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ORIGINAL PAPER

# Colony nutritional status modulates worker responses to foraging recruitment pheromone in the bumblebee *Bombus terrestris*

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**Abstract** Foraging activity in social insects should be regulated by colony nutritional status and food availability, such that both the emission of, and response to, recruitment signals depend on current conditions. Using fully automatic radio-frequency identification (RFID) technology to follow the foraging activity of tagged bumblebees (*Bombus terrestris*) during 16,000 foraging bouts, we tested whether the cue provided by stored food (the number of full honeypots) could modulate the response of workers to the recruitment pheromone signal. Artificial foraging pheromones were applied to colonies with varied levels of food reserves. The response to recruitment pheromones was stronger in colonies with low food, resulting in more workers becoming active and more foraging bouts being performed. In addition to previous reports showing that in colonies with low food successful foragers perform more excited runs during which they release recruitment pheromone and inactive workers are more prone to leave the nest following nectar influx, our results indicate that evolution has shaped a third pathway that modulates bumblebee foraging activity, thus preventing needless energy expenditure and exposure to risk when food stores are already high. This new feedback loop is intriguing since it involves context-dependent response to a signal. It highlights the integration of information from both forager-released pheromones (signal) and nutritional status (cue) that occurs within individual workers before making the decision to start foraging. Our results support the emerging view that responses to pheromones may be less hardwired than commonly acknowledged.

**Keywords** Activity pattern · Context dependence · Cue · Feedback · Honeypot · Signal · Social insect

## Introduction

In social insects, information flow between individuals enables a coherent allocation of the worker force to a variety of essential tasks. This information can be directly and actively transmitted to nestmates by sending signals of various kinds, including visual, acoustic, or chemical modalities (Smith and Harper 2003; Billen 2006), e.g., ant trail pheromones (Beckers et al. 1992). However, information can also be indirectly transmitted by cues left by individuals as unintentional by-products of their activity, e.g., bumblebee scent marks (Saleh et al. 2007). The difference between cues and signals is that signals serve a specific communication function as a result of natural selection, whereas cues are information unintended (from the sender's point of view) to serve a specific communication purpose (Seeley 1998). While information transmission via signals has been studied extensively, the use of cues is more difficult to assess, since potential cues in the

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environment are much more abundant than signals. In addition, both signals and cues can interact. For instance, in honeybees, queen substance (9-oxodecenoic acid) has distinct effects on workers depending on context, i.e., either regulation of reproduction in the nest or attraction at the time of swarming (Slessor et al. 2005). In ants, the same pheromone can be used for both trail marking and sex attraction (Hölldobler 1971). Finally, scent marks left by bumblebees lead to distinct responses by other workers depending on where they are found (Saleh and Chittka 2006).

The way cues can modulate signals is particularly interesting in the context of recruitment for foraging. In bumblebees, floral nectar collected by foragers is stored in honeypots in the nest (Sladen 1912). Hence, full honeypots represent a cue to workers, i.e., a by-product of foragers' activity and an index of colony nutritional status. The presence of full honeypots in the nest inhibits two mechanisms of forager recruitment (Dornhaus and Chittka 2005), as shown in Fig. 1. Firstly, it reduces the number of excited runs performed by successful foragers returning to the nest, thus limiting the release of recruitment pheromone (Dornhaus et al. 2003) and/or its proper diffusion within the nest. Secondly, it prevents the activation of additional foragers following nectar influx into honeypots. When resources are scarce, the opposite pattern is observed (Dornhaus and Chittka 2005). These two feedback loops guarantee a quick and efficient adjustment of colony activity to its nutritional needs. However, a third potential pathway remains unexplored, namely, that workers' responsiveness to recruitment pheromones may also depend on food reserves. Thus, the amount of food stored in the colony's honeypots could act as a cue to modulate the workers' response to a signal: recruitment pheromone. Here we tested how colony nutritional status affects the workers' response to artificial recruitment pheromones. Previous work has already shown that workers react to artificial pheromone by increasing their foraging efforts (Mena Granero et al. 2005). The occurrence of a third feedback loop would emphasize the elaborate integration of information about both colony nutritional status and food availability outside the nest that ultimately regulates foraging activity. We used radio-frequency identification (RFID), a method that is particularly suited to the study of social insects, since it enables large amounts of data about individual activity to be collected automatically.

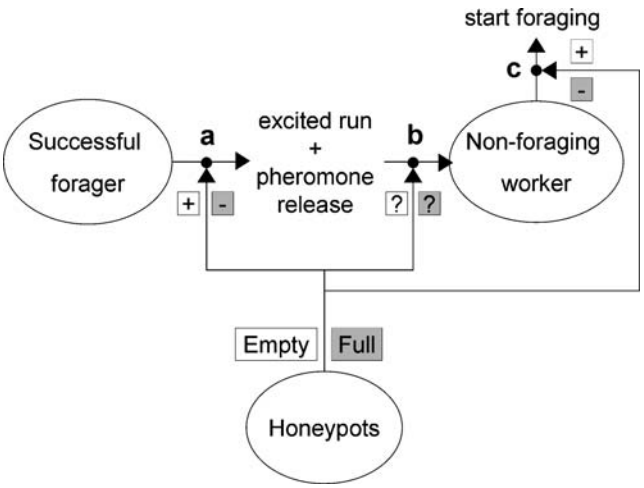


**Fig. 1** Feedback loops based on colony nutritional status regulate forager recruitment. A successful forager returns to the nest, releases foraging pheromones, and performs excited runs (**a**). This promotes recruitment of non-foraging workers to foraging (**b**). The influx of nectar to honeypots also triggers a switch to foraging among non-foraging workers (**c**). Mechanisms (**a**) and (**c**) are known to be modulated by the amount of nectar stored in honeypots (Dornhaus and Chittka 2005) via negative feedback from large food reserves (*gray boxes*) and positive feedback from low reserves (*white boxes*). We study whether the behavioral response of non-foraging workers to recruitment pheromone (**b**) is subject to similar regulation

## Materials and methods

### Bumblebee colonies

In our experiments, we used eight queenright *Bombus terrestris dalmatinus* (Dalla Torre) colonies, supplied by Syngenta Bioline Bees (Weert, Netherlands), which were tested in two distinct cohorts ($N$=3 and 5 colonies per cohort respectively). Colonies of the first cohort (containing 83, 131, and 69 workers at the start of experiments) were each housed in a bipartite wooden nest-box (28×16×11 cm) connected to a foraging arena (116×31×71 cm) via a transparent Plexiglas tube (Fig. 2). Colonies of the second cohort (containing 30, 29, 24, 30, 49 workers at the start of experiments) were housed in larger bipartite nest-boxes (40×28×11 cm) each connected to a foraging arena (larger nest-boxes were used because counting honeypots became more difficult as colonies grew large in small nest-boxes of cohort 1). Colonies grew to sizes of up to approximately 200 workers during the experiments. Colonies were fed on 50% sucrose solution (*v*/*v*) placed in a gravity feeder in the foraging arena. Feeders were always removed during the experiments to avoid the release of recruitment pheromones by successful foragers. Defrosted pollen (Koppert B.V., Berkel en Rodenrijs, Netherlands) was added directly into the nest every other day after experiments were completed.

### Automatic recording of foraging activity

In order to record individual bee activity, we used radio-frequency identification (RFID: Streit et al. 2003; Sumner

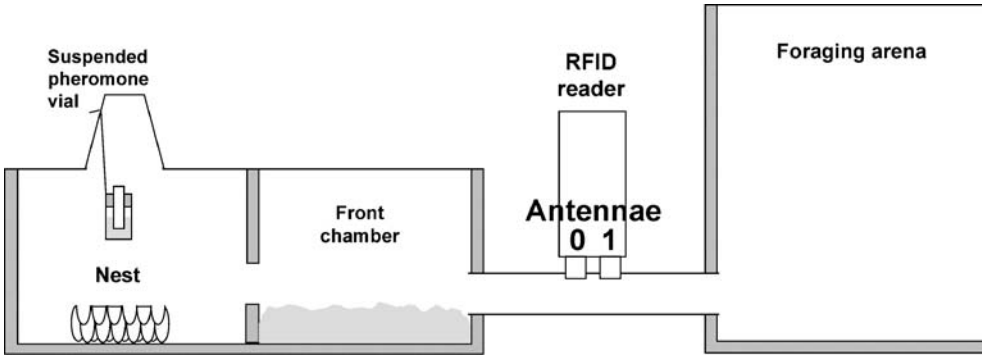


**Fig. 2** Experimental setup for recruitment pheromone trials. The bipartite wooden nest-box is divided into a rear nest chamber containing the wax cells and a front chamber in which the bees deposit their waste. The nest-box is connected to a wooden foraging arena (only partly represented) via a Plexiglas tube. The RFID reader, with two antennae (0 and 1), is located above the tube. During test phases, a vial containing pheromones that evaporate via a wick is suspended above the nest

et al. 2007). Each bee was tagged shortly after pupal eclosion with a mic3®-TAG 64 bit RO (iID2000, 13.56 MHz system: Microsensys GmbH, Erfurt, Germany) programmed with a unique 19-digit code for individual identification. These tags were small enough (1.0×1.6×0.5 mm) to be glued to the bees' pronotum without impeding their behavior (Streit et al. 2003). An RFID tag-reader (iID2000, 2k6 HEAD: Microsensys GmbH, Erfurt, Germany) was placed above the tube connecting the nest to the foraging arena to monitor bee foraging activity (Fig. 2). Each reader had two antennae (0 and 1) which recorded the tag identification (ID) number and the time as the bee passed underneath. The sequence in which the antennae read the tag told us the direction in which the bee was traveling: bees moving from the nest to the foraging arena (0 followed by 1) or from the foraging arena to the nest (1 followed by 0). Accordingly, foraging bouts were identified as '0110' antenna read sequences, and we computed bout duration (time between the two consecutive reads from antenna '1') and the time spent in the nest between bouts (time between two consecutive reads from antenna '0'). This information was associated with the bees' identity. The foraging activity of colonies could thus be precisely recorded. Data were downloaded from the RFID readers to a computer using a dedicated program (Streit et al. 2003) and processed using a Microsoft Excel macro.

We performed preliminary tests to assess the reliability of the RFID system. During 24 tests, each lasting 15 min, we observed 393 bees passing below the RFID reader. Of those bees 99.4% were detected by at least one of the RFID antennae, and 90.8% were detected by both. On only two (of 393, i.e., 0.5%) occasions, the bee was not detected at all. Accordingly this method is a very good alternative to observation or filming, and it records bee identity with 100% accuracy.

## Responses to recruitment pheromone depending on colony nutritional status

Each experimental test consisted of two phases during which bee activity was recorded: a 30-min control phase, during which colonies were unmanipulated, followed by a 30-min test phase, during which colonies were exposed to artificial foraging pheromones in the nest. During the test phase a glass vial containing pheromones was suspended above the nest from which the pheromone evaporated via a cotton roll wick (DE Healthcare Products, Gillingham, U.K.). The pheromone used was either eucalyptol (400 $\mu l\, l^{-1}$ acetone) or a mixture of eucalyptol, farnesol, and ocimene (400 $\mu l\ l^{-1}$ acetone for each molecule), the three major bioactive components of the *B. terrestris* recruitment pheromone (Mena Granero et al. 2005). This allowed us to test if a pheromone mixture, which more closely mimics the natural pheromone, is more effective than a single chemical at recruiting nestmates. Each component was released in the nest at a rate of 0.24 $\mu l\ h^{-1}$ (i.e., similar to the application rate used by Mena Granero et al. 2005). The solvent alone (acetone) was not used during the control phase because Mena Granero et al. (2005) showed that it has no effect on foraging activity.

Before running each experimental test, the nutritional status of each colony was assessed by counting the percentage of full honeypots. Honeypots were categorized as full whenever they contained nectar. This is a reasonable approximation since nectar is concentrated in a few full pots instead of being distributed across many part filled pots (Free and Butler 1959 p.98). Specific measurements showed that among five colonies, the ratio of part filled pots relative to full pots was 1:12, 0:7, 0:4, 2:14, and 0:9. These data confirm that, on average, 95.5% of all pots containing any food are full. Part full pots are rare and probably in the process of being filled by foragers or being

fed from by workers. We did not measure the actual quantity of stored nectar because emptying all pots with a syringe and then replacing the nectar before running experiments disturbs the colonies, which may alter their response to treatments. In contrast, counting full honeypots is a reliable method that does not require opening the nest box, as counting can be performed through the transparent Plexiglas lid.

### Statistical analyses

Regression analyses were performed to assess the continuous effect of colony nutritional status on the response of workers to artificial recruitment pheromone. We subsequently divided our continuous data into two subsets ("low" and "medium to high" food) to carry out multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) analyses that could take into account all three factors. The dependent variables were the percentage increase following pheromone application in number of foragers, number of bouts, bout duration, and time spent inside the nest between bouts. The independent variables were colony nutritional status (either "low" or "medium to high" food), pheromone composition (eucalyptol alone or eucalyptol/ocimene/farnesol mixture), and cohort (1 or 2). Colonies were considered to be in a "low food" state when the percentage of full honeypots was below 5% and "medium to high food" state above this threshold. To put this in context, levels of nectar storage in field bumblebee colonies are quite low (22% to 45% full honeypots depending on habitat: Goulson et al. 2002), and consistent with the maximum storage level achieved among our colonies (49% full honeypots after prolonged exposure to ad libitum feeders). Our nutritional status data are continuously distributed from 0% to 49% and not clustered around 5% (see electronic supplementary material S1). To ensure that our results did not depend on our choice of the 5% threshold, we repeated our analyses for a lower threshold (2%) and a higher threshold (12.6%, the median percentage of full pots) level. The total number of honeypots was counted each day. Tests were run at different times of day to avoid artefacts from colony daily activity cycle (Pelletier and McNeil 2004). More than 16,000 foraging bouts were recorded during a total of 178 tests (20–28 tests per colony): 81 tests with eucalyptol alone and 97 with a eucalyptol/ocimene/farnesol mixture, and 108 tests on medium to high food and 70 on low food states. We computed the percentage increase between control and test phases for the number of foragers, number of foraging bouts, bout duration and time spent in the nest between bouts. All analyses were performed using Statistica 7.1 (StatSoft, www.statsoft.com).

## Results

Colonies typically responded to the application of artificial pheromones by increasing their foraging activity, both in terms of the number of foragers active (mean±SD: from 13±7 (control) to 19±11 (treatment), pairwise $t$ test: $t_{177}$= 11.01, $P$<0.0001; example in Fig. 3) and number of foraging bouts recorded per 30 min (from 41±28 to 49± 29, $t_{177}$=7.31, $P$<0.0001). However, the magnitude of this response was highly dependent on the treatment received (Fig. 4; see following paragraph and Table 1 for statistical analyses). Colonies responded more strongly to pheromone application when they had low food reserves, and the response to the mixture of three molecules was greater than to eucalyptol alone. Accordingly, the smallest increase in foraging activity was observed following application of eucalyptol alone in medium to high food colonies, while exposing low food colonies to the three molecule mixture produced the largest increase. The two other combinations, namely, eucalyptol in low food colonies and the three molecule mixture in medium to high food colonies, produced intermediate increases in activity levels. Pheromone application did not lead to any change in foraging bout duration (from 137±51 s to 142±56 s: $t_{177}$=1.24, $P$= 0.22) or time spent in the nest between bouts (from 141± 72 s to 149±64 s, $t_{176}$=1.52, $P$=0.13), irrespective of the nutritional status or molecules used.

As each colony contains a finite number of foragers, potential changes in activity from the control to the test phase are strongly dependent on the basal activity level

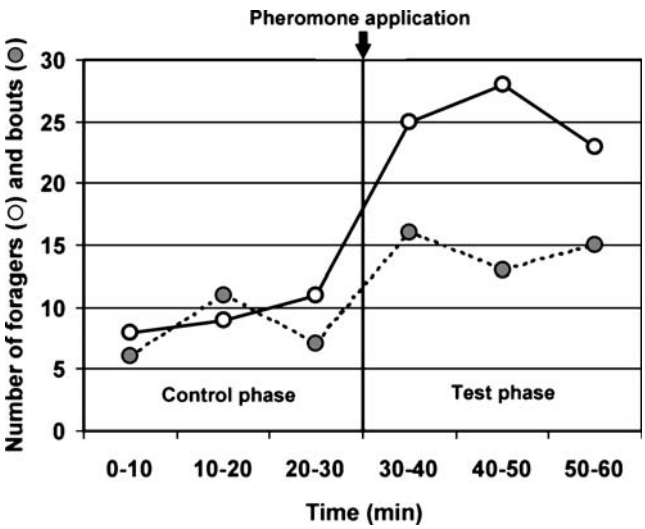


**Fig. 3** Typical colony response to pheromone application (here, a mixture of eucalyptol, farnesol, and ocimene). Once the pheromone vial is placed above the nest after 30 min (*arrow and vertical line*), the number of active foragers (*open circles*, *solid line*) and foraging bouts (*gray circles*, *broken line*) increases

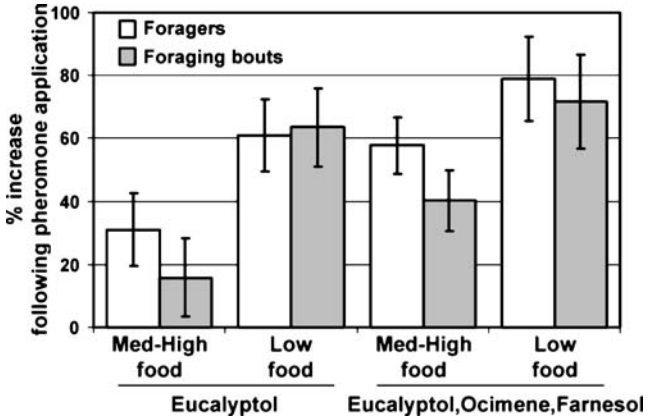


**Fig. 4** Effect of colony nutritional status and pheromone composition on the percentage increase in the number of foragers (*white*) and foraging bouts (*gray*) following pheromone application. Column heights indicate mean (±1 SE) values averaged across 178 tests. Significance of the two factors in ANOVA analyses (pheromone composition and colony nutritional status) can be found in Table 1

**Table 1** Results of the ANOVAs and MANOVA analyzing the effect of colony nutritional status (low food versus medium to high food: threshold = 5% full pots), pheromone composition (eucalyptol alone versus mixture of eucalyptol, ocimene, and farnesol), and cohort (1 versus 2) on response of workers to pheromones. The response variables are the residuals of the percentage increases for number of foragers, number of foraging bouts, bout duration, and time spent inside the nest between bouts. Means are not reported as they correspond to residuals and therefore have no direct meaning. Actual mean percentage increases for both the number of foragers and foraging bouts are reported in Fig. 4. None of the interactions between factors are significant

| Factors | Variable (residuals) | $F_{1,1}$ | $P$ |
|---|---|---|---|
| Nutritional status | Number of foragers | 7.83 | 0.006 |
| | Number of bouts | 3.97 | 0.048 |
| | Bout duration | 0.07 | 0.797 |
| | Time spent inside nest between bouts | 2.34 | 0.128 |
| | All (MANOVA: Wilk's $\lambda$ and $F_{1,4}$) | 0.92 and 3.80 | 0.006 |
| Pheromone composition | Number of foragers | 5.92 | 0.016 |
| | Number of bouts | 0.68 | 0.411 |
| | Bout duration | 1.84 | 0.177 |
| | Time spent inside nest between bouts | 2.21 | 0.139 |
| | All (MANOVA: Wilk's $\lambda$ and $F_{1,4}$) | 0.94 and 2.49 | 0.045 |
| Cohort | Number of foragers | 26.12 | <0.001 |
| | Number of bouts | 2.07 | 0.152 |
| | Bout duration | 0.94 | 0.333 |
| | Time spent inside nest between bouts | 0.16 | 0.688 |
| | All (MANOVA: Wilk's $\lambda$ and $F_{1,4}$) | 0.85 and 7.47 | <0.001 |

during the control phase (Fig. 5). In extreme cases, a high level of basal activity during the control phase could exclude any potential increase during the test phase because all foragers were already active. The relationship between levels of activity in the control and test phase is a decreasing exponential function. Thus, we corrected each raw dataset (percentage change in the number of foragers, number of foraging bouts, bout duration and time spent in the nest between bouts) by fitting an exponential curve using a quasi-Newton nonlinear estimation (Bishop 1995). These exponential functions accounted for 16%, 18%, 22%, and 17% of the variance in each of our respective datasets (number of foragers, number of bouts, bout duration, and time spent inside the nest), meaning that once this artefact was removed, our data still had 78–84% variance that could be attributed to treatment effects. The following tests were carried out on the residuals of this relationship instead of the raw data.

The percentage of full honeypots was an accurate predictor of the percentage increase in the number of foragers following pheromone application when used as continuous variable (linear regression: $R^2$=0.03, $F_{1,176}$= 5.71, $P$=0.018), but data were highly dispersed. Although the same trend was found concerning the relationship between percentage increase in number of foraging bouts and full pots, this was not statistically significant (linear regression: $R^2$=0.013, $F_{1,176}$=2.40, $P$=0.12). Pooling data

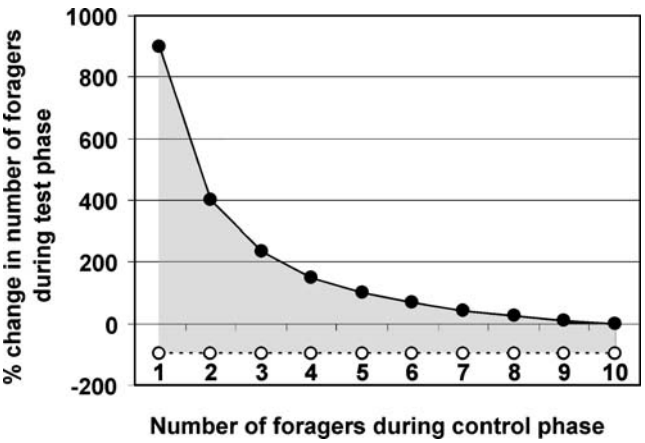


**Fig. 5** Relevance of residuals of foraging activity depending on baseline activity and treatment. As all colony have a fixed number of foragers (here $N$=10), the maximum possible increase in number of active foragers during the test phase (*black circles*, *solid line*) depends on the number active during the control phase. For instance, for a colony containing ten foragers in which four were active during the control phase, a maximum of six more foragers could become active during the test. Thus, the percentage of increase is $100 \cdot \frac{10-4}{4} = 150\%$. The maximal decrease (*open circles*, *broken line*) is always 100%, corresponding to a complete cessation of foraging activity. Accordingly, the possible values are located in the *shaded area*, thus biasing the results if no correction for control activity level is applied. One can avoid this potential artefact by fitting a decreasing exponential function to the raw data and analyzing the residuals

into two discrete subsets ("low food" ≤5% full honeypots or "medium to high food" >5% full honeypots) revealed that all three factors (colony nutritional status, pheromone composition, and cohort) had a significant effect on the percentage increase in colony foraging activity following pheromone application (all four variables—foragers, bouts, bout duration and time in the nest between bouts—analyzed simultaneously in a MANOVA, Table 1). Colony nutritional status had a significant effect on both number of foragers and number of foraging bouts (ANOVAs, Table 1), with low food colonies showing higher responses. This result was robust to the threshold chosen (see electronic supplementary material S2). The percentage change in foraging bout duration and time spent within the nest between bouts was not dependent on colony nutritional status (Table 1).

Applying a mixture of three pheromone molecules (eucalyptol, ocimene, and farnesol) instead of one (eucalyptol) resulted in a higher percentage increase in the number of foragers, but had no effect on the percentage change in number of foraging bouts, bout duration, or time spent in the nest between bouts (Table 1).

There was also a significant cohort effect on the percentage increase in the number of foragers (Table 1). Colonies in cohort 2 ($N$=5) were less responsive to pheromones than cohort 1 ($N$=3 colonies), probably because of differences in colony age and size (cohort 2 consisted of smaller, younger colonies, housed in larger boxes). No cohort effect was found for the percentage change in number of foraging bouts, foraging bout duration, or time spent within the nest between bouts (Table 1).

None of the interactions between factors were significant, making interpretation of the MANOVA/ANOVA results straightforward. Colony effects were not included in analyses because the large number of colonies effectively decreased the sample size for each combination of treatments, thus dramatically lowering the chances of detecting potential effects even with a robust sample size (178 experimental tests). Sample size was often smaller for the low food group because starving colonies was potentially dangerous for bees and was therefore not maintained for prolonged periods. Consequently, pairwise tests for individual colonies did not detect any significant effect of pheromone type or food level after Bonferroni correction for multiple comparisons. The cohort effect is a good approximation of colony effect at a larger scale that allows us to avoid this sample size issue.

## Discussion

### Context-dependent response to recruitment pheromone

By using RFID technology to collect large amounts of behavioral data, this study revealed a new feedback loop that regulates colony foraging activity depending on nutritional status (Fig. 1). Although food stores are known to regulate both the readiness of workers to leave the nest following nectar influx and the release of pheromones by successful foragers (Dornhaus and Chittka 2005), this is the first time in bumblebees that a behavioral response to recruitment pheromone is shown to be modulated by a cue, i.e., colony nutritional status. The response of colonies with low food reserves to our artificial pheromone mixture of three molecules is 78% higher than that of colonies with medium to high food reserves for the number of foraging bouts, and 37% higher for the number of foragers. In addition to the two feedback loops found by Dornhaus and Chittka (2005), this third feedback loop gives bumblebee colonies a high degree of behavioral plasticity in their responsiveness to food availability both within and outside the nest. The existence of multiple regulatory pathways to prevent needless worker activity outside the nest is probably an evolutionary consequence of the high costs associated with foraging, both in terms of energy expenditure (in honeybees: Neukirch 1982) and exposure to predators and parasites (Pouvreau 1974); see also Raubenheimer and Gäde (1996); Simpson et al. (2006) for related processes in other species.

Our results also highlight the integration of different signals and cues within each individual. Indeed, in order to decide if it will leave the nest to forage, a bumblebee worker takes into account the presence of recruitment pheromone as well as the recent influxes of nectar and balances this information with the amount of food stored in the colony. The nutritional status of the colony is a key bit of information for an individual's decision, since it also influences a forager's choice to abandon a flower when threatened by a predator (Cartar 1991), to focus collection efforts towards the most needed resource, namely, nectar or pollen (Cartar 1992), or to accept lower quality food and to fill its crop more (in ants: Josens and Roces 2000). The actual mechanism for assessing the percentage of full honeypots probably involves the detection of own or larval hunger level (Den Boer and Duchateau 2006), although comprehensive sampling of honey storage cannot be excluded since bumblebee nests tend to remain relatively small (Dornhaus and Chittka 2005): our largest colony had 75 pots, of which no more than 29 were ever full.

### Foraging trip characteristics

Recruitment pheromones could affect the foraging behavior of bees in a number of ways. For instance, each bee might spend more time looking for food outside the nest, increasing their bout duration, and/or limiting the time spent in the nest between bouts to maximize the number of trips (although the time spent in the nest could also increase

if they perform recruitment runs). Alternatively, recruitment pheromones might only influence a bee's motivation to leave the nest, but not their subsequent propensity to explore the environment. We did not detect any significant changes to foraging trip characteristics (bout duration or time spent in the nest between bouts) in response to application of recruitment pheromones, but we cannot extrapolate these results to natural conditions. Indeed, the foraging bouts recorded in our small arenas were short (2–3 min) relative to those reported in the field (mean per colony 22–30 min: Cartar 1992; mean per habitat 18–55 min: Ings et al. 2006; mean per colony 66–82 min and range 10–209 min: Westphal et al. 2006; mean per colony: 31–51 and range 6–502 min: Raine and Chittka 2008), probably because it only takes a short time for foragers to determine that no food is available. Bouts would probably have been longer if feeders had been in the foraging arenas during experiments, but foragers would then have released recruitment pheromone when they returned to the nest, thus interfering with our manipulation. Changes in bout duration may be easier to detect when bouts are longer and food is available in the environment; further field experiments could address this issue.

### Importance of multi-compound pheromones

Using eucalyptol increased the number of foraging bouts by 16%. The three molecule blend, which is closer to the pheromone produced by bumblebees, was 154% more efficient than eucalyptol alone, taking the increase in number of foraging bouts to 40%. This suggests that the activity of the three components is complementary and that the message carried by the pheromone is incomplete unless all components are present (similarly to the honeybee queen retinue pheromone: Keeling et al. 2003).

Bumblebee colonies are used in very large numbers to pollinate greenhouse crops (Velthuis and Van Doorn 2006). Yet, they often lack motivation to forage, and our results suggest that this may be because they receive abundant food (particularly artificial nectar) within the nest, which interferes with their recruitment system. Using artificial recruitment pheromones might help compensate for the detrimental effects of overfeeding on foraging motivation, provided that bumblebee responses to pheromones remains high despite long-term application. This question is currently under investigation.

### Plastic responses to pheromones

Our study highlights the importance of context in the recruitment and decision mechanisms of bumblebees. Although nutritional status was already known to influence foragers' motivation to recruit nestmates and workers' readiness to switch to foraging following nectar influx to the colony (Dornhaus and Chittka 2005), we show that the recruitment pheromone does not trigger a stereotyped response by bumblebee workers, but is instead interpreted with respect to context, namely, the amount of stored food acting as a nutritional cue. Our findings support the emerging view that animal responses to pheromones are less hardwired and unalterable than commonly acknowledged. For instance, the honeybee queen mandibular pheromone has different effects on workers depending on whether they are foragers or in-hive workers (Grozinger and Robinson 2007). Moreover, bumblebees workers have distinct responses to scent marks left by others depending on where they are found (Saleh and Chittka 2006). In addition, the alarm pheromone in a grass-cutting ant elicits a lower response when foraging trails are less crowded, maybe because such trails lead to low-value resources less worth defending (Hughes and Goulson 2001). Finally, in mammals, response to pheromones can depend on context (e.g., the sexual pheromone triggering attraction or aggression in mice: Stowers and Marton 2005). Accordingly, future studies of responses to pheromone signals should take into account the integration of other external information. Compounds that elicit an entirely stereotyped response (i.e., pheromones sensu stricto) might be less common than previously thought.

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