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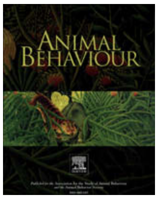

Communication signals used in worker–drone interactions in the honeybee, *Apis mellifera*

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Although the use of communication signals during worker–queen interactions in honeybees have been studied in detail, signals involved in worker–drone interactions have received less attention. We determined whether workers perform piping and the vibration signal on drones, and whether drones respond in ways that potentially enhance their mating success. Workers did not produce piping on drones, but they did perform vibration signals. Approximately 50% of the drones examined received vibration signals, and drones were more likely to be vibrated when they were sexually immature than when they were mature. Drones responded to the vibration signal by increasing movement and by interacting more with workers, which contributed to an increase in the proportions of time that they received trophallaxis and grooming. Because trophallaxis supplies nutrients necessary for sexual maturation, and because grooming may also contribute to drone health, workers could potentially use the vibration signal to promote development and mating performance in drones. We found no evidence that the vibration signal was associated with drone flight. The majority of signals were performed on drones in the morning several hours before flight activity, there was no relationship between the level of vibration activity experienced by drones in the morning and flight activity later that same day, and drones were rarely vibrated when returning to the nest between consecutive mating flights. In many social insect species, reproductive males are fed through trophallaxis and workers perform vibrational signals to influence activity of nestmates. Tactile, vibratory signals may therefore provide mechanisms for investigating the role of worker–drone interactions in colony reproductive decisions for a wide array of species.

Sterile workers in social insect colonies primarily gain reproductive benefits indirectly through the actions of reproductive castes. Natural selection should favour any mechanism that enables workers to influence the behaviour of reproductive individuals in a manner that promotes colony reproductive success. Such interactions provide insights into the formulation of colony reproductive decisions as well as the different levels of selection that have shaped social evolution (Tarpy et al. 2004).

Communication among castes has been extensively studied in the honeybee, *Apis mellifera*, especially during worker–queen interactions associated with reproductive swarming and queen replacement. Workers use at least two communication signals, the vibration signal and worker piping, to influence queen behaviour during colony reproductive events. The vibration signal, which consists of a worker grasping a recipient with its legs and rapidly vibrating its body dorsoventrally for 1–2 s, is performed on both laying queens and virgin queens. It exerts a nonspecific modulatory influence that may help to prepare laying queens for flight and departure from the natal nest (Allen 1959; Fletcher 1978; Schneider 1991; Pierce et al. 2007), and may influence the development and survival of virgin queens during queen replacement (Schneider et al. 2001; Schneider & DeGrandi-Hoffman 2003, 2008). Worker piping consists of a worker producing a high-pitched, pulsed sound while pressing its body against another bee, which causes the recipient to warm its flight muscles in preparation for flight (Seeley & Tautz 2001). Workers perform piping on laying queens at high rates immediately before the departure of the swarm from the natal nest and inside the swarm cluster before liftoff for the new nest cavity, which may provide the immediate trigger for queen flight (Pierce et al. 2007). In combination, the vibration signal and worker piping help coordinate queen behaviour with the different stages of the swarming process, which in turn contributes to the successful completion of swarm production and movement, the continuation of the parental colony and worker inclusive fitness.

Workers also gain inclusive fitness benefits through males (drones) and are therefore expected to show mechanisms that

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influence drone success. Workers contribute to drone production through the construction of drone comb and the feeding of drone larvae (Winston 1987; Pratt 2004). After drones emerge as adults, workers could potentially contribute to their success by influencing two main aspects of their biology: (1) sexual development and (2) flight activity. Drones typically reach sexual maturity around 12 days of age, and the maturation process depends in part upon nutrients supplied by workers through trophallaxis (Ruttner 1966; Rueppell et al. 2005). As drones age, they are fed less by workers and obtain the energy for mating flights primarily by feeding directly from honey cells (Free 1957; Ruttner 1966). Workers also groom drones, which may further contribute to health and development (Ohtani 1974). Thus, any actions by workers that influence the tendency to receive feeding and grooming could potentially enhance drone maturation and mating performance.

Drone flight activity occurs during the afternoon hours and consists of orientation flights, which are taken by younger drones and last 1–6 min, and mating flights, which are performed by sexually mature drones and last 20–30 min (Taber 1964; Ruttner 1966; Witherell 1971). Mature drones may take 2–4 separate mating flights per day and fly up to 7 km from their hive (Currie 1987). Between flights, they return to the nest for brief periods, during which they feed from cells or receive trophallaxis in preparation for subsequent flights. Drone flight behaviour is under strong endogenous control, and the timing of flight activity may be more dependent upon a drone's circadian rhythm than on interactions with workers (Koeniger et al. 1994). However, in the ant species *Pogonomyrmex maricopa* and *Camponotus herculeanus*, workers influence the initiation of male flight within the time frame of the male's circadian rhythm (Hölldobler & Maschwitz 1965; Hölldobler 1976). It is unknown whether honeybee workers similarly affect drone flight.

Workers could potentially use the vibration signal and piping to influence drone maturation and flight behaviour. Vibration signals performed on workers and queens stimulate increased movement rates, contribute to greater trophallaxis and cell inspection activity, and enhance the likelihood of flight, whereas piping may serve as an immediate trigger for takeoff (Schneider et al. 1986; Seeley & Tautz 2001; Donahoe et al. 2003; Cao et al. 2007, 2009; Pierce et al. 2007; Rangel & Seeley 2008). If drones also receive vibration signals and piping, and respond similarly, then workers could use the signals to facilitate the trophallactic activity necessary for sexual development, the feeding behaviour associated with flight preparations and mating flight activity. Workers will vibrate drones (Milum 1955); however, the influence of the signal on drone behaviour has never been investigated, and worker piping directed towards drones has not been reported.

We investigated worker–drone interactions by examining the use of the vibration signal and of piping to influence drone behaviour. We had four main objectives. First, we estimated the proportion of drones that received vibration signals and worker piping, and determined whether the likelihood of receiving the communication signals was influenced by a drone's sexual maturity. Second, we examined the daily patterns of signal production on drones to assess whether temporal changes in the use of vibration signals and of piping were associated with temporal patterns of drone flight activity. Third, we examined the behaviour of individual drones receiving vibration signals and piping to determine whether the signals were associated with the tendency to engage in trophallaxis, grooming, feeding from cells, or flying from the nest. Fourth, we examined the behaviour of drones returning from mating flights to assess whether worker interactions were associated with consecutive flight attempts.

METHODS

Colony Set-up and Establishing Drone Populations

We examined worker–drone interactions in three four-frame observation hives (colonies A, B and C) maintained on the campus of the University of North Carolina at Charlotte during May–June 2008. Each observation colony was set up from a five-frame 'nucleus' colony, which was maintained in the field until the day before observations began. The glass walls of the observation hives were marked off in a grid of 4 × 4 cm squares to facilitate monitoring drone behaviour.

Within each study colony, we established a population of 150–160 individually marked drones of known age. To obtain drones for marking, we introduced one frame of empty drone comb into each of three large, unrelated field colonies maintained in 45-litre box hives. We initiated drone rearing in the large field hives rather than in our nucleus colonies to ensure sufficient drone production. After the drone brood was capped, we transferred a single frame of drone brood from each of the field colonies into one of the three nucleus colonies. Thus, although the drones were unrelated to the workers in the nucleus colonies, the completion of rearing occurred in the nucleus hives, which enhanced the acquisition of colony odours and facilitated drone acceptance after emergence. When the drone brood was nearing emergence, each frame of drone comb was removed from its nucleus colony, placed in a prelabelled, nylon-mesh cage and transferred to an incubator (33.5 °C; 50% RH). Emerged drones were collected and marked with plastic tags glued to the thorax (Opalithplätchen; Graze, Germany). Each tag had a unique number–colour combination to allow for individual drone identification. Drones were tagged within 24 h of emergence and all drones added to a colony were marked on the same day.

Immediately after marking, the drones were reintroduced into their nucleus colonies. For each nucleus colony, less than 1% of the drones (0–2 tagged individuals) were expelled after reintroduction. Each nucleus colony also produced a limited number of their own drones during the study period (approximately 150–200 cm^2 of drone brood/colony), which may have contributed to a rearing environment that further facilitated the acceptance of the tagged drones.

The day after the tagged drones were reintroduced into the nucleus hives, each colony was moved into an observation hive by transferring four frames filled with brood and food, the queen and 6000–8000 bees, including the tagged drones. Observations of worker–drone interactions were initiated the following day and continued for approximately 3 weeks, during which time the drones in each observation colony ranged in age from 2 to 25 days. On a given day, all the drones in a colony were the same age. We observed drones before and after they were sexually immature ($<$12 days old and $\geq$12 days old, respectively). Although there is natural variation in the age of drone sexual maturation, 12 days is the mean age at which drones mature (Ruttner 1966; Winston 1987; Rueppell et al. 2005) and provides a biologically relevant delineation for establishing maturation categories.

Determining the Proportion of Drones that Received Vibration Signals and Worker Piping

To determine the proportion of drones that received the different signals, we monitored worker–drone interactions continuously during 0800–1700 hours, 5 days each week throughout the study period, for a total of 160 h of observation for each colony. The three observation colonies were monitored simultaneously each day. For each drone that was observed to receive a vibration signal or worker piping, we recorded its identity and age. At the end of the study period, we determined the number of different drones in each colony that were vibrated or piped at least once and expressed these values as proportions of the total tagged drones added. Each individual drone was counted only once in the calculations, regardless of the number of times that it received each signal during the study period.

Determining the Influence of Sexual Maturity on Worker–Drone Interactions

After drones reach sexual maturity and begin taking mating flights, their attrition increases dramatically because of predation and because they die after ejaculation (Winston 1987). Therefore, to assess the influence of sexual maturity on a drone's likelihood of receiving vibration signals and piping, we used the following approach to account for attrition as the study progressed. On each day of observation we conducted hourly counts of the number of tagged drones on each of the four combs and determined the maximum total number present in the colony that day. We then determined the total number of different individual drones that received vibration signals or piping throughout the day, and expressed the values as proportions of the maximum number of tagged drones present. We then compared the daily proportions within and between colonies during the periods of sexual immaturity and sexual maturity.

Determining Daily Patterns of Worker–Drone Interactions and Drone Flight Activity

To assess possible associations between temporal patterns of worker–drone interactions and flight activity, we scanned each observation hive for 5 min each hour during 0800–1700 hours, 5 days each week throughout the 3-week study period. During each scan, the two sides of an observation hive were scanned simultaneously by separate observers and the number of vibration signals and piping signals performed on the tagged drones were counted and summed for both sides, to give a total number for each hourly period. Each hourly value was divided by the total number of tagged drones present in the colony for that hour, giving the number of signals per drone per 5 min. In this manner, we could account for hourly changes in the number of drones present in a colony on a given day due to drone flight, and changes in the number of drones on different days due to attrition as the study period progressed. Immediately following each 5 min scan, we conducted two 1-minute counts of the number of tagged drones flying from the hive. Subsequently, we determined hourly means for the number of vibration signals per drone per 5 min, the number of piping signals per drone per 5 min, and flights from the hive per min over all days of observation during the period of sexual immaturity and sexual maturity.

Assessing the Behaviour of Drones Receiving Vibration Signals and Worker Piping

We used two approaches to examine the effects of vibration signals and piping on drone activity. First, we compared the behaviour of tagged drones that did and did not receive signals. Throughout the study period, vibrated and piped drones were selected at random and observed for 20 min. For each drone that received a signal, we also monitored a tagged control drone that was of the same age and in a similar initial location of the nest. If a control drone received a vibration or piping signal during the observation period, a new control was selected and observed for 20 min. To the extent possible, we monitored the behaviour of each drone and its control counterpart within 1 h of one another, although both drones were often monitored simultaneously by separate observers. Each drone was monitored only once. Because of drone attrition as the study progressed, it was not always possible to obtain controls that had not already been monitored, especially towards the end of the study period. As a result, the number of recipient and control drones in some of our colonies differed.

For each recipient drone and its control, we recorded the following using digital stopwatches when necessary: (1) age, (2) number of grid squares crossed, (3) total signals received, (4) time spent receiving trophallaxis and grooming from workers, (5) time spent feeding from cells and (6) whether it flew from the hive. Subsequently, we classified each drone as sexually immature or sexually mature and calculated its movement rate (grid squares crossed/min), interaction rate (total interactions/min, comprising the combined total of all signals received and all incidences of trophallaxis and grooming) and the proportion of time spent receiving trophallaxis, grooming and feeding from cells.

Our second approach to assess the effects of the signals on drone behaviour was to compare the activities of individual drones before and after receiving a vibration signal or piping. If, while observing a control drone, it was vibrated or piped, we recorded the observation time that had elapsed before the signal was received and then continued to monitor the drone's behaviour for up to 20 min after the signal occurred. Subsequently, we calculated before and after values for movement rate, interaction rate, the proportion of time spent receiving trophallaxis and grooming and the proportion of time spent feeding from cells.

Examining Worker–Drone Interactions during Mating Flight Activity

Throughout the periods of mating flight activity, we randomly selected tagged drones as they returned to the observation colonies and monitored their behaviour for 10 min each, or until they left the nest on another flight. We determined for each drone the number of vibration and piping signals received, the total number of interactions with workers, the proportion of time spent in trophallaxis and the proportion of time spent feeding from cells.

Statistical Analyses

We used repeated measures ANOVA (Sokal & Rohlf 1995) to compare the daily proportions of drones that received each signal during the periods of sexually immaturity and sexually maturity. Proportional data were arcsine transformed prior to analysis to achieve normality.

We used the following approach to assess the daily patterns of worker–drone interactions. First, we compared hourly values for signalling rates and drone flight among the three colonies. Because of the large number of zero values, the data could not be normalized, and thus, we used Kruskal–Wallis tests for our between-colony comparisons. If the colonies did not differ in their activity levels, we combined the data for the subsequent analyses; otherwise, we examined each colony separately. Second, we used Kruskal–Wallis tests to compare the mean hourly vibration and piping rates between the periods of sexual immaturity and maturity. Third, to assess the possible association between signalling rates and drone flight activity, we used Model I regression (Sokal & Rohlf 1995) to examine the relationships between the maximum hourly values for vibration and piping rates on a given day and the maximum number of drones flying from the nest/min that same day during the periods of sexual immaturity and sexual maturity.

To compare the movement rates of drones that received signals and their controls, we used mixed-model ANOVA that used colony as a random factor and generated a drone type (recipient versus control) × sexual-maturity interaction. Movement rates were square-root transformed prior to analysis to normalize the data. For all other recipient–control comparisons (interaction rates; proportion of time spent receiving trophallaxis, grooming and feeding from cells), and for all of our before/after comparisons, we had numerous zero values, such that the data could not be normalized. As a result, we conducted the comparisons using nonparametric Kruskal–Wallis tests (Sokal & Rohlf 1995). We first compared among the three colonies to determine whether the data could be combined and, if so, analysed the data for each variable over all colonies.

All mean values are reported as ±one SE. All statistical tests were two tailed. The sequential Bonferroni adjustment (Rice 1989) was used to determine significance levels for the multiple comparisons made between recipient and control drones, and for the before–after comparisons of individual drones.

RESULTS

In 480 h of observation on our three colonies, we observed only one incident of piping on drones. Thus, piping was excluded from all results reported.

Proportion of Drones that Received Vibration Signals

When viewed over the three observation colonies, 53.3 ± 7.88% of the tagged drones were vibrated during the study period. However, the likelihood that a drone would receive vibration signals varied with its sexual maturity. The mean proportion of drones that were vibrated each day when sexually immature (14.6 ± 0.02%) was more than double the 6.8 ± 0.02% observed during the period of sexual maturity (ANOVA: $F_{1,44} = 11.19$, $P = 0.0017$). This trend was exhibited strongly in colonies A and B, but less so in Colony C, resulting in a significant sexual maturity × colony interaction (ANOVA: $F_{2,44} = 3.64$, $P = 0.035$; Fig. 1).

Daily Patterns of Vibration Signal Activity and Drone Behaviour

The observation colonies did not differ in the hourly values for number of vibration signals per drone per 5 min (Kruskal–Wallis test: $\chi^2_2 = 5.65$, $P = 0.059$) or drone flights/min ($\chi^2_2 = 2.95$, $P = 0.229$). Consequently, the examination of the daily patterns for worker–drone interactions and drone activity were based on the combined data for the three colonies.

The mean hourly vibration rates experienced by drones were quite low and typically did not exceed three to four signals/100 drones in a given 5 min period (Fig. 2a). Despite these low levels of signalling activity, three distinct tendencies emerged from the daily patterns for vibration signals performed on drones. First, for both sexually immature and mature drones, vibration activity was always greatest in the morning and declined or ceased in the afternoon (Fig. 2a). Second, the vibration rates experienced by sexually immature drones were greater than those of mature drones (Kruskal–Wallis test: $\chi^2_1 = 27.09$, $P < 0.0001$; Fig. 2a). Third, there was no clear association between the daily patterns of vibration activity and drone flight. Drone flight was restricted almost entirely to 1400–1700 hours (Fig. 2b). For the sexually immature drones, this afternoon flight activity consisted of orientation flights, whereas for the mature drones it consisted of mating flights. For both the immature and mature drones, the vast majority of vibration signals preceded flight by several hours and fell to near zero during the periods of flight performance (Fig. 2a). There was no relationship between the maximum vibration rate experienced during the morning hours and the maximum flight activity recorded later that same day when the drones were sexually immature (regression analysis: $F_{1,18} = 0.34$, $P = 0.569$, $R^2 = 0.0184$) or sexually mature ($F_{1,28} = 1.36$, $P = 0.254$, $R^2 = 0.0463$). There was a small increase in vibration activity on immature drones at 1300 hours, immediately before the onset of orientation flight behaviour (Fig. 2a). However, there was no relationship between the level of signalling at 1300 hours and the maximum number of drones leaving the hive/min during orientation flights (regression analysis: $F_{1,18} = 1.85$, $P = 0.191$). In combination, these results suggest that there was no strong, immediate association between the vibration signal and drone flight, nor was the signal performed on drones in the morning hours to prepare them for flight later in the day.

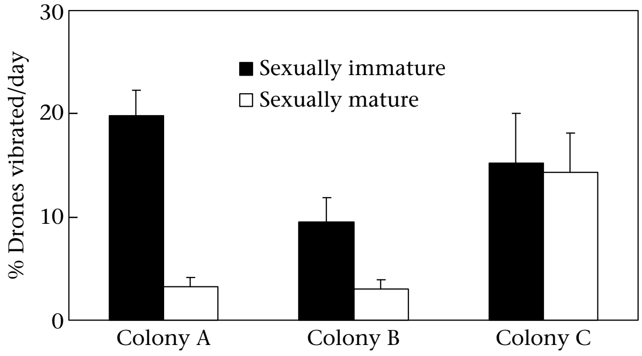

Figure 1. Mean ± SE proportion of honeybee drones in each observation colony that received vibration signals each day before and after they reached sexual maturity.

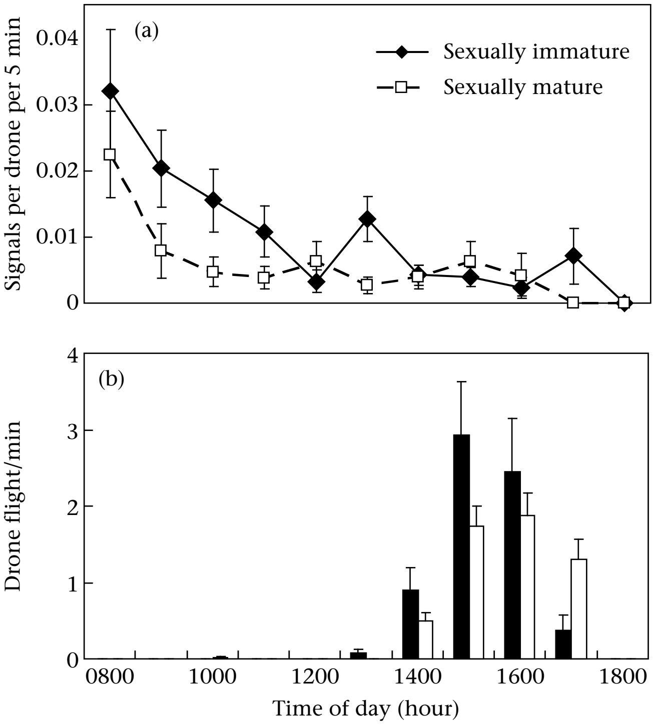

Figure 2. Mean ± SE (a) hourly vibration rates and (b) flight activity for sexually immature and sexually mature honeybee drones.

The Influence of the Vibration Signal on Drone Behaviour

We monitored the behaviour of 76 vibrated drones (25.3 ± 3.71/colony) and 68 same-age, nonvibrated controls (22.7 ± 3.18/colony). Of the 144 total drones observed, 98 were sexually immature and 46 were sexually mature. The drones monitored in the three observation hives did not differ in their movement rates (ANOVA: $F_{2,143} = 0.51$, $P = 0.599$), interaction rates (Kruskal–Wallis test: $\chi^2_2 = 0.38$, $P = 0.82$), or the proportions of time receiving trophallaxis ($\chi^2_2 = 4.84$, $P = 0.089$), grooming ($\chi^2_2 = 4.56$, $P = 0.102$), or feeding from cells ($\chi^2_2 = 0.77$, $P = 0.68$). As a result, we combined the data among colonies and focused our comparisons on the behaviour of recipient and control drones when sexually immature and mature.

Immature and mature drones received 2.7 ± 0.25 and 2.0 ± 0.33 signals/20 min observation period, respectively (Student's *t* test: $t_{74} = 1.53$, $P = 0.13$; Fig. 3). Thus, although overall vibration rates were quite low and immature drones were vibrated at higher rates, the drones that were vibrated tended to receive multiple signals during a given period of time.

Vibrated drones were more active and had more interactions with workers than did nonvibrated controls. Although drones tended to move relatively little during our observations, vibrated drones showed greater rates of locomotion compared to nonvibrated controls (ANOVA: $F_{1,140} = 17.60$, $P < 0.0001$), regardless of whether drones were sexually immature or mature ($F_{1,140} = 2.41$, $P = 0.123$; Fig. 4).

Vibrated drones interacted with workers at higher rates compared to controls, both when sexually immature (Kruskal–Wallis test: $\chi^2_1 = 41.78$, $P < 0.0001$) and sexually mature ($\chi^2_1 = 18.03$, $P < 0.0001$; Fig. 4). Compared to controls, vibrated drones spent greater proportions of time receiving trophallaxis (Kruskal–Wallis test: immature drones: $\chi^2_1 = 10.66$, $P = 0.0011$; mature drones: $\chi^2_1 = 7.03$, $P = 0.008$; Fig. 5). Immature vibrated drones spent more time receiving grooming compared to controls (Kruskal–Wallis test: $\chi^2_1 = 3.88$, $P = 0.0487$; Fig. 5a). Vibrated mature drones also tended to receive more grooming than controls, although the difference was not significant (Kruskal–Wallis test: $\chi^2_1 = 1.74$, $P = 0.188$; Fig. 5b). Vibrated drones and nonvibrated controls did not differ in the proportion of time spent feeding from cells either when immature (Kruskal–Wallis test: $\chi^2_1 = 1.00$, $P = 0.316$) or mature ($\chi^2_1 = 0.18$, $P = 0.669$; Fig. 5). Only two of the vibrated recipients and one control drone flew from the nest during the observation periods, which further suggests that the signal was not associated with stimulating drone flight.

When we compared the behaviour of individual drones before and after they received the vibration signal, the results were similar to those observed for control and vibrated drones. We conducted before/after comparisons for 23 tagged drones (7.7 ± 1.45/colony), which were observed 8.6 ± 0.66 min before and 16.5 ± 0.88 min after receiving the signal. Drones showed little or no activity before they were vibrated, but most of the behavioural aspects monitored increased after the signal was received. Vibrated drones showed slight, significant increases in movement rate after they received the signal compared to their rate before they received the signal (Kruskal–Wallis test: $\chi^2_1 = 4.86$, $P < 0.027$; Fig. 6a). Likewise, after receiving the vibration signal, drones interacted with workers at higher rates (Kruskal–Wallis test: $\chi^2_1 = 17.38$, $P < 0.0001$; Fig. 6a) and spent more time receiving trophallaxis ($\chi^2_1 = 5.72$, $P = 0.0167$; Fig. 6b). Drones also tended to receive more grooming and spent more time feeding from cells after receiving vibration signals, and these differences

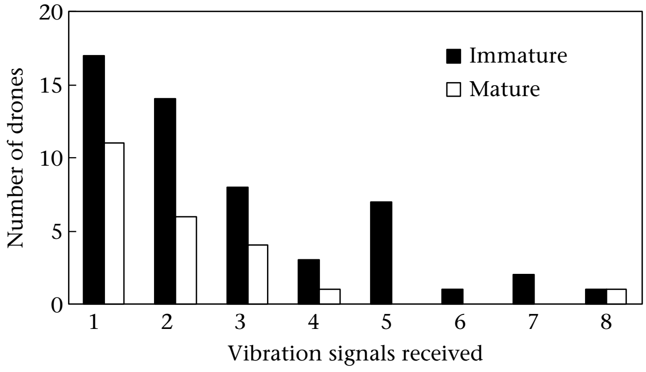

Figure 3. Distribution of the number of vibration signals received by sexually immature and mature honeybee drones.

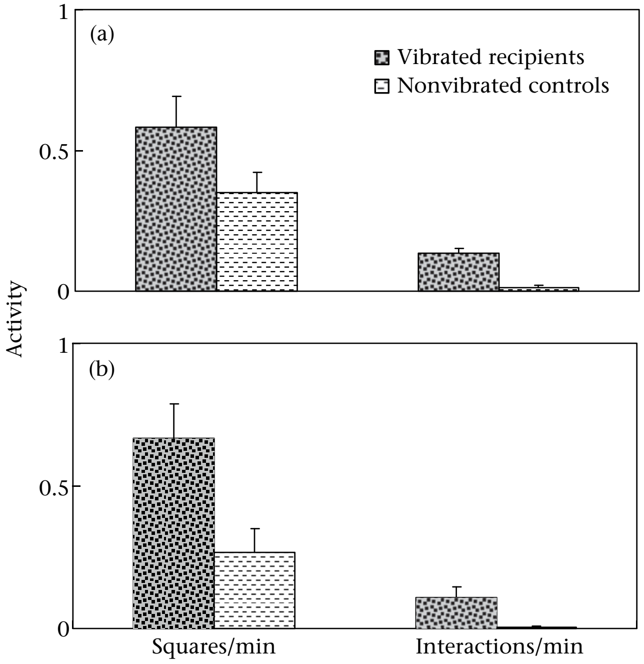

Figure 4. Mean ± SE movement rates (squares crossed/min) and interaction rates (total interactions with workers/min) for (a) sexually immature and (b) mature vibrated honeybee drones and their same-age nonvibrated controls.

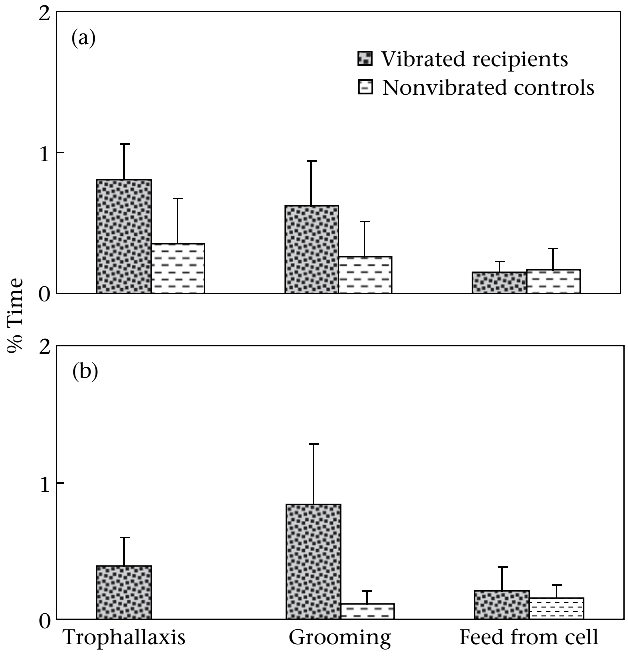

Figure 5. Mean ± SE proportions of time spent receiving trophallaxis, grooming and feeding from cells for (a) sexually immature and (b) mature vibrated honeybee drones and their same-age nonvibrated controls.

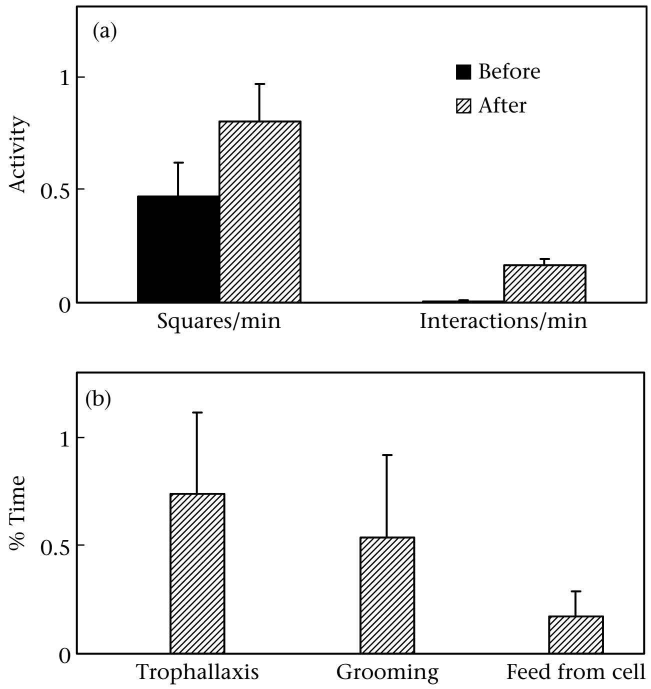

Figure 6. (a) Mean ± SE movement rates (squares crossed/min) and interaction rates (total interactions with workers/min) for individual honeybee drones before and after receiving the vibration signal. (b) Mean ± SE proportions of time that individual honeybee drones engaged in trophallaxis, grooming and feeding from cells before and after receiving the vibration signal.

approached significance (Kruskal–Wallis test: grooming: $\chi^2_1 = 3.62$, $P = 0.057$; feeding from cells: $\chi^2_1 = 3.14$, $P = 0.077$; Fig. 6b). None of the drones for which we conducted before/after comparisons flew from the nest after receiving the vibration signal.

The Behaviour of Drones during Mating Flight Periods

We monitored 89 sexually mature drones (29.7 ± 6.23/colony) that returned to the colonies during mating flight activity. Each drone was observed for 5.0 ± 0.49 min, and during this time they crossed 18.6 ± 1.31 grid squares/min, engaged in 1.5 ± 0.23 interactions with workers, spent 4.5 ± 0.73% of their time receiving trophallaxis, 13.3 ± 1.57% feeding from cells and 3.1 ± 1.26% receiving grooming. Of these returning drones, 88.0% left the hive for another flight. Only four of the 89 drones received vibration signals between consecutive mating flights. Thus, the signal was not associated with drone behaviour during mating flight activity.

DISCUSSION

Whereas communication signals involved in worker–queen interactions in social insects have been extensively studied, those occurring between workers and reproductive males have received less attention, partly because males do not contribute actively to colony labour and partly because the most noticeable activity of males (mating flight) can occur with relatively little worker involvement (Winston 1987; Hölldobler & Wilson 1990; Koeniger et al. 1994). However, our results suggest that honeybee workers may use the vibration signal to influence drone behaviour in a manner that could potentially enhance mating performance. In particular, two lines of evidence suggest that workers may use the vibration signal to promote drone sexual maturation.

First, our results suggest that drones may be more likely to receive vibration signals when they are sexually immature. Drones were vibrated at higher rates when immature and, in two of our three colonies, the proportions of drones receiving signals when immature were double those observed during the periods of sexual maturity. These results must be interpreted cautiously, however, because we did not have drones of different ages in the colonies at the same time. Thus, the greater vibration activity on immature drones in our study may have resulted simply because levels of signalling behaviour in the colony were greater during periods of sexual immaturity, independent of drone developmental state. However, colony vibration activity shows relatively moderate day-to-day variation during the period in which the present study was conducted (Hyland et al. 2007). It therefore seems unlikely that the different vibration rates observed on immature and mature drones were simply artefacts of colony-level signalling activity. Although we cannot fully evaluate the relationship between the vibration signal and drone maturity, our results suggest that workers may have directed their signalling activity more strongly towards immature drones.

Second, immature drones that were vibrated showed increased movement and had more interactions with workers, which in turn, may have contributed to greater amounts of time spent receiving trophallaxis and grooming. Trophallaxis supplies the proteins and nutrients necessary for sexual maturation, and grooming may also contribute to drone health. Thus, workers may use the vibration signal to help ensure that young drones receive the care necessary for proper sexual development. Additionally, workers may use the signal to help maintain drone condition after sexual maturation, because mature drones that were vibrated also tended to spend more time receiving trophallaxis and grooming. Worker honeybees that receive vibration signals show slight, significant increases in juvenile hormone (JH) titers (Schneider et al. 2004), but it is unknown whether drones show a similar hormonal response. However, in many insect species, male sexual development is influenced by JH (Gillott 1995). It is therefore possible that the vibration signal could also contribute to drone development by affecting endocrine responses that promote sexual maturation.

The factors that influence the performance of vibration signals on drones are unclear. Signalling activity on drones was consistently highest during the early morning hours, which is also the period of the greatest vibration activity on workers (Schneider et al. 1986; Biesmeijer 2003; Matilla et al. 2008). Drones may therefore not have been actively selected as recipients, but rather were vibrated incidentally during periods of signal production. On the other hand, several observations suggest that workers may specifically direct signals towards certain drones. Drones may have been vibrated more when sexually immature, suggesting that drone age and developmental state influence signal performance. Also, the drones that were vibrated tended to receive multiple signals during a given 20 min period, suggesting that signalling activity was focused on a subset of drones in the colony. Workers and queens tend to be vibrated more when they are inactive than when they are active (Schneider 1991; Lewis et al. 2002; Hyland et al. 2007), raising the possibility that drone activity also influences the likelihood of receiving signals. Thus, if the vibration signal promotes trophallaxis and grooming (and perhaps hormonal responses), then workers may direct their signals towards particular drones based on some assessment of physiological or behavioural condition.

We cannot fully explain why some workers may use the vibration signal to enhance the feeding and care of drones indirectly, rather than directly engaging in these interactions with drones themselves. This may be related to the age of workers that vibrate reproductive castes. Vibration signals performed on queens are produced primarily by older, foraging-age workers (Painter-Kurt & Schneider 1998). Older bees typically have little contact with queens and do not produce the proteinaceous secretions that are distributed to reproductives through trophallaxis (Winston 1987). If the workers that vibrate drones are also older bees, then perhaps they cannot provide proteins through trophallaxis directly, even if they can assess and respond to drone physiological and developmental state. Thus, although some workers can feed and groom drones directly, others may enhance these interactions indirectly through the vibration signal. In this manner, the signal may be used to fine-tune the care and development of more drones, resulting in greater colony reproductive output. However, we did not determine the characteristics of the workers that vibrated drones and, at present, our understanding of worker–drone interactions is too rudimentary to allow for definitive conclusions about the role of the vibration signal in drone sexual development.

We found no strong evidence that workers used the vibration signal to promote drone flight. Few of the drones monitored flew from the observation colonies after receiving vibration signals. Most vibration activity preceded drone flight by several hours and there was no association between the maximum signalling activity each morning and drone flight activity later that same day. Vibrated drones did not significantly increase their rate of feeding from cells, which is the primary source of energy for flight. Also, there was virtually no vibration activity on drones during periods of mating flight. In contrast, the results of previous studies showed that the vibration signal is used to enhance flight in workers and queens (Allen 1959; Schneider 1991; Lewis & Schneider 2000), and that vibration activity in the morning and worker foraging activity later in the day are positively correlated (Schneider et al. 1986). Furthermore, whereas piping is the immediate trigger for flight in workers and queens during swarming (Seeley & Tautz 2001; Donahoe et al. 2003; Pierce et al. 2007; Rangel & Seeley 2008), workers did not perform this signal on drones. Taken together, these observations support the hypothesis that endogenous rhythms are primarily responsible for orchestrating flight activity in honeybee drones (Koeniger et al. 1994), and that worker communication signals have little influence on drone orientation or mating flights. Similarly, male flight in many species of ants shows strong circadian rhythms that do not involve communication with workers (McCluskey 1965; Wilson 1971; Hölldobler & Wilson 1990).

It is unclear why the vibration signal and piping are not used to influence drone flight, given their strong effects on flight activity in other honeybee castes. However, these differences may reflect the contexts in which reproductive flights occurs and the number of individuals involved, and may provide insights into the different levels of selection that have shaped caste interactions in honeybees. Flight by queens occurs within the social contexts of reproductive swarming, in which the behaviour of one or a few reproductive females must be coordinated with colony-level actions to achieve a group-level response. In contrast, drone flight involves hundreds of different individuals acting independently within an appropriate time frame during the day. It is conceivable that worker involvement during drone flight could limit the variation among individual drones in the timing and number of mating flights, which may be important components of colony reproductive success. Thus, colony-level selection may have contributed to the use of the vibration signal and piping to organize queen flight within a social context, whereas drone flight may have been shaped more by selective pressures associated with individual reproductive behaviour.

Our results suggest that the vibration signal may contribute to sexual maturation of honeybee drones primarily by increasing trophallaxis and grooming activity. The feeding of male reproductives through trophallaxis is common in the social insects and has been reported for a wide array of ants, bees, wasps and termites (Hölldobler 1964; Montagner 1964; Wilson 1971; Hölldobler & Wilson 1990). Many of these species also perform vibratory tactile displays that modulate recipient activity (Hölldobler & Wilson 1990; Schneider & Lewis 2004; Jeanne 2009). Although these signals have been studied primarily for their effect on worker behaviour (Fuchs 1976; Brillet et al. 1999; Schneider et al. 2004; Cao et al. 2009), these signals are sometimes used to influence the activity of reproductives (West-Eberhard 1978; Hölldobler et al. 1996; Schneider et al. 2001; Schneider & DeGrandi-Hoffman 2003, 2008). Thus, vibration-like displays may provide fruitful avenues for investigating worker regulation of male behaviour in many social insect species, and for understanding the role of these interactions in formulating colony-level reproductive decisions.

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