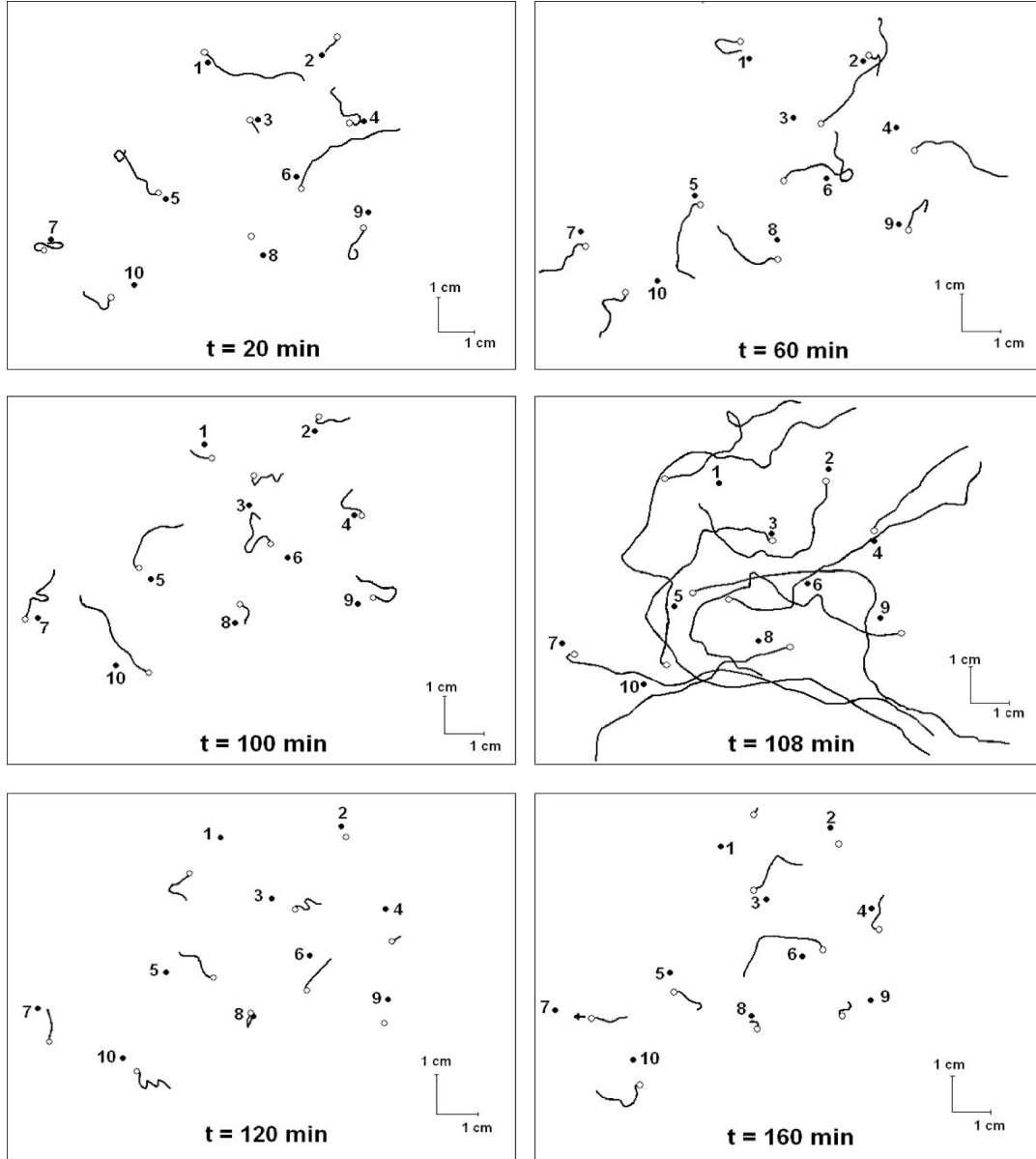
Figure 1.2. Distances traveled by ten randomly-chosen bees before, during, and after the swarm departure of colony 1. Each bee followed was the bee closest to one of the points numbered from one to ten at each time interval. The open circles indicate the position of the bees' thoraces at the start of a 3-second period, and the lines indicate the paths taken by the bees during that time interval. Different bees were observed in each sampling period. Departing bees left the hive through the entrance at lower right corner. Video was taken for a total of 160.5 min, and the distances traveled were measured every 5 min. Here we show travel patterns before (t = 20 min, 60 min, and 100 min), during (t = 108 min) and after (t = 120 min and 160 min) the swarm left the hive.

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Table 1.2. Circular histograms and statistics for the movement directions of bees before and during swarm departure in three observation colonies. If bees had moved mainly toward the entrance, the mean vector bearing would have been 135°

	Colony 1	Colony 2	Colony 3
N (angles)	241	253	301
MVB (°)	346.1	327.0	68.5
MVL	0.05	0.09	0.12
Rayleigh's test (Z)	0.53	2.12	4.09
P-value	> 0.1	> 0.1	> 0.1
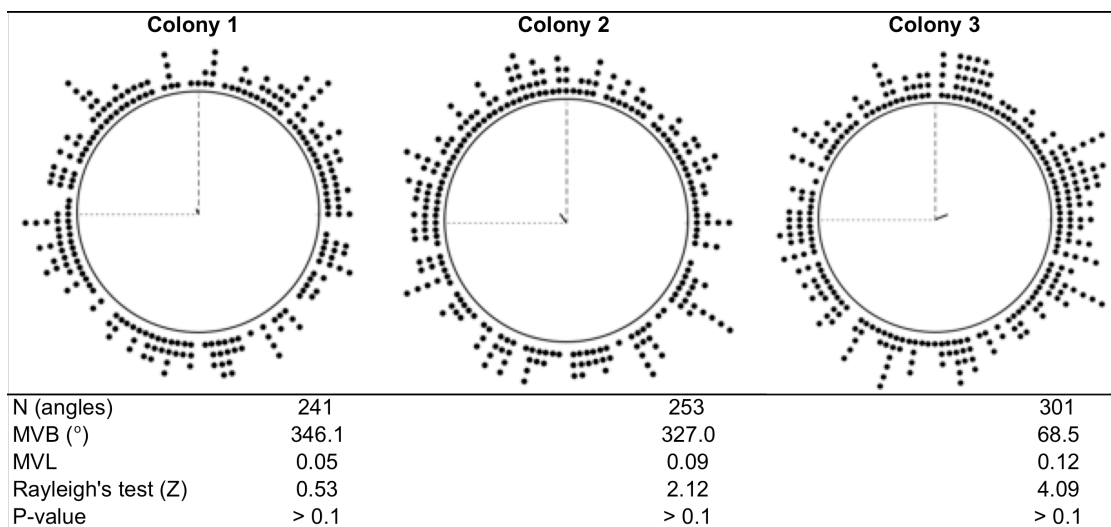
MVB: mean vector bearing; MVL: mean vector length. MLV is unitless and measures the scatter of the angles.
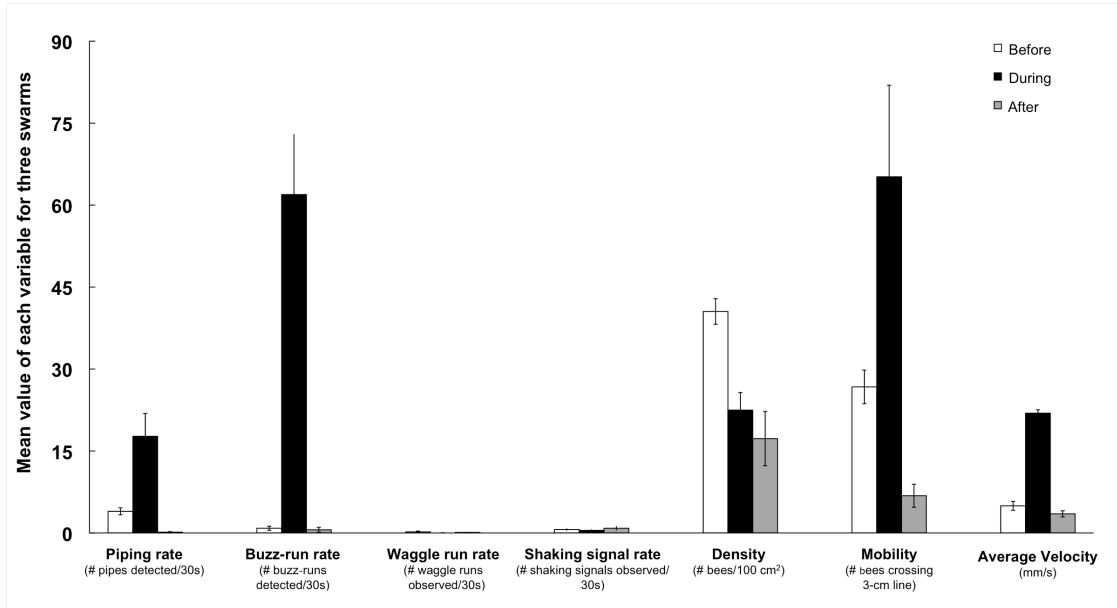
Figure 1.3. Mean ± S.E. values of honey bee density, mobility variables, and triggering signals for before, during, and after three departures of swarms from their nests. t-tests were conducted to test for a difference between the 3-swarm means for before and during swarm departure. Horizontal lines and asterisks indicate in which pairs of means there were significant differences ($p < 0.05$).
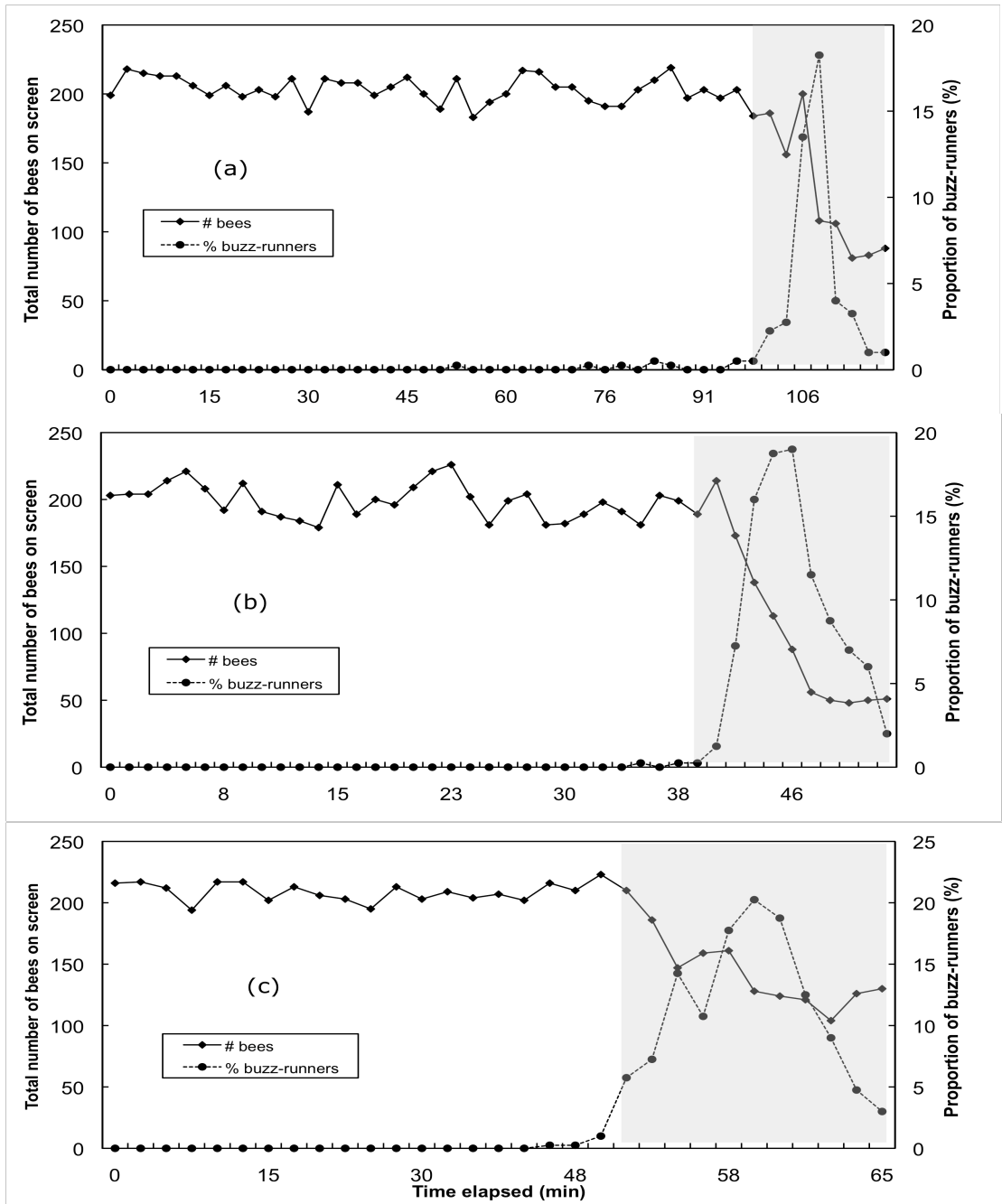
Figure 1.4. Total number of bees seen on video screen and the proportion of those bees that produced the buzz-run signal over time, for three swarms. Buzz-runners were observed at the beginning and throughout the swarm's exodus (grey box). Measurements were taken every (a) 2.5 min for colony 1, (b) 1.25 min for colony 2, and (c) 2.5 min for colony 3.

directionality in bees moving across the comb before or during swarm departure, indicating that the distribution of movement angles came from a uniform circular distribution (Rayleigh's Z test, Table 1.2).

To test for significant changes in the triggering signals and mobility variables during the exodus, we pooled the mean values for each measure from the three swarming events aforementioned, and determined whether there was a difference between the overall mean values associated with before and during swarm departure. Fig. 1.3 shows the values of the overall means for each variable. We found that there was an increase in the piping signal rate ($p = 0.04$), as well as a significant increase in the buzz-run rate ($p = 0.02$) during the swarm departure. We found no significant change in either the waggle run rate ($p = 0.84$) or the shaking signal rate ($p = 0.80$) when comparing the periods before and during the exodus. For the aspects of mobility, we found that there was a significant drop in the density ($p = 0.0078$) as bees left the nest. Moreover, there was a nearly significant increase in mobility ($p = 0.09$), and a significant increase in both mobility index ($p = 0.02$) and average velocity ($p = 0.0003$) during the swarm departure compared to before the departure.

When we measured the proportion of the bees that initiates a swarm's departure, we found that the total number of bees on the video screen was about 200 bees before departure, but that less than one percent of those bees had begun to buzz-run at the beginning of the exodus (Fig. 1.4). The proportion of buzz-runners increased to about 20 percent of the total number of bees appearing on the video screen in the middle of the swarm's departure, but this number is high due to the low density of bees remaining in the colony as most bees had exited at that time.

DISCUSSION

Although it has been known for centuries that the departure of a honey bee swarm from its nest involves a sudden and massive exodus of bees, to our knowledge, this is the first report that quantitatively documents this striking phenomenon and provides evidence regarding the signals that trigger this explosive event. In all three swarming colonies, we found that worker piping started 60+ min prior to swarm departure, gradually increased, and then peaked during swarm departure. The buzz-running signal, however, was produced strongly only during the exodus process; it increased from nearly zero before departure to over 100 buzz-runs observed in 30 s during departure. Both signals disappeared after the swarm had left. We also found that in all three colonies, the density of bees sharply decreased at the time of the exodus as a result of most bees evacuating the hive. Also, the movement of the bees in the hive skyrocketed over the course of 5 min, with average mobility jumping as much as four-fold, and the average velocity shooting up as much as six-fold, compared to the period before departure (Table 1.1). Within 12-18 min of the start of swarm departure, the high activity had ended and the density of bees had fallen markedly, from about 40 bees per 100 cm^2 to only 10-20 bees per 100 cm^2.

The patterns of signal production that we observed for initiating the departure of a swarm from its nest are nearly identical to those previously reported for priming and triggering the take-off of a swarm from its bivouac site, when it begins its flight to a new nest site (Seeley & Tautz 2001, Rittschof & Seeley 2008). During both processes, the first signal produced by bees is the piping signal, which evidently stimulates bees to warm their flight muscles in preparation for rapid flight. It is likely that the piping signal also serves as a primer for swarm exodus, stimulating the bees to warm up in preparation for their explosive departure from the nest. The evidence that piping

actually causes bees to warm up, and is not simply correlated with their warming up, comes from Seeley and Tautz (2001). When they prevented a small group of bees in a swarm from getting piped prior to swarm take-off, they found that these bees did not warm themselves, and fell to the ground when the rest of the swarm bees launched into flight. It would be interesting to document with a thermovision camera whether bees inside the nest increase their thoracic temperature after being piped, as do bees outside the nest in a swarm cluster (Seeley & Tautz 2001), and whether workers might pipe the queen to prime her to leave with the swarm.

The second signal that is used in both swarm departure contexts is the buzz-run. Buzz-runners begin to appear about 10 min before a swarm's exodus from the nest, and they appear in high numbers 5 - 10 min before a swarm's take-off from the bivouac site. Evidently, the small minority of bees that produce the buzz-run function as the "releaser" of departure in both contexts. Rittschof and Seeley (2008) report that bees standing motionless in a small group are stimulated to activity when a buzz-running bee pushes through them. Forsyth (1981) reports a remarkably similar process of triggering immobile individuals to become active by buzz-running wasps in colonies of *Polybia occidentalis*.

It is interesting to note that the exodus of swarm bees from inside the nest is slower than the take-off of swarm bees from the bivouac site: the former takes about 10 min, whereas the latter takes only about 1 min (Seeley et al. 2003). Probably, this difference exists because in the first scenario the bees must funnel out a small entrance opening whereas in the second they can simply launch into flight in the open air, not because the bees are differently stimulated for departure in the two scenarios.

Our finding that the buzz-run is a critical signal during the swarm departure process is consistent with Martin's (1963) observation that buzz-runners appeared near the entrance of an observation hive approximately 15 min before the swarm left the

nest. He found that the buzz-runners tried to contact as many bees as possible by pressing into clusters of bees and making zig-zag motions while actively buzzing their wings. We too observed this pattern of cluster break-up by buzz-runners during the beginning stages of the departure process inside the hive. Curiously, Martin did not report piping prior to swarm departure, and he even mentioned that the buzz-runners were silent while signaling to other bees. Because he did not plant microphones inside his observation hives, and because it is almost impossible to hear piping signals through the glass walls of an observation hive, it seems that Martin missed an important part of the pre-swarm behavioral sequence, namely, the production of piping signals for an hour or so before the exodus of the swarm.

We also found that neither the worker-worker shaking nor the waggle-run signals increased before or during the swarm departure. This result indicates that they are not directly involved in stimulating swarm bees to depart. It is probably not surprising that the waggle dance is not used in this context, since it is mainly used as a mechanism to communicate the locations of important resources, i.e. rich food sources and suitable nest sites (Lindauer 1955, Seeley 1995). Nevertheless, it is valuable to report for the first time that this important signal is evidently not used by bees to initiate swarm exodus.

Likewise, the worker-worker shaking signal does not appear to play a major role in triggering the mass exodus of a swarm. We found that the level worker-worker shaking was low before the swarms departed, did not increase during the departures, and did not change after the swarms had left their nests. It is clear that worker-worker shaking functions as an activational signal that boosts the activity level of worker bees (Winston 1987, Nieh 1998) engaged in brood rearing, foraging, food processing, nest construction, and nest maintenance (Allen 1959a, b, Schneider & Lewis 2004, Cao et al. 2007, Hyland et al. 2007). Likewise, in the context of swarming, worker-worker

shaking acts as a non-specific modulatory signal that causes a general activation of worker bees (Visscher et al. 1999, Lewis & Schneider 2000, Donahoe et al. 2003). A recent study reports an increase in shaking signal production before a swarm's departure from the parental nest that is specifically targeting the activation of the queen, with workers shaking her more and more often two to three days before swarm departure (Pierce et al. 2007). Presumably, this increased shaking of the queen serves to notify her of the imminent departure. Another recent study reports that during the queen-replacement process, virgin queens were shaken by workers 25 times more often in colonies that produced afterswarms than in colonies that did not, further indicating the role of the shaking signal in activating queens for departure (Schneider & DeGrandi-Hoffman, 2007). Thus, while the shaking signal evidently plays several important roles during swarming, it seems clear that at least worker-worker shaking is not a main signal for triggering a swarm's exodus from its nest.

Although this study reports the conspicuous use of mechanical modes of communication for triggering swarm departure (i.e. the piping and buzz-running signals), honey bees may also employ other signal modalities to initiate swarm departure, including chemical signals. Such signals are used by other social insect species for triggering group departures. For example, the carpenter ant *Camponotus herculeanus* relies on strong chemical signals to synchronize the departure of males and females when they swarm out on mating flights (Hölldobler 1965). Males begin the swarming process by "sun-bathing" for a few hours just outside the nest entrance. Once they have reached a threshold body temperature, they release a mandibular pheromone that attracts females to emerge from the nest entrance and crawl up the tree trunk to an elevation above the males. The swarm of reproductives takes off with both sexes combined in a cloud. Also, a recent study of honey bee signaling by Thom et al. (2007) has shown that the famous waggle dance is not simply a mechanical/acoustical

signal, but also has a chemical component. We wonder if one or both of the signals that we have found important in initiating swarm departure –worker piping and buzz-running– might likewise have a chemical component along with their conspicuous mechanical components.

Some tantalizing puzzles remain to be solved about swarm departure in honey bees. Which bees produce the piping and buzz-running signals? Are the pipers and buzz-runners the same individuals? Presumably the bees that signal to the rest of the group that it is time to leave home must be informed about the weather conditions outside, for swarms only depart when the weather is sunny and warm (Winston 1987). This observation makes us suspect that the pipers and buzz-runners are either foragers or nest-site scouts. Prior studies of the swarm's house-hunting process provide us with some appealing leads. Lindauer (1955) reports that some nest-site scouts are engaged in exploring for nest sites even before their colony swarms. Also, when Visscher and Seeley (2007) labeled nest-site scouts at nest boxes on an island in Maine, it was found that the pipers on a swarm cluster are the scouts from the site chosen for the swarm's new home. Rittschof and Seeley (2008) reported that the buzz-runners on a swarm cluster are the same bees as the pipers; the bees that start the piping on a swarm cluster gradually add buzz-running to their signaling repertoire and ultimately perform mainly buzz-runs just before a swarm's take-off to fly to its new home. Does the same thing happen inside a nest when a swarm prepares for departure? To find out if it is one group of bees that produces both the piping and buzz-run signals inside a nest prior to a swarm's exodus, and if so, to determine who these bees are, one could label foragers at feeders or scout bees at nest boxes shortly before swarm departure (both forager bees and scouts would know when the weather conditions are ideal for swarm bees to leave the protection of the parental nest). Video analysis of the swarm exodus process could reveal whether the same bees produce

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both the piping and buzz-run signals, and whether it is foragers or nest-site scouts that produce the two signals.

The departure of a honey bee swarm from its parental nest provides a prime example of how a small minority of individuals in a social insect colony can operate as an oligarchy to make an important decision, i.e., when to leave the nest. We found that only a few percent of the bees in a colony appear to trigger the exodus on an entire swarm. Presumably, the individuals that initiate the mass departure of a swarm have special access to information about the conditions necessary for the group to leave the nest safely.

ACKNOWLEDGEMENTS

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CHAPTER 2

AN OLIGARCHY OF NEST-SITE SCOUTS TRIGGERS A HONEY BEE
SWARM'S DEPARTURE FROM THE HIVE *

ABSTRACT

Animals that travel in groups must coordinate the timing of their departures to assure cohesion of the group. While most activities in large colonies of social insects have decentralized control, certain activities (i.e. colony migration) can have centralized control, with only a special subset of well-informed individuals making a decision that affects the entire colony. We recently discovered that a small minority of individuals in a honey bee colony (an 'oligarchy') decides when to trigger the departure of a reproductive swarm from its hive. The process begins with some bees producing the worker piping signal (the primer for departure), followed by them producing the buzz-run signal (the releaser for departure). In this study we determined the identity of these signalers. Using a nest box and colonies in observation hives, we found that a swarm's nest-site scouts search for potential nest cavities prior to the departure of the swarm from its hive. Furthermore, we found that the predeparture nest-site scouts are the sole producers of the worker piping signal and that they are the first producers of the buzz-run signal. The control of the departure of a honey bee swarm from its hive is an example of how a small minority of well-informed individuals in a large social insect colony can make important decisions about when a colony should take action.

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INTRODUCTION

Animals that migrate in groups from one location to another need to synchronize the timing of their communal departure to assure group cohesion (Conradt & Roper 2005, Conradt & List 2009). The decision of when to move may be despotic, whereby one individual triggers the group's departure. This is the case in white-faced capuchins (*Cebus capucinus*), where an individual located in the edge of a stationary group initiates the troop's movement by producing specific trill vocalizations (Boinski & Campbell 1995). At the opposite extreme, the decision is reached through a democratic process by which a majority of the group's members agrees on when to move (Conradt & Roper 2003). For example, in herds of the African buffalo (*Syncerus caffer*) a unified gaze in a specific direction by the majority of adult females triggers the group's initiation of movement in that direction (Prins 1996). While most studies regarding group travel have shown that the process is usually initiated by a despotic leader or a democratic majority (Conradt & Roper 2003, 2005, 2007), a few reports have shown that the decision of when to move may be reached through an oligarchic process, in which a small minority of well-informed individuals initiates the move only when the conditions are ideal (Conradt & Roper 2003, Sumpter 2006). For example, in shoals of the fish *Notemigonus crysoleucas*, a small group of individuals determines when to move to find suitable foraging sites (Reebs 2000). Because much of the research on collective movement has focused on vertebrate species (reviewed in Couzin 1996, Boinski & Garber 2000, Conradt & Roper 2003, 2005, Conradt & List 2009), large gaps remain in our knowledge of group travel in invertebrate species (but see Franks et al. 2002, Pratt 2005, Buhl et al. 2006, Simpson et al. 2006). And regarding group travel by invertebrates, one especially intriguing mystery is whether oligarchic control of the timing of movement operates in the large societies of insects,

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where democratic (decentralized) control of group behavior is typical.

Social insects are attractive for the study of group movements because their large colonies need a high degree of group integration in order to move as a cohesive unit (reviewed by Dyer 2000). Inside a large colony of social insects there is usually little, if any, centralized control over daily activities. Instead, individuals respond to local information to make their own decisions (reviewed in Anderson & Mc Shea 2001, Camazine et al. 2001, Jeanne 2003). But while the mechanisms of group integration in daily colony tasks have been widely studied, more research is needed on how social insects initiate and coordinate their group movements (Forsyth 1981, Dyer 2000, Schultz et al. 2008, Latty et al. 2009). In this study, we investigated a striking example of group travel: the departure of a honey bee swarm from its hive.

Honey bee (*Apis mellifera*) colonies reproduce by fissioning, whereby a swarm, composed of the old mother queen and roughly two thirds of the worker population, leaves its hive en masse while the remaining workers and a new daughter queen stay behind (Winston 1987, Martin 1963). The swarm then clusters temporarily on a tree branch where it undertakes a complex house-hunting process. In a recent study of the signals that initiate a swarm's exodus, Rangel and Seeley (2008) discovered that starting about one hour before departure a few dozen bees start producing the worker piping signal, which involves a bee pressing her thorax against other bees while producing a high-pitched vibration of her flight muscles (Seeley & Tautz 2001, Seeley & Visscher 2004). This worker piping signal primes the swarm bees for flight. Then, starting about five to ten minutes before the swarm's departure, a few bees start producing the buzz-run signal, which involves a bee randomly butting into other bees while buzzing her wings (Martin 1963, Rittschof & Seeley 2008). This buzz-run signal triggers the bees to fly out of the hive. Having determined what signals are

used to initiate a swarm's exodus, we wanted to determine the identity of the bees that produce these signals and thus control the timing of swarm departure.

Once the bees in a swarm have left their hive and settled into a temporary cluster hanging from a tree branch, they show two markedly different patterns of behavior. The vast majority of the bees are relatively inactive and serve as food reservoirs that keep the swarm supplied with energy (Combs 1972). However, a small minority (only about five percent, Seeley et al. 1979), composed of workers with foraging experience, are active and serve as nest-site scouts that discover and inspect potential nest cavities (Gilley 1998). If a scout finds a high-quality cavity, she will return to the swarm and perform a waggle dance to recruit other bees to that location (Lindauer 1955, Seeley et al. 2006). The collective decision of where to move is reached through a process of competition among scouts visiting different sites, with scouts from the highest quality site producing the strongest waggle dances and thus attracting the strongest support for their site (reviewed in Seeley & Visscher 2004, Seeley et al. 2006, Visscher 2007). When a new home has been chosen, the nest-site scouts start producing the worker piping signal to prime the inactive bees in the swarm for flight (Seeley & Tautz 2001, Seeley et al. 2003, Visscher & Seeley 2007). When all the swarm bees have warmed their flight muscles to a flight-ready temperature (35°C), the nest-site scouts begin producing the buzz-run signal to release the liftoff of the swarm (Visscher & Seeley 2007, Rittschof & Seeley 2008).

Given the similarities in the behaviors used to initiate a swarm's departure from its hive, and those used to initiate a swarm's liftoff from its clustering site, we wanted to address four questions about the process of swarm departure. First, do nest-site scouts begin the house-hunting search before a swarm leaves its hive? Second, if the scouts start searching before a swarm leaves its hive, do they also begin recruiting other bees to potential home sites before leaving the hive? Third, do the scouts produce the

signals that trigger a swarm's departure from its hive? And finally, do most of the bees in a hive raise their flight muscle temperature to at least 35°C prior to swarm departure?

To figure out who are the bees that trigger a swarm's departure from its hive, we went to a location devoid of natural nest sites and installed in observation hives honey bee colonies that were preparing to swarm. We then provided an attractive nest box near the hives to see whether nest-site scouts would begin searching for a future home site prior to their swarm's departure. When we found that they would do so, we labeled them at the nest box and observed their behaviors both at the nest box and at the hive prior to the departure of the swarm, to see if they would produce the signals that trigger a swarm's sudden exodus from its hive. We also recorded the temperatures inside and outside the hive as the swarm bees prepared for and then performed their departure.

METHODS

Study Site

All work was done at the Shoals Marine Laboratory on Appledore Island, Maine (42°58'N, 70°37'W). This 39-hectare island lacks large trees with cavities, hence it has no natural nest sites for honey bees. We were thus confident that nest-site scouts from our observation hives would discover and show interest in the nest box we provided.

Set Up of Observation Hives

Three medium-sized honey bee colonies were brought from the mainland (Ithaca, New York), all headed by naturally mated New World Carniolan queens (*Apis mellifera carnica*; Strachan Apiaries, Yuba City, California, USA). On 1 July 2008, each colony was installed in a three-frame observation hive (described by Seeley 1995). The three frames chosen for each colony were covered with adult bees (approximately 6 000 workers plus the queen), and were roughly half full of brood and half full of pollen and honey. At least one frame had one or more queen cells, which the bees had started to produce in preparation for swarming back in June, the time of year when most swarms occur around Ithaca (Fell et al. 1977). Each hive was installed inside a barn and connected to the outside through an exit tube.

Before the glass sides were installed on the observation hives, we placed an electret condenser microphone (Radio Shack Model 33-3013, 70-16,000 Hz frequency response) at the center of the bottom frame so that we could hear the worker piping signals made by bees in preparation for swarming. We monitored the colonies daily, listening for piping signals every 15 min from 08:00 to 16:00 hours, and when the piping rate inside a colony became higher than 3 signals in 30 sec, we followed the colony closely until its swarm departed.

We also placed a copper-constantan thermocouple in the center of the middle frame to monitor the temperature inside each hive. We placed another similar thermocouple outside each hive to record ambient temperature. Both thermocouples were connected to a digital logging thermocouple thermometer (Model HH611A-PL4, Omega Engineering, Stamford, Connecticut). When the ambient temperature was high, many of the workers clustered outside their hive's entrance opening in a "beard" to provide more room in the hive for ventilation (Winston 1987). These beards

enabled us to monitor the sounds produced by bees when their colony was preparing to swarm.

Set Up of Nest Box

A 40-L nest box with a 12.5 cm^2 entrance hole (described by Seeley & Morse 1978) was located approximately 225 m from the observation hives. The nest box, which had most of the characteristics favored by nest-site scouts during the house-hunting process, was placed inside a small hut for protection from the sun and wind. It was located in a direction in which there were no other potential nest cavities so that we could distinguish dances for this nesting site from dances for other sites that the scouts might find. On the front wall of the nest box we placed a vial containing the blend of pheromones produced by the Nasonov gland, which bees normally release at their nest entrance as an olfactory guide to their home (Winston 1987), to expedite their fellow scouts' discovery of the nest box. The lure was removed after the first scouts arrived at the nest box.

Scout Marking and Data Collection at Nest Box

Once the observation hives and the nest box were established, we waited at the nest box for the arrival of the first scouts. After scouts began visiting the nest box, they were caught at its entrance in an insect net (15-cm diameter, 20-cm depth) either by placing the net over the entrance opening to catch bees as they exited, or by catching them in flight around the entrance. Every captured bee was labeled on the thorax with a dot of shellac-based paint (von Frisch 1967) and then was released from the net. We used a different paint color every day. Even though we had planned on

labeling every bee that visited the nest box, this goal was not achieved fully when scouts arrived in high numbers. Thus, not all visitors to the nest box were labeled. After the bee labeling begun, the person at the observation hives began checking each hive for labeled bees to know which colony to follow closely. Only one colony at a time had scouts at the nest box. The labeling continued until the colony of interest cast its swarm.

The following data were collected every 15 min at the nest box: (a) the number of bees labeled in the previous 15 min, (b) the number of bees seen outside the nest box, and (c) the proportion of the bees seen outside the nest box in a "snapshot" moment that were labeled. To measure the number of bees outside the nest box, we recorded the maximum number of bees visible in each of six consecutive 10-s blocks, and we took the mean of the six values. This census estimated the visitation rate of scouts throughout the day. The measure of the proportion of labeled bees gave us an estimate of the proportion of scout bees that returned to the observation hive unmarked. However, many of the bees that were unlabeled in one time interval were labeled subsequently.

Data Collection at the Observation Hives

Once the labeling of scout bees at the nest box begun, we monitored the activities of bees at the observation hives. We focused our attention on the colony whose scouts had begun scrutinizing the nest box. Throughout the day we scanned the surface of the colony's beard to take the following measurements every 15 min: (a) the number of bees producing the worker piping signal, (b) the number of bees performing the waggle dance indicating the location of our nest box, (c) the number of bees running rapidly, and (d) the number of bees producing the buzz-run signal. For all of the above

measurements, we noted which bees were labeled, which ones were unlabeled, and for which ones the possession of a color label was unknown (because we could not always see their color mark clearly, i.e., when the focal bee was moving under other bees). Additionally, we automatically recorded the temperatures inside and outside the focal observation hive every minute from approximately 1000 until 1700 hours.

Video and Audio Recording

Because the number of buzz-running bees increases dramatically during the final minutes prior to swarm departure (Rangel & Seeley 2008), we used video recordings to estimate the number of buzz-runners at the peak of the swarming process. To do this, we positioned directly in front of the beard a digital video camera (Sony DCR-TRV50), and connected to the camera an electret condenser microphone (Radio Shack Model 33-3013, 70-16,000 Hz frequency response) mounted in a Tygon tube of 40 mm length and 8 mm internal diameter that made the microphone directional. When we detected with the microphone at least two piping bees in the beard, we turned on the video camera and recorded the bees in the beard until the swarm's mass exodus was underway. The start of the swarm's departure was defined as the moment at which the bees on the surface of the beard began to take off. This was followed by a rapid outflow of bees from the hive.

Video and Data Analysis

The video analysis focused on the final minutes before the beard took off. We sampled each video recording at 1.0 min intervals (unless otherwise noted) and analyzed it at slow speed or frame-by-frame using a video editing deck (Sony DSR-

30). We scanned the entire screen of the video monitor from top-left to bottom-right to count (a) the number of buzz-runners, and whether these bees were labeled or unlabeled, or whether a bee's possession of a color mark could not be determined; and (b) the total number of bees on the video screen, for calculating what proportion of these bees were producing the buzz-run signal before and during the swarm's exodus. We scanned for buzz-running bees at slow speed from one second before to one second after each sampling time. We did so to see whether only a small minority of individuals in the colony triggered the departure of its swarm, and to see whether the bees that produced the buzz-run signal had previously been labeled at the nest box, hence were nest-site scouts.

RESULTS

Scouting Activity at the Nest Box

Scouts from colony 1 began to visit the nest box on 1 July, the evening of the day that the observation hives were set up. A total of 41 bees were labeled on 1 July, 155 bees were labeled on 2 July, and 266 bees were labeled on 3 July, the day of swarm departure. Thus, nest-site scouts from this colony searched for potential nest cavities prior to swarm exodus, and the scouts that found the nest box recruited other bees to this site before the swarm left the observation hive. Over the course of the day that the swarm departed, 3 July, the number of bees labeled at the nest box, and the average number of bees seen around the nest box, increased drastically (Fig. 2.1a, b). However, as the number of visitors increased, the task of labeling all of them became harder and by 10:30 some of the bees counted at the snapshot moments were unlabeled. The number of unlabeled bees seen at the nest box increased over time

Figure 2.1 Records of activity at the nest box and of the signals and aspects of mobility at the hive that changed in preparation for swarm departure in colony 1. Data were collected from the nest box (a-c), and from the surface of the colony's "beard" (d-f). Stacked bars (c-f) represent the number of bees counted that were either labeled (black) or unlabeled (white) when the data were collected. The number of bees seen around the nest box (b) is given as mean ± S.E bars. In this colony, the swarm departed from the hive at 12:52. The abbreviation "n.d." indicates that no data are available for a given sampling interval.

until the swarm departure begun (Fig. 2.1c). After the swarm bees had settled outside the observation hive and had been captured in a small hive, we opened the nest box to look for scout bees that may have stayed inside the nest box while the swarm was issuing from the observation hive. To our surprise, we found approximately 200 labeled bees inside the nest box. They flew off in the direction of the observation hives after they were shaken from the nest box.

Scouts from colony 2 begun to appear at the nest box on July 5. From this colony, we labeled 2 bees on 5 July, 4 bees on 6 July, and 79 bees on 7 July, the day that colony 2 swarmed. Although the number of visitors to the nest box was smaller for colony 2 than for colony 1 in the days prior to swarm departure, nest-site scouts did recruit bees to the nest box in high numbers on the day of swarm departure.

Scouts from colony 3 discovered the nest box on 8 July. We labeled 351 bees on that day and 221 bees on 9 July, the day of swarm departure. In this colony we did not observe a crescendo of visitors to the nest box like what we saw on the day of swarm departure in colony 1 (Fig. 2.2a-c). However, most of the bees that were counted at the snapshot were labeled (Fig. 2.1c), perhaps because fewer bees from colony 3 visited the nest box compared to colony 1.

Signaling Activity at the Observation Hive

The activity of bees on the surface of colony 1's beard increased throughout the day on 3 July, until the swarm initiated its departure at 12:52. The number of bees producing waggle dances advertising the nest box rose over time, although many of the dancers were unlabeled, especially early in the day (Fig. 2.1d). The number of bees running across the beard escalated dramatically from fewer than 4 per scan before 10:00, to nearly 20 per scan in the hour prior to swarm departure. The vast majority of
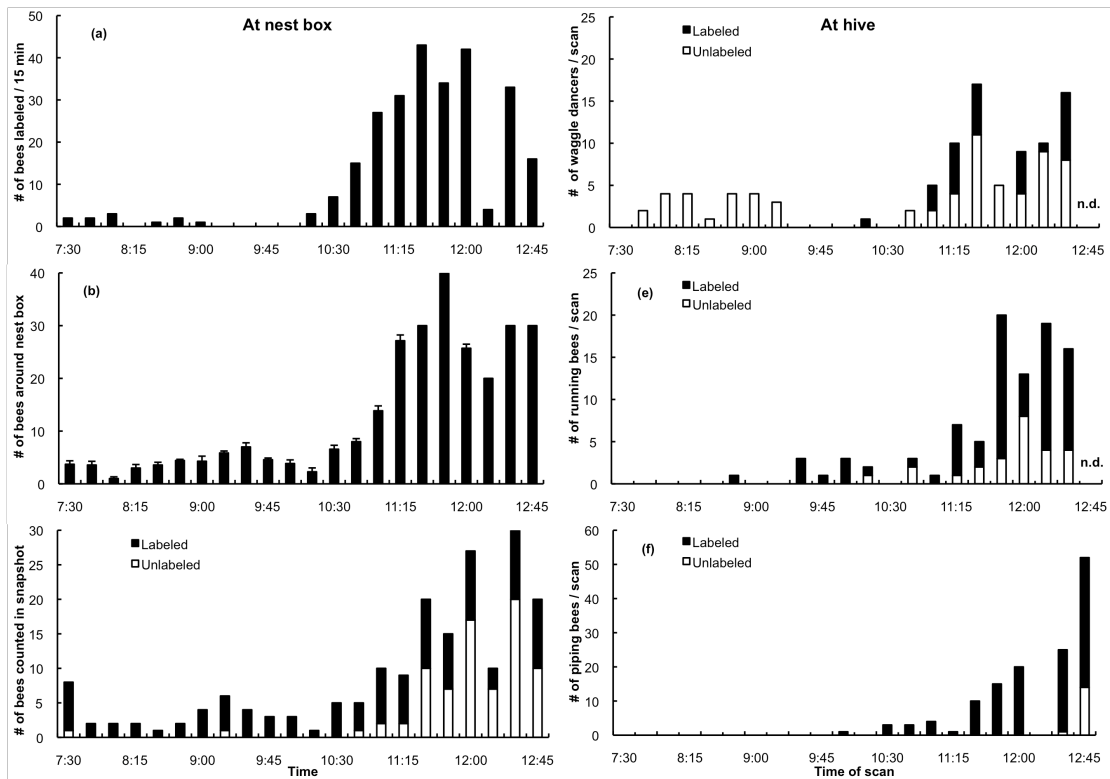
41
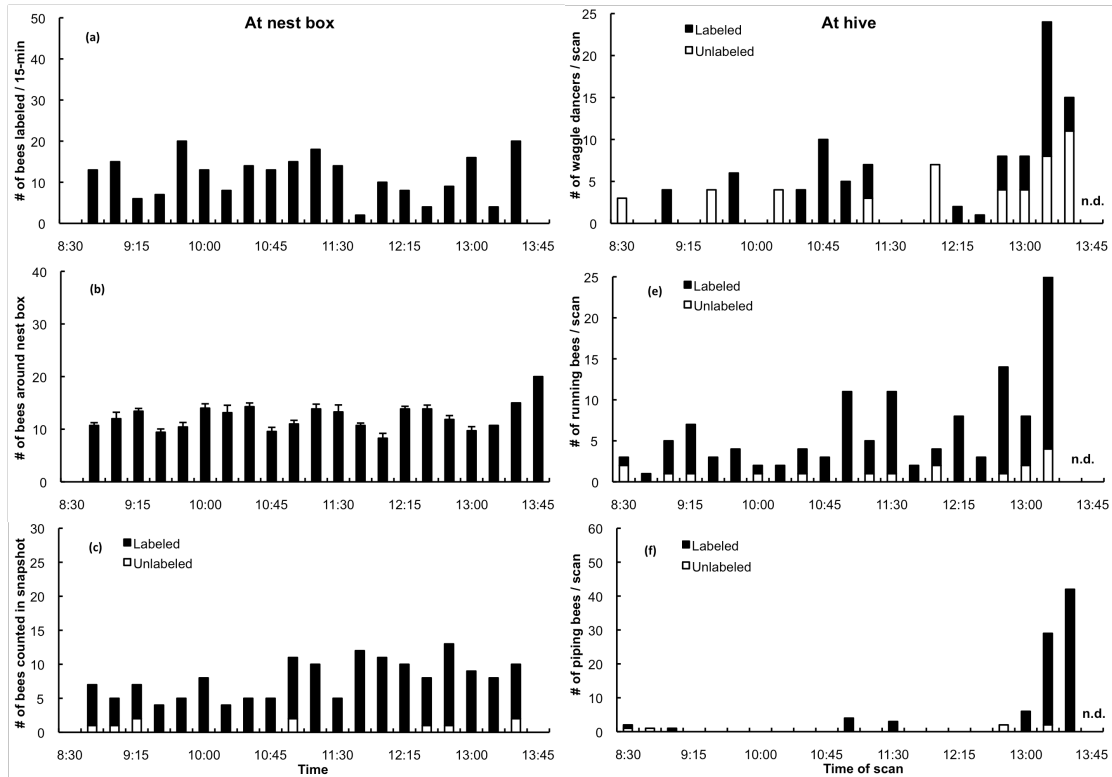
Figure 2.2 Records of activity at the nest box and of the signals and aspects of mobility at the hive that changed in preparation for swarm departure in colony 3. Data were collected from the nest box (a-c) and from the beard's surface (d-f). Stacked bars (c-f) represent the number of counted bees that were either labeled (black) or unlabeled (white). The number of bees seen around the nest box (b) is given as mean ± S.E bars. The swarm departed from the hive on 9 July 2008 at 13:45. The abbreviation "n.d." indicates that no data are available for a given sampling interval.

bees running on the surface of the beard were labeled (Fig. 2.1e). Piping bees first appeared at 10:00, more than two hours before the swarm's exodus, and their numbers rose to 52 at 12:45, the last scan time before the swarm departed at 12:52. For each scan time, all the piping bees were labeled except for the last sampling interval in which 14 out of 52 of the pipers were unlabeled (Fig. 2.1f).

Colony 2 swarmed on 7 July at 10:58. Because colony 2 was less populous than the other two colonies it did not create a beard outside the observation hive, so we were unable to watch for dancing bees and listen for piping bees on the surface of a beard.

In colony 3, waggle dancers were observed on the surface of the beard as early as 08:30 on 9 July, the day of swarm departure, and their numbers increased over time until the swarm left the observation hive at 13:45 (Fig. 2.2d). The number of bees running across the beard stayed relatively low (no more than 11 per scan) throughout the day until a half hour before swarm departure, when it rose rapidly to 25 bees at 13:15. The vast majority of the bees running on the beard's surface were labeled (Fig. 2.2e). Finally, the piping signal was heard sparingly as early as 08:30. Almost every piping bee was labeled. The number of piping bees rose to 42 at 13:30, the last scan time before the swarm departed at 13:45 (Fig. 2.2f).

When we measured the proportion of the bees that triggered the swarm's departure, we found that the total number of bees visible on the video screen before the exodus was about 300 bees in colony 1 and nearly 600 bees in colony 3. We also found that there were fewer than 15 buzz-runners in colony 1 and fewer than 20 buzz-runners in colony 3, when the swarm departure started. Therefore, less than five percent of the total number of bees seen on the video screen had begun to buzz-run before the start of the exodus (Fig. 2.3). For both colonies, the proportion of buzz-runners increased to nearly 60 percent of the bees appearing on the video screen in the

middle of the swarm's departure. The first producers of the buzz-run appeared about 10 minutes prior to the swarm's departure, and many bees in this small minority were labeled individuals. As the takeoff progressed, a higher number of unlabeled individuals started to buzz-run, so that by the peak of the exodus, the vast majority of individuals performing the buzz-run were unlabeled (Fig. 2.3).

Temperature Changes Inside the Colony

Fig. 2.4 shows the patterns of temperature change inside and outside the three observation hives used in this study. On 3 July, the day of swarm departure for colony 1, the outside temperature remained relatively unchanged at about $25^{\circ}C$ throughout the recording period. However, the temperature inside the hive increased from about $35^{\circ}C$ to about $38^{\circ}C$ over the half hour prior to the swarm's departure, with a temperature of $38.5^{\circ}C$ when the bees were exiting the hive en masse (Fig. 2.4a).

The outside temperature on 6 July, the day of swarm departure for colony 2, increased from $23.8\,^{\circ}C$ over an hour prior to the exodus to no higher than $25.1\,^{\circ}C$ during the departure. In contrast, the temperature inside the hive increased from $31.9\,^{\circ}C$ at 09:40, to $35.6\,^{\circ}C$ at the beginning of the swarm's departure at 10:58, reaching a maximum temperature of $37.3\,^{\circ}C$ at the end of the mass exodus.

The air was warmer on 9 July, the day of swarm departure for colony 3. On this day, the temperature outside the hive was $26.9\,^{\circ}C$ at 11:30, and rose to almost $29^{\circ}C$ during the swarm's exodus. Inside the hive, the temperature rose from $34.6\,^{\circ}C$ to $36.9^{\circ}C$ over the two hours prior to the beard's take off, reaching a maximum temperature of $37.8\,^{\circ}C$ in the minutes following the start of the swarm's departure (Fig. 2.4c).
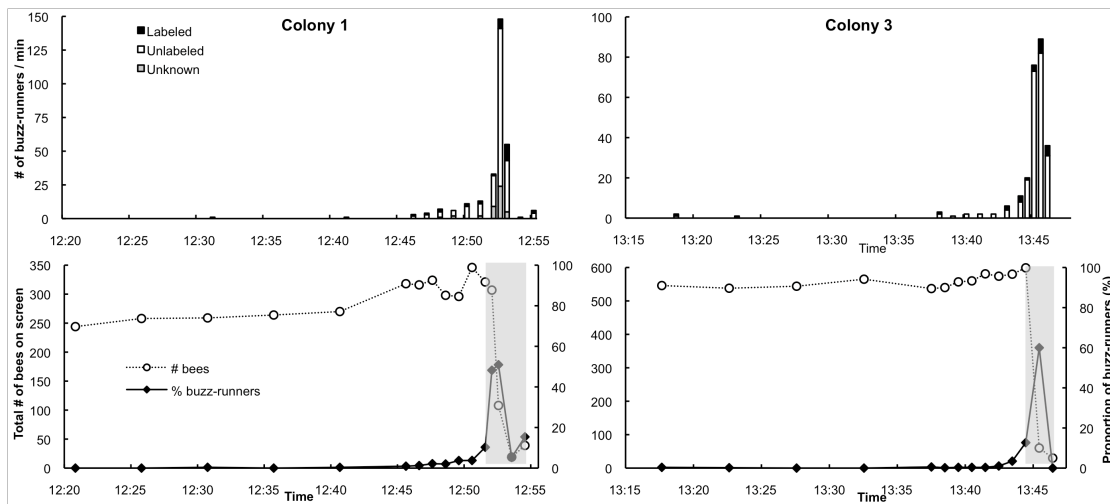
Figure 2.3. The number of buzz-runners before and during the swarm's departure in colonies 1 and 3 (top graphs). Stacked bars represent the number of buzz-runners observed on the surface of the beard that were labeled (black), unlabeled (white) or unknown (gray, if we were unable to detect the presence/absence of a color mark on the buzz-runner). Also shown are the total number of bees seen on the video screen before and during (grey box) the swarm's departure, and the proportion of these bees that produced the buzz-run signal over time for colonies 1 and 3 (bottom graphs).
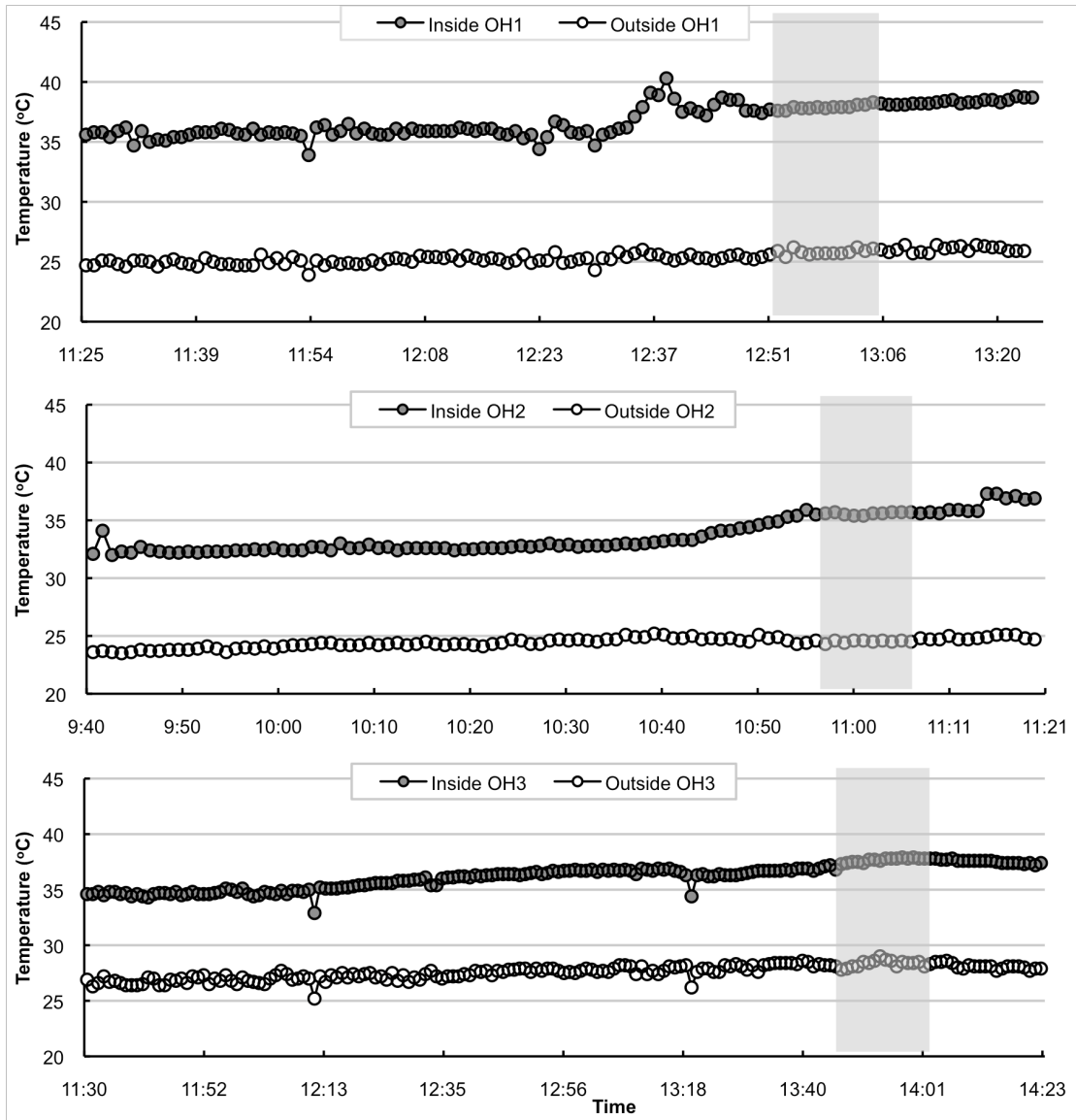
Figure 2.4. Patterns of temperature change inside (gray circles) and outside (white circles) three honey bee observation hives (a-c) starting approximately two hours before a swarm departed from each hive. Black arrows indicate the time at which each swarm initiated its departure. Gray boxes indicate the duration of each swarm's departure.

DISCUSSION

Nest-Site Scouts Become Active Before the Swarm's Departure

Our results indicate that nest-site scouts belonging to honey bee colonies that are preparing to swarm may start the house-hunting process prior to a swarm's departure from the parental hive. Our observations also show that when nest-site scouts are interested in a discovered site, they return to the hive and recruit nestmates to that site by performing waggle dances.

Similar observations of nest-site scouts starting the house-hunting process a few days before their swarm departed its parental hive were previously reported by Lindauer (1955). He performed two experiments to answer the question "when does a bee become a scout?" The first experiment was conducted on a North Friesian island where only artificial nest sites were available, much like the conditions of our experiment on Appledore Island. Lindauer brought to the island a hive of bees that was ready to swarm and watched to see if nest-site scouts would start inspecting his nest boxes even before the swarm issued from the hive. The swarm departed from the hive four days after it was brought to the island, and a few scouts were observed exploring the nest boxes three days before the swarm emerged from the hive. Lindauer further noticed that nest-site scouts were not only inspecting his nest boxes, but were also examining other possible nest sites around the island: holes leading to cavities between rocks. He concluded that scouts may begin the search for nest sites prior to a swarm's departure, but that they do not choose their future home site before the swarm leaves the nest.

In a second experiment in which Lindauer watched nest-site scouts spring into in action before their swarm left its hive, he discovered that after nest-site scouts had

successfully found the nest box he provided, many performed waggle dances inside the hive to recruit other bees to the box. He reported that 95 scout bees were seen dancing for a particular nest box the day of swarm departure, and that after the swarm left the hive it clustered for only 15 min before it launched into flight and moved into the nest box. Thus, Lindauer found that in certain situations, such as having discovered a high-quality nest site in a location lacking many options, the nest-site scouts in a colony may develop a strong preference for a particular future nesting site even before the swarm departs its home hive. Lindauer noted that this might be especially likely to occur with afterswarms (i.e. smaller swarms that issue from the hive in the weeks following a primary swarm's departure), which often fly off soon after clustering on a tree branch.

Scout Bees Produce the Signals that Initiate the Swarm's Departure

Our study shows that when a swarm leaves its parental hive, it is the nest-site scouts who produce the signals that trigger the exodus, being the sole producers of the worker piping signal that primes the bees for departure, and the first producers of the buzz-run signal that triggers the bees to swarm out of their hive.

Similar to our results, in a study of how a bivouacked swarm initiates its flight to a new nesting cavity, Seeley and Visscher (2003) found that when at least 10-15 bees are together outside a possible home site, the scouts there sense that a quorum has been reached at that site and when they return to the swarm, these bees start producing the piping signal to prime the swarm bees for liftoff and flight to the chosen site. The piping signal serves to activate quiescent bees on the swarm to raise their flight muscle temperature to at least 35°C, the minimum temperature needed for rapid flight (Heinrich 1981, Seeley & Visscher 2003). In a follow up study, Visscher & Seeley

(2007) discovered that nest-site scouts from the chosen site are the only producers of the piping signal. To perform this study, they set up an artificial swarm and nest boxes on Appledore Island, and they labeled nearly every scout visiting the chosen nest box. Once the liftoff preparations began and the first piping bees appeared on the swarm, they saw that the proportion of piping bees that were labeled matched or exceeded the proportion of the nest-site scouts at the chosen nest box that had been labeled. Thus they concluded that nest-site scouts from the chosen site produce the signals that initiate a swarm's liftoff.

Our results are also consistent with those of Rittschof and Seeley (2008), who discovered that on clustered swarms that are preparing their liftoff to fly to a new home, the bees that produce the piping signal later produce the buzz-run signal. Thus, nest-site scouts are not only the sole producers of the piping signal (Visscher & Seeley 2007), but they are also the producers of the buzz-run signal (Rittschof & Seeley 2008). Once the swarm takes flight, it forms a cloud of swirling bees in which scouts act as "streakers" who guide the rest of the swarm in the direction of the chosen site (Beekman et al. 2006, Schultz et al. 2008, Latty et al. 2009).

Colony Temperature Rises Prior to Swarm Departure

We also found that the temperature inside a colony that is preparing to swarm increases to above 35 $^{\circ}$C shortly before it casts a swarm. Colony 2 provided a clear example of this, increasing its internal temperature by as much as 5°C (from 32 $^{\circ}$C to 37 $^{\circ}$C) as its bees prepared to leave and then many of them left in a swarm. In all three colonies the temperature inside the hive was lower than, or had barely reached, 35 $^{\circ}$C in the hour prior to the swarm's departure, but increased by at least 3 $^{\circ}$C by the time the swarm had begun its departure. In each colony, the rise in internal temperature

reached its peak when the level of activity in the colony began to climax. Given that piping bees begin to appear in the hour or so prior to the swarm's departure, which coincides with the time at which the temperature inside the colony begins to rise, it is likely that the swarm bees start to warm up when they are contacted by pipers in preparation for the swarm's departure from the hive.

We know that the piping signal is produced by nest-site scouts to warm up quiescent swarm-mates as they initiate their liftoff from the clustering site. As reported by Seeley and Tautz (2001), the increase in production of the piping signal on swarm clusters over time clearly matches the increase in the swarm's temperature. In fact, when piping bees were prevented from contacting a subset of bees in the surface of a swarm, it was found that the uncontacted bees did not warm up to the threshold temperature of $35^{o}C$. In a separate study, Seeley et al. (2003) used a thermographic camera to measure the thoracic temperatures of all the bees on the surface of a swarm cluster, and they found that in the 10 min before the swarm's liftoff, 100 percent of the bees had warmed their thoraces (by shivering) to at least $35^{o}C$. Our results are consistent with these findings, for in all three colonies the swarm did not depart until the colony had reached an internal temperature of at least $35^{o}C$. However, we can only suggest that bees about to swarm shiver to increase their body temperature as a result of being contacted by pipers, because we did not measure the thoracic temperatures of individual bees. Other factors, such as the increased production of heat by bees moving faster across the comb, cannot be ruled out as possible contributors to the observed rise in the colony's internal temperature shortly before swarming.

Swarm Departure is a Process Coordinated by an Oligarchy

This study provides evidence that inside a honey bee colony that is preparing to swarm, a tiny minority of well-informed individuals, the nest-site scouts, initiate the swarm's departure from the parental nest. This oligarchic mechanism of decision-making ensures that only individuals who are knowledgeable about the conditions both inside and outside the hive make the important decision of when is the right time for the swarm to leave the parental hive.

Even though a few of the piping bees observed on the beard surface were not labeled, we are confident that all the piping bees were nest-site scouts, for we were unable to label all the nest-site scouts. We did notice, however, that unlike in the study by Rittschof and Seeley (2008), which found that all the piping bees turned into buzz-runners and both of these signals were performed by the same nest-site scouts, our study suggests that in the context of a swarm's departure from the parental hive, the buzz-run signal is used in a slightly different manner. It seems likely that the explosive departure of the swarm is first released by nest-site scouts that produce both the piping signal and the buzz-run signal, but that the colony later implements "relay communication" to expedite the departure process. In this type of information transfer, first suggested by Wilson (1971, p. 270), the receivers of a signal start producing the signal themselves in a chain reaction that grows exponentially, thus propagating the signal such that the threshold level that is needed to take action (in our case, the departure of a swarm from its hive) is reached in a short time. Similarly, the bees in a colony that is preparing to cast a swarm may show a "quorum response" (Sumpter & Pratt 2009) such that the probability that a worker starts performing the buzz-run behavior is a nonlinear function of the number of other workers already producing this signal. This could explain why at first many of the buzz-runners in our

study were labeled and thus were nest-site scouts, but as the preparations for departure progressed, the vast majority of the buzz-runners were unlabeled and thus probably were not nest-site scouts.

Few studies have reported an oligarchic control of group travel. One example comes from a study of domestic cattle (*Bos taurus*). Ramseyer et al. (2009) reported that a group's movement is preceded by a 30-min period of preparation in which the activity level (i.e. head movements, number of awake heifers, and alignment of bodies) of a small number of individuals increased. The initiation of a departure depended on the behavior of a few first movers, and on how well they relayed this information to other group members. This led to a higher number of individuals behaving similarly, ultimately releasing the entire group's move.

Even fewer studies have reported instances in which a group's decision is made by an oligarchy in a social insect species. For example, during nest-site selection in rock ants (*Temnothorax albipennis*), a few knowledgeable scouts make the decision of when their colony should migrate. When a site of good quality is discovered, scouts begin to recruit nestmates by conducting tandem runs, followed by the active transportation of brood and nestmates to the new location. Thus, nest-site selection is centralized in that a small minority of the colony's population (i.e. nest-site scouts) assesses the discovered sites, recruits a higher number of nestmates to the locations of higher quality, and ultimately initiates the colony's migration when it senses that the conditions needed to move are right (Mallon et al. 2001, Pratt et al. 2002). Another interesting example occurs during display tournaments for territorial disputes between colonies of the honey ant *Myrmecocystus mimicus*. In this species, it appears that a few individuals conduct large-scale assessments of their colony's relative strength, and if they sense that their colony is outnumbered, then they will leave the tournament area

to return to their colony's nest and recruit other workers as reinforcements (Hölldobler 1981, Lumsden & Hölldobler 1983).

The present study reports how, in the context of colony fissioning in honey bees, an oligarchy of workers exerts control over a colony-wide action. It shows that a relatively small group of nest-site scouts searches for, and recruits to, potential nest sites prior to a swarm's exodus from the parental hive. It also shows that these nest-site scouts control the timing of the swarm's departure by producing the signals that initiate the mass exodus. To better understand the importance of oligarchic control, we need more studies that focus on the possibility that, when appropriate, a small minority of individuals in a social insect colony monitors the state of their colony and makes the decision of when to take action.

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CHAPTER 3

NEST-SITE DEFENSE BY COMPETING HONEY BEE SWARMS DURING

HOUSE-HUNTING *

ABSTRACT

Cavity-nesting animals must often defend their homes against intruders, especially when the availability of suitable cavities is limited. Competition for nest sites is particularly strong when multiple groups of the same species migrate synchronously to found a new home. This may be the case for honey bees during the reproductive season, because neighboring colonies often cast swarms simultaneously, leading to potential competition for high-quality nesting cavities. To test the idea that honey bee swarms may compete for and defend potential nest sites as they search for a new home, we twice observed a pair of artificial swarms that were house-hunting concurrently. Workers from one swarm in each pair carried a gene influencing body color, so that the bees from the two swarms were easily distinguished. We set up a high-quality nest box and waited for nest-site scouts from each swarm to explore and recruit swarm-mates to it. We recorded all the interactions between competing scouts at the nest box and found that, when scouts from both swarms explored the box simultaneously, they behaved agonistically against bees from the other swarm. The level of aggression depended on the number of scouts from each swarm present at the nest box. When only 1-3 scouts from each swarm were at the box, they rarely fought. But when the scouts from one swarm outnumbered those from the other swarm (4-20 vs. 1-3 bees), those in the majority advertised their presence with a buzzing behavior at the entrance opening, and started mobbing and killing those in the minority. When one swarm gained clear control of the nest box (20+ vs. 0-1 bees), some of its scouts guarded the box's entrance, preventing entry by foreign scouts. Our study exemplifies how cavity-nesting animals may compete for and defend suitable nesting sites.

INTRODUCTION

Competition for nest sites in cavity-nesting species can be strong, especially when the availability of suitable sites is low and multiple individuals are seeking a home in a given region with similar qualities. Competition for limited nest sites is widely observed across the animal kingdom. For example, burrow-dwelling fish show strong male-male competition for suitable nesting burrows to increase a males' breeding success (Kroon et al. 2000, Lindström & Pampoulie 2004). This is the case of the smallmouth bass, *Micropterus dolomieu*, where strong interactions between resident males and other male intruders are costly due to the high risk of injury (Wiegmann & Baylis 1995, Iguchi et al. 2004). In many hole-nesting birds, competition for limited cavities is so strong that the population density trends of competing species are inversely related such that the density of a subordinate species increases as the density of the dominant species declines (Newton 1994, Banda & Blanco 2009). For example, in the collared flycatcher, *Ficedula albicollis*, competition with birds of other species for nest sites is substantially greater than competition with them for food (Krist 2004). Some communally nesting reptiles, such as the green iguana (*Iguana iguana*), compete aggressively for nesting burrows (Rand 1968) and invest considerable energy in disputes over nest sites not only against conspecifics (Rand & Rand 1976), but also against other reptilian intruders (Dugan et al. 1981).

Competition for nest sites is also common in insects. In the digger wasp *Cerceris binodis*, resident males defend pre-existing burrows containing nesting females by standing adjacent to the cavities and excluding conspecific non-resident males (Banks 1995), while in *Cerceris fumipennis*, females engage in aggressive interactions during nest usurpation, with larger females typically displacing smaller ones from their burrows (Mueller et al. 1992). In the burrowing bee, *Amegilla dawsoni*, nest

usurpation is common and female residents usually antagonize and outcompete female intruders regardless of their body size (Alcock et al. 2006). And in the ant *Temnothorax nylanderi*, suitable nest sites are typically taken over by extremely aggressive colonies, since nest sites are a highly limited resource (Foitzik & Heinze 1998).

Honey bees (*Apis mellifera*) live in colonies that reproduce by fissioning, whereby roughly two thirds of the worker population leaves the nest with the mother queen as a swarm, while the remaining one third of the workers stays behind with a new queen in the old nest (Martin 1963, Winston 1987). In a process that takes less than an hour, the swarm prepares for and then performs its departure with a special subset of workers, the nest-site scouts, producing the signals that initiate the swarm's mass exodus (Rangel & Seeley 2008, Rangel et al. 2009). Prior to the swarm's departure, the nest-site scouts, who comprise at most five percent of the swarm (Seeley et al. 1979), begin the house-hunting process by discovering and inspecting potential nesting cavities (Gilley 1998, Rangel et al. 2009). Once the swarm leaves the hive, it settles and then temporarily hangs from a tree branch where the search for a new nesting site continues with scouts exploring various sites of different qualities. When a scout finds an attractive cavity, she returns to the swarm and performs a waggle dance to recruit swarm mates to that site (Lindauer 1955, Seeley et al. 2006). The collective decision of where the swarm will move is reached through a process of competition among scouts that are visiting sites of different qualities, with the more attractive sites stimulating scouts to perform stronger waggle dances, leading to the fastest recruitment of uncommitted scouts to the site of highest quality (reviewed in Seeley and Visscher 2004, Seeley et al. 2006, Visscher 2007). When a decision of where the swarm will move has been reached, the nest-site scouts start producing the working piping signal, which primes the inactive bees for flight (Heinrich 1981, Seeley and

Tautz 2001, Seeley et al. 2003, Visscher and Seeley 2007). Once all the bees in the swarm have warmed their thoracic muscles to at least 35°C, the temperature needed for flight, the scouts start producing the buzz-run signal, which releases the swarm's liftoff to fly to its new home (Visscher and Seeley 2007, Rittschof and Seeley 2008).

Because in temperate latitudes swarming occurs mainly in late May or early June (Winston 1987), and usually on days with warm and sunny weather, it is very common that neighboring honey bee colonies cast swarms at approximately the same time. For this reason, it is likely that bivouacked swarms have to compete for nesting cavities with other nearby swarms. In this study we wanted to see whether multiple honey bee swarms that are house-hunting concurrently compete for and defend nest sites.

In a recent study, we used honey bee colonies housed in observation hives to determine the identity of the signalers that initiate a swarm's exodus from its hive. By labeling nest-site scouts at a nest box located a few hundred meters from the hive, and watching closely the scouts back at the hive, we found that it is nest-site scouts who perform the signals that initiate the swarm's mass exodus (Rangel et al. 2009). In two of the three trials conducted in this study, we also observed that over two hundred nest-site scouts remained inside the nest box while the swarm was leaving its hive. When we opened the box, we noticed that some of these bees were assuming the characteristic stance of bees that guard a nest entrance (described by Seeley 1985). These observations prompted us to ask in the present study the following questions regarding nest-site defense between competing swarms: During the house-hunting process, do scouts from multiple swarms ever inspect the same nest site at the same time? If they do, what types of interactions are observed at the site? What levels of aggression, if any, are observed between scouts from different swarms at the same nest site?

To determine whether honey bee swarms compete for and defend high-quality

nesting cavities, we went to a small island devoid of natural nest sites and set up a pair of artificial swarms whose members were visually distinguishable. We then provided the two swarms with one attractive nest box and observed whether nest-site scouts from both swarms would explore the nest box at the same time. When we found that they would, we recorded all the interactions at the nest box between scouts from both swarms. Over several days, we noted the number of individuals from each swarm present at the nest box and the types of behaviors they performed, identifying the attacker(s) and the victim(s) in all agonistic encounters.

METHODS

Study site and bees

All observations were made at the Shoals Marine Laboratory on Appledore Island, Maine (42°58'N, 70°37'W). This wind-swept, 39-hectare island lacks large trees with cavities and thus is devoid of natural nest sites for honey bees. This gave us confidence that nest-site scouts from our swarms would discover and become interested in the nest box we provided. Four medium-sized honey bee colonies were brought from the mainland (Ithaca, New York) in small hives. Two colonies were headed by naturally mated New World Carniolan queens (*Apis mellifera carnica*; Strachan Apiaries, Yuba City, California) whose workers had a dark brown body color. The other two colonies were headed by cordovan queens that were artificially inseminated with sperm from cordovan drones using standard queen insemination procedures (Glenn Apiaries, Fallbrook, California). The workers of these queens were homozygous for the cordovan allele and so were visually distinctive; a bee that is homozygous for the recessive cordovan allele has a light-brown or "blonde" body

color, unlike the dark-brown or black body color of non-cordovan, wild-type bees (e.g. see Taber 1955).

Swarm preparation

We prepared an artificial swarm from each of the colonies brought from the mainland, but used only two colonies (one cordovan, one wild-type) at a time. To make an artificial swarm, we first located the colony's queen and placed her in a small cage (3.2x10x1.6 cm). Then, we used a metal funnel to shake 1.0 kg of bees (approximately 7 700 workers, Mitchell 1970) into a swarm cage (15x25x35 cm) made of wood and covered on its two largest sides by wire screen to give the bees access to food and air. We placed the caged queen inside the swarm cage before sealing it, and kept the swarm cage in the shade. We fed each swarm ad libitum with a 50% (vol/vol) sucrose solution for 48-72 hours, when copious wax scales were deposited by the bees on the floor of their cage. After this, the swarm cage was unsealed, the queen cage was attached to a swarm mount (see "Set up of swarms"), and the workers were shaken onto the base of the mount. In less than an hour the workers were clustered around the queen cage in the center of the swarm mount. Like in natural swarms, scouts from our artificial swarms began searching for prospective nesting cavities.

Set up of nest box

A nest box like that described by Seeley and Visscher (2003) was established 225 m from the swarms. The nest box was specially designed to enable us to observe the behaviors of bees both outside and inside the box. It was built with 2.0-cm-thick

plywood, and was shaped as a cube that provided a 27-l cavity. The box had a 2.5-cm diameter entrance hole at the center of one of its side walls, and had another side wall left open. The nest box was bolted by its four corners to the outside of an observation hut so that the open wall of the nest box was aligned with a same-size opening on one of the hut's sides. A sheet of 3.2-mm-thick glass was placed over the opening so we could sit inside the hut and watch scout bees inside the nest box without disturbing them. The observation hut (244x113x113 cm) was constructed of plywood and assembled with bolts. Because all of the hut's interior surfaces were painted black, it provided a dark room inside which we observed bees in the nest box. Enough light for observing bees came in the nest box's entrance opening, but occasionally a flashlight was used to observe certain behaviors in better detail. Outside the entrance of the nest box, to expedite its discovery by nest-site scouts, we hung a vial containing the blend of pheromones produced by the honey bee's Nasonov gland (Bee Lure, Brushy Mountain Bee Farm, Moravian Falls, North Carolina), which bees usually release at their nest entrance to attract nest mates to their home (i.e. "scenting behavior," Winston 1987). The lure was removed as soon as scouts began visiting the nest box.

Set up of swarms

For each trial, we set up two swarms, one cordovan and one wild-type. Each swarm was placed on a swarm mount as described previously by Seeley and Buhrman (1999, Fig. 1), except that we did not use a wire screen to cover the swarm's surface. Each mount consisted of a flat vertical board large enough for the bees to spread out upon as they clustered around the queen cage. This allowed us to monitor easily the bees on the swarm's surface. The swarms were set up beside the front and the back porches of the island's old Coast Guard building, approximately 20 m apart.

Data collection at the nest box

After the two swarms and the nest box were set up, one of us waited at the nest box for the arrival of the first scout bees. Once scouts started to appear at the nest box, the observer there radioed to the person monitoring the swarms the body color of the scouts at the box. For each trial, the date and time of arrival of the first scouts from each swarm were noted. Once scouts from one or both swarms began to appear at the nest box, we recorded every 15 min (unless otherwise noted) the number of scouts from each swarm seen both outside and inside the nest box. To help us better describe the behaviors performed by scouts at the nest box, we videotaped these behaviors using a digital video camera (Sony DCR-TRV50).

When two or more bees interacted at the nest box, we recorded the dates and times of the encounters and whether the interactions were: (a) between swarm-mates, when scouts from the opposite swarm were not present at the nest box, (b) between swarm-mates, when scouts from the opposite swarm were present at the nest box, or (c) between members of opposite swarms. When agonistic interactions occurred, we collected the following data: (a) the date and time of each interaction, (b) the type of the interaction, with a detailed description of all the behaviors, (c) the number of bees from each swarm involved in the interaction, and (d) to which swarm the attacker(s) and the victim(s) belonged. If bees were killed inside the nest box, the date, time, and other details about the death were recorded. We concluded each trial when sufficient interactions between scouts from both swarms had been recorded at the nest box.

Experimental manipulations at the swarms

Because scout bees that find potential nest sites return to the swarm cluster and

perform waggle dances to recruit other bees to their discoveries (Lindauer 1955), we checked for bees producing the waggle dance on the surface of each swarm every 15 min from approximately 0800 to 1700 hours (unless otherwise noted) on each day of the study. If a bee was dancing for a site other than the nest box, she was plucked off the swarm with forceps and killed, to prevent recruitment of bees to the non-nest-box site. When a scout appeared at the nest box, the person at the swarms watched to see if she would later produce a waggle dance advertising the nest box. If the scout danced, the person at the nest box was informed that recruits were likely to appear there soon.

On days when bees belonging to one swarm began to appear in high numbers (more than 10) at the nest box before bees from the other swarm had discovered the box, the person monitoring the swarms temporarily shut down the first swarm's scouting activity by sealing it off. To do this, an "envelope" made of mesh fabric was placed over the swarm, preventing bees from returning to or leaving from the swarm. The envelope was kept 5 cm from the surface of the swarm by a wire frame that was attached to the swarm mount. The envelope was removed when bees from the other swarm began to appear at the nest box. After this, scouts from both swarms visited the nest box simultaneously. When we concluded a trial, each swarm was shaken into a hive at dusk and taken to a beeyard several hundred yards from the location of the swarms. Each swarm ceased its scouting after it was installed in a hive.

Data analysis

The information that we report comes only from days on which we observed interactions at the nest box between scouts from both swarms. Video recordings were made to help us describe the scout bees' behaviors. Each video recording was made

by following an individual bee and was analyzed at slow speed or frame-by-frame using a video editing deck (Sony DSR-30). For each video recording, we noted the duration of each behavior, the distance traveled by the bee (in mm), and information about the context in which the behavior was produced.

RESULTS

We performed two trials with a different pair of swarms in each trial. In each trial, we recorded interactions at the nest box between scout bees from both swarms. The types of interactions and their durations depended on the number of bees from each swarm present at the time of the encounter; more aggressive interactions occurred when one swarm's scouts strongly outnumbered those from the other swarm and thus had control over the nest box. The types of agonistic behaviors observed at the nest box are described in Table 3.1. The less aggressive behaviors were guarding and chasing; the more aggressive behaviors were leg-pulling, grappling, stinging, and mobbing (Table 3.1). The interactions that occurred in each trial are summarized below.

Trial 1

We set up the nest box and the first pair of swarms on 20 June 2009. For the next 4 days the weather was rainy, cold, and windy, and scout bees did not fly from either swarm's cluster. On 25 June the weather finally improved and scouts from the wild-type swarm began to visit the nest box. When only 2 or 3 wild-type bees were at the nest box, each one explored the box without interacting with her swarm mates. When more than 3 bees were present, some of them began to produce a curious nest-site buzzing behavior, which we describe below. When the number of wild-type scouts at

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the nest box grew large (i.e. >26 bees inside, 5 bees outside), we observed guarding behavior. At the end of the day, the number of wild-type bees inside the nest box had increased to above 60 and the nest-site buzzing behavior had stopped.   After dusk, we found one wild-type bee and one cordovan bee dead inside the nest box. Possibly, they had stung each other.

We recorded additional interactions between scouts from the two swarms on 26 June. Early on 26 June, we sealed off the wild-type swarm using the swarm cover described above, to facilitate the discovery and exploration of the nest box by cordovan scouts. Some wild-type scouts had begun flying to the nest box before we sealed off their swarm, however, and by mid-morning we observed numerous wild-type scouts at the nest box (approx. 30 bees), with at least 6-8 bees guarding the entrance. At 1020 h we noticed one cordovan bee approaching the nest box and attempting to enter it, but wild-type scouts guarding the box repeatedly repelled her. At approximately 1130 h we began removing wild-type bees from the nest box with an aspirator. This worked and by 1200 h one cordovan bee had entered the nest box, at which time there was only one wild-type bee there.   Later that day, when there were equally low numbers of bees from both swarms at the nest box (i.e. 1-3 individuals), one-on-one aggressive interactions were observed.   All the attacks were started by wild-type bees (Fig. 3.1a, b). At 1352 h, a wild-type bee stung and killed a cordovan bee.

After 8 days of bad weather (27 June – 4 July), the swarms reinstated their scouting activities on 5 July. On this day, two cordovan bees were visiting the nest box by 1100 h, and one wild-type bee was being chased away constantly from the entrance by a cordovan bee. When the first wild-type bee managed to enter the nest box at 1215 h, she was repeatedly chased and grabbed by the legs by one or two

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Table 3.1. Description of agonistic behaviors performed at the nest box by nest-site scouts from competing swarms during their house-hunting process.

	Behavior	Description of behavior
Increased level of aggression	Guarding	When approached, a bee standing at the nest box entrance raises her front legs, projects her antennae forward, flares her wings, and often chases the intruder away
	Chasing	A bee rushes toward another bee from behind as the chased bee runs away
	Leg pulling	A bee grabs the hind leg of another bee with her mandibles or front legs, and pulls her across the substrate, often for several minutes
	Grappling	A bee grabs another bee from underneath the abdomen to avoid getting stung. They lock in position and move around trying to sting each other
	Stinging	A bee protrudes her stinger while grappling with another bee, and inserts her sting in the other bee's abdomen. Venom is released. The soft inter-segmental membrane of the victim allows the stinging bee's sting to come out intact, thus the stinging bee may survive the attack and sting the victim repeatedly
	Mobbing	A prolonged attack by two or more bees against another bee, often involving chasing, leg pulling, grappling, and occassionally stinging

Figure 3.1. Records of aggressive interactions between scouts from competing swarms at the nest box during trial 1. The top plots represent the number of bees from each swarm observed at the nest box. The bottom plots show the types of agonistic interactions observed between non-swarm mates. The identity of the attacker (cordovan or wild-type scout) is represented by either an open circle or a black diamond. The abbreviation "n.d." indicates that no data are available for a given sampling

cordovan bees at a time, until she fled the nest box and did not return (Fig. 3.1c, d). Over the rest of the day, the number of cordovan bees increased to nearly 20, and all the attacks consisted of mobbing (i.e. 2-3 bees against an individual bee) performed by cordovan bees against the one wild-type bee. When we made a night check up of the nest box (at 1945 h), we found one cordovan bee and one wild-type bee twitching and dying, and two cordovan bees dead, inside the box.

On 6 July the cordovan swarm retained control of the nest box the entire day. By 0745 h there were more than 20 cordovan bees inside the nest box and several were guarding the entrance. Nevertheless, one wild-type bee managed to get past the cordovan guards and enter the box at 0745h. At 0755 one cordovan bee was stung and killed by the wild-type bee. Immediately after, the remaining cordovan bees mounted several mobbing attacks against the wild-type bee (Fig. 3.1e, f), but she was not killed. She left the nest box around 0930 h, did not return, and did not recruit any swarm mates to the nest box. By the end of the afternoon, over 50 cordovan bees were at the nest box. At 1800 h we placed both swarms into hives and so ended the first trial.

Trial 2

We set up a second pair of swarms on 9 July, swapping the locations of the two swarm types from the first trial to exclude the possibility that one type of swarm was advantaged by a superior location for discovering the nest box. Having mastered our technique of sealing off a swarm to allow the opposite swarm to discover the nest box, we were more successful at getting scouts from both swarms to visit the nest box at the same time. On 9 July, cordovan scouts began visiting the nest box after 1100 h, and by 1220 h we counted over 20 cordovan bees at the box, which prompted us to seal off their swarm to allow recruitment by the wild-type scouts. This kept most, but

not all (due to a leakage of bees during the sealing process), of the cordovan scouts from visiting the nest box. At 1618 h we observed two wild-type bees and one cordovan bee at the nest box, with occasional chasing and grappling interactions.

The cordovan swarm was kept sealed off in the morning of 10 July, though a few bees had leaked out overnight. By 0930 h there was one cordovan bee inside and one wild-type bee outside the nest box. We uncovered the cordovan swarm at 1050 h and five minutes later there were three cordovan bees and one wild-type bee at the nest box entrance. Throughout the day we saw a maximum of three wild-type bees at the nest box, while the number of cordovan scouts fluctuated from zero to eight. The majority of the attacks were made by cordovan bees against wild-type bees, and the behaviors ranged from chasing to stinging and mobbing (Fig 3.2a, b). Neither swarm increased its presence at the nest box to the high numbers seen the day before, but we observed many instances of mobbing by cordovan bees against wild-type bees (Fig 3.2a, b). One of these mobbing events ended with a cordovan bee getting stung and killed by a wild-type bee, followed by the wild-type bee getting stung repeatedly, and ultimately killed, by cordovan bees. The many fights observed throughout the day resulted in another cordovan bee and four wild-type bees getting stung and killed. We sealed off both swarms at the end of the day to better control their presence at the nest box the next morning, but when we uncovered them, neither swarm visited the nest box that day.

Two days later, at 0945 h on 12 July, we observed two cordovan bees and one wild-type bee inside the nest box. When the wild-type bee approached a cordovan bee at the nest box entrance, the cordovan bee chased the wild-type bee without hurting her (Fig. 3.2c, d). The number of bees inside the nest box remained low throughout the morning, fluctuating between zero and one for either swarm, and we saw no fighting. In the afternoon, the number of wild-type bees at the nest box increased to
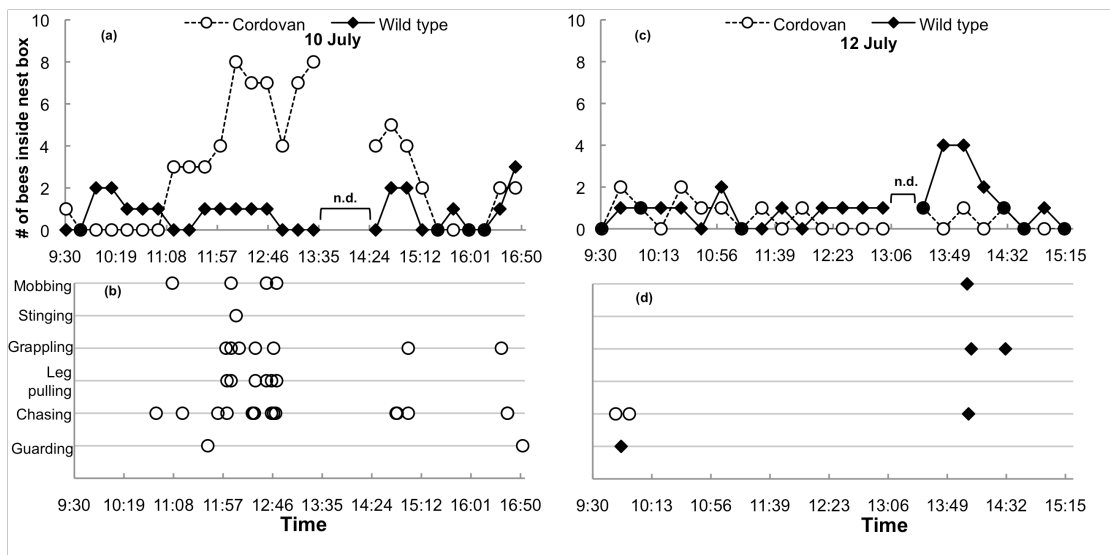
Figure 3.2. Records of aggressive interactions between scouts from competing swarms at the nest box during trial 2. The top plots show the number of bees from each swarm observed at the nest box. The bottom plots show the types of agonistic interactions observed between non-swarm mates. The identity of the attacker (cordovan or wild-type scout) is represented by either an open circle or a black diamond. The abbreviation "n.d." indicates that no data are available for a given sampling interval.

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four, while only one cordovan bee remained interested in the nest box. At this point, wild-type bees performed attacks against cordovan bees, which included chasing, grappling, and mobbing (Fig. 3.2c, d). At the end of the day, the swarms were installed in hives, thus concluding the second trial.

The nest-site buzzing behavior

When one swarm had control of the nest box (i.e. its scouts strongly outnumbered those from the other swarm) but the total number of visitors remained rather low (i.e. around 8 to 20 bees), we often observed bees performing a curious behavior near the entrance opening whenever a flying bee approached the nest box, regardless of what swarm the visitor belonged to. In performing this behavior, when the flying bee attempted to land at the nest box entrance, the other bee would run rapidly about near the entrance hole, moving in a zig-zag motion, partially opening her wings, and head-butting the newcomer while performing a buzzing behavior (Fig. 3.3). The buzzing bee often moved inside the nest box while remaining close to the entrance hole.   The buzzing bee also spent time (a) buzzing while contacting other bees, (b) buzzing without contacting any bee, (c) contacting other bees without buzzing, (d) flying off from the nest box entrance, or (e) standing inside the nest box near the entrance hole. Fig. 3.4 shows the percentages of time spent in each activity by bees performing the nest-site buzzing behavior.

DISCUSSION

Our results demonstrate that when multiple honey bee swarms are searching for a home at the same time, they may explore the same potential nest sites, especially if the
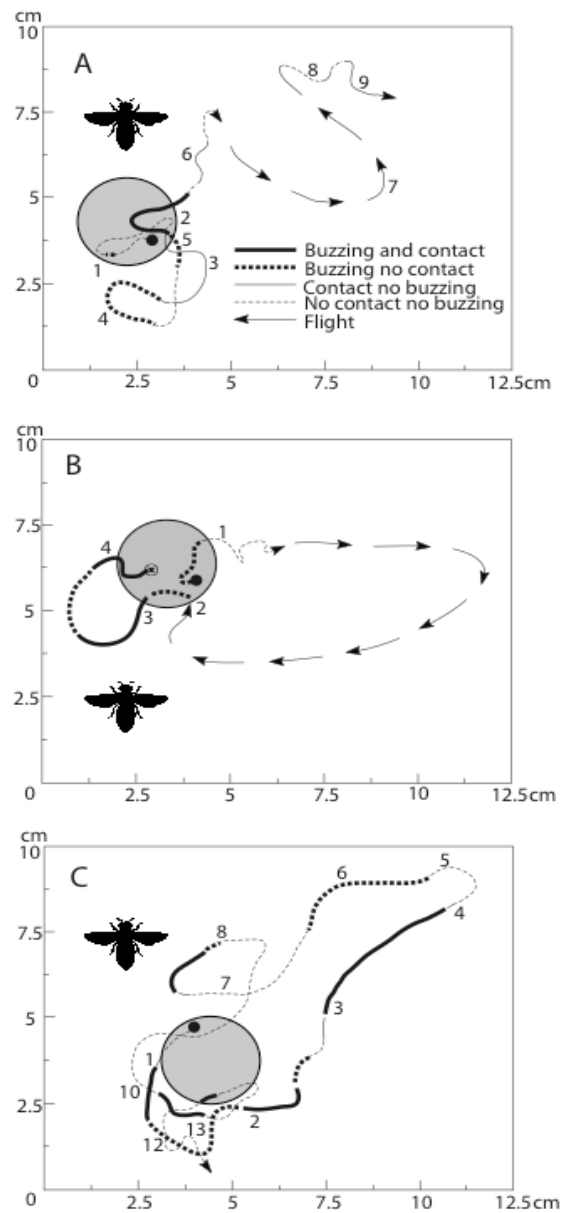
Figure 3.3. Detailed view of the travel patterns, taken from videorecordings, of nest-site scouts producing the buzzing behavior near the entrance opening of the nest box. Each record depicts a different bee (a-c). The numbers next to the line denote the time elapsed (in seconds) as the bee moved across the front of the nest box. In each diagram, the large grey circle represents the entrance hole, the black circle indicates the starting point of the behavioral sequence, and an open circle with an "x" represents when the bee entered the nest box and disappeared from the camera's view.
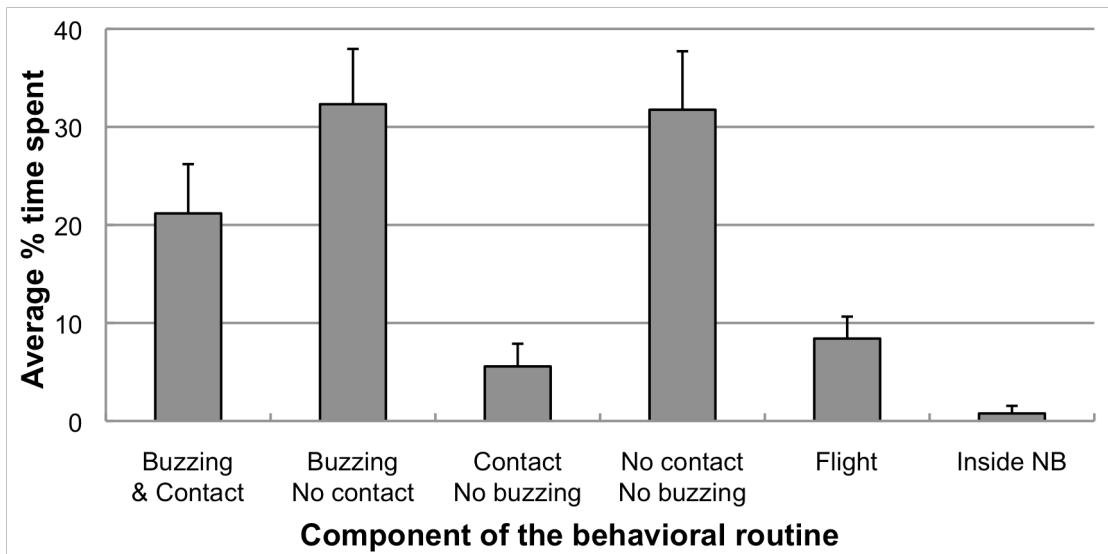
Figure 3.4. Average percentage (± S.E.) of time spent producing each component of the nest-site buzzing behavior by nest-site scouts (n=17).

Summary of interactions observed at the nest site

Level I	Level 2	Level 3	Level 4
A B	A B	A B	A B

# of bees at the nest site

Level I	Level 2	Level 3	Level 4
3 ↑ 1 3 ↑ 1	20 ↑ 4 0	20-30 ↑ 4 3 ↑ 1 A≥B+2	20-30+ ↑ 0-1

Types of interactions observed

No guarding	No guarding	No guarding	Guarding
No buzzing	Buzzing	Buzzing	No buzzing
No scenting	Scenting	Scenting	Scenting
Brief 1-on-1 fighting	No fighting	Mobbing is common	Fighting is rare

Figure 3.5. Summary of the interactions observed between nest-site scouts from two different swarms while competing for control of a nest site. The types of agonistic interactions observed depend on the number of bees from each swarm present at the nest site.

availability of these sites is low. Our findings also demonstrate that when this concurrent exploration happens, the nest-site scouts from competing swarms may defend a nest site by engaging in agonistic interactions that vary in the level of aggression depending on the number of nest-site sco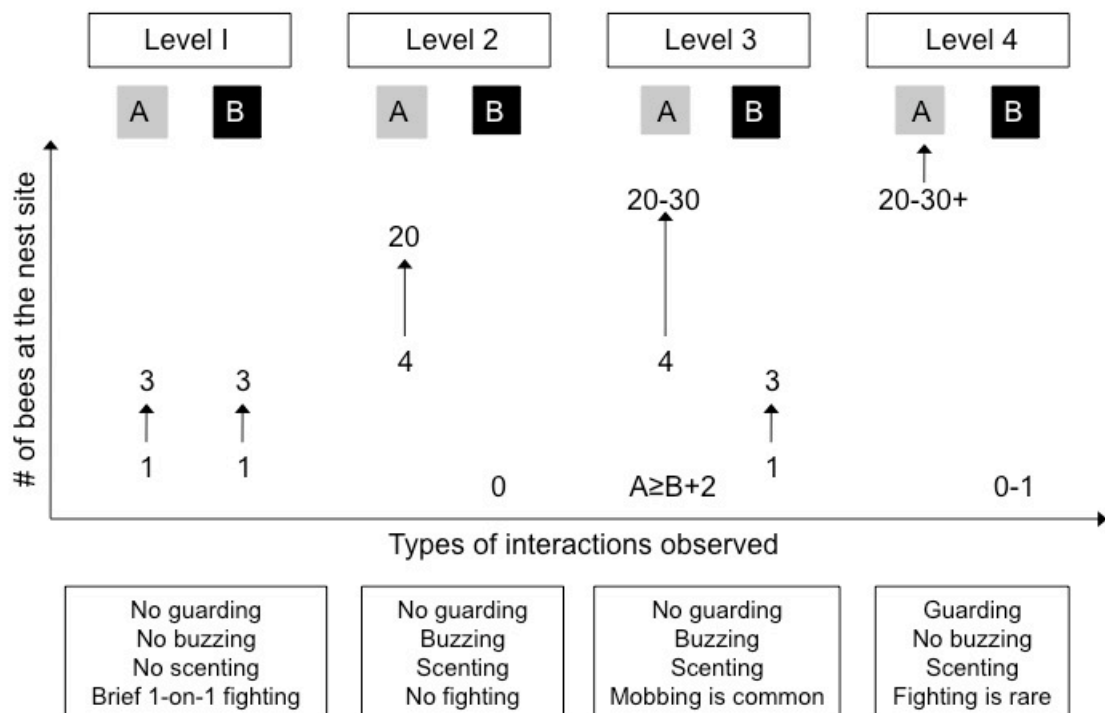uts belonging to each swarm at the time of the encounters. While the interactions at the nest box between scouts from both swarms varied between trials and days, we observed consistent patterns of behavior depending on the number of bees from each swarm present together at the nest box. As shown in Fig. 3.5, we distinguished four levels of nest-site control, each one characterized by a particular set of behaviors and a certain number of bees from each swarm. The four levels are as follows:

Level 1: Neither swarm has control of the nest site and scouts from both are present in low numbers. At this level, each swarm has 1 to 3 bees at the site, and there are few violent interactions between non-swarm mates. These consist of one-on-one fights that last briefly, for just a few seconds, and that are rarely lethal. The nest-site buzzing behavior is not produced, and newly arriving scouts explore the site peacefully. When a bee encounters a swarm mate, they interact without aggression for several seconds.

Level 2: One swarm has weak control of the nest site with 4-20 bees there, and members of the other swarm are not present. The nest-site buzzing behavior is sometimes observed. Heavy scenting is done by bees at the entrance. The interactions between bees are peaceful.

Level 3: One swarm has weak control of the nest site, and members of the other swarm are present in low numbers. At this level, there are 4 to 30 bees from the swarm in the majority, and only 1 to 3 bees from the swarm in the minority. When the

scouts in the majority outnumber those in the minority by at least two bees, aggression toward bees from the swarm in the minority is common. The fights can still be one-on-one, but when the majority group outnumbers the other by a higher amount (i.e. 8 against 1), mobbing becomes prevalent. The nest-site buzzing behavior is common. Guarding at the nest box is not observed, although occasional attacks are seen in the air outside the entrance. The majority of fights do not end in deaths, and most aggressive interactions involve chasing and grappling directed toward bees in the minority.

Level 4: One swarm has strong control of the nest site. When one swarm has more than 20 bees at the nest site, and the other swarm has no bees there, the nest-site buzzing behavior is stopped and guarding is started. When there are 20 to 30 scouts outside, they react defensively toward visitors from both swarms, allowing entrance to swarm mates, but chasing away non-swarm mates. Usually, bees from the competing swarm do not approach the nest site closely. If there are few scouts guarding the entrance, and a bee of the minority swarm enters the nest site, she will be mobbed and so has a low likelihood of escaping alive.

Theoretical models of agonistic contests between two individuals predict that an actor's sense of its competitive advantage should strongly influence its choice among aggressive behaviors (Parker 1974, Parker & Rubenstein 1981). Thus we suspected that in honey bees, a nest-site scout's sense that her swarm does or does not have control of a contested site might strongly influence her decision of whether or not to aggressively fight off scouts from other swarms. Our observations support this idea: when scouts from one swarm had weak control of the nest box, they generally performed the less aggressive chasing and leg pulling behaviors. But when the scouts

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from one swarm had full control of the nest box, they more frequently performed the more aggressive stinging and mobbing behaviors. Because many of these agonistic encounters between scouts from competing swarms resulted in death or injury, it seems likely that honey bees, like other species that compete for territories, mates, or food, will assess their competitive advantage before deciding whether to engage in combat against other conspecific opponents (Bradbury & Vehrencamp 1998).

One of the behaviors performed at the nest box entrance by scouts, the nest-site buzzing behavior, has not been previously described. This behavior is similar in form to the buzz-run that nest-site scouts perform to release a swarm's take off from the bivouac site (Visscher & Seeley 2007). In this behavior, a bee encounters lethargic bees in the swarm cluster and rouses them to greater activity by moving in a zig-zag motion with her wings spread as she buzzes through them. After a few seconds the bee breaks contact with the cluster of quiescent bees while she continues to buzz and ultimately runs off the swarm (described in Rittschof & Seeley 2008). The message of this signal is now known: it is "time to go." In contrast to the bees producing buzz-runs to release a swarm's take off, nest-site buzzing bees contact swarm mates and non-swarm mates alike, and their behavioral routine typically starts with the buzzing bee coming out of the nest box entrance. Bees might produce the buzzing behavior signal near the entrance opening to provide visual beacons to other swarm mates approaching the nest box, or they might produce the signal to appear larger than they really are and thus discourage non-swarm mates from attempting to land at an already dominated nest box. Future work needs to examine nest-site buzzers more closely to determine the exact message of their (presumed) signal.

Even though in this study we easily witnessed two swarms competing for the same scarce resource sites, it is possible that this phenomenon is not common. Research in other Hymenoptera species has shown that once a nesting cavity is occupied it

becomes less desirable to other individuals in search of a similar new home.   In their study of nest site usurpation, Tepedino and Torchio (1994) showed that females of the xylophilous bee *Osmia lignaria* only usurped nest holes during nest-site selection when they encountered active, but unattended nests. This was because the current occupants of nest holes were always successful in defending the hole, apparently due to the fighting advantage they obtained from their position at the nest entrance. Even though founders were usually unsuccessful in regaining control of a nest that had been usurped, they contested ownership with intruders by engaging in assaults that often resulted in injury (Tepedino & Torchio 1994). Thus, nest usurpation is highly costly and is rarely attempted in this species.

In another example, laboratory experiments have shown that when given a choice of multiple nest sites during colony emigration, an emigrating colony of the ant *Temnothorax albipennis* preferred to move into a new nest site that was furthest away from a nest site that was already occupied by a resident colony (Franks et al. 2007). The emigrating colony's selection was not simply the result of aggressive blockage of the available sites near the resident colony's site, but was also due to the odor cues that were left around the unoccupied sites by ants from the resident colony. The repulsive effects of a resident colony increase the competition for nest sites suitable for *Temnothorax* ants, making such sites a highly limiting resource. Indeed, the spatial distribution of colonies is constrained by the distribution of available nest sites (Foitzik & Heinze 1998).

This study provides the first description of an interesting aspect of the natural history of honey bees. It demonstrates that under certain conditions (i.e. when suitable nesting cavities are scarce), neighboring honey bee swarms may compete for and defend nest sites of high quality. The level of aggression between scouts from different swarms depends on the number of scouts from each swarm present at the

time of the encounters, such that strong aggression is only observed when scouts from one swarm highly outnumber the other. Thus, scouts seem to assess their swarm's control of a site and adjust their guarding and fighting behaviors accordingly. Furthermore, we report a curious behavior, the nest-site buzzing behavior, which scouts seem to produce as part of their repertoire of aggressive behaviors to defend a high-quality nest site. Is this a signal used by scout bees to indicate resource-holding potential? The answer must await further study.

ACKNOWLEDGEMENTS

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CHAPTER 4

NO INTRACOLONIAL NEPOTISM DURING COLONY FISSIONING IN HONEY

BEES*

ABSTRACT

Most species of social insects have singly mated queens, but in some species each queen mates with numerous males to create a colony whose workers belong to multiple patrilines. This colony genetic structure creates a potential for intracolonial nepotism. One context with great potential for such nepotism arises in species, like honey bees, whose colonies reproduce by fissioning. During fissioning, workers might nepotistically choose between serving a young (sister) queen or the old (mother) queen, preferring the former if she is a full sister but the latter if the young queen is only a half sister. We examined three honey bee colonies that swarmed, and performed paternity analyses on the young (immature) queens and samples of workers who either stayed with the young queens in the nest or left with the mother queen in the swarm. For each colony, we checked whether patrilines represented by immature queens had higher proportions of staying workers than patrilines not represented by immature queens. We found no evidence of this. The absence of intracolonial nepotism during colony fissioning could be because the workers cannot discriminate between full-sister and half-sister queens when they are immature, or because the costs of behaving nepotistically outweigh the benefits.

INTRODUCTION

Although queens in most social insect species do not mate with multiple males
(Strassmann 2001), polyandry is prominent in certain taxa including yellow jacket
wasps (*Vespula*, Ross 1986), leaf-cutter ants (*Atta*, Fjerdingstad et al. 1998;
Acromyrmex, Boomsma et al. 1999), army ants (*Eciton*, Denny et al. 2004; *Dorylus*,
Kronauer et al. 2004), harvester ants (*Pogonomyrmex*, Rheindt et al. 2004, Wiernasz et
al. 2004, Pol et al. 2008), desert ants (*Cataglyphis*, Timmermans et al. 2008), and
honey bees (*Apis*, Estoup et al. 1994, Tarpy & Nielsen 2002). As a result of this
polyandry, a colony's females (queens and workers) are not all full sisters. Instead,
they constitute several patrilineal groups, with females in the same patriline related as
full sisters (r=0.75) and those in different patrilines related as half sisters (r = 0.25).

The genetic structure of multi-patriline colonies creates a potential for
intracolonial nepotism in various contexts, including food-sharing and brood-rearing,
though there is no convincing evidence that workers behave nepotistically in these two
particular contexts (Breed et al. 1994, Tarpy et al. 2004, and Châline et al. 2005). A
third context with great potential for intracolonial nepotism arises in species, such as
honey bees and army ants, whose colonies reproduce by fissioning (Wilson 1971).
During this process of colony multiplication, the workers rear several young queens,
all of whom are the workers' sisters. Eventually, once the original colony divides
itself, one of these young (sister) queens will head one of the derivative colonies and
typically the old (mother) queen heads the other derivative colony. Thus the workers
in a colony that is fissioning might choose between serving a young queen or the old
queen. And in making this choice, a worker might act nepotistically, preferring to
serve a young queen if she is likely to be a full sister (r = 0.75) or preferring to serve
the old queen (r =0.50) if all the young queens are half sisters (r = 0.25).

To date, two studies with honey bees have investigated whether workers nepotistically choose between a young (sister) queen and the old (mother) queen during colony fissioning, but neither study provides a definitive answer. Getz et al. (1982) established colonies each of which was headed by a queen who was homozygous for a recessive body color marker (cordovan) and was instrumentally inseminated with semen from one wild-type drone and one cordovan drone. Thus the patriline membership of each worker was indicated by her body color. From each of the two colonies that fissioned ("swarmed"), samples of workers were collected from the swarm and the nest, and the young queens were collected from the nest (in honey bees, the old queen leaves in the swarm). All the young queens were cordovan, and yet in both colonies the proportion of cordovan workers was higher in the group that left with the old queen than in the group that stayed with the young queen. These results contradict the prediction that workers should prefer to stay with the young queen if she is their full sister. However, the use of the cordovan marker gene—which may be linked to genes conferring a propensity for leaving in a swarm (Breed et al. 1994)—and the use of colonies with only two patrilines—honey bee colonies typically contain ten or more patrilines (Tarpy & Nielsen 2002)—make it difficult to draw firm conclusions from this study regarding intracolonial nepotism during colony fissioning.

The second study was done using colonies without the cordovan marker and with a natural number of patrilines (Kryger & Moritz 1997). This study looked at how the workers who stayed behind in the nest behaved when the remnant colony was strong enough to fission again, casting a second swarm ("afterswarm") that would be headed by one of the young queens. The authors predicted that workers are more likely to leave in the afterswarm than to stay in the nest if the afterswarm is headed by a full-sister queen rather than a half-sister queen. To test this prediction, they studied two colonies that produced both a prime swarm (containing the old, mother queen) and an

afterswarm (containing a young, sister queen). Workers were sampled from both the prime swarm and the afterswarm. In both colonies, there was no difference in patriline composition between the prime swarm and the afterswarm, which suggests that in both colonies the workers in the patriline of the young queen heading the afterswarm had not increased their likelihood of leaving the nest between the prime swarm context and the afterswarm context. Unfortunately, the authors did not determine the patrilines of the queens in the afterswarm and in the nest, and they did not sample the workers who stayed behind in the nest, hence they were unable to make a full test of their hypothesis. Additionally, once the authors sampled the workers in the prime swarm, they returned the prime swarm to its hive (minus the roughly 200 workers collected from it) to encourage the production of an afterswarm. One wonders whether the worker-assortment patterns were the same for the prime swarm and the afterswarm because the authors returned the prime swarms to their nests, and the prime swarm bees left again in the afterswarms.

Because the evidence about intracolonial nepotism during colony fissioning in honey bees remains ambiguous, we examined three honey bee colonies that were headed by naturally mated queens and that were allowed to swarm naturally, to test the hypothesis that a worker bee is more likely to stay in the nest (with a young, sister queen) than to leave in the swarm (with the old, mother queen) if at least one of the young queens being reared in the nest is her full sister. If this hypothesis is true, then patrilines that are represented by immature queens will have higher proportions of staying workers than will patrilines that are not represented by immature queens. The null hypothesis is that worker bees do not decide to stay or leave based on their genetic relatedness to the young queens being reared in the nest. If so, then the two groups of patrilines—those that are and are not represented by immature queens— should not differ in the proportion of workers who stay in the remnant colony.

METHODS

Study Site and Bees

We conducted our study at the Liddell Field Station of Cornell University in Ithaca, New York (42^o26'N, 76^o30'W). Three medium-sized honey bee colonies were used, all headed by naturally mated New World Carniolan queens (*Apis mellifera carnica*; Strachan Apiaries, Yuba City, California, USA). On 13 May 2008, each colony was installed with its original queen in a three-frame observation hive (described by Seeley 1995). The three frames chosen for each colony were covered with adult bees (approximately 6,000 workers), and were roughly half full of brood and half full of pollen and honey to simulate the conditions present in a natural colony that is preparing to swarm (Winston 1987). The observation hives were set up in a light-proof room to simulate the darkness inside natural honey bee nests, leaving only the hive entrance as a source of light. Two weeks after the colonies were established, the bees started to produce queen cells in preparation for swarming in late May to early June, the time of year when most swarms are issued in the Ithaca area (Fell et al. 1977).

Before placing the glass walls on each observation hive, we installed an electret condenser microphone (Radio Shack Model 33-3013, 70-16,000 Hz frequency response) at the center of the bottom frame so we could hear worker piping, the mechanical-acoustic signal produced by a few dozen bees in colonies that are preparing to swarm (Rangel & Seeley 2008). We checked each observation hive daily, listening every 30 min for piping signals from 10:00 to 16:00 hours. Once a colony's piping rate was higher than 3 signals in 30 s, we monitored that colony closely until its swarm departed.

Collection of Samples

Once a swarm departed its nest, we waited until the swarm bees had settled on a tree branch and no more bees were exiting the hive to join the swarm, whereupon we collected workers from both the swarm and the remnant colony so that the workers' patriline memberships could be determined through genotyping. At the swarm, workers were collected randomly by gently carving out a side of the swarm from bottom to top so that the workers fell into a vial containing ethanol. At the remnant colony, we opened the glass walls of the observation hive and collected workers from both sides of the frames of comb at random, placing the bees in vials with ethanol. At least 120 workers were genotyped from both the swarm and the remnant colony (range = 120 -131 workers per group across colonies). All immature queens were placed in individual vials with ethanol, and the developmental stage of each one was noted (i.e. larva, pupa, or adult).

DNA Extraction and Microsatellite Analysis

We used polymorphic DNA microsatellite markers to determine the patriline composition of the swarm and remnant-colony bees. Paternity was determined by analyzing seven microsatellite loci (Ap033, Ap068, A079, A113, Ap226, Ap256, and Ap289), which are highly variable and sufficient to assign a worker to a patriline in colonies with ten or more patrilines (Solignac et al. 2003, Schlüns et al. 2005). For each marker, the forward primer was labeled with one of four fluorescent phosphoramidites so that the polymerase chain reaction (PCR) products could be separated by size and fluorescence. We extracted DNA from the hind legs and thoraces of workers, and from the whole bodies of immature queens with a DNeasy

Blood and Tissue Kit (Qiagen, Valencia, CA). PCR reactions were performed in a thermal cycler (Thermo Electron Corporation, Milford, MA) using a 10-µL mixture that contained 1 µL of DNA in solution, 5 µL of pre-mixed PCR reagents from a multiplexing kit (Qiagen, Valencia, CA), 1.2 µL of water, and 0.2 µL of each primer (for a total primer concentration of 2.0M). The thermocycler was programmed at 95 $^{\circ}$C for 15 min, 94 $^{\circ}$C for 50 s, 57 $^{\circ}$C for 45 s, and 72°C for 90 s. The annealing temperature was dropped one degree per cycle for the first seven cycles, then the reactions were cycled 28 more times at 94 $^{\circ}$C for 50 s, 50 $^{\circ}$C for 45 s, and 72 $^{\circ}$C for 90 s. The PCR products were visualized with a 3730xl DNA analyzer (Applied BioSystems, Foster City, CA) at the Cornell University Core Laboratories Center using GeneMapper, version 3.0 (Applied BioSystems, Foster City, CA).

For each colony, the queen's genotype for each locus was inferred by comparing the genotypes of immature queens and workers. Genotyped workers were assumed to belong to the same patriline if their profile of drone-derived alleles was the same.

Statistical Analysis

For each patriline represented in a colony, we calculated the proportion of workers who stayed in the nest ("stayers") by dividing the number of stayers in that patriline by the total number of workers (stayers and "leavers") in the patriline. For each colony, we tested whether the proportion of stayers was higher in patrilines with immature queens than in those without immature queens. We used a one-sided t-test because our data met the assumption of normality for parametric tests. Also, for the one colony that contained adult virgin queens, we used a chi-square test to determine whether having an adult full-sister queen present in the nest increased a worker's tendency to stay. For this test, we compared the frequency of staying workers between patrilines

with and without adult full-sister queens. Data are reported as mean proportions ± s.d. We set the level of significance of all tests at α=0.05.

RESULTS

Colony 1 contained 15 patrilines, 10 of which were represented by immature queens (Fig. 4.1a). Colony 2 contained 13 patrilines, 3 of which were represented by immature queens (Fig. 4.1b). Colony 3 contained 19 patrilines, 6 of which were represented by immature queens (Fig. 4.1c). When we determined, for each patriline in each colony, the proportion of the sampled bees that stayed in the nest, we did not find higher proportions of stayers in patrilines that did, relative to those that did not, have immature queens developing in the nest (Table 4.1).

We found two newly emerged virgin queens roaming inside the nest of colony 3 after the swarm had issued; they belonged to patrilines 2 and 5 (Fig. 4.1c). The worker bees in patriline 2 had fewer stayers than leavers (7 versus 12), while those in patriline 5 had more stayers than leavers (24 versus 9). In this colony, the workers who had an adult full-sister queen in the nest prior to swarming did not show a higher tendency to stay compared to workers who had an immature full-sister queen or no full-sister queen inside the nest (χ^2=2.24, d.f.=1, p=0.1341).

DISCUSSION

Our results show that honey bee workers are not more likely to stay in the nest rather than to leave in the swarm if at least one full sister is being reared as a young queen prior to swarming. This finding indicates that workers do not show intracolonial nepotism during colony fissioning.
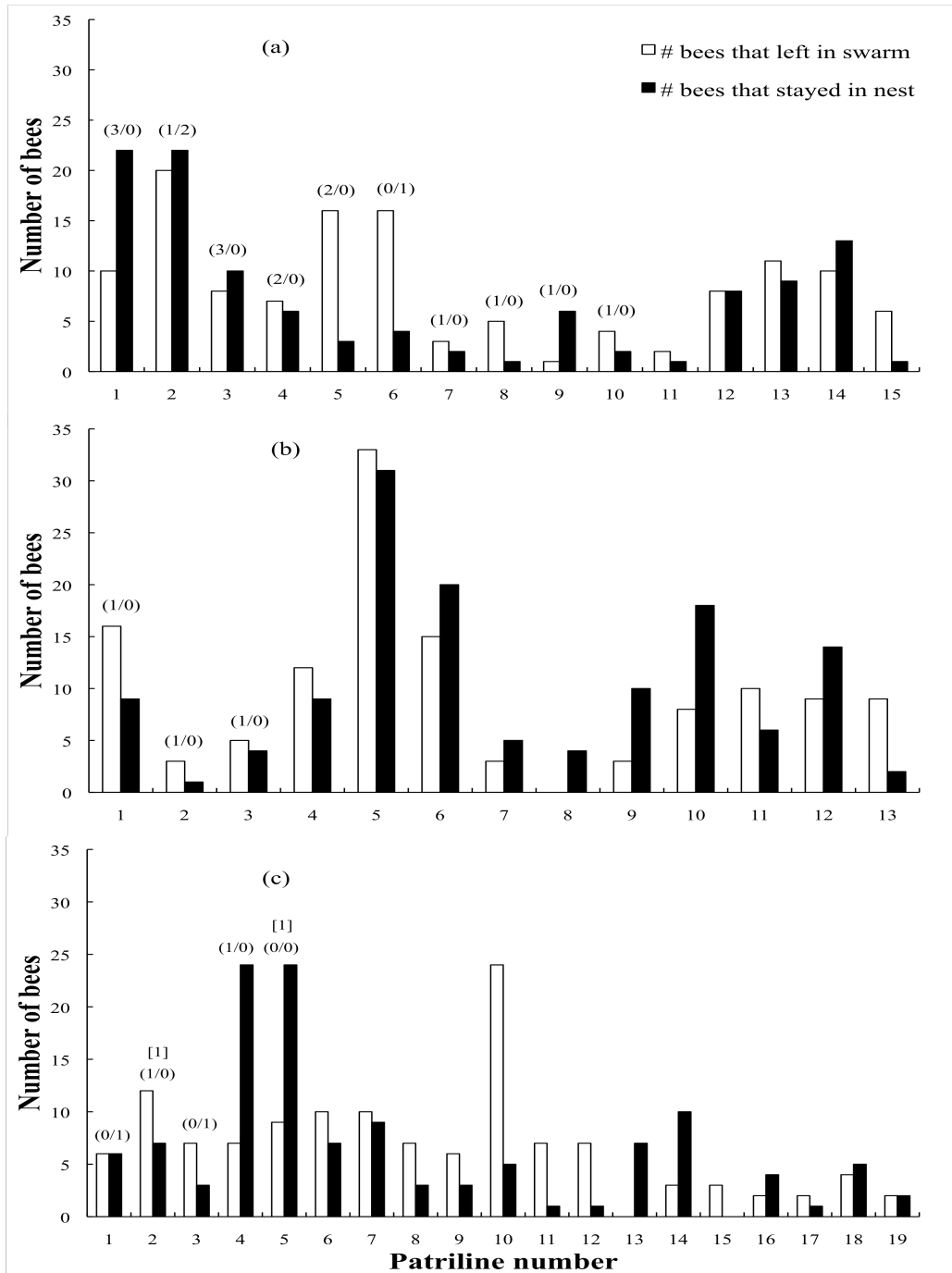
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Figure 4.1. Comparison between the number of bees who stayed in the nest (black bars) and the number of bees who left in the swarm (white bars) for each patriline. The number of immature queens belonging to a patriline is noted above the bars in parentheses as: (number of larvae/number of pupae). Numbers inside brackets above the parentheses denote queens that had emerged as adults and were roaming the nest after the swarm departed.

Table 4.1. Summary of the proportions of workers who stayed in the nest for both the patrilines that were represented by immature queens and the patrilines that were not represented by immature queens. Proportions are given as mean ± s.d.

Colony	Number of immature queens	Patrilines with immature queens		Patrilines without immature queens		t	d.f.	p-value
		Number of patrilines	Proportion of stayers	Number of patrilines	Proportion of stayers			
1	18	10	0.46 ±0.21	5	0.40 ± 0.17	0.61	13	0.28
2	3	3	0.35 ± 0.10	10	0.62 ± 0.23	-1.63	11	0.87
3	6	5	0.57 ±0.17	14	0.43 ± 0.25	1.16	17	0.13

Our results are consistent with those of two previous studies that reported preliminary results suggesting an absence of intracolonial nepotism in honey bees during colony fissioning. Kryger and Moritz (1997) found no significant difference in patriline compositions between the prime swarm and the afterswarm in two colonies. Their results are similar to our results in that the workers in their two colonies showed no sign of deciding to leave in the swarm versus stay in the nest based on their genetic relatedness to the queen that they will serve. However, the study by Kryger and Moritz differs from ours in several aspects. Most importantly, after each colony produced the afterswarm, Kryger and Moritz did not sample any adult workers from the remnant colony to determine for each patriline the proportion of workers who stayed in the nest versus the proportion that left in the afterswarm. Thus, the proportion of adult workers who stayed in the nest was not compared between patrilines with and without full-sister queens in the nest. There is also the complication that the authors returned the prime swarm from each colony back to its hive to encourage the production of an afterswarm, and this manipulation by itself could have caused the similarity in patriline distributions between prime swarms and afterswarms.

The other study that attempted to look for nepotism during colony fissioning in honey bees was performed with two colonies headed by cordovan queens that were artificially inseminated with semen from one wild-type and one cordovan drone (Getz et al. 1982). After each of the two colonies swarmed, there was actually a higher proportion of cordovan workers in each swarm than in each remnant colony, even though all the virgin queens found in the two remnant colonies were cordovan. Evidently, workers did not show intracolonial nepotism. The results of this early study support the current view that cordovan workers may have a higher propensity to swarm relative to wild-type workers, and that colonies headed by queens artificially

mated with a low number of drones are unnatural and their use may yield unrealistic results (Breed et al. 1994).

The present study is, to our knowledge, the first to test for intracolonial nepotism at the fissioning stage of the swarming process in undisturbed colonies headed by naturally mated queens. We are the first to identify the patrilines of adult workers in both the swarm and the remnant colony, and of all immature queens present in the nest. Also, we avoided using special genetic lines or returning swarms to colonies to encourage further swarming. Our negative results regarding intracolonial nepotism by workers during colony fissioning are similar to those from most studies of workers' intracolonial nepotism during queen rearing, which report no worker tendency to favor full-sister queens at the egg, larval, or adult stage (see Breed et al. 1994 for review).

The question remains whether workers are unable to discriminate among full-sister and half-sister queens, or whether they have not been selected to make this discrimination because the costs of discrimination outweigh the gains, or both.

In theory, honey bee workers are predicted to use self-referent phenotype matching based on genetically-based odor cues to discriminate between full-sister and half-sister immature queens at the time of swarming (Visscher 1986). It has been shown that, at least under certain experimental conditions, workers can discriminate full-sister from half-sister workers (Getz 1991), and a few laboratory studies have shown that cuticular hydrocarbons that provide odor cues may indicate a queen's patriline membership and may be used by workers to discriminate full-sister from half-sister queens (Moritz & Crewe 1988, Page et al. 1991, Getz & Page 1991, Arnold et al. 1995). However, in colonies kept in natural settings there seems to be weak discrimination between full-sister and half-sister queens, perhaps because of a low allelic diversity of genetic odor cues used in recognition (Ratnieks 1991). It is also possible that the weak discrimination between full-sister and half-sister queens reflects selection for the

muting or scrambling of kin recognition cues by the queens to prevent half-sister workers (the vast majority) from withholding resources (Reeve 1998).

Workers may not discriminate among full-sister and half-sister queens before swarming for other reasons. Ratnieks and Reeve (1991) proposed that high colony-level costs of kin discrimination (i.e. reduction in the colony's total production of queens) may outweigh the benefit that a worker gains from her selfish interest to help support a full-sister queen. Another possibility is that extreme polyandry results in so many patrilines in a colony that a worker's probability of encountering and detecting a full-sister queen is low. This last assertion is especially likely when one considers the highly congested environment inside a colony that is preparing to swarm.

Our finding of a lack of intracolonial nepotism during colony fissioning in honey bees is consistent with the negative results reported in most studies on intracolonial nepotism in species of social insects whose colonies are composed of multiple patrilines or matrilines. Although little is known about whether individuals behave nepotistically during colony fissioning in other polyandrous species (e.g. army ants), several studies of polygynous ant and wasp species have failed to detect nepotism by workers toward the brood of particular queens (see Carlin et al. 1993, DeHeer & Ross 1997, and Holzer et al. 2006 for ants; and Queller et al. 1990, Solis et al. 1998, Strassmann et al. 2000, for wasps). What are especially needed now are studies of intracolonial nepotism during colony fissioning in other species of social insects with polyandrous queens, most notably the army ants.

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