Age matters: pheromone profiles of larvae differentially influence foraging behaviour in the honeybee, *Apis mellifera*

Kirsten S. Traynor a, *, Yves Le Conte b, Robert E. Page Jr. a

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How a colony regulates the division of labour to forage for nutritional resources while accommodating for size and demographic composition is a fundamental question in the sociobiology of social insects. In honeybees, *Apis mellifera*, young and old larvae produce pheromones that differ in composition. Nurses differentially regulate larval nutrition, feeding young worker larvae a surplus diet that parallels queen larvae in protein composition and food availability, while old larvae are restrictively fed a diet with similar sugar content as queens. The presence of larvae affects division of labour, but it is unknown whether foragers regulate resource collection based on larval age or pheromone production in the nest. We studied how larval age demography and the larval pheromone e-beta ocimene affect foraging activity and foraging load. Our results suggest that workers recognize larval age, probably by detecting changes in the pheromones emitted by larvae as they mature, and adjust the foraging division of labour (pollen versus nectar) to meet the nutritional needs of the colony’s brood. For younger larvae, this results in a bias towards pollen collection.

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Foraging and the dynamic mechanisms that regulate food collection are evolutionarily critical attributes of all organisms. Honeybees, *Apis mellifera*, show a temporal division of labour partitioned between young bees (nurses), which care for and feed immature nestmates inside the hive, and older workers (foragers), which collect water, resin, pollen and nectar outside the hive (Robinson, 1992; Seeley, 1995; Winston, 1987). We know a great deal about how honeybees regulate this temporal division of labour, allocating foragers to nectar collection (Seeley, 1989, 1995; Seeley, Camazine, & Sneyd, 1991), but how colonies collect sufficient stores of pollen is still poorly understood.

Nectar, converted into honey, is the main food source of colonies, enabling them to survive winter, while pollen provides the colony with the proteins that are channelled into colony growth. Colonies dynamically adapt to resource availability and changing colony conditions (Seeley, 1995). Stored pollen inhibits additional pollen collection (Fewell & Page, 1993; Fewell & Winston, 1992). This stored pollen is consumed by young nurse bees, which convert the protein into proteinaceous glandular secretions fed to developing larvae (Crailsheim, 1992); thus, larvae are the indirect consumers of pollen. The presence of larvae in turn stimulates additional pollen foraging (Free, 1967).

During the 6-day period of the fifth-instar larval stage, larvae are confined to individual cells and remain dependent on the care of nurse bees. The larvae emit pheromones that influence worker physiology, suppressing ovary development (Arnold et al., 1994; Maisonnasse et al., 2009; Mohammedi, Paris, Crauser, & Le Conte, 1998) and stimulating hypopharyngeal gland development in nurse bees, which produce the protein-rich larval food (Mohammedi, Crauser, Paris, & Le Conte, 1996). Chemical signals produced by larval change during development (Le Conte, Sreng, & Trouiller, 1994). From the first through the third larval instar, larvae produce volatile e-beta ocimene (eβ), which has been shown to suppress ovary activation (Maisonnasse et al., 2009) and accelerate behavioural maturation in worker bees (Maisonnasse, Lenoir, Beslay, Crauser, & Le Conte, 2010). This volatile pheromone begins to diminish by the fourth larval instar, when larvae predominantly emit the nonvolatile brood ester pheromones (BEP), a blend of 10 ethyl and methyl fatty acid esters (Le Conte, Arnold, Trouiller, & Masson, 1990; Trouiller, 1993). While the behavioural effects of BEP on the regulation of foraging behaviour have been well studied (Pankiw, 2004a, 2004b; Pankiw, Page, & Fondrk, 1998; Pankiw & Rubink, 2002), little is known about the effects of eβ on the foraging division of labour.
Larvae produce $e^b$ during a period of developmental totipotency (Jung-Hoffmann, 1966; Rhein, 1933), when the protein-rich larval food remains unrestricted in quantity (Haydak, 1943). The late third and early fourth instars mark a change in pheromone profile to predominantly BEP (Maisonnasse et al., 2009, 2010; Trouiller, 1993) that corresponds with a shift in the diet provided by nurses. This suggests that larval pheromones can orchestrate the feeding behaviour of the adult workers that provision larvae (Le Conte, Seng, & Poitout, 1995). Although sugar content is quadrupled in the brood food of older larvae, overall food availability (Asencot & Lensky, 1988; Rhein, 1956; Shuel & Dixon, 1968), including protein content (Kunert & Crailsheim, 1987), is reduced. After nurses seal the cells, the worker larvae starve through to the preupal stage (Leimar, Hartfelder, Laubichler, & Page, 2011; Wang, Kaftanoglu, Fondrk, & Page, 2014), but they continue to produce minute amounts of both $e^b$ and BEP (Maisonnasse et al., 2010).

Because the changes to brood pheromone emissions are stage specific, nurses probably use them to guide how they care for the larvae (Le Conte et al., 1995). Development is thus likely to be orchestrated by the interplay of larval signals and nurse responses. Although it has been widely established that immature honeybees directly influence the division of labour among workers, modification of pollen and nectar foraging (Amdam, Rueppell, Fondrk, Page, & Winston, 1992; Free, 1967; Pankiw, 2001; Pankiw et al., 1998; Tsuruda & Page, 2009), previous research on the effect of brood on worker foraging behaviour generalized larvae into a single category of larvae/open brood (Dreiller et al., 1999; Dreiller & Tarpy, 2000; Pankiw et al., 2001; Pankiw et al., 1998). Therefore, it was not possible to determine the relationship between brood pheromone release at different life stages and worker food collection. We predicted that young larvae would stimulate more pollen foraging by workers than old larvae, as younger larvae require more protein to complete development. To determine the influence of larval age on the division of foraging labour, we thus compared the releaser effects of pheromones from young larvae, old larvae and capped pupae as well as the absence of brood. We also tested whether $e^b$ alone produces the same foraging behaviour in workers as the presence of young larvae, hypothesizing that the presence of concentrated $e^b$ is sufficient to determine the foraging behaviour of workers.

METHODS

Experiment 1: Releaser Effects of Young Larvae, Old Larvae and Capped Brood

To determine whether workers perceive the different nutritional needs of immature honeybees and adjust their foraging accordingly, we compared the foraging releaser effects of three immature stages of brood against a control of no brood.

Bees

All colonies used in this experiment contained naturally mated *Apis mellifera* L. queens purchased from commercial beekeepers in California. We collected 6 kg of mixed-age worker honeybees from 10 full size colonies by shaking frames of bees into a ventilated box. The bees were placed in a cool room (35–40 °C) for 4 h, then were equally divided into four 1.5 kg units and placed in small ‘nucleus’ hives (nucs) in the local apiary adjacent to our research facilities in Mesa, Arizona, U.S.A. Each nuc received a mated queen, one synthetic queen pheromone strip (PseudoQueen; ConTech, Victoria, BC, Canada), one comb of honey, one empty comb and one treatment comb. Synthetic queen pheromone is often used in experimental hives to control for possible differential queen pheromone effects across individual colonies, as pheromone quality is influenced by numerous physiological factors.

Treatments

Workers were exposed to a comb containing one of four stimuli treatments: (1) young larva; (2) old larva; (3) pupae (capped brood); or (4) an empty comb (no brood). To generate these treatment combs, queens from additional colonies were caged on empty combs for 18 h. Each brood treatment consisted of ~2000 cells containing larvae or pupae. When foraging activity was measured, young larvae were 5 days postoviposition, predominantly second-instar larvae that emit up to 1008 ng of volatile $e^b$ as their pheromone in 24 h (Maisonnasse et al., 2009, 2010). Old larvae were 8 days postoviposition, predominantly fifth-instar larvae that emit up to 564 ng of nonvolatile brood ester pheromone (BEP) and minute amounts of $e^b$ (up to 30 ng) (Trouiller, 1993; Trouiller, Arnold, Le Conte, & Masson, 1991). The capped brood were pupae at 13 days post egg laying that emit small amounts of both $e^b$ and BEP underneath a semipermeable wax cap (Maisonnasse et al., 2010; Trouiller, 1993). The experiment was replicated four times over a 10-day interval. New nucs were established for each replicate.

Foraging activity

Full-size colonies, maintained in the same apiary as the experimental colonies, collected abundant pollen and nectar resources from autumn-blooming plants, indicating sufficient resource availability to compare nectar versus pollen foraging. Hive entrances of the experimental nucs were partially blocked for 5 min with a wire-mesh screen that allowed colony odours to pass through, thus helping foragers orient to the entrance, but restricted returning foragers to re-enter via a small opening. During this time, an observer sat at the side of the entrance with hand-held counters and recorded all returning foragers as either pollen foragers or nonpollen foragers. Pollen foragers had visible pollen loads on their hindlegs; nonpollen foragers had no noticeable loads. Pollen foragers included those that collected both pollen and nectar, while nonpollen foragers included foragers that collected nectar or water or had no load. Total foraging activity was determined by summing the foragers for each 5 min interval (4–5 intervals per hive) and comparing these foraging intervals across treatments. Pollen foraging activity was determined by calculating the proportion of total foragers that collected pollen (pollen foragers/total foragers). Foraging activity was monitored in the morning during 0900–1100 hours and across all treatment groups at the same time.

Foraging loads

After foraging activity was recorded, entrances were completely blocked with mesh screens so that returning foragers landed at the entrance but could not enter the hive. From each of the four test colonies, 50 random foragers were collected. Each bee was placed in an individual cage so that it could not exchange food via trophallaxis. Bees were anaesthetized with CO₂, then sacrificed to obtain their nectar and pollen loads. The nectar load from their honey stomachs was expressed into a 50 μl capillary tube (Kimble, Vineland, NJ, U.S.A.) by gently squeezing the abdomen (Gary & Lorenzen, 1976). The volume of nectar was measured using a millimetre-scale ruler. The sucrose concentration was then determined using an electronic Brix refractometer (MISCO Palm Abbe, Cleveland, OH, U.S.A.). The pollen load from one leg of each pollen forager was removed and weighed. Because bees carry a balanced pollen load (Winston, 1987), the weight of the single pellet was doubled to represent total pollen load collected. Foraging loads were classified as empty, water, nectar or pollen. Empty foragers
had no visible pollen load and no more than 3 μl of contents in their crop; water foragers had crop contents containing less than 5% sucrose. Bees that had collected both pollen and nectar were counted as pollen foragers, as there were too few to include an additional foraging category (2–7/treatment).

**Experiment 2: Releaser Effects of Young Larvae and eβ**

To determine whether the pheromone signal alone can stimulate increased pollen foraging or whether workers must interact with live larvae, we compared the releaser effects of young larvae and eβ on foraging behaviour to a no-brood control.

**Bees**

We established 12 colonies from 1.5 kg packages purchased from a commercial beekeeper. Each colony contained one honey comb and two empty combs on which the queens laid eggs. Colonies developed for 3 days prior to beginning the experiment, after which the queen was confined in a small cage. All combs were removed from each colony and replaced with one comb of honey, one empty comb and one treatment comb.

**Treatments**

Workers were exposed to one of three stimulus treatments: (1) young larvae; (2) a mixture of ocimene isomers including eβ (Sigma-Aldrich, St Louis, MO, U.S.A.) or (3) no brood. Young larvae and no brood treatments were prepared as in experiment 1. Since eβ and no brood treatments do not require live brood, workers were exposed to an empty comb. Young larvae and no brood in the control treatment received 1 ml of paraffin oil, while those in treatment eβ received 10 000 larval equivalents of ocimene in 1 ml of paraffin oil (Sigma-Aldrich). Larval pheromones are described in terms of larval equivalents (Leq), which indicate the known amount of pheromone emitted by one developing larva over 24 h (volatile pheromones) or rinsed off the cuticle (nonvolatile) of one larva (Le Conte et al., 1990; Maisonnasse et al., 2009, 2010). Pheromone treatments were presented in a mesh-screened glass petri dish below the brood nest area 2 h prior to measuring foraging activity. Bees could not contact the pheromone directly (Maisonnasse et al., 2010).

**Foraging activity**

Nucs were monitored for foraging activity in 5 min intervals, as in experiment 1. Instead of replicating the experiment four times over 10 days, we monitored foraging activity across all 12 nucs, four per treatment group, on the same day between 0900 and 1200 hours. Foraging activity fluctuates with temperature, which affects pollen and nectar availability throughout the day. To compensate for fluctuations in resource availability, foraging activity was measured in five rounds. A round consisted of one 5 min foraging interval for each of the 12 experimental hives; subsequent rounds were spaced by approximately 30 min. One hive was discarded from the analysis because it had fewer than 60 total foragers during the entire experiment, while all other colonies had between 177 and 680 foragers during the same time frame.

**Statistics**

To account for factors of replicate/round and treatment, we used two-way ANOVA to analyse total foraging activity, pollen foraging activity and individual foraging loads; we conducted LSD Student’s t test post hoc analyses on significant results (Sokal & Rohlf, 1995) using JMP Pro v. 10 (SAS, Cary, NC, U.S.A.). Because foraging load data are categorical, we analysed the distributions of foraging loads collected with three-way and two-way contingency tables using a custom chi-square contingency table (http://vassarstats.net/newcs.html). Replicates were conducted on different days over a 10–day interval and thus encompassed variance across days and individual nucleus colony differences. Significant differences in foraging activity across replicates in experiment 1 may be due to differences in foraging availability, as replicates were conducted on different days over a 10-day period. Pollen/nectar availability can also fluctuate over time as different plants come into flower.

**RESULTS**

**Foraging Activity**

**Experiment 1: releaser effects of young larvae, old larvae and capped brood**

We monitored foraging activity in 5 min intervals, counting every bee that returned to the hive from a foraging trip. Total foraging activity differed by replicate (ANOVA: F_{3,67} = 18.72, P < 0.001), but not by treatment (ANOVA: F_{3,67} = 2.606, P = 0.062; Fig. 1a). If a returning forager carried a visible pollen load, it was classified as a pollen forager. Pollen foraging activity differed significantly by replicate (ANOVA: F_{3,52} = 9.35, P < 0.001; Fig. 2a). Young larvae had twice as many pollen foragers as capped brood (paired t test: t_{52} = 4.841, P < 0.001) or no brood (t_{52} = 4.266, P < 0.001) and 1.5-fold more than old larvae (test: t_{52} = 2.827, P = 0.007).

**Experiment 2: releaser effects of young larvae and eβ**

Total foraging activity, measured as in experiment 1, differed by round (ANOVA: F_{4,40} = 9.65, P < 0.001) and treatment (ANOVA: F_{2,40} = 17.16, P = 0.023; Fig. 1b). Differences across rounds were expected, as resource availability and hive needs fluctuate throughout the day. Hives treated with eβ showed 1.5-fold more total foraging than either young larvae (paired t test: t_{40} = 2.366, P = 0.023) or no brood (t_{40} = 2.692, P = 0.010), which did not differ from each other (t_{52} = 0.352, P = 0.727). Pollen foraging activity differed significantly by treatment (ANOVA: F_{2,40} = 3.79, P = 0.031; Fig. 2b), but not by round (ANOVA: F_{4,40} = 2.137, P = 0.094). Exposure to eβ and young larvae stimulated twice as many pollen foragers as exposure to no brood alone (paired t test: eβ: t_{40} = 2.542, P = 0.015; young larvae: t_{40} = 2.114, P = 0.041), comparable to the results of experiment 1. The two brood treatments, young larvae and eβ, did not differ from each other (t_{40} = 0.585, P = 0.562).

**Foraging Loads**

**Experiment 1: releaser effects of young larvae, old larvae and capped brood**

Entrance counts did not provide details on nonpollen foragers (i.e. loads of nectar or water, or empty foragers). We collected foragers and expressed the contents of their crops to determine the effects of brood age on foraging load. Weather conditions were warm during the 10 days of the experiment (range 15–35 °C, mid-October 2012, Mesa, AZ). The frequency distribution of foraging load sucrose concentrations was strongly bimodal. Peaks occurred at 0% and around 50%. The peak at 0% was likely a consequence of water foraging activity to cool the hive.

Each bee was classified as one of four types of foragers, based on their foraging load: pollen, nectar, water or empty (Fig. 3). To determine interaction effects of foraging load with treatment and replicate, we conducted a three-way contingency table analysis (Sokal & Rohlf, 1995) (Table 1). There was no significant interaction of
treatment and replicate ($G_9 = 0.02, P = 1.0$). However, there was a significant interaction of treatment and type of foraging load collected ($G_9 = 43.4, P < 0.001$) and a significant interaction of treatment, foraging load and replicate ($G_{54} = 158.36, P < 0.001$). Since there was no significant interaction of treatment and replicate, we pooled the count data across replicates (Fig. 4) and conducted a 4×4 contingency table analysis. Treatment significantly influenced the type of load collected (chi-square test: $\chi^2_9 = 44.2, P < 0.001$).

We assessed total foraging activity and pollen foraging activity to examine treatment effects at the colony level. We also quantified individual foraging loads of nectar and pollen to determine whether treatment influenced the load size or sucrose concentration collected. Individual loads did not differ between treatments for nectar volume (ANOVA: $F_{3,148} = 0.63, P = 0.60$), nectar sucrose concentration ($F_{3,148} = 1.80, P = 0.15$), or pollen mass ($F_{3,226} = 1.42, P = 0.24$). However, the number of empty foragers returning to the hive varied significantly by treatment (chi-square test: $\chi^2_3 = 20.55, P < 0.001$). Treatments exposed to no brood had the greatest number of empty foragers; those exposed to young larvae had the fewest number of empty foragers; and those exposed to capped brood and old larvae had intermediate numbers of empty foragers. The number of pollen foragers also varied significantly ($\chi^2_3 = 35.1, P < 0.001$): no brood and capped brood had
Figure 3. Foraging loads from 50 foragers were measured for each treatment and replicate. Bees were classified as ‘empty’ (dark grey) if they carried no foraging load, ‘pollen’ (orange) if a visible pollen pellet was found on their hindleg, ‘nectar’ (light grey) if they had no pollen load and their crop contained at least 3 μl with >5% sucrose solution and ‘water’ (blue) if their crop contained a solution with <5% sucrose. Treatments: no brood (NB); capped brood (CB); old larvae (OL); young larvae (YL); e-beta ocimene (eβ). Replicates are indicated at the bottom of each column.

Foraging loads were pooled across replicates so that foraging loads from 200 individuals were measured for each treatment. Foraging loads were classified as in Fig. 3. Positive and negative values indicate the percentage deviations from expected if foraging load is independent of treatment.

Table 1

| Source                          | C²   | df   | P     |
|--------------------------------|------|------|-------|
| Treatment•load•replicate        | 158.36 | 54   | <0.001|
| Treatment•load                 | 43.36 | 9    | <0.001|
| Treatment•replicate            | 0.02  | 9    | 1     |
| Load•replicate                 | 56.98 | 9    | <0.001|
| Treatment•load (replicate)     | 101.38 | 36   | <0.001|
| Treatment•replicate (load)     | 58.04  | 36   | 0.011 |
| Load•treatment (replicate)     | 114.98 | 36   | <0.001|

Foraging loads from experiment 1, represented in Fig. 3, were analysed in a three-way contingency table for foraging load, treatment and replicate. The last three rows of the analysis results represent the two-way interactions for each pair of variables when controlling for the effects of the third variable (parentheses).

Figure 4. DISCUSSION

Pheromones regulate complex interactions in insect societies and enable the colony to adapt to changing environments. Our experimental results demonstrate that honeybee colonies actively regulate their foraging allocation efforts for pollen and nectar in response to the signals produced by larvae and pupae of different ages. The data we present provide additional evidence that total foraging (Fig. 1), pollen foraging (Fig. 2) and foraging load (Fig. 5b) are regulated by integrating in-hive stimuli of the brood nest to allocate forager collection of nectar, pollen and water resources.

Young larvae are the principal recipients of pollen-derived protein resources within the colony (Sagili & Pankiw, 2007); accordingly, we found that they stimulated foragers to collect more pollen (Figs 2, 4). Our experimental results also demonstrate that eβ, the young larval pheromone, alone is sufficient to induce increased pollen foraging (Fig. 2b). This pheromone may also serve as a signal to stimulate provisioning behaviour by nurse bees, although this remains to be tested. The resultant increase in protein demand by nurses may further enhance forager bias towards pollen. In contrast, nurses feed old larvae a diet with reduced protein...
content and a higher proportion of carbohydrate-rich nectar (Haydak, 1970; Huang & Otis, 1991; Jung-Hoffmann, 1966). This restrictive feeding decreases the rate of larval growth and results in the development of the worker phenotype. Correspondingly, we found that old larvae stimulated intermediate levels of pollen foraging and slightly increased nectar foraging in comparison to young larvae (Figs 2, 4). This change in the proportion of pollen and nectar collected may result directly from the reduced release of e\(_f\) in older larvae, or it may be due to the increase in BEP, stimulating foragers to respond to the new nutritional demands of older larvae.

The change in pheromone signals of larvae as they mature may have evolved concurrently with the regulated feeding regime imposed by nurse bees on worker larvae. Larvae benefit from a highly proteinaceous diet only during a limited developmental window, which rapidly closes after the third instar (Haydak, 1943, 1970; Rangel, Keller, & Tarpy, 2013; Woyke, 1971), as the larvae lose their totipotency and can no longer be shunted into the queen development trajectory. As a direct result of diet, larvae change dramatically in size during larval development, increasing their weight up to 1500-fold during 6 days (Snodgrass, 1925), in large part due to protein synthesis in the fat body. Through the first 48 h of larval development, when larvae emit only e\(_f\), worker- and queen-destined larvae grow at similar rates, with worker larvae slightly outpacing queen larvae in weight gain (D. Wang, 1965; cf. Rembold & Kremer, 1980; Stabe, 1930). By 72 h, just as e\(_f\) production decreases and larvae start to emit BEP, worker-destined larvae substantially outweigh queen larvae, weighing 1.5–1.75-fold as much (Stabe, 1930; Wang, 1965). However, larval weight gain slows to approximately one-half to one-fourth of the growth rate that occurs at 48 h (Himmer, 1927; Wang, 1965). Larvae remain bipotent through the third larval instar under normal conditions, although gene and protein expression of worker- and queen-bound larvae begin to diverge almost immediately after hatching (Cameron, Duncan, & Dearden, 2013).

Worker larval growth rate slows after the third larval instar and the developmental pathways of hive-reared workers and queen-destined larvae diverge. Queen larvae continue to gain weight at a faster rate and achieve a substantially larger body size by the time their cells are capped (Wang, 1965). The third instar thus marks a shift from a stage of rapid and generalized growth to a stage during which development becomes canalized into a specific adult phenotype. A concomitant shift in pheromones, from volatile e\(_f\) to nonvolatile BEP, occurs as nurses start reducing larval food, suggesting that e\(_f\) may be a ‘feed me protein’ signal.

After this time point, rerouting the phenotypic trajectory from worker to queen is correlated with reduced ovariole number, queen weight and semen storage capacity, all signs of reduced queen fecundity (Rangel et al., 2013; Woyke, 1971). This larvae–nurse bee signalling via larval pheromones thus enables workers to rear bees of the worker phenotype without queen characteristics through restrictive feeding during late larval development (Linksvayer et al., 2011; Page, 2013).

Young larvae are not the only emitters of e\(_f\); well-mated queens that are exclusively fed a diet of proteinaceous royal jelly also emit e\(_f\) (Gilley, Degrandi-Hoffman, & Hooper, 2006). Virgin queens and queens that are rejected after introduction into a hive (superseded), perhaps due to insufficient mating, lack this pheromone signal (DeGrandi-Hoffman, Gilley, & Hooper, 2007; Huang, DeGrandi-Hoffman, & LeBlanc, 2009). Fecund queens and young larvae that maintain the ability to develop into reproducitives thus share the same pheromone signature, suggesting, perhaps, that one of the two co-opted the ‘feed me protein’ signal from the other and gained access to more food.

The shifting pheromone profiles of larvae may also provide a colony-level cue about seasonality, in addition to informing workers about the age and reproductive potential of developing larvae. Brood nests in colonies typically consist of a mixture of eggs, young and old larvae and capped brood, so that workers are exposed to a complex chemical bouquet of pheromones. Although a mixture of differently aged brood exists in the hive environment, the age distribution changes with the season (Bodenheimer, 1937; McLellan, 1978; Winston, 1987) and can thus inform and coordinate the division of labour to ensure that foragers return with the nutritional resources required for sustaining healthy and environmentally appropriate hive development. Young larvae dominate in the late winter and early spring; this is a climatically unpredictable time in temperate climates when it is crucial that the hive collects protein-rich pollen (Dustmann & von der Ohe, 1988; Farrar, 1934; Mattila & Otis, 2006; Seeley & Visscher, 1985). Old larvae and capped brood dominate the hive environment in the late spring and summer (Bodenheimer, 1937; Seeley; Visscher, 1985), when the colony has its most substantial weight gain in honey (Seeley & Visscher, 1985), amassing a surplus of food stores in preparation for winter to minimize winter mortality (Seeley, 1978). The queen’s

**Figure 5.** Effect of (a) replicate and (b) brood treatment on mean ± SE pollen load in experiment 1. Replicates were conducted on 4 different days over a 10-day interval, during which pollen availability can fluctuate widely. Significant differences (\(p < 0.05\)) are indicated by different letters. Treatments: no brood (NB); capped brood (CB); old larvae (OL); young larvae (YL). Sample sizes are indicated.
egg-laying rate decreases and the brood nest where larvae were previously raised is filled with nectar during the summer; with reduced larvae there is a diminishing drive for pollen collection. This seasonal cycle in brood nest composition may enable the colony to integrate the changing pheromone signal into successful foraging decisions and exploit environmental resources in a seasonally appropriate manner, potentially an emergent property of normal colony development. The chemical communication system of the complex social environment in a honeybee colony is in need of further decoding, but our present results demonstrate that young and old larvae release very different chemical signals that strongly influence the feeding and foraging behaviour of the colony. Now we know that pollen foraging is dynamically regulated and directly influenced by brood nest composition. The immediate impact of young larvae and their pheromone $e$ is a shift in the number of successful foragers and a bias towards pollen collection that provides needed proteins for the developing young emerging the signal.

The ‘road to insect sociality was paved with pheromones’ (Blum, 1974, page 197), and honeybee colonies offer a plethora of pheromones to study, many of which have both releaser and priming properties. We have demonstrated the releaser effects of young and old larvae: Od of the young larval pheromone $e$ on the division of foraging labour of adult workers. Current models predict additional priming effects, proposing that young larvae and their pheromones accelerate behavioural maturation of workers so that they transition to outside foraging precociously, while old larvae prolong nursing and thus delay maturation (Maisonnasse et al., 2010). Additional studies are still needed to investigate the priming influence of young and old larvae on the physiology of the caregiving nurses and their ensuing developmental maturation from in-hive tasks to outside foraging.

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