UNIVERSITY OF CALIFORNIA, SAN DIEGO

Bumblebee vibration activated foraging:

A Thesis submitted in partial satisfaction of the requirements for the degree
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in

Biology

by

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2009

The Thesis of Dan Kuan-Nien Su is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2009

DEDICATION

To my parents, who are always making sure I'm on track with my studies, even though they never remember what I'm studying (Bees?).

To all the people I've met in San Diego that made the last 5 years enjoyable and memorable.

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ABSTRACT OF THE THESIS

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The ability use vibrational signals to activate nestmate foraging is found in the highly social bees, stingless bees and honey bees, and has been hypothesized to exist in the closely related, primitively eusocial bumble bees. We provide the first strong and direct evidence that this is correct. Inside the nest, bumble bee foragers produce brief bursts of vibration (foraging activation pulses) at 594.5 Hz for 63±26 ms ($velocity_{RMS}$=0.46±0.02mm/s, $force_{RMS}$=0.8±0.2 mN. Production of these vibrations significantly increased by 1.5 fold inside the nest when a forager successfully returned with artificial nectar (2.5 M sucrose solution) and the number of foragers exiting and entering the colony into a foraging arena significantly increased by 1.8 fold and 1.8 fold respectively as compared to when the colony was offered water. The nest is well suited to transmitting these vibrations, and the wax food pots and brood cells exhibit a transmission resonance at 300-700 Hz (1.9-2.3 fold increase in

dB relative to other frequencies from 10 Hz to 10 kHz). Playbacks of foraging activation pulses at natural amplitudes (force=1mN, velocity=0.4 mm/s) results in a 1.2 fold increase in bees entering the foraging arenas as compared to playbacks of white noise adjusted to be equal in vibrational amplitude, force, and duty cycle to the natural signals. Thus, vibrational foraging activation signals may be a basal trait shared by primitively eusocial and highly social bees.

Introduction

Cooperative signals involved in social foraging have evolved to remarkable degree in social insects. Multiple sensory modalities are involved in such social insect foraging communication (Nieh 2009). However, vibrational signals have received particular attention because they play a key role in, arguably, one of the most sophisticated examples of animal communication, the honey bee dance language, a functionally referential communication system that encodes distance and direction to a food source (von Frisch 1923). Honeybees produce vibrations and airborne sounds as a key component of the waggle dance, and researcher have proposed that this evolved from a simpler acoustic signal that alerted nestmates to the presence of good food in the environment. Indeed, vibrational movements also play a key role in the recruitment of the closely-related stingless bees (Nieh 2004; Hrncir et al. 2006a). However, molecular evidence suggests that bumble bees are the most closely-related group to the honey bees (Cameron 1993), and thus researchers have long speculated that similar vibrational signals may exist in bumble bees (Katayama 1998; Dornhaus and Chittka 2001; Oeynhausen and Kirchner 2001).

The corbiculate bees (Apidae) that live in extended social groups (bumble bees, stingless bees, and honey bees) coordinate colony foraging using a variety of signals whose effects can range from simply activating nestmates to forage (foraging activation) to communicating a specific food location. Honey bees and many species of stingless bees can communicate food location. Honey bees (Apini) use the waggle dance in which the waggle phase communicates food distance and direction and has a vibrational component detectable either through the substrate or, most strongly, through direct antennal contacts (von Frisch 1923; Wenner 1962; Rohrseitz and Tautz 1999; Nieh and Tautz 2000). Stingless bees

(Meliponini) also produce sounds and vibrations during recruitment (Nieh and Roubik 1998; Aguilar and Briceño 2002; Nieh et al. 2003; Hrncir et al. 2006a). Vibrations potentially related to recruitment have also been recorded during stingless bee trophallaxis between forager and receiver (Hrncir et al. 2006b). In some stingless bee species, the durations of these vibrational recruitment pulses are correlated to food quality, with longer pulses being produced for food with higher sugar concentrations (Nieh et al. 2003; Hrncir et al. 2004).

Relatively little is known about foraging activation in bumble bees. There are over 250 species of bumble bees that inhabit vastly different habitats in arctic, palaearctic, nearctic, and tropical habitats (Goulson 2003). Bumble bees live in annual, not perennial colonies (like the highly social bees), are not known to communicate food location (Dornhaus 2002), and their foraging largely consists of individuals finding good food, not group foraging in which groups of individuals from the same colony exploit a food patch (Dornhaus and Chittka 2004). However, bumble bee foragers can activate nestmates to exit the nest when a successful forager returns. This foraging activation can use an activation pheromone (Dornhaus et al. 2003) and involve direct contact with successful foragers (Dornhaus and Chittka 2001; Renner and Nieh 2008). These activated foragers can exhibit a preference for food with the same scent as that brought back by the activating forager (Dornhaus and Chittka 1999; Molet et al. 2009).

Unlike the highly social bees, bumble bees do not engage in trophallaxis. Thus, information about colony need and foraging success seem to be largely gleaned, with the exception of excitatory jostling contacts, from the nest. Like honey bees (Camazine et al. 1998; Nakamura and Seeley 2006), bumble bee nests act as "information centers" in which

foragers can directly inspect food pots to determine colony carbohydrate (Dornhaus and Chittka 2005) and protein (Kitaoka and Nieh 2009) needs.

The role of vibration communication in bumble bee foraging is relatively unexplored, and, thus far, the foraging of relatively few bumble bee species have been studied. Studies on recruitment information flow have mainly been focused in *B. terrestris* (a European species) and *B. transversalis* (a neotropical species) Dornhaus and Cameron 2003; Dornhaus and Chittka 2005. Katayama (1998) reported vibration communication inside the nest between larvae and workers that feed the larvae. The workers produce vibrational pulses outside of larval cells before feeding the larvae. The larvae then reposition themselves to facilitate feeding. Successful *B. terrestris* foragers have also reported to run in a zig-zag motion combined with fanning bouts that create vibrational pulse sounds inside the nest, although these sounds have not been quantitatively described (Oeynhausen and Kirchner 2001). In this species, playbacks of simulated sounds, described as "leaving sounds" significantly increased the number of bees exiting the nest by more than 2-fold, although it is unclear if this was specific to the leaving sounds or a disturbance artifact (Oeynhausen and Kirchner 2001).

We therefore tested the hypothesis that bumble bees can produce excitatory vibrational signals that activate nestmates to forage. We chose a species of New World bumble bee, *Bombus impatiens* (Apidae, Bombini), because most research on bumble bee communication has focused on the European bumble bee, *Bombus terrestris*. *Bombus impatiens* occurs in Canada and the Eastern United States where it ranges from Ontario and Maine to Florida and west to Michigan, Illinois, Mississippi and Kansas (Heinrich 1979). We performed three experiments. First, we tested if bumble bees produce substrate-borne

vibrational signals during foraging for rich food. We provided bumble bee foragers with water or sucrose solution and recorded acoustic vibrations made upon the return of bees inside the nest. *Bombus impatiens* foragers can activate nestmates to forage for artificial nectar (Renner and Nieh 2008). However, *B. impatiens* and other bumble bee species are not known to activate foraging for water (Dornhaus and Chittka 2004), although they will individually collect water for the nest, as needed (Heinrich 1979).

We recorded the number of bees entering and exiting the nest as an indicator of foraging activity. In the second experiment, we measured the vibrational transmission characteristics of bumble bee nests to determine if they are tuned to transmit the recorded foraging-related vibrations. In the third experiment, we played back previous recorded foraging sounds from the first experiment to determine if these sounds will increase colony foraging as compared to white noise playbacks (to control for a playback disturbance effect).

Methods and Materials

Colonies and Study Site

The experiments were conducted in a temperature-controlled lab (30°C to maintain colony warmth) at UCSD, La Jolla, California, USA (N32°52.690′, W117°14.464′). We used nine *B. impatiens* colonies containing approximately 100 bees per colony from BioBest (Leamington, Ontario, Canada). We used one colony at a time. The colony was placed in a wood nest box (32 x 20 x 15 cm) covered with a clear plastic cover. Inside the box, the colony wax pots and cells were placed on of two layers of thin plastic membrane comprised of heat-shrink polyolefin film (75 gauge, LS-2475, BCU Plastics, San Diego, CA, USA) to facilitate vibrational recordings. This material has excellent vibrational transmission characteristics as measured through laser Doppler vibrometry. The nest box was connected with a tubing Y-manifold to two foraging arenas (transparent plastic boxes of 35 x 78.5 x 33 cm, Renner and Nieh 2008). The foraging arenas were covered with clear plastic covers with one mesh panel (25.5 x 21cm) on the top to allow ventilation. Plastic gates placed throughout the tubing allowed us to control ingress and egress from foraging arenas. The foraging arenas were illuminated with halogen lights from 07:00 to 14:00 each day. We conducted one trial per day beginning at 10:00.

General Methods

We labeled bees by capturing them individually in small plastic vials, chilling them at 0°C for 2-3 minutes, and attaching a small numbered plastic tag (The Bee Works, Orillia, Ontario, Canada) to the thorax with cyanoacrylate adhesive. In the foraging arenas, we provided 1.5 M unscented sucrose solution (43%) w/w *ad libitum* in multiple small plastic

Petri dishes at 16:00, several hours after the trial. Inside the nest, we provided freshly ground honey bee-collected pollen *ad libitum* in Petri dishes. In experiments with rewarded foraging, we used 2.5 M (65% w/w sucrose solution). Floral nectars occur at a variety of concentrations, and generalist bee foragers collect nectars ranging from 10-70% sugar w/w (Roubik et al. 1995).

Recording Experiments

The unrewarded arena was always empty (no sucrose solution or water). During each experiment, one randomly chosen foraging arena held a dish of unscented 2.5M sugar solution or water ("rewarded" arena) and the other had no food (unrewarded arena). Before the trial began, we randomly selected 3-5 foragers and placed them in the rewarded arena, blocking their path back to the nest with a plastic gate.

The experiment is divided into three time periods: one 15 min, one 40 min, and one 15 min interval in which we censused foraging activity each 5 min by counting the total number of bees going in and out of the nest over 5 min. In the first 15 min, the foragers from the nest could only go freely between the unrewarded arena and the nest. During this period, foragers inside the rewarded arena could collect sucrose solution and water, but could not return to the nest.

After 15 min, one randomly-selected forager (focal forager) from the rewarded arena was allowed to leave the arena, to either return to the nest or enter the unrewarded arena. If the first focal forager did not leave the nest to resume foraging, another forager that had collected sucrose solution or water was allowed to leave the foraging arena and enter the nest. Filming and sound recording of the nest began during this 40 min experimental period.

During this time, only the focal forager (controlled by access gates) could return to the rewarded arena. No other bees were allowed access, and thus their foraging activation must have been due to information provided by the focal forager, not their own direct experience with food or water. The filming and sound recording stage lasted for 40 minute. In the final 15 min, only the number of bees entering and exiting the nest was censused.

To record intranidal behavior and sounds, we placed a digital video camera (Canon 3CCD Digital Video Camcorder) over the nest to record behavior. We recorded vibrations with a accelerometer (Brüel and Kjaer Miniature accelerometer type 4393, Skodsborgvej, Denmark) attached to the nest floor and amplified through a Nexus conditioning amplifier (B&K 2691-A-0S2) whose signal was digitally recorded, along with the video, into a iMac G4 computer using iMovie software (Apple Corporation, Cupertino, CA, USA). In a subset of trials, we measured the force generated by bees producing these vibrations. To estimate this force, we placed a force meter (B&K impedance head type 8001) underneath and in contact with the center of the nest floor and recorded vibrational force. The complete setup was tested and calibrated (flat frequency response from 10-10,000 Hz) with a laser Doppler vibrometer (OFV512 head OFV2200 vibrometer control, Polytec, Hopkinton, Massachusetts, USA) recording a signal from a vibrational calibrator (B&K Calibration Exciter type 4294) measured with a digital oscilloscope (Tektronix TDS2024B, Beaverton, Oregon, USA). Vibrations were also measured with RavenPro v1.3 software (Cornell Lab of Ornithology, Ithaca, NY, USA).

Measuring Vibration Transmitting Properties of the Nest

To determine how vibrations propagate through the nest surface, we used a mini-shaker (B&K Mini Shaker type 4810) providing pure sine waves at 0.4 mm/s and 1 mN (corresponding to natural signal strengths). We applied these vibrations to a 1 mm^2 point applied at different areas of four fresh nests with no bees on the surface at ambient nest temperature (30°C). The amplitude of the transmitted vibrations was then measured with the laser vibrometer at 1 and 2 cm from the contact point, (approximately corresponding to one and two bee body lengths away from the vibrational stimulus). The setup was calibrated as previously described.

Playback Experiments without Food

We used the same dual arena setup for the measurement and playback experiments. However, in the playback experiments, foragers were allowed to go into both foraging arenas unimpeded. We conducted two types of playback (natural foraging activation sounds and white noise) in two experiments (no food in foraging arenas and 2.5 M sucrose in one foraging arena). Each playback trial lasted for 40 min and we censused the number of bees entering and exiting the nest each 5 min. During the first 5 min, we played back vibrations. We then recorded nest sounds (see above) for 15 min. Next, we repeated this procedure (playbacks from 20-25 min, nest sound measurements from 25-40 min). It is not possible to record non-playback sounds during the playback periods. Thus, we designed the experiment to include two 15 min intervals during which natural nest sounds, potentially in response to playbacks, could be recorded.

Natural foraging activation sounds consisted of three representative sound pulses (50 ms duration, duty cycle=0.3%) randomly selected from the prior recording experiments. To

determine if there was a specific effect of the foraging sounds, not simply a disturbance effect of playing back any sound, we also generated white noise sound pulses (bandpass filtered to 10-10,000 Hz, 50 ms duration, duty cycle=0.3%, B&K 1405 white noise generator). To vibrate the nest floor in a controlled way, we placed the B&K 8001 impedance head (to measure playback force) in contact with the bottom center of the nest floor and mounted on top of a B&K 4810 mini-shaker. We delivered an amplified signal (B&K power amplifier WQ 1105) from the computer using our sound analysis software. We calibrated these playbacks such that natural and white noise pulses delivered signals at a natural force of 1 mN and natural average pulse amplitude of 0.4 mm/s (as measured with the laser vibrometer from the nest with bees during playbacks).

Statistical Analysis

We use JMP (v. 5.1) to perform all statistical tests. All data met parametric assumptions and thus we performed repeated-measures analysis with time as repeated-measures factor in a nested design multivariate analysis of variance (MANOVA) or in repeated-measures ANOVA, as appropriate for the data. For the recording experiments, we analyzed colony as a random effect (Standard Least Squares with an EMS algorithm) and treatment (sugar concentration) as a fixed effect. Time is a repeated measure. We tested the effect of different treatments on foraging activity level and vibrational pulse production in the 15 min period after each playback. For the playback experiments, we analyzed colony as a random effect and treatment (natural foraging activation pulses or white noise) as fixed effects. Time is a repeated measure. We report all averages as mean±1 standard error (SE).

All amplitude measurements (displacement, velocity, acceleration, and force) are root mean square (RMS) values.

Results

Foraging activation pulses

After a forager returned to the nest from 2.5 M food, a distinct vibrational pulsing was occasionally heard inside the nest (Fig. 1). These pulses had a different dominant frequency (595±305 Hz) and were quite brief (63±23 msec) in comparison to the buzzing of thermoregulating bees inside the nest (dominant frequency = 241.2±42.2, duration 123±26 msec, N=5).

Recording experiment

We therefore investigated the effect of food versus water collection on vibrational pulse production. The number of bees entering and exiting the nest were significantly 1.8 fold higher for 2.5 sucrose as compared to water (bees exiting: $F_{1, 511}$=49.1, p<0.0001, bees entering: $F_{1,511}$=55.4, p<0.0001, Fig. 2a). There is a significant effect of colony ($F_{8, 520} \geq$ 20.0, p < 0.001) and a significant interaction between treatment and time ($F_{1, 520} \geq$ 6.3, $p \leq$ 0.01) such that the number of bees exiting and entering the nest increase over time for sucrose but not for water (Fig. 2a).

The number of pulses also increased as foraging activation increased. We therefore call these pulses foraging activation pulses. We recorded significantly more pulses (1.5 fold more, $F_{1,193}$=22.41, p<0.0001) when a forager returned from sucrose collection as compared to water collection (for sucrose: 12.71$\pm$ 0.85 pulses per 5 min, for water: 8.32$\pm$ 0.86 pulses per 5 min, Fig. 2b). There is a significant effect of colony ($F_{8, 190}$=6.99, p = 0.001), but no effect of time ($F_{13, 511}$=0.5, p = 0.94) and no significant interaction between time and treatment($F_{13, 511}$=0.7, p = 0.75).

Other measures of signaling intensity also increased for sucrose as compared to water. The amplitude and duration of pulses are significantly greater for 2.5 M sucrose as compared to water (duration: $F_{1,151}$=6.41, p=0.0021; amplitude: $F_{1,151}$=4.16, p=0.017). The amplitude of pulses elicited by sucrose feeding is 2.1 fold higher than for water collection (2.5 sucrose pulse amplitude=1728.4 mm/s; water pulse amplitude=840.4 mm/s). The duration of pulses elicited by sucrose feeding is 1.6 fold higher than for water collection (2.5 sucrose pulse duration=70 msec; water pulse duration=43 msec).

Nest vibrational transmission characteristics

Different nests varied in their vibrational transmission characteristics (colony effect: $F_{6,287}$=21.90, p<0.0001), primarily in the degree to which they attenuated certain frequencies, but all nests showed the same frequency response. Frequencies from 300-700 Hz were transmitted better than frequencies above or below this range (Fig. 3a). This is reflected in the significant frequency effect ($F_{1, 287}$=213.42, p<0.0001). There is no significant difference in the nest frequency response between distances of 1 and 2 cm ($F_{1, 287}$=0.30, p=0.607). The foraging active pulse vibrations range in frequency with an average dominant frequency of 595±305 Hz. Thus, *B. impatiens* nests transmits vibrations at these frequencies quite well, demonstrating a classic tuning between signal frequency and environmental transmission properties.

Playback experiments without food

The playback of recorded bumble bee sounds (Fig. 4) to the *Bombus impatiens* colony significantly affected the foraging levels and sound pulse production (bees exiting: $F_{1,135}$=107,

$p< 0.001$; bees entering: $F_{1,135}=28$, $p< 0.001$; sound pulse production: $F_{1,105}=3.87$, $p=0.005$) as compared to white noise playbacks. The number of bees entering and exiting the foraging arena increased by 1.2 fold and 4.4 fold respectively for foraging activation pulses as compared to white noise pulses. Sound pulse production inside the nest increased by 1.5 fold after foraging activation pulse playbacks as compared to white noise playbacks.

There is no colony effect on foraging activity and sound pulse production (bees exiting: $F_{1,181}=1.03$, $p= 0.31$; bees entering: $F_{1,135}=0.657$, $p= 0.42$; sound pulse production: $F_{1,98}=1.93$, $p= 0.17$). There is no significant effect of time on foraging level and sound pulse production (bees exiting: $F_{14,181}=0.65$, $p= 0.82$; bees entering: $F_{14,181}=0.61$, $p= 0.85$; sound pulse production: $F_{12,98}=1,11$, $p= 0.36$). There is no significant interaction between time and foraging levels (Bees exiting: $F_{1, 181}=1.03$, $p=0.31$; bees entering: $F_{1, 181}=0.66$, $p= 0.42$). There is no significant interaction between time and pulse production ($F_{1, 98}=1.11$, $p= 0.36$).

Playback experiment with food

However, there was no significant effect of playbacks on actively foraging colonies. There was no significant effect of foraging pulse versus white noise pulse playbacks on foraging levels (bees exiting: $F_{1,131}=3.60$, $p= 0.06$; bees entering: $F_{1,131}=0.59$, $p= 0.44$) or sound pulse production ($F_{1,97}=0.65$, $p=0.42$). There was a significant colony effect on foraging level and sound pulse production (bees exiting: $F_{1,131}=78.9$, $p< 0.0001$; bees entering: $F_{1,131}=66.6$, $p< 0.0001$; sound pulse production: $F_{1,97}=31.3$, $p< 0.0001$). Different colonies had markedly different levels of foraging activity (Fig. 5). Nonetheless, there was no significant effect of playback type in the full models that accounted for colony effects. There was no significant effect of time on foraging level and sound pulse production (bees exiting:

$F_{2,131}$=3.04, p= 0.05; bees entering: $F_{2,131}$=1.88, p= 0.16; sound pulse production: $F_{2,97}$=0.46, p= 0.63).

Discussion

Our results show that *B. impatiens* colonies produce foraging activation pulses that are correlated with increased colony foraging activity. Significantly more bees entered the foraging arena and returned to the nest (1.8 fold increases) when one focal forager was allowed to collect a rich sugar solution (2.5 M sucrose) as compared to collecting water. Upon the forager's return inside the nest, we recorded brief vibrational pulses (594.5 Hz for 63±26 ms (velocity=0.46±0.02 mm/s, force=0.8±0.2 mN). The number of such pulses increased by 1.5 fold for sucrose as compared to water. Moreover, pulse characteristics associated with signaling intensity increased for sucrose relative to water collection. The amplitude, duration, and frequency of the sound pulses recorded in the presence of high quality food is higher, typical of a more excited response toward a higher quality food source. When colonies were quiescent and not actively foraging because no food was available, playbacks of natural foraging activation pulses significantly increased foraging levels and resulted in a 1.2 fold increase in the number of bees entering foraging arena as compared to white noise playbacks. Thus, there is good evidence that the pulses we measured are linked to foraging activation.

Nest Vibrational Characteristics

There was a good match between the average frequency of the foraging activation pulses (594.5 Hz) and the frequency response of the comb (450-600 Hz vibrations transmitted well). Such tuning of sound production frequencies to sound transmission medium is a widely observed adaptation, facilitating longer-distance transmission, increased signal clarity, and (in some cases) reduced metabolic cost of signaling (Bradbury and

Vehrencamp 1998). In honey bees, a similar tuning exists because the honey comb amplifies frequencies between 150 Hz and 250 Hz and bees produce waggle dance vibrations at 250 Hz (Sandeman et al. 1996). The extent to which substrate vibrations produced by bumble bees travel through the comb is unclear, but these vibrations were 5.8 fold higher in amplitude than vibrations by the honey bee waggle dance measured from the comb (average of 0.079 mm/s measured 2 cm from the dancer, Nieh and Tautz 2000). In the stingless bee, *Melipona seminigra*, vibrations produced by recruiting foragers had amplitudes of 0.3 mm/s at a dominant frequency of approximately 500 Hz. Thus, the foraging activation vibrations of *B. impatiens* are, on average, 1.5 fold higher in amplitude and occupy a similar frequency range to those of *M. seminigra*.

Sound pulse properties

The foraging activation pulses (FAP, 594.5 Hz for 63 ms at an amplitude of 0.5 mm/s) are significantly different in frequency and pulse duration from previously reports of bumble bee sounds. *Bombus terrestris* reacts to disturbance by producing a broadband hissing sound produced by wing vibrations that is stimulated by vibrational disturbances of the nest, air currents, and CO_2. These hisses had an amplitude of 350-780 mm/s (two orders of magnitude louder than FAP) and a fundamental frequency of 193±13 Hz (lower than FAP). The hiss extended into the ultrasonic frequency range, with broad distribution of frequencies up to 60 kHz (Kirchner and Röschard 1999). Duchateau (1989) reported on sounds performed by *B. terrestris* queens and workers that have been called humming, buzzing, and fanning sounds. Katayama (1998) recorded bursts of buzzing by foragers feeding larvae (duration of 182 ms for *B. diversus* and 30.4 ms for *B. ussurensis* workers, frequencies not

reported). Oeynhausen and Kirchner (2001) reported that *B. terrestris* produced vibrational foraging signals, but did not describe the physical properties of these signals. In general, many of these acoustic behaviors have not been quantitatively described. Thus, it is difficult to compare them with the foraging activation pulse that we describe. However, we also recorded buzzing sounds inside the nest and found that these had a much lower frequency (241.2±42.2 Hz) and longer duration (123±26 ms) than the foraging activation pulses.

The sound pulses showed a change in response to different quality food sources, with more pulses, longer pulses and at a higher velocity for high quality food as compared to water (Table 1). A similar effect of food quality on recruitment sounds is found in the closely related stingless bees. In the stingless bees, *M. mandacaia*, *M. bicolor*, and recruitment sound pulse duration increases for high quality food to which bees recruits (2.5 M) as compared to lower sucrose concentration (Nieh et al. 2003). In *M. seminigra*, foragers similarly produce longer sound pulses for high quality food as compared to lower quality food (Hrncir et al. 2004), in accordance with general principles of animal signaling motivation (Bradbury and Vehrencamp 1998).

The dominant frequency of the foraging activation pulse (595 Hz) in *B. impatiens* is within the same frequency range as that reported for other bee sounds. *Melipona mandacaia* produces sounds at a fundamental frequency of 551±8 Hz, while *M. bicolor* produces sounds at a fundamental frequency of 538±6 Hz. While waggle dancing, honey bees produce recruitment vibrations at lower frequencies 200-300 Hz (mean 244± 28 Hz, Nieh and Tautz 2000).

The ability of bumble bees to perceive vibrations has not been well-studied, but they were clearly able to sense the vibrations generated, at natural amplitudes and frequencies,

during our playbacks (Figs. 4, 5). If their vibration-detection if similar to that of honey bees, they may detect vibrations through their subgenual organ (Autrum and Schneider 1948) which has good sensitivity to vibrations from 150 to 900 Hz (average response threshold from 0.06 and 0.15 mm/s peak-peak (Kilpinen and Storm 1997; Rohrseitz and Kilpinen 1997). If *B. impatiens* subgenual organs have a similar response threshold, then average foraging activation pulses should exceed the average response threshold by 4.4 fold.

Effect of playback

Foraging activation is adaptive when it enables a successful foragers to rouse a colony which is not actively foraging or foraging at low levels (Dornhaus and Chittka 2001). The playback experiments using recorded bee vibrations show colony foraging levels and sound pulse production significantly increased for recorded bee vibrations as compared to white noise when the colony is in a quiescent foraging state (Fig. 5). However, if a colony is already actively foraging and collecting rich food, we would not expect foraging activation to exert a strong effect. Thus, playbacks did not significantly alter colony foraging when the colony was already collecting rich food (2.5 M sucrose, Fig. 5), although the number of bees entering and exiting the nest is slightly higher for bee vibration playbacks as compared to white noise (both colonies, playbacks with food, Fig. 5). These results complement the report that significantly more *B. terrestris* bees (double) left the nest during the playbacks as compared to a control period before the playbacks of sounds synthesized to match natural departure sounds (Oeynhausen and Kirchner 2001).

Intranidal behavior

There is strong evidence that the vibrational pulses that we measured are a foraging activation signal, behavior that has been modified by natural selection to convey information. The numbers of pulses increased upon a forager's return to the nest and increased with foraging activity (Fig. 2). Pulses were acoustically distinct from other forms of intranidal vibration such as bees thermoregulating. Pulse duration, amplitude, and frequency were correlated with and thus conveyed information about food quality (Table 1). There is evidence that foragers acted upon the information provided by pulses because playbacks of natural pulses increased foraging activity, unlike white noise.

Colonies also responded to playbacks by producing more pulses after playbacks of natural as compared to white noise pulses (Fig. 5). This type of "chorusing" has been reported in some stingless bees (Kerr 1969), but has not been studied in detail for any bee. It is possible that such chorusing could help intensify and propagate the foraging signal throughout the colony, but this requires further study.

The behavior of bees producing the foraging activation sound also requires further elucidation. Although we videotaped the behavior of returning foragers inside the nest, foragers often moved within and under nest structures such as food pots and cells, making it difficult to determine precisely how and when they produced vibrational pulses. Sound pulses were not exclusively produced during foraging. We also detected them, at a low level, inside quiescent colonies. Future research should clarify which bees are producing these sounds, but we speculate that a low level of foraging activation pulses may be related to colony need. This phenomenon may also reflect, as is observed in honey bee vibratory

signals, a low level of basal signaling that is only exceeds receiver response thresholds when signaling levels are elevated (Nieh 1993; Schneider and Lewis 2004).

Evolution of Communication

Given that the highly social corbiculate bees (honey bees and stingless bees) that are closely related to the bumble bees (Cameron 2003) also produce vibrational pulses during recruitment, it is possible that this ability was present as an ancestral state. Thus, our findings shed light upon the evolution of acoustic recruitment communication in the Apidae, a group in which honey bees have evolved the remarkable ability to encode location information into a foraging activation signal (von Frisch 1967). However, even if these abilities have evolved independently within Apidae, the existence of vibratory signals as a common thread is revealing. Researchers have long speculated that the ability to activate nestmates through excitatory behaviors ritualized from cues associated with successful foraging. A successful forager returns to the nest with the odor of the food she has visited borne on her body hairs, she may be moving rapidly to deposit her food, and she may also buzz her wing muscles upon departure to generate heat for efficient flight (Esch 1967; von Frisch 1967). The existence of vibratory recruitment pulses in all social corbiculate bees now suggests that the ritualization of acoustic cues, whether in a common ancestor or multiply among the social lineages, played an important role in the evolution of bee recruitment communication.

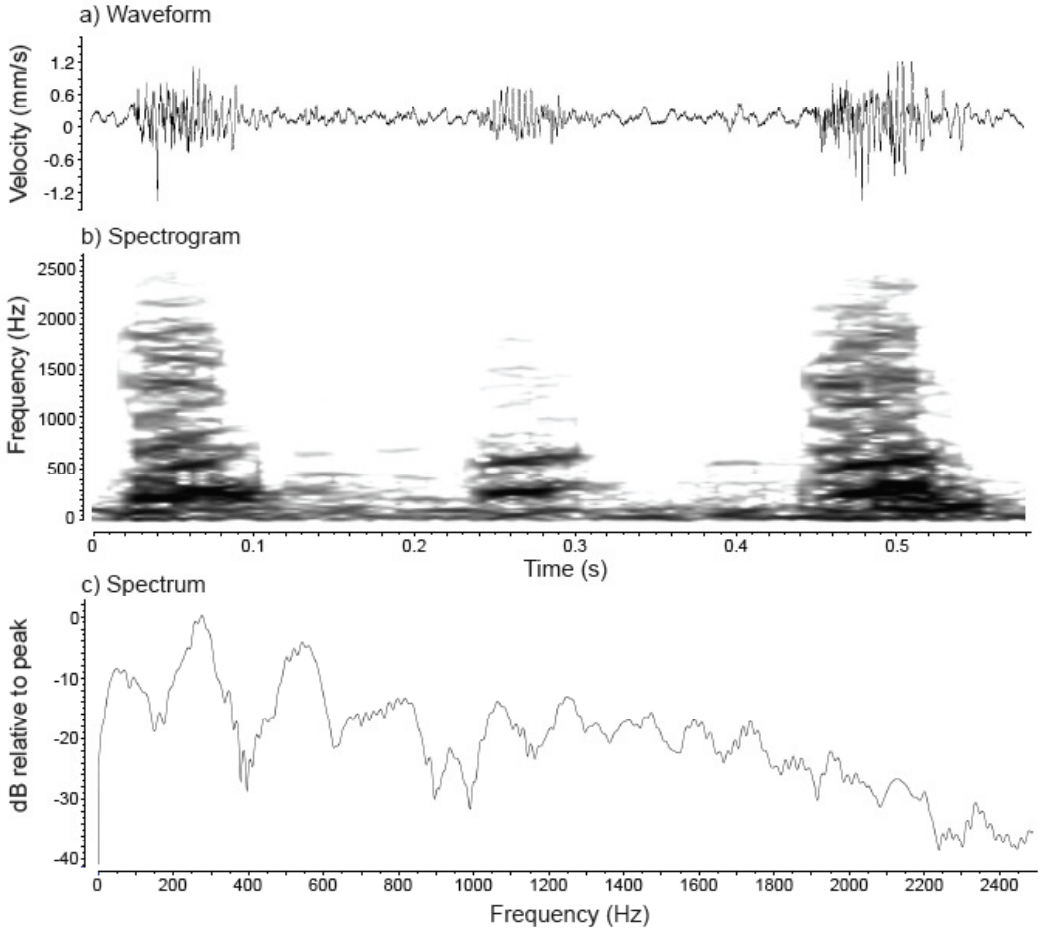

Figure 1. Acoustic properties of foraging-related bumble bee sounds. The (a) waveform, (b) spectrogram (analysis resolution: 3 dB filter bandwidth=31.8 Hz, rectangular window function), and (c) spectrum are shown for three characteristic vibrational pulses. The spectrum shown is based upon an average of all three pulses (analysis resolution: 3 dB filter bandwidth=23.3 Hz, rectangular window function).

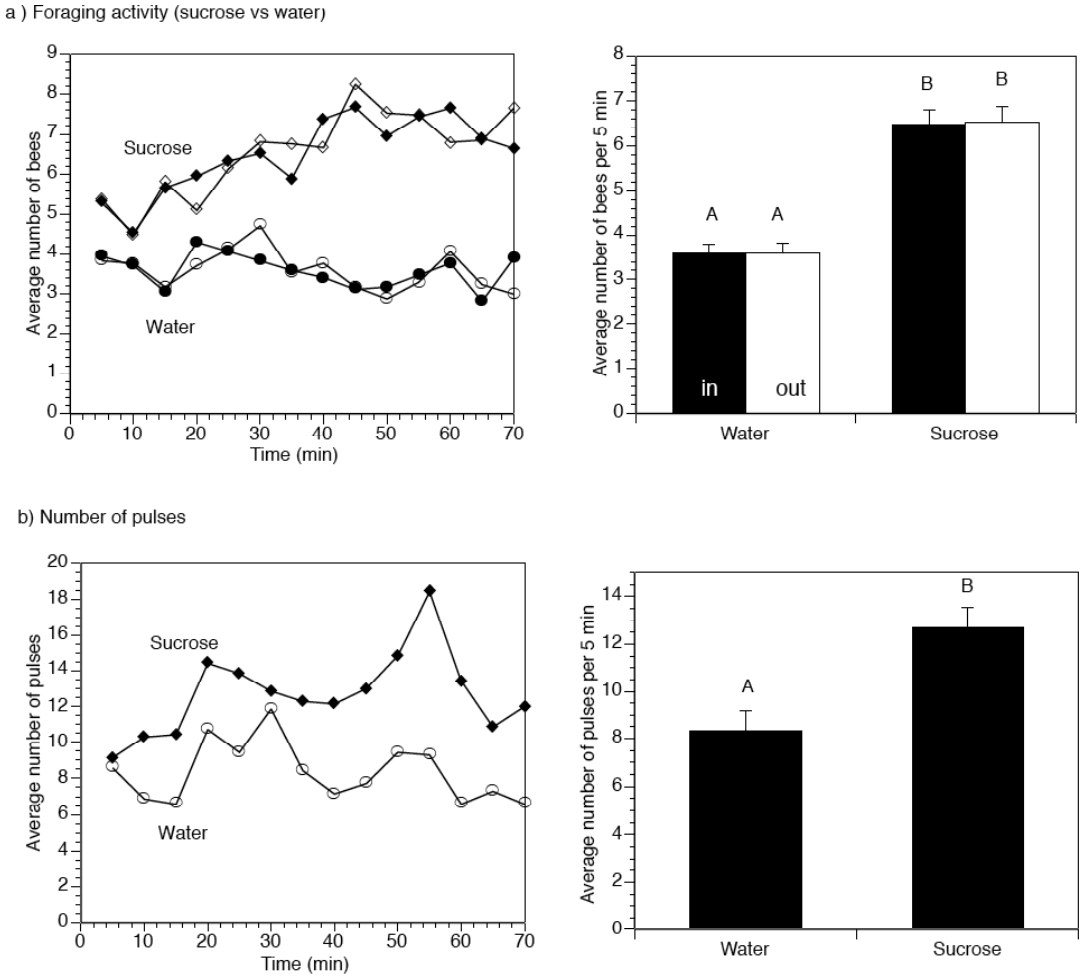

Figure 2. Results of the foraging-activation sound experiment. Pooled data from all colonies are shown. Bar graphs show averages and standard errors, with significant differences indicated by different letters above each bar. (a) Foraging activity in response to sucrose solution or water over time (line plot) and overall (bar graph). The line plot shows the number of bees entering (filled circles=water, filled diamonds=sucrose) and exiting (open circles=water, open diamonds=sucrose) over time. The bar graph shows the average number of foragers entering (black bars) and exiting (white bars) over all time intervals. (b) Number of vibrational pulses produced over time (line plot) and overall (bar graph). The line plot shows the number of pulses produced each 5 min for sucrose solution (filled diamonds) and water (filled circles). The bar graph shows the average number of pulses per five min interval.

Properties of foraging activation vibrational pulses measured for high quality sucrose solution (2.5 M) and water.

Measurement	Average +/- standard error	Max	Min	N
2.5M sucrose pulse duration	70± 5.4 msec	200 msec	34 msec	40
Water pulse duration	43± 5.6 msec	89 msec	26 msec	16
2.5M sucrose pulse frequency	624.5± 62.8 Hz	2239.5 Hz	172.3 Hz	40
Water pulse frequency	746.5± 90.0 Hz	1378.1 Hz	344.5 Hz	16
2.5M sucrose pulse velocity	0.46 ± 0.02 mm/s	2.08 mm/s	0.14 mm/s	40
Water pulse velocity	0.23 ± 0.03 mm/s	0.42 mm/s	0.010 mm/s	16
Mean Pulse Frequency	594.5± 304.8 Hz	2239.5 Hz	172.3 Hz	153
Mean RMS velocity	0.3677 ± 0.2469 mm/s	2.8813 mm/s	0.027 mm/s	153
Mean RMS acceleration	1062.5 ± 899.9mm/ s^2	6236.7 mm/ s^2	196.7 mm/ s^2	153
Mean RMS displacement	0.1562 ±0.2468 μm	1.4874 μm	0.0031μm	153
Mean pulse duration	63 ± 26 msec	198 msec	22 msec	153
Mean Force	0.792 ± 0.151 mN	4.772 mN	0.343 mN	28

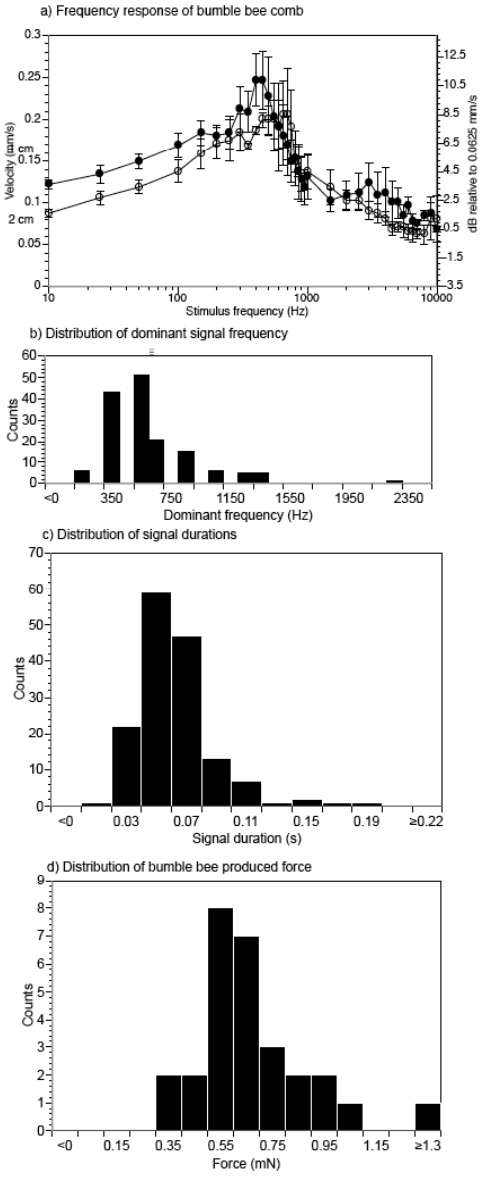

Figure 3. Nest vibrational characteristics and corresponding signal properties. (a) Velocity of nest vibrations in response to the stimulation produced by the vibrational exciter. The line plot shows the average velocity of the comb at the stimulus frequency, and shows the standard errors. (b) The figure shows the distribution of the dominant signal frequency from vibrational pulses produced by the bumble bees. The mean dominant frequency is 594 Hz. (c) The figure shows the distribution of the signal duration from the vibrational pulses produced by the bumble bees. The average duration is 63±26 ms. (c) The figure shows the distribution of force of the bumble bee produced vibrational pulses. The average force is 0.792± 0.151 mN.

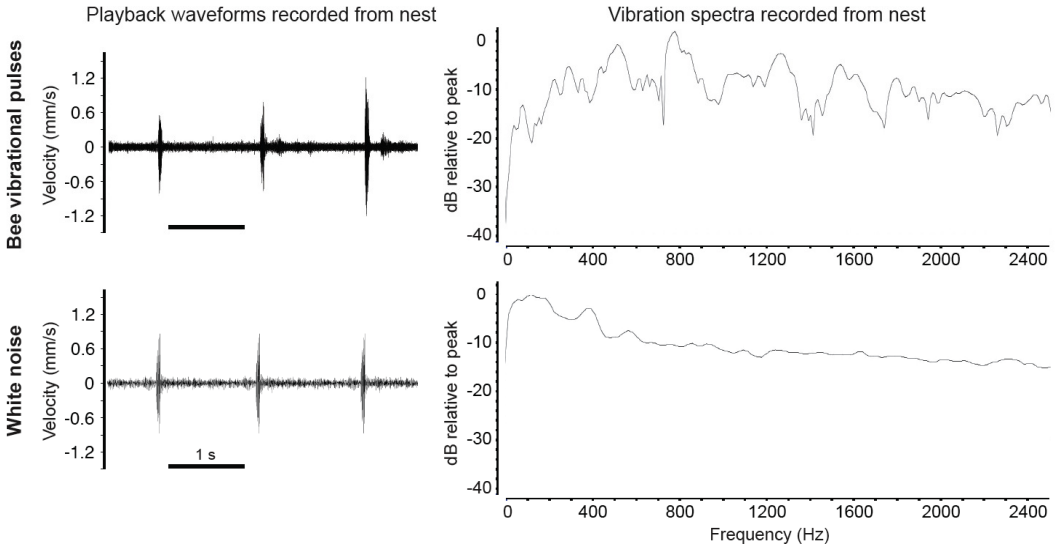

Figure 4. Sound properties of bee vibrational pulse and white noise playbacks as measured from the nest substrate. Playback wavefo and spectra are shown. Both bee vibrational pulses and white noise playback consist of 50 msec pulses delivered each 1.3 s (force=1 mN, velocity=0.4 mm/s, duty cycle=0.3%).

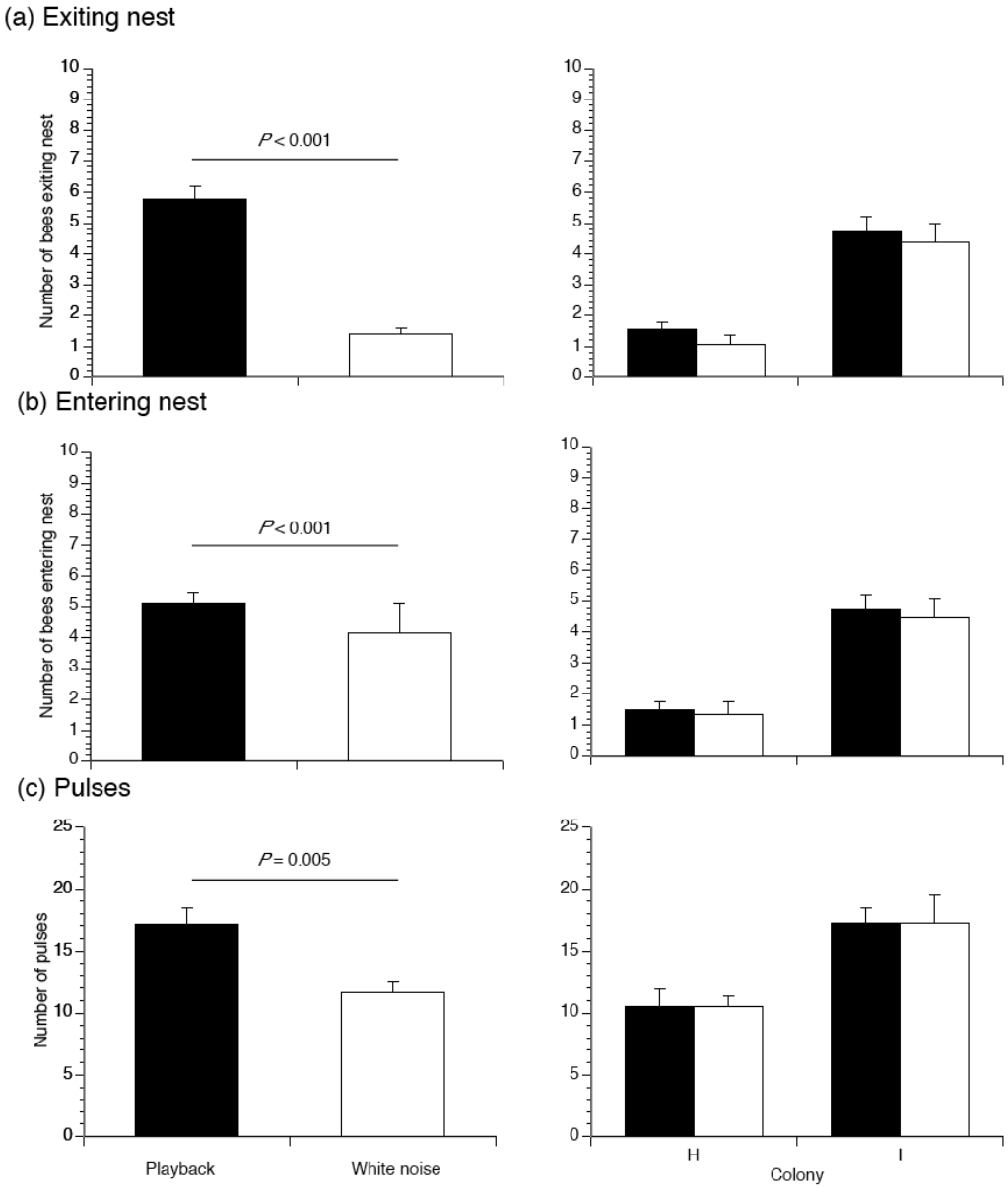

Figure 5. Results of the playback experiment. Results of playback with no food on the left column, and playback with food on the right column, filled bars indicate playback vibrations while white bars indicate white noise vibrations. Colony effect significant in playback with food so the colonies tested are presented separately. Pooled data from all colonies are shown. Bar graphs show averages and standard errors, with significant differences indicated by p-values. (a) Number of bees exiting the nest in response to playback and white noise vibrations. (b) Number of bees entering the nest in response to playback and white noise vibrations. (c) Number of sound pulses produced in response to playback and white noise vibrations.

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