



# Vibration signal behaviour and the use of modulatory communication in established and newly founded honeybee colonies

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We investigated the effects of colony condition on the use of modulatory communication in social insects by examining vibration signal behaviour in established and newly founded colonies of the honeybee, *Apis mellifera*. Compared to the established colonies, the newly founded colonies had smaller population sizes, built more comb, devoted proportionately more comb space to brood rearing and less to food storage, gained weight, and had higher per capita rates of foraging. In conjunction with the different growth and foraging patterns, the newly founded colonies also had higher per capita vibration rates and slightly but significantly greater proportions of workers that produced the signal. Individual vibrators in the newly founded colonies performed signals on slightly but significantly more of the workers they contacted and focused their vibration activity more strongly on less active recipients than did vibrators in the established colonies. Vibrated recipients showed increased task performance compared to nonvibrated controls, suggesting that the signal increased individual labour rates in both colony types. However, the levels of task performance did not differ between newly founded and established colony recipients, suggesting that the degree to which workers responded to the signal was not altered by colony developmental state. Thus, the vibration signal may have helped to adjust worker activity to the resource and labour demands associated with nest founding primarily by activating larger proportions of the less active work force, rather than by increasing the individual work efforts of recipients in the newly founded colonies relative to those in established colonies.

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A primary goal in the study of social insects is to understand how communication signals adjust worker activity to changing colony conditions. Increasing social complexity in insects is frequently associated with an

increased use of modulatory communication signals to organize cooperative behaviour (Anderson & McShea 2001). Modulatory signals do not elicit specific responses in themselves, but rather operate in a general manner to alter the probability that workers will respond to other stimuli, thereby simultaneously influencing many different tasks (Hölldobler & Wilson 1990; Partan & Marler 1999; Schneider & Lewis 2004). Modulatory signals therefore provide opportunities to investigate the mechanisms that help to coordinate broad aspects of cooperative labour with changes in colony state. An example of a modulatory signal is the vibration signal of the honeybee, *Apis mellifera*, which causes a nonspecific increase in activity

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that enhances the performance of a variety of tasks, including brood care, nest maintenance, food processing and foraging (reviewed in Schneider & Lewis 2004). Vibration signals are produced primarily by successful foragers, who often move through large areas of the nest while performing long series of signals that are preferentially directed towards less active bees of all ages (Painter-Kurt & Schneider 1998; Lewis et al. 2002). This behaviour may help to coordinate an array of tasks that must be adjusted to changing colony food intake and energy needs (Schneider & Lewis 2004). However, the effects of colony state on the use of modulatory communication in honeybees are poorly understood.

We investigated the effects of colony condition on vibration signal behaviour by comparing signal use in newly founded and established honeybee colonies. When a swarm first moves into a new nest cavity, it has a reduced population size and limited energy reserves, yet workers must quickly build combs and initiate brood rearing and food storage to amass the resources necessary for winter survival (Seeley & Visscher 1985; Pratt 2004). Thus, compared to established colonies that are buffered by large worker populations, fully constructed combs and large food stores, newly founded colonies may require more carefully balanced allocations of resources and labour among the different colony activities. This, in turn, may necessitate altered task performance and perhaps a greater coordination of behaviour among worker groups that perform interdependent tasks. Indeed, compared to large established colonies, small and newly founded colonies devote greater proportions of comb space to brood rearing, have a greater emphasis on pollen collection, distribute foragers more broadly among food patches, and show greater foraging efforts by individual workers (Fewell et al. 1991; Eckert et al. 1994; Schneider & McNally 1994; Beekman et al. 2004). The vibration signal influences many of these tasks, so the different activity and growth patterns of established and newly founded colonies may arise, in part, from differences in vibration signal use. Examining vibration signal behaviour under different stages of colony development, therefore, provides opportunities to investigate the function of the signal in cooperative labour in honeybees, and to explore the function of modulatory communication in animal social systems in general.

We examined three hypotheses for the relationships between the vibration signal and colony developmental state. Hypothesis 1: in conjunction with differences in the allocation of resources and labour, newly founded colonies will have greater vibration signal activity than established colonies. We predicted that newly founded colonies should show greater per capita vibration rates and greater proportions of workers that perform the signal. Hypothesis 2: compared to workers that perform vibration signals in established colonies, those in newly founded colonies will alter their signalling behaviour to activate a greater proportion of the work force. Specifically, individual vibrators in newly founded colonies should produce signals at a greater rate, perform signals on a greater proportion of the workers they contact, and focus their signals more strongly on workers of certain

ages or those showing particular levels of activity. Hypothesis 3: compared to workers that receive vibration signals in established colonies, recipients in newly founded colonies will show increased labour. We predicted that individual recipients in newly founded colonies would spend more time engaged in tasks and perform more tasks to help meet the labour demands associated with nest founding.

## METHODS

### Experimental Plan and Colony Set-up

The basic plan of our study was to monitor vibration signal behaviour in pairs of four-frame observation hives, one of which contained an established colony and the other a newly founded colony. All colonies were selected from field colonies maintained in five-frame nucleus hives. For a given trial, a pair of nucleus colonies was selected and matched for population size and amounts of brood and food comb. One nucleus colony was randomly designated to become the established colony. The other, which was designated to become the newly founded colony, was used to create an artificial swarm (Lewis & Schneider 2000; Donahoe et al. 2003). The queen was removed from the nucleus colony and confined in a small cage that was suspended inside a large screen-mesh swarm cage (56 × 56 × 38 cm). Next, the workers from the nucleus colony were shaken into the swarm cage and quickly clustered about the queen. The swarm cage was then closed and the bees were fed a sucrose solution (50% by volume) *ad libitum* for 4–5 days, which stimulated wax scale production and simulated the preparations that normally precede swarming and nest founding. In this manner, the swarm was prepared to initiate nest founding and comb construction when moved into an observation hive.

After the swarm cluster was producing large amounts of wax scales, it was transferred to an observation hive that contained four wooden frames. Each frame was empty except for a 42 × 12-cm strip of wax foundation, which ensured that combs were built with the proper orientation within the hive. Thus, to simulate the construction and energy requirements associated with founding a new nest, each newly founded colony was required to build its entire complement of combs (except for the wax foundation strips) and initiate brood rearing and food storage. In contrast, each established colony was set up by transferring from a nucleus hive the queen, 6000–8000 workers and four frames of fully constructed comb that were filled with food and brood in all stages of development. During a given trial, the established and newly founded colonies were transferred into the observation hives simultaneously. The glass walls of the observation hives were marked off in grids of 4 × 4-cm squares to facilitate monitoring of worker behaviour.

Each pair of observation colonies was monitored for 3 weeks, which corresponded to the first complete brood-rearing cycle for the newly founded colony (Winston 1987). We conducted four trials during the spring and

summer of 2004 and 2005 on the campus of the University of North Carolina at Charlotte, involving a total of four established and four newly founded colonies. During a trial, both colonies were monitored simultaneously to ensure that each experienced the same climatic and foraging conditions, although conditions varied between trials and years. The colonies were not fed during the trials, and all food harvested during the observation periods was collected through the colonies' own foraging efforts.

### Establishing Populations of Known-age Workers

We established populations of marked workers of known age in each colony to facilitate observing bees that performed or received vibration signals. On each day during the 2-week period that preceded the onset of a trial, we added cohorts of newly emerged, marked bees to the established and newly founded nucleus hives. Thus, by the time the colonies were transferred to the observation hives, each contained populations of known-aged workers (age range 1–14 days). During the days that the newly founded colony was inside the swarm cage, we continued to add marked workers through a covered opening in the cage lid. To obtain workers for marking, we removed combs of emerging bees from field hives that were unrelated to the established and newly founded colonies and maintained them in an incubator (35°C; 50% RH). All workers were marked within 24 h of emergence so that their exact age (in days) was known throughout the study period. In a given trial, all marked workers added to the study colonies came from the same field colony.

We created two populations of marked workers in each colony. The first consisted of cohorts of 100 workers added every day and labelled with plastic tags (Opalithplättchen, Chr. Graze, Endersbach, Germany) glued to the thorax. The tags provided different colour-number combinations that, in conjunction with paint marks applied to the edges, allowed for individually recognizable marked workers. We added 1100–1300 tagged bees to each colony. The second population of marked workers consisted of cohorts of 200–400 bees, added every 1–2 days that had been marked on the thorax or abdomen with a dot of Testors paint (Rockford, Illinois, U.S.A.). All bees within an age cohort received the same colour and placement of paint mark, but each successive cohort was painted with a different colour and mark location. We added 2000–2500 paint-marked bees to each colony before transfer into the observation hives. We selected workers that performed and received vibration signals from the tagged population to ensure that each worker was monitored only once, although we occasionally monitored painted bees whose markings were individually recognizable. The paint-marked workers were used primarily to provide large populations of known-age bees to assess the effect of worker age on the selection of recipients by vibrating bees. To ensure that a full age range of marked workers was present in the colonies throughout the 3-week observation periods, we continued to add cohorts of paint-marked bees to each observation hive every 2–3 days during a trial.

### Estimating Colony Growth and Foraging Activity

To gauge possible differences in resource and labour allocations between the established and newly founded colonies, we monitored colony growth and foraging activity throughout each trial. Growth patterns were estimated every other day by determining colony population size, comb areas and weight. Worker population size was estimated using the grid squares drawn on the observation hive walls (Seeley 1995). On each side of an observation hive, we counted the number of workers in 20% of the grid squares that contained comb, calculated an average number of bees per square, and then multiplied by the total number of squares of comb present. We focused our counts only on areas of constructed comb, because in the newly founded colonies, few workers gathered on sections of wax foundation that were not being built into comb. Inclusion of these grid squares in our counts would have underestimated population sizes for the newly founded colonies. We also used the 4 × 4-cm grids to estimate, every other day, total comb area and the proportion of comb space that contained nectar, honey, pollen and brood in all stages of development. Colony weight-gain patterns were estimated using a Kubota KA-10 digital platform scale graduated in 5-g increments. Weights were recorded at the end of the day after foraging activity had ceased and all workers had returned to the nest. The weight of the empty hive and wooden comb frames was subtracted from each day's weighing, resulting in a value for the combined weight of all workers, brood, food and comb in the colony. We subtracted each weight value from that of the preceding day's measurement to obtain estimates of fluctuations in colony growth and energetic condition over the course of a trial.

On each day that we monitored colony growth patterns, we also estimated foraging activity. During each hour from 0700 to 1700 hours, we determined: (1) the mean number of bees returning to the hive in two 1-min counts (flight in/min); (2) the mean number of workers returning with pollen loads in two 1-min counts (pollen in/min); and (3) the mean number of waggle dances observed during two 1-min scans of the lower two combs (one scan on each side of an observation colony). We subsequently determined a daily mean for each foraging variable by averaging the counts over all hourly periods. We used the population estimates to express the daily foraging and recruitment rates/1000 bees, to adjust for differences in population size between the established and newly founded colonies on a given day of observation. Examination of the adjusted rates for flight in/min allowed us to compare the total foraging effort between the two colony types. We excluded from our counts periods of orientation flight by young bees, to ensure that we monitored only flight activity that was associated with food collection (Schneider et al. 1986). Comparisons of the adjusted rates for pollen in/min provided an estimate of the degree to which established and newly founded colonies allocated foraging effort towards acquiring protein, which is used primarily for brood production but also for the nutritional needs of adult workers (Winston 1987). The adjusted rates

for waggle dances/min allowed us to examine the influence of colony state on foraging recruitment activity.

### Estimating Colony Vibration Signal Activity

Levels of vibration signal activity were examined in the established and newly founded colonies by determining daily signalling rates and the proportion of workers that performed the signal during the course of a 3-week trial. Using the  $4 \times 4$ -cm grids, we counted the number of vibration signals observed during 3-min scans conducted each hour from 0700 to 1700 hours every other day. A separate 3-min scan was conducted simultaneously on both sides of an observation hive by different observers assigned randomly to the established or newly founded colonies. The results of the scans were averaged over all hourly readings to provide a daily mean for the number of vibration signals/3 min for each colony. All scans of vibration activity were conducted on the same days that we estimated colony growth patterns and foraging activity. Vibration rates were expressed per 1000 bees to adjust for differences in population size, and the adjusted daily rates were used to compare signalling activity in the established and newly founded colonies.

We estimated the proportion of workers that performed vibration signals in the two colony types by determining the total number of tagged bees that vibrated during a trial. We monitored the established and newly founded colonies continuously each day during 0700–1700 hours and recorded the identity of every tagged bee observed to perform vibration signals. Workers could perform signals on more than one day, so we excluded from the data set all but the first observation for each individual vibrator, to ensure that we counted each tagged bee only once. Workers typically begin to perform vibration signals when they are 3 days old, and approximately 90% of tagged bees survive to this age over a broad range of colony conditions (Painter-Kurt & Schneider 1998). We therefore estimated the proportion of vibrating bees in each colony by dividing the number of different vibrators observed by a value corresponding to 90% of the total tagged bees added.

### Monitoring the Behaviour and Selection of Recipients by Workers that Performed Vibration Signals

Throughout a 3-week trial, tagged bees that performed vibration signals were selected at random in the established and newly founded colonies and filmed for 15 min each, or until they were lost within the colony or left the hive. The filming was conducted using a SONY DCR-VX 2000 video camera, which allowed for low-light videorecording inside the observation hives without the use of external light sources. The videotapes were transcribed using a Mitsubishi HS U748 VCR with variable-speed playback capabilities. For each vibrating bee monitored, we recorded: (1) her age; (2) the total time observed; (3) whether she was associated with foraging (e.g. carried

pollen loads, performed or followed waggle or tremble dances, or left the hive during the filming period); (4) the total number of signals produced; (5) the total number of grid squares crossed; and (6) the total number of workers contacted. Subsequently, we determined for each vibrator her signalling rate (signals produced/min), movement rate (grid squares crossed/min) and contact rate (bees contacted/min).

For each worker contacted by a vibrating bee, we recorded: (1) whether she received a vibration signal or was bypassed (antennated but not vibrated); (2) her age, if she was marked; and (3) whether she was active or inactive. A contacted bee was classified as active if she was walking, running, engaged in trophallaxis (mouth-to-mouth exchange of liquid food), grooming another bee, or performing the tasks of brood care, food processing, comb manipulation, ventilating, attending the queen or foraging (carrying pollen loads or performing or following waggle dances). A worker was considered to be engaged in brood care or food processing if, at the time she was contacted, she had at least her head inside a brood cell and was moving in the cell (Lewis et al. 2002). A worker was classified as inactive if she was standing stationary or sitting motionless inside a cell when contacted by a vibrating bee. We subsequently determined for each vibrating bee: (1) the total proportion of contacted workers that received vibration signals; (2) the mean age of vibrated versus bypassed bees; (3) the proportion of active and inactive workers contacted; and (4) the proportion of active and inactive workers that were selected as recipients of the signal.

### Monitoring the Behaviour of Workers that Received Vibration Signals

To study the responses of workers that received vibration signals, we randomly selected vibrated, tagged recipients throughout a trial and monitored each for up to 20 min or until she left the hive. For each recipient, we recorded her age, the total time she was observed and whether she flew from the nest during the observation period. We used digital stopwatches to determine the time spent by each recipient in the tasks of grooming another bee, attending the queen, exchanging food with another worker, ventilating, manipulating comb, performing or following waggle dances or tremble dances, and engaging in brood care and food processing (defined as being active in a brood or food cell for more than 5 s; Schneider 1987; Lewis et al. 2002). For each vibrated recipient monitored, we observed a nonvibrated control that was selected to be of the same age, in the same initial location of the nest, and showing the same initial level of activity as her vibrated counterpart. Each control was selected within 1 h of observing the vibrated recipient and, whenever possible, was monitored by a separate observer simultaneously with its vibrated counterpart. If a control received a vibration signal, observations were terminated and a new control was chosen. Each vibrated recipient and control worker was monitored only once. We subsequently determined for each recipient and control: (1) whether

she was associated with foraging (e.g. flew from the hive or performed or followed recruitment dances); (2) the total number of tasks performed; (3) the proportion of time spent in all tasks combined; and (4) the proportions of time spent in the specific tasks of brood care, food processing and comb manipulation.

## Statistical Analyses

With respect to colony growth and activity patterns, we used paired *t* tests (Sokal & Rohlf 1995) to compare initial and final population sizes between the established and newly founded colonies. We used two-way repeated measures ANOVA (SAS Institute 1997) to compare the two colony types for the proportions of comb area devoted to brood rearing and food storage, daily changes in weight, adjusted foraging and recruitment rates, and adjusted rates of vibration signal activity. Log-linear models were used to compare the total number of tagged bees that performed vibration signal in the two colony types.

To examine the behaviour of individual vibrators in the established and newly founded colonies, we used two-way ANOVA with replication (SAS Institute 1997) to compare, between the colony types: (1) age, signalling rates, movement rates and contact rates for the vibrators; (2) the difference in the mean ages of vibrated and bypassed bees; (3) the difference in the proportions of active and inactive bees contacted by vibrators; and (4) the difference in the proportions of contacted active and inactive workers that were selected as recipients of vibration signals. Log-linear models were used to compare the number of vibrators in each colony type that engaged in foraging.

With respect to the behaviour of vibrated recipients, we used three-way ANOVA with replication (SAS Institute 1997) to compare the difference between recipients and controls within and between the established and newly founded colonies for: (1) the number of tasks performed; (2) the proportion of time spent performing all tasks combined; and (3) the proportions of time spent performing the individual tasks of brood care, food processing and comb manipulation. We compared the number of recipients and controls that flew from the nest or performed or followed a waggle dance or tremble dance using  $4 \times 2 \times 2$  contingency tables (4 trials  $\times$  2 colony types  $\times$  2 bee types, recipient or control) analysed by log-linear models. In the ANOVA and log-linear models, the first-order interactions (bee type  $\times$  colony type) allowed us to assess whether the magnitude of the difference between recipients and controls varied with colony developmental state. The second-order interactions (trial  $\times$  bee type  $\times$  colony type) allowed us to determine whether the effect of colony type on the magnitude of recipient responses differed between trials.

In all the ANOVAs, between-subjects factors were trial and colony type, and the within-subject factors were day of observation (for the comparisons of colony growth and activity patterns), individual vibrator (for the comparisons of vibrator behaviour) and individual recipient or control (for the comparisons of recipient behaviour). If significant interaction terms were present, we conducted post hoc

paired comparisons using Tukey's HSD test (Sokal & Rohlf 1995). To achieve normality, proportional data were arcsine-transformed and colony activity rates and the activity rates for individual recipients and controls were square-root-transformed prior to analysis. For the behaviour of individual vibrators, vibration signalling rates were cube-root-transformed and movement rates were log-transformed before analysis. The sequential Bonferroni adjustment (Rice 1989) was used to determine significance levels for the multiple comparisons made between colonies and trials for the different growth and activity parameters, the behaviour of vibrators, and the behaviour of recipient and control bees. All statistical tests were two tailed; mean values are reported as  $\pm$  one SE.

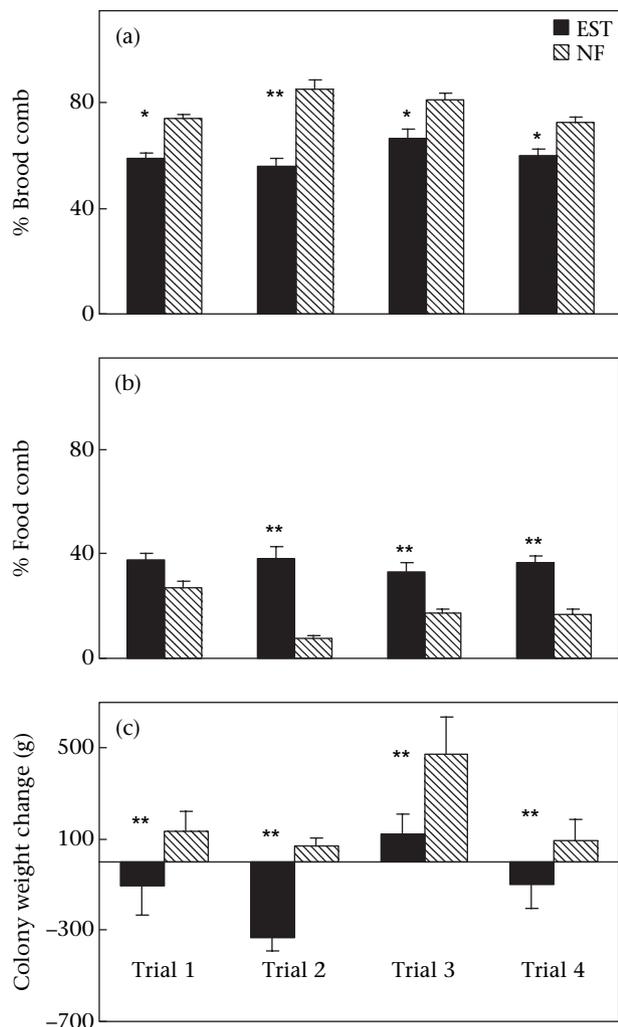
## RESULTS

### Colony Growth Patterns and Foraging Activity

When first transferred into the observation hives, the population sizes of the newly founded colonies ( $4875 \pm 863$  bees,  $N = 4$  colonies) were smaller than those of the established colonies ( $6225 \pm 170$  bees,  $N = 4$ ), but this difference was not statistically significant (Student's *t* test:  $t_3 = 1.04$ ,  $P = 0.40$ ). However, at the end of the trials, the final population size of the newly founded colonies ( $4765 \pm 878$  bees) was approximately half that of the established colonies ( $10\,475 \pm 649$  bees;  $t_3 = 10.67$ ,  $P = 0.002$ ). These differences occurred because there was no interruption of brood rearing in the established colonies, whereas in the newly founded colonies, brood production ceased while the colonies were in the swarm cages, and new workers did not begin to emerge until the end of each 3-week trial.

Comb-building activity, comb usage and weight-gain patterns also differed between the two colony types. The established colonies were set up with four frames of completely built comb, and their comb areas remained virtually constant at approximately  $7050 \text{ cm}^2$  during the study periods. In contrast, the newly founded colonies constructed an average of  $3424 \pm 1197 \text{ cm}^2$  of comb during the trials. The newly founded colonies devoted greater proportions of available comb space to brood rearing than did the established colonies (ANOVA:  $F_{1,52} = 78.49$ ,  $P < 0.0001$ ; Fig. 1a), and this trend was expressed similarly in all trials ( $F_{3,52} = 3.98$ ,  $P = 0.013$ , adjusted alpha level: NS). Concomitantly, the newly founded colonies devoted smaller proportions of comb space to food storage than did the established colonies ( $F_{1,60} = 110.56$ ,  $P < 0.0001$ ; Fig. 1b). This difference was highly significant in trials 2, 3 and 4, but not in trial 1 (Fig. 1b), which resulted in a trial-by-colony type interaction ( $F_{3,60} = 6.58$ ,  $P = 0.0006$ ). Throughout the 3-week trials, the newly founded colonies gained weight, whereas the established colonies tended to lose weight ( $F_{1,52} = 15.94$ ,  $P < 0.001$ ; Fig. 1c), and this trend was similar among trials ( $F_{3,52} = 0.41$ ,  $P = 0.75$ ).

When adjusted for differences in colony population size, the newly founded colonies had greater foraging activity than the established colonies. Compared to the

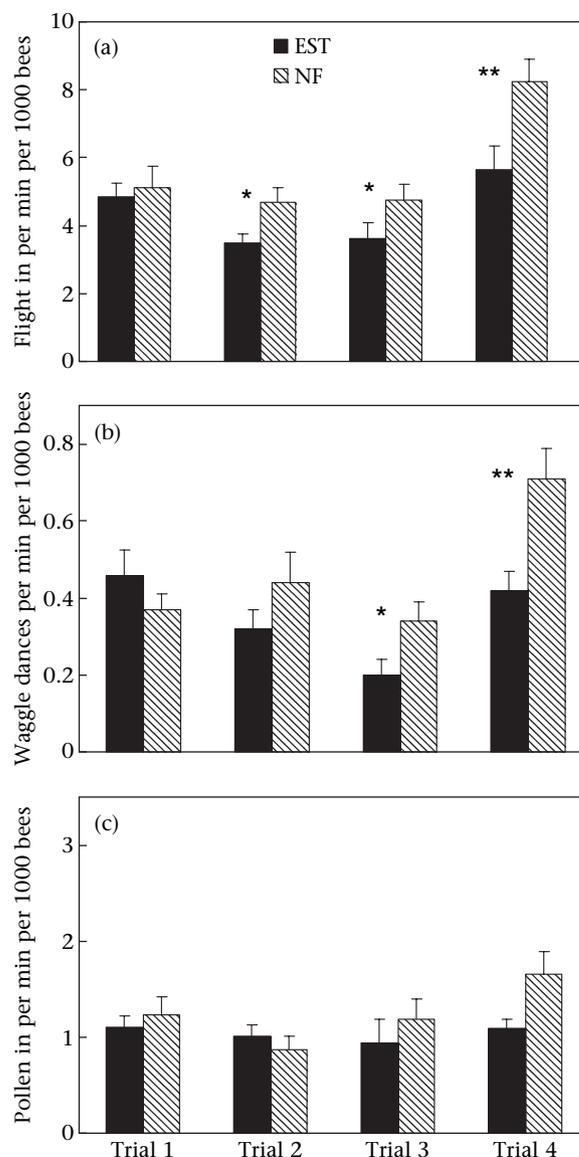


**Figure 1.** Mean  $\pm$  SE proportions of comb space devoted to (a) brood rearing and (b) food storage, and (c) changes in weight gain in the established (EST) and newly founded (NF) colonies during each trial. \* $P < 0.05$ ; \*\* $P < 0.01$ , with Tukey's test.

established colonies, the newly founded colonies had greater flight activity/1000 bees ( $F_{1,60} = 8.71$ ,  $P = 0.0045$ ; Fig. 2a) and waggle dance activity/1000 bees ( $F_{1,60} = 7.08$ ,  $P = 0.010$ ; Fig. 2b), and both trends were shown similarly among trials (flight activity/1000 bees:  $F_{3,60} = 2.02$ ,  $P = 0.121$ ; waggle dance activity/1000 bees:  $F_{3,60} = 2.53$ ,  $P = 0.065$ ). Pollen foraging activity did not differ statistically between the two colony types ( $F_{1,60} = 1.38$ ,  $P = 0.244$ ), although on average, the newly founded colonies had 21% more pollen foragers per min per 1000 bees (Fig. 2c).

### Colony Vibration Signal Activity

The two colony types differed in both vibration signalling rates and the proportion of workers that performed signals. The signalling rates/1000 bees for the newly founded colonies were, on average, 96% greater than those of the established colonies ( $F_{1,60} = 69.33$ ,  $P < 0.0001$ ; Fig. 3). The difference between the two colony

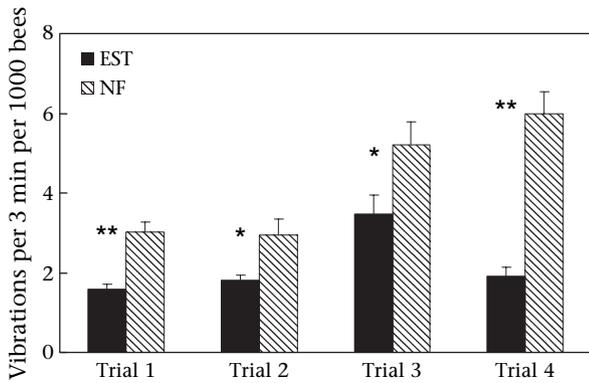


**Figure 2.** Mean  $\pm$  SE foraging rates for the established (EST) and newly founded (NF) colonies during each trial. Rates were adjusted for differences in colony size. \* $P < 0.05$ ; \*\* $P < 0.01$ , with Tukey's test.

types was significant within each trial (Fig. 3), but was more strongly expressed in trials 1 and 4, which resulted in a trial-by-colony type interaction ( $F_{3,60} = 5.72$ ,  $P < 0.0016$ ). A slight but significantly greater proportion of tagged workers produced vibration signals in the newly founded colonies than in the established colonies (log-linear analysis:  $\chi^2_1 = 6.82$ ,  $P = 0.009$ ). On average,  $11.0 \pm 0.53\%$  of the total tagged bees that were added to the newly founded colonies vibrated during a trial; this value for the established colonies was  $8.7 \pm 1.02\%$ .

### Behaviour and Recipient Selection of Individual Vibrators

We videorecorded 401 individual vibrators ( $48.2 \pm 8.2$  vibrators per established colony;  $52.0 \pm 15.1$  vibrators



**Figure 3.** Mean  $\pm$  SE vibration signalling rates for the established (EST) and newly founded (NF) colonies during each trial. Rates were adjusted for differences in colony size. \* $P < 0.05$ ; \*\* $P < 0.01$ , with Tukey's test.

per newly founded colony). On average,  $7.5 \pm 0.25$  min of videorecordings were transcribed for each vibrator monitored. We found no trial-by-colony type interactions for any of the variables examined for workers that performed vibration signals (Table 1). We therefore focused our results on the main effect of colony type.

Vibrators in the established and newly founded colonies did not differ in age (ANOVA:  $F_{1,393} = 4.28$ ,  $P = 0.04$ , adjusted alpha level: NS), signalling rates ( $F_{1,393} = 0.82$ ,  $P = 0.37$ ) or the likelihood of flying from the nest (log-linear analysis:  $\chi^2_1 = 1.05$ ,  $P = 0.305$ ), carrying pollen loads ( $\chi^2_1 = 0.33$ ,  $P = 0.564$ ), or performing waggle dances or tremble dances ( $\chi^2_1 = 0.01$ ,  $P = 0.922$ ) during the observation periods (Table 2). However, vibrators in the newly founded colonies moved at a slower rate ( $F_{1,393} = 8.97$ ,  $P = 0.003$ ) and contacted workers at a lower

**Table 1.** The results of the trial-by-colony type interactions for the variables examined for the behaviour of workers that performed vibration signals and the characteristics of the bees they contacted and selected as recipients of the signal

Trial $\times$ colony type interaction	Statistic	$P$
Vibrator age	$F_{3,393} = 0.57$	0.635
Signalling rates	$F_{3,393} = 0.04$	0.989
Movement rates	$F_{3,393} = 2.38$	0.070
Contact rates	$F_{3,393} = 1.46$	0.226
Proportion of contacted bees vibrated	$F_{3,393} = 1.14$	0.332
Proportion of contacted bees that were active versus inactive	$F_{3,393} = 0.82$	0.366
Age of vibrated versus bypassed bees	$F_{3,107} = 1.68$	0.175
Proportion of active versus inactive bees selected as recipients of vibration signal	$F_{3,393} = 0.31$	0.819
Vibrators that flew from nest	$\chi^2_3 = 3.72$	0.293
Vibrators that carried pollen	$\chi^2_3 = 1.41$	0.703
Vibrators that performed recruitment dances	$\chi^2_3 = 1.37$	0.712

None of the interaction terms was significant, suggesting that patterns of vibrator behaviour were similar in the established and newly founded colonies.

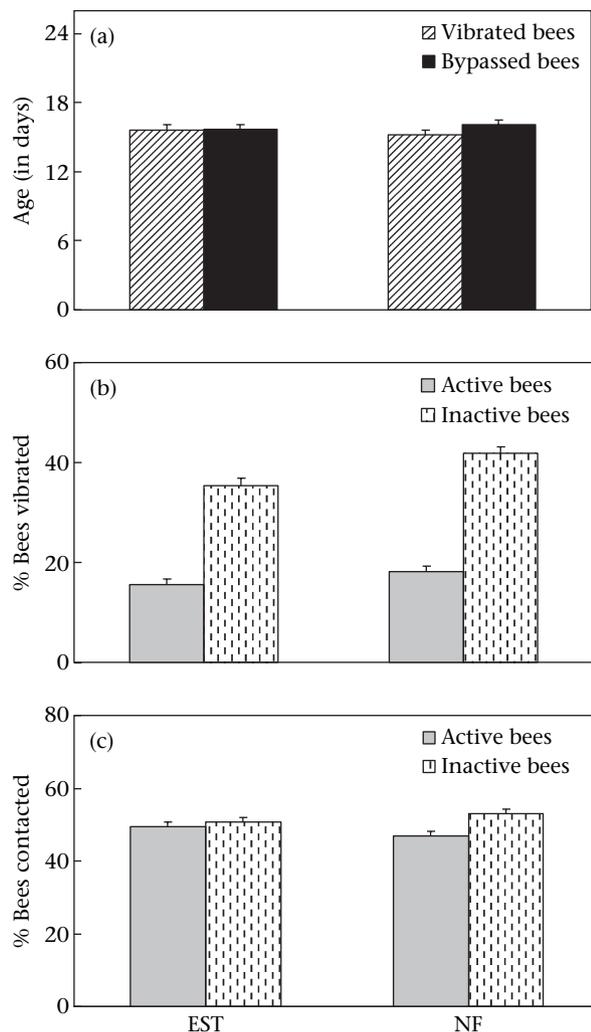
**Table 2.** Mean  $\pm$  SE age and behaviour of workers that performed vibration signals in the established (EST) and newly founded (NF) colonies

	Colony vibrators	
	EST	NF
	( $N=193$ )	( $N=208$ )
Vibrator age (in days)	$18.1 \pm 0.47$	$17.1 \pm 0.42$
Signals performed/min	$5.9 \pm 0.54$	$5.8 \pm 0.26$
Fly from nest	31	33
Carry pollen	12	10
Perform waggle dances or tremble dances	6	4
Squares crossed/min	$5.3 \pm 0.43$	$4.1 \pm 0.22$
Workers contacted/min	$19.7 \pm 1.9$	$15.3 \pm 0.56$
% Contacted workers vibrated	$27.0 \pm 0.90$	$32.0 \pm 0.85$

rate ( $F_{1,393} = 5.63$ ,  $P = 0.0182$ ), but performed signals on a slightly but significantly greater proportion of the workers contacted ( $F_{1,393} = 13.10$ ,  $P = 0.0003$ ) than did the established colony vibrators (Table 2).

Worker age did not influence a vibrator's likelihood of selecting contacted bees as recipients. Of the 401 vibrators videorecorded, 115 contacted enough marked bees to allow for statistical analysis of recipient age. This sample of vibrators revealed that, in both the established and newly founded colonies, signals were performed on workers of all ages, and there was no difference in the mean age of vibrated versus bypassed bees ( $F_{1,107} = 0.14$ ,  $P = 0.71$ ; Fig. 4a). In contrast, the selection of recipients was strongly influenced by the activity level of contacted workers. Although vibrators produced signals on bees of all activity levels, a significantly greater proportion of inactive versus active workers was selected as recipients in both colony types ( $F_{1,373} = 10.49$ ,  $P = 0.0013$ ; Fig. 4b). However, as vibrators moved through the nest, there was no difference in the proportion of inactive and active bees contacted in either colony type ( $F_{1,393} = 0.44$ ,  $P = 0.51$ ; Fig. 4c). Thus, the greater proportion of inactive workers selected as recipients did not result simply because vibrators contacted larger numbers of inactive bees. Furthermore, the tendency to preferentially vibrate inactive workers was slightly but significantly more pronounced in the newly founded colonies ( $F_{1,367} = 9.00$ ,  $P = 0.0029$ ; Fig. 4b). On average,  $23.7 \pm 1.3\%$  more inactive than active workers were selected as recipients in the newly founded colonies; this value in the established colonies was  $19.9 \pm 1.1\%$ .

In summary, the results suggest that colony developmental state did not influence the age or rate at which individual workers produced vibration signals, or the likelihood that vibrators were engaged in foraging. However, compared to vibrators in the established colonies, those in the newly founded colonies showed slight but significant alterations to their movement patterns and selection of recipients, which may have distributed their signals more widely among the less active workers that were contacted as they moved through the nest.



**Figure 4.** Mean  $\pm$  SE (a) age of contacted workers that were vibrated and bypassed (antennated but not vibrated), (b) proportion of active and inactive workers that received vibrations signals and (c) proportion of active and inactive workers contacted by individual vibrators as they moved throughout the established (EST) and newly founded (NF) colonies.

### The Behaviour of Vibrated Recipients

We monitored 894 recipient and control bees during the study period ( $114.0 \pm 3.1$  bees per established colony;  $109.6 \pm 3.9$  bees per newly founded colony). Each recipient and control was observed for  $17.9 \pm 3.94$  min. We found no trial-by-bee type-by-colony type interactions for any aspect of behaviour examined (Table 3) and therefore focused our results on the main effect of colony type.

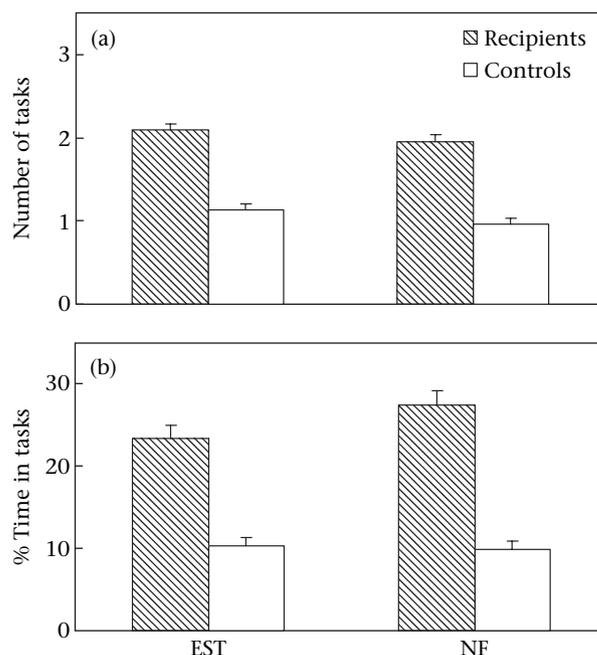
In both the established and newly founded colonies, recipients of the vibration signal showed increased task performance. Compared to nonvibrated controls, recipients performed more tasks ( $F_{1,878} = 178.0$ ,  $P < 0.001$ ; Fig. 5a) and spent a greater proportion of time performing all tasks combined ( $F_{1,878} = 152.9$ ,  $P < 0.001$ ; Fig. 5b). Recipients also spent a greater proportion of time in brood cells ( $F_{1,878} = 54.08$ ,  $P < 0.001$ ), food cells ( $F_{1,878} = 24.86$ ,  $P < 0.001$ ) and comb manipulation ( $F_{1,878} = 56.64$ ,  $P < 0.001$ )

**Table 3.** The results of the trial-by-bee type-by-colony type interactions for the comparisons of the differences between vibrated recipients and nonvibrated controls in the established and newly founded colonies

Trial $\times$ bee type $\times$ colony type interaction	Statistic	<i>P</i>
Number of tasks performed	$F_{3,878} = 2.28$	0.077
Proportion of time spent performing all tasks combined	$F_{3,878} = 0.98$	0.404
Proportion of time in brood cells	$F_{3,878} = 1.83$	0.140
Proportion of time in food cells	$F_{3,878} = 2.52$	0.057
Proportion of time spent in comb manipulation	$F_{3,878} = 0.77$	0.509
Number that flew from nest	$\chi^2_3 = 0.36$	0.551
Number that followed waggle or tremble dances	$\chi^2_3 = 0.93$	0.819

None of the interaction terms was significant, suggesting that patterns of recipient behaviour were similar in both colony types.

than did controls in both colony types (Table 4). Recipients and controls did not differ in their likelihood of flying from the nest (log-linear analysis:  $\chi^2_1 = 0.44$ ,  $P = 0.51$ ), but recipients were significantly more likely to follow waggle dancers or tremble dancers during the observation periods ( $\chi^2_1 = 6.59$ ,  $P = 0.01$ ; Table 4). Too few recipients and controls performed waggle dances or tremble dances to warrant statistical comparisons for these aspects of foraging behaviour. Thus, under both colony conditions, vibrated recipients increased their labour for in-hive tasks and were more likely to encounter recruitment signals that potentially influenced foraging.



**Figure 5.** Mean  $\pm$  SE (a) number of tasks performed and (b) proportion of time spent in all tasks combined for vibrated recipients and nonvibrated controls in the established (EST) and newly founded (NF) colonies.

**Table 4.** Mean  $\pm$  SE proportion of time that vibrated recipients and nonvibrated controls spent in brood care, food processing and comb manipulation, and the number of recipients and controls that engaged in foraging-related tasks in the established (EST) and newly founded (NF) colonies

	EST colonies		NF colonies	
	Recipients	Controls	Recipients	Controls
	(N=228)	(N=228)	(N=219)	(N=219)
% Time in brood cell	7.6 $\pm$ 1.11	2.6 $\pm$ 0.55	10.7 $\pm$ 1.29	2.9 $\pm$ 0.60
% Time in food cell	8.0 $\pm$ 1.06	4.6 $\pm$ 0.76	8.0 $\pm$ 1.10	3.7 $\pm$ 0.68
% Time in comb manipulation	4.3 $\pm$ 0.75	1.4 $\pm$ 0.43	5.0 $\pm$ 0.82	1.1 $\pm$ 0.29
Fly from nest	20	7	18	7
Follow waggle dancer or tremble dancer	26	12	31	11

Colony condition did not influence the degree to which recipients responded to the vibration signal. The magnitude of the difference between recipients and controls did not differ between the two colony types for the number of tasks performed ( $F_{1,878} = 0.00$ ,  $P = 0.969$ ), the proportion of time spent engaged in all tasks combined ( $F_{1,878} = 1.32$ ,  $P = 0.251$ ) or the proportion of time spent in brood cells ( $F_{1,878} = 0.45$ ,  $P = 0.716$ ), food cells ( $F_{1,878} = 0.06$ ,  $P = 0.808$ ) and comb manipulation ( $F_{1,878} = 1.23$ ,  $P = 0.268$ ), or the likelihood of flying from the nest ( $\chi^2_1 = 0.46$ ,  $P = 0.497$ ) or following waggle or tremble dancers ( $\chi^2_1 = 0.33$ ,  $P = 0.564$ ; Fig. 5, Table 4).

In summary, under both colony conditions, vibrated recipients showed increased labour relative to nonvibrated controls. However, the extent to which recipients responded to the signal did not differ between the newly founded and established colonies. Thus, we found no evidence that colony developmental state altered the magnitude of vibrated recipients' responses.

## DISCUSSION

We observed patterns of growth and activity that suggested differential allocations of resources and labour in the established and newly founded colonies. Compared to the established colonies, our newly founded colonies had smaller population sizes, but constructed more comb, devoted greater proportions of comb space to brood rearing and less to food storage, and had greater per capita flight and foraging recruitment rates during the study periods. Similar patterns of population growth, comb use and foraging activity have been reported for newly founded and small honeybee colonies (Fewell et al. 1991; Eckert et al. 1994; Schneider & McNally 1994; Beekman et al. 2004), which suggests that our methods accurately simulated the conditions associated with nest founding. Thus, although the established colonies had greater total amounts of brood and food comb, larger populations and perhaps more total foragers per unit time than the newly founded colonies, the two colony types differed in the relative extents to which efforts were distributed among the various cooperative activities. Furthermore, our newly founded colonies gained weight

throughout the 3-week trials, whereas the established colonies tended to lose weight. Although we cannot fully explain the different weight-gain patterns, they further suggest differences in the allocations of resources and labour, especially considering that, within a trial, both colonies experienced the same foraging environment.

As we predicted, the different growth and activity patterns of the established and newly founded colonies occurred in conjunction with differences in vibration signal activity and modifications to the behaviour of vibrating bees. Compared to the established colonies, the newly founded colonies had greater per capita vibration rates and slightly but significantly greater proportions of workers that performed the signal. Vibrators in the two colony types did not differ in their age, signalling rates or the degree to which they were associated with foraging. However, individual vibrators in the newly founded colonies moved through the nest more slowly and contacted fewer workers/min, but vibrated a greater proportion of the total workers contacted and directed their signals more strongly towards less active bees. Although all of these differences were significant, they were slight, and thus their influence during colony development must remain speculative. Nevertheless, in the collective, the slight modifications to the behaviour of individual vibrators may have resulted in higher levels of signal activity focused on larger portions of the less occupied work force. This, in turn, may have helped to adjust labour to the demands associated with nest founding. Such adjustments may be particularly important during the initial phase of starting a nest, when comb areas and the space available for brood rearing and food storage may increase rapidly, and thus the number of workers needed for the different tasks can change quickly. During this period, increased vibration signal activity preferentially directed towards inactive workers may help to fine-tune labour allocations at a time when colony activities may be rapidly expanding.

Although the likelihood of receiving vibration signals was strongly influenced by worker activity level, it was unaffected by worker age. As vibrating bees roamed through the colonies, they performed signals on workers of all ages, and there was no evidence that signalling activity was directed towards particular age groups of

recipients in either the established or newly founded colonies. To ensure a broad age range of potential recipients at all times, we continued to add newly emerged, marked bees to the study colonies throughout the 3-week observation periods. However, new workers normally do not emerge in newly founded colonies until 3 weeks after brood rearing has been initiated (Winston 1987). Thus, the ages of workers selected as recipients in our newly founded colonies may not have reflected what typically occurs during the initial stage of nest founding. Nevertheless, our results suggest that, even when given an atypical opportunity to choose among workers of different ages, vibrators in the newly founded colonies showed no preference for particular age classes of recipients. Vibrators in the established colonies also showed no age preferences in the selection of recipients. Thus, worker age probably had little effect on the selection of recipients in either colony type, which may have allowed the signal to simultaneously influence a wide age range of bees and thus a broad array of tasks.

In both colony types, recipients responded to the vibration signal with increased task performance relative to same-age, nonvibrated controls. Thus, regardless of colony developmental state, the vibration signal enhanced worker labour. However, we found no evidence to support our hypothesis that recipients in newly founded colonies increase the magnitude of their response to the signal compared to established colony recipients. Therefore, any influence of the vibration signal on the labour patterns of newly founded colonies may have resulted primarily from the activation of larger numbers of inactive bees, who then worked at the same rate as recipients in the established colonies, rather than through heightened individual labour responses by recipients in the newly founded versus established colonies. Honeybees may maintain large numbers of inactive individuals at all times to provide a pool from which workers can be quickly recruited in response to changing colony needs (Kolmes 1985; Anderson 2001; Johnson 2002). Our results suggest that the vibration signal may be important in activating bees from the reserve work force to help adjust task performance to the labour requirements associated with different stages of colony development.

To our knowledge, this is the first study to demonstrate that colony developmental state can affect the performance of a modulatory communication signal, which, in turn, may help to coordinate a broad spectrum of tasks with changing colony conditions. Modulatory signals are widespread in the social insects and are frequently involved in the multicomponent communication systems used to adjust cooperative activities to colony needs (Hölldobler 1999; Partan & Marler 1999; Anderson & McShea 2001; Schneider & Lewis 2004; O'Donnell 2006). However, the exact manner in which modulatory signals influence worker task performance is often unclear (Schneider et al. 2004). A more complete understanding of the organization of labour in social insects may therefore require detailed knowledge of the factors that influence modulatory signal use and how the signals may influence worker assessments of colony state.

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