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The Vibration Signal and Juvenile Hormone Titers in Worker Honeybees, *Apis mellifera*

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Abstract

We examined the association between the vibration signal and juvenile hormone (JH) titers of honeybees by comparing vibrated recipients and non-vibrated control workers that had been matched for age, colony of origin, and time of collection. Recipients collected at the moment they received vibration signals (0-min bees) did not have higher JH titers compared with controls, which suggests that a worker's initial JH level did not influence its likelihood of receiving signals. In contrast, JH titers in workers collected 15–30 min after receiving vibration signals were slightly, but significantly higher than those of controls monitored for the same amount of time. These trends were consistent among colonies, despite the fact that we collected different age ranges of workers and observed pronounced variation in JH titers within and between the 0- and 15–30-min groups of bees. Thus, over a broad age range of workers the vibration signal may contribute to elevated JH levels, and this effect does not occur because recipients have higher titers at the moment they receive signals. Because JH affects response thresholds in honeybees, increased titers elicited by the vibration signal may allow the signal to influence the performance of a variety of tasks in different worker age groups.

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Introduction

A primary objective in the study of highly social insects is to understand how communication signals help organize cooperative activities within and among different worker groups. Much of the research conducted on social insect communication has focused on determining the behavioral responses elicited by specific signals. However, to fully assess the function of signals, it is also necessary to understand the proximate physiological mechanisms underlying the observed behavioral responses. For example, honeybee queen mandibular pheromone

(QMP) influences worker–queen interactions, queen rearing and the age-related division of labor (Pankiw et al. 1998; Winston & Slessor 1998; Pankiw 2004). QMP changes the expression of a wide array of genes, which in turn may affect endocrine and physiological processes that change stimulus response thresholds and alter the behavioral state of workers (Grozinger et al. 2003). In contrast, we do not have a good understanding of the physiological mechanisms through which most other communication signals affect the behavior of recipients.

We examined the physiological basis underlying the responses of worker honeybees to the vibration signal, which consists of a bee rapidly vibrating her body dorso-ventrally for 1–2 s while grasping a worker with her legs (Schneider & Lewis 2004). The vibration signal is associated with many cooperative activities in honeybees, including foraging, brood care, queen rearing, colony reproduction and swarm movement (Nieh 1998; Schneider et al. 2001; Schneider & DeGrandi-Hoffman 2002; Donahoe et al. 2003; Schneider & Lewis 2004). Vibrating workers roam throughout large areas of the nest and preferentially direct their signals toward less active recipients of all ages (Lewis et al. 2002). Workers respond to the vibration signal with increased activity, although this response is delayed and often does not become detectable until 15–30 min after the signal has been received (Schneider et al. 1986; Schneider 1987; Nieh 1998). The effect of the signal is most often expressed as heightened locomotion (Schneider et al. 1986; Nieh 1998; Lewis & Schneider 2000), but recipients can also respond with increased task performance. Older workers that are vibrated move into the 'dance area' of the nest, where they are more likely to contact the odor cues and waggle dances that stimulate food collection (Schneider et al. 1986). In contrast, younger recipients spend more time performing in-hive tasks such as brood care, food processing and comb manipulation (Schneider 1987; Schneider & McNally 1991). Thus, the vibration signal acts in a non-specific 'modulatory' manner to enhance a wide array of specific actions, depending in part upon recipient age (Nieh 1998; Schneider & Lewis 2004). The signal may therefore help to coordinate labor among worker groups that perform different, but interrelated tasks (Lewis & Schneider 2000; Schneider & Lewis 2004).

The vibration signal could influence worker behavior by affecting the production of juvenile hormone (JH). JH contributes to the changing response thresholds associated with the age-related division of labor in honeybees, and much research has concentrated on the role of JH in long-term behavioral development that occurs over days or weeks (Robinson 1992; Robinson & Vargo 1997). However, JH can also influence the behavior of honeybee workers and other insects on time scales that can be measured in hours or minutes (Trumbo et al. 1995; Elekonich et al. 2001; Maleszka & Helliwell 2001; Scott et al. 2001; Lin et al. 2004). If the vibration signal is associated with a short-term change in JH titers, then it might cause a temporary, general alteration of response thresholds, which in turn could contribute to the performance of a variety of specific actions depending upon recipient age and contextual cues. However, to date there has been no investigation of the possible physiological effects of the vibration signal on worker bees.

Our study had two main objectives. First, we examined if a worker's initial JH level influenced its likelihood of receiving vibration signals. Secondly, we examined changes in the JH titers of workers 15–30 min after receiving vibration signals, to determine if the signal induced an endocrine response.

Materials and Methods

Colony Setup and Collection of Vibrated Recipients

The study was conducted on the campus of the University of North Carolina at Charlotte using three colonies (A, B, and C) maintained in four-frame observation hives. The Plexiglas® walls of each hive contained four hinged access ports (each 18 × 40 cm) through which workers could be removed. We established in each colony a population of bees of known age by adding cohorts of 100–200 newly emerged workers marked on the thorax or abdomen with a distinguishing color of paint. Workers for marking were obtained from combs maintained in an incubator (34°C; 50% relative humidity) and paint marked within 24 h of emergence. Cohorts of marked workers were added every 2–3 d for 4 wk.

An observation hive was scanned until a paint-marked worker received a vibration signal. For each recipient, we recorded its age and activity at the time the signal was received (e.g. standing stationary, walking, or engaged in a task such as manipulating comb, ventilating, attending the queen, brood care or food processing). The recipient was then removed through the hinged access ports using padded forceps. The forceps were rinsed with distilled water after each collection to minimize the accumulation of any pheromones that may have been released during capture. We collected two groups of vibrated bees: those removed immediately after they received a vibration signal (0-min bees) and those that were monitored for 15–30 min after receiving vibration signals and then removed (15–30-min bees). Because of time constraints, we did not collect both categories of workers from all three observation colonies. 0-min bees were collected from colonies A and B, and 15–30-min bees were collected from colonies A and C and were monitored for 17.3 ± 0.13 min before removal from the observation hives. For each vibrated recipient collected, we collected within 1 h a non-vibrated control bee from the same colony. To the extent possible, each control was of the same age and exhibited the same initial level of activity as its vibrated counterpart. The 0-min controls were collected immediately upon selection, whereas the 15–30-min controls were monitored for the same amount of time as their vibrated counterparts before collection. If a 15–30-min recipient or control could not be collected at the end of the monitoring period because it had moved beyond the area of an observation port, then it was abandoned and a new marked bee was selected.

Vibrated bees were selected at random, in that we collected the first paint-marked worker observed to receive a signal during a scan of an observation colony. As a result, we did not collect the same age ranges of workers for the 0- and 15–30-min bees from the different colonies.

Obtaining Hemolymph Samples

Each collected worker was placed in a pre-labeled Ziploc® (SC Johnson & Son, Inc., Racine, WI, USA) plastic bag and immediately buried in ice pellets. The cold-anesthetized workers were then immobilized with strips of plasticine in a chilled dissecting dish and placed under a dissecting microscope. A hole was pricked on the inter-segmental membrane between the second and third abdominal segments and hemolymph was collected in a capillary tube that had been pre-baked at 500°C for 3.5 h to minimize JH adsorption (Strambi et al. 1981). A minimum of 2 μl of hemolymph was collected from each bee, measured to the nearest 0.1 μl, immediately expelled into 500 μl acetonitrile to precipitate blood proteins and JH-degrading enzymes, and then stored at −20°C for later JH analysis. All hemolymph samples were gathered within 30 min of placing the bees on ice. This amount of time in chilling does not affect JH levels (Lin et al. 2004). We occasionally failed to successfully collect a hemolymph sample and thus did not have exactly the same number of samples for recipient and control bees within each of our worker groups.

For each worker, we measured the hemolymph titer of JH III (the only form of JH found in honeybees; Hagenguth & Rembold 1978) using the chiral-specific radioimmunoassay technique described by Hunnicutt et al. (1989) and validated for adult honeybees by Huang et al. (1994). JH in the hemolymph samples was extracted twice with hexane and evaporated using a vacuum centrifuge linked to a condenser that trapped the solvent at −98°C (Savant SS21). Dried JH was re-dissolved in 50 μl methanol and incubation was started by adding a 5-μl aliquot of the methanol solution to a 200 μl mix of JH antiserum (1:28 000) and 10 000 DPM of [10-^{3}H(N)]-JH (MEM, 629 Gbq/mmol). Liquid scintillation counting was performed using a Packard Tricarb 2100TR (Downers Grove, IL, USA). All solvents were HPLC grade, obtained from Burdick and Jackson (Muskegon, MI, USA).

Statistical Analyses

The JH titers were compared using two-way analyses of variance, which had one between-subject factor (colony), one within-subject factor (bee type: vibrated vs. control worker), and a colony-by-bee type interaction. A separate analysis was conducted for the 0- and 15–30-min bees. A Box-Cox transformation (Sokal & Rohlf 1995) was used to normalize the data for JH titers prior to analysis. The sequential Bonferroni adjustment was used to determine significance levels for the multiple comparisons made between recipient and control bees. All values are reported as untransformed $\bar{x} \pm 1$ SE.

Results

We gathered hemolymph samples from a total of 86 0-min bees and 53 15–30-min bees (Table 1). Although we collected workers of all ages, the random process by which vibrated recipients were selected resulted in our examining

Table 1: Sample size and $\bar{x} \pm$ SE age (in days) of the recipient and control workers for the 0- and 15–30-min bees for which hemolymph samples were collected. 0-min bees were not collected from colony C; 15–30-min bees were not collected from colony B

	0-min Bees				15–30-min Bees			
	Recipient		Control		Recipient		Control	
Colony	n	Age	n	Age	n	Age	n	Age
A	17	24.9 ± 2.8	19	23.6 ± 2.6	8	16.1 ± 2.7	8	16.1 ± 2.7
B	25	15.7 ± 2.3	25	15.6 ± 2.2				
C					19	6.5 ± 0.9	18	7.5 ± 0.8

different age ranges of workers for the 0- and 15–30-min bees in our three colonies (Table 1). We observed pronounced variation in JH titers between colonies and among workers within the same colony (Figs 1 and 2).

A worker's likelihood of receiving vibration signals was not strongly associated with its JH titer. The JH titers of recipient 0-min bees were slightly higher than those of controls in both colonies examined (Fig. 1). However, this difference was not significant ($F = 0.86$; $df = 1, 85$; $p = 0.356$; Fig. 1). There was no colony-by-bee type interaction ($F = 1.02$; $df = 1, 85$; $p = 0.315$), despite the fact that the bees collected from colony A were on average 9–10 d older than those from colony B (Table 1). Thus, recipients did not have higher JH titers at the moment they received vibration signals, and this trend was consistent between colonies and over a broad age range of workers.

Vibration signals may have contributed to elevated JH levels. On average, the JH titers of workers 15–30 min after receiving vibration signals were 9–26 ng/ml higher than those of non-vibrated, same-age control bees monitored for the same amount of time ($F = 9.42$; $df = 1, 52$; $p = 0.0035$; Fig. 2). There was no colony-by-bee type interaction ($F = 0.06$; $df = 1, 52$; $p = 0.809$), despite the fact that

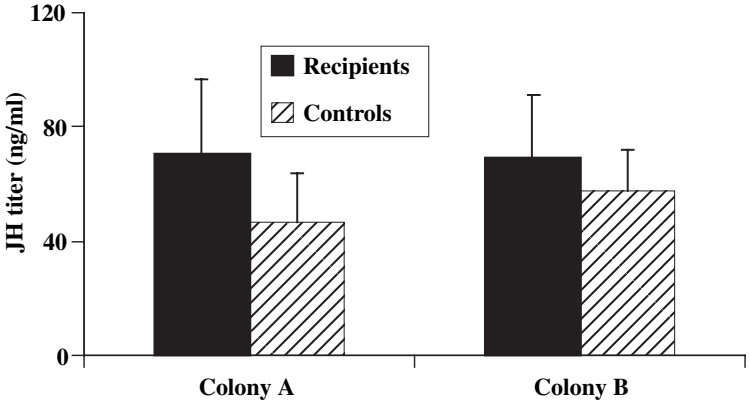

Fig. 1: $\bar{x} \pm$ SE JH titers for vibrated and control 0-min bees in colonies A and B. The difference between the two groups is not significant ($p = 0.356$; two-way ANOVA)

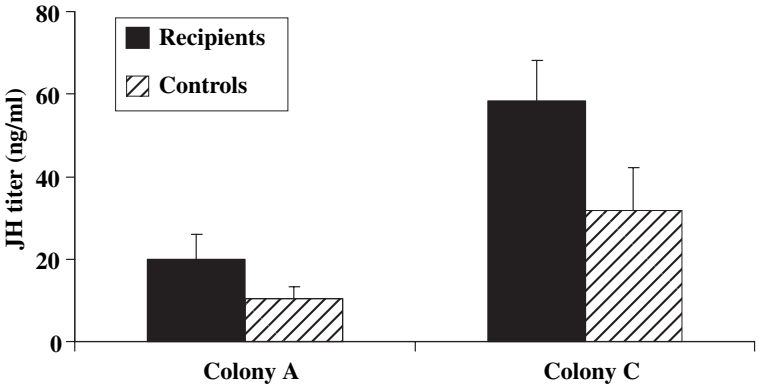

Fig. 2: $\bar{x} \pm$ SE JH titers for vibrated recipients and non-vibrated control bees after 15–30 min of observation in colonies A and C. The difference between the two groups is significant ($p = 0.0035$; two-way ANOVA)

the 15–30-min bees collected from colony A had only one-third the levels of JH ($F = 22.51$; $df = 1, 52$; $p < 0.0001$; Fig. 2) but were on average 10 d older than those collected from colony C (Table 1). Thus, regardless of the initial levels of JH, titers showed a slight, but significant increase within 15–30 min after receiving vibration signals over a broad age range of workers.

Discussion

The JH titers in this study exhibited considerable variation within and between the 0- and 15–30-min groups of bees, and this may have arisen from two main factors. First, we examined different age ranges of workers in our colonies and JH titers vary with age. JH titers are usually lower in younger workers, increase shortly before the onset of foraging behavior and then remain higher in older, foraging-age bees (Huang & Robinson 1996; Robinson & Vargo 1997; Elekonich et al. 2001; Lin et al. 2004). Workers in our observation hives typically begin foraging at 19–26 d of age, but can begin as early as 5–6 d or as late as 35–40 d old (Painter-Kurt & Schneider 1998). The mean ages of the workers that we collected suggested that the 0-min bees from colony B and the 15–30-min bees from colonies A and C were primarily of pre-foraging age (Table 1). Indeed, the JH titers measured for these workers were consistent with those previously reported for nurse bees and other young workers before the onset of foraging (Huang et al. 1998; Pankiw et al. 1998; Elekonich et al. 2001; Lin et al. 2004). In contrast, the 0-min bees from colony A were of foraging age (Table 1). Their JH titers were lower than those typically reported for foragers (Huang et al. 1998; Lin et al. 2004), although Pankiw et al. (1998) found similar low levels of JH in foraging-age bees. Thus, some of the variability in JH levels that we observed may have resulted from the differing ages of workers collected and the variability in the age at which foraging began within and between colonies. Secondly, JH titers

fluctuate diurnally and are typically lower in the morning and higher in the afternoon hours (Elekonich et al. 2001). We collected workers throughout the day, which may have further contributed to the observed variability in JH levels.

We attempted to control for these factors by matching recipients and controls for age, colony of origin and time of collection. Despite the age ranges of workers examined and the variability in JH levels, we found two trends in JH titers that were consistent among colonies. First, JH titers were not higher in vibrated recipients compared with non-vibrated controls at the moment the signal was received. This trend was observed for both colony A, from which we collected primarily 0-min bees of foraging age, and colony B, from which the 0-min bees were mostly of pre-foraging age. Secondly, JH titers were significantly elevated 15–30 min after receiving vibration signals and this trend was observed over a wide age range of workers. In combination, these results suggest that for several different age groups of bees the vibration signal contributes to increased JH levels, and that this effect does not result from recipients already having higher titers when they receive the signal. We do not know if the increase in JH after vibration signals also occurs in foraging-aged workers, because the 15–30-min bees that we examined were primarily of pre-foraging age (Table 1). Nevertheless, the available evidence suggests that elevated JH titers may occur within 15–30 min of receiving vibration signals, at least among the age groups of younger workers that perform in-hive tasks. Changes in JH titers within 30 min have been previously reported for honeybees (Elekonich et al. 2001), and changes within 10 min have been observed for burying beetles (Trumbo et al. 1995; Scott et al. 2001).

It is unclear exactly how the vibration signal may contribute to elevated JH titers and alter worker behavior. The effect could be indirect, in that the signal elicits increased activity, which in turn may trigger increased JH release. Alternatively, the signal could cause a direct increase in JH titers within 15–30 min, which could alter response thresholds and contribute to greater activity and task performance. In a recent study, Sullivan et al. (2003) found evidence that higher JH titers may promote increased metabolism in honeybees. This finding is consistent with our suggestion that the vibration signal may increase worker activity by eliciting a slight, temporary increase in JH levels. Such an endocrine response would be consistent with several aspects of the vibration signal, including its delayed mode of action, the non-specific increase in activity it elicits, and the performance of many different tasks by recipients depending upon their age, work history and other contextual cues. Furthermore, an endocrine response that causes a general alteration of metabolism and response thresholds could help to explain how the same signal can be used in many different contexts and directed toward such diverse recipients as young in-hive bees, older foraging-age workers, virgin queens and laying queens. However, before the proposed function of the signal can be fully evaluated many questions must be addressed, including to what extent JH titers and activity levels are associated in workers and queens, how long the elevated JH levels persist, and if JH production and recipient behavior are influenced differently when bees receive single vs. multiple vibration signals.

Božič & Woodring (2000) recently found that the JH titers of foraging-age workers that followed waggle dancers were slightly, but significantly higher than those of the dancers themselves. The authors suggested that dance following may contribute to increased JH biosynthesis, which may help motivate foraging activity. Thus, the effect of several communication signals on honeybee behavior may be mediated by endocrine responses involving JH. Additionally, communication signals could influence the production of brain amines, such as octopamine, which are known to increase JH biosynthesis in both larvae (Rachinsky 1994) and adult honeybees (Kaatz et al. 1994) and also affect foraging ontogeny and honeybee division of labor (Schulz & Robinson 1999; Wagener-Hulme et al. 1999; Barron et al. 2002; Schulz et al. 2002, 2003). Future research should focus on determining brain amine biosynthesis in response to signals such as the vibration signal and the waggle dance.

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