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SHORT COMMUNICATION

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Worker honey bee pheromone regulation of foraging ontogeny

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Abstract The evolution of sociality has configured communication chemicals, called primer pheromones, which play key roles in regulating the organization of social life. Primer pheromones exert relatively slow effects that fundamentally alter developmental, physiological, and neural systems. Here, I demonstrate how substances extracted from the surface of foraging and young pre-foraging worker bees regulated age at onset of foraging, a developmental process. Hexane-extractable compounds washed from foraging workers increased foraging age compared with controls, whereas extracts of young pre-foraging workers decreased foraging age. This represents the first known direct demonstration of primer pheromone activity derived from adult worker bees.

Introduction

A primary characteristic of eusocial life is an age-related division of labor. The temporal patterning of behavior is known as temporal, or age, polyethism and is expressed as apparent changes in probabilities that workers perform different behavioral tasks (Winston 1987). In general, when workers are in about their third week of life they cease performing tasks within the nest and begin foraging outside (Winston 1987). Factors known to change foraging onset probabilities include genotype (Page et al. 2000), social (Huang et al. 1998), and pheromone environments (Pankiw et al. 1998; LeConte et al. 2001).

Only two social insect primer pheromones have been chemically characterized; honey bee queen mandibular (QMP) and brood (BP) pheromones, communicating queen and larva presence, respectively (Slessor et al. 1988). Primer pheromones are defined as communication chemicals that change individuals to an altered behavioral repertoire. Change occurs through putative response threshold shifts to different stimuli by altering reproductive, endocrine, developmental, and neural systems (Hölldobler and Wilson 1990; Pankiw and Page 2003). As a primer pheromone, QMP inhibits colony-level reproduction by inhibiting queen rearing behavior of workers (Winston et al. 1990; Pankiw 1997), partially inhibits worker ovary development (Hoover et al. 2003), decreases proboscis extension response thresholds on antennal stimulation with sucrose (Pankiw and Page 2003), and delays foraging ontogeny (Pankiw et al. 1998). BP also partially inhibits worker ovary development (Mohammedi et al. 1998), and has dose-dependent effects on sucrose response thresholds and foraging ontogeny (LeConte et al. 2001; Pankiw and Page 2001a).

Adult worker bees also show possible primer pheromone effects on foraging ontogeny. Huang and Robinson (1996) demonstrated that foraging ontogeny of honey bees may be accelerated, delayed, or reversed by skewing the worker age distribution structure of colonies. Removing the forager class accelerated foraging ontogeny, adding foragers delayed foraging ontogeny, and removing nurses reversed foragers to nursing tasks. Subsequently, Huang et al. (1998) partially and completely restricted physical contact between young and forager bees with single and double screens, respectively. Foraging ontogeny was regulated under single-screen separation but lost with double-screen separation (Huang et al. 1998). Combined, the results of these studies suggest that adult workers possess pheromone(s) regulating foraging ontogeny. In this study, the pheromone hypothesis was tested directly by measuring the effect of hexane-extractable compounds washed from the surface of forager and young pre-forager bees on foraging ontogeny.

There is a robust association between honey bee sucrose response threshold and foraging behavior (Pankiw and Page 2001b; Pankiw et al. 2002; Pankiw 2003). Pankiw (2003) measured the sucrose response thresholds of newly emerged Africanized and European bees prior to feeding or social experience. Race of bee significantly constrained sucrose response threshold, such that African-

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ized bees were significantly more likely to respond to lower concentrations of sucrose. Africanized bees foraged at significantly younger ages than European bees. A model of sucrose response threshold and age of first foraging showed that for each unit decrease in sucrose response threshold, probability of foraging behavior increased by 14.3% (Pankiw 2003). The primer pheromones of the queen and brood, known to regulate foraging ontogeny, also show an associated modulation of sucrose response thresholds (Pankiw and Page 2001a, 2003). QMP increases sucrose response threshold and delays foraging ontogeny (Pankiw et al. 1998; Pankiw and Page 2003). BP has dose-dependent effects on sucrose response threshold modulation and regulation of foraging ontogeny. Relatively low amounts of BP decrease sucrose response threshold and accelerate foraging ontogeny, whereas a relatively high amount of BP has the opposite effect (LeConte et al. 2001; Pankiw and Page 2001a). I hypothesized that worker pheromone induced sucrose response threshold modulation would predict the direction of worker pheromone regulated foraging ontogeny.

Materials and methods

Pheromone preparation

Combs from multiple colony sources containing pupae were placed in an incubator (50% RH and 34–35° C). Bees that emerged over a 12-h period were color coded with paint (Testors) applied to the thorax. Four thousand bees per cohort were introduced to a common foster colony. Additional cohorts were added weekly. Known-aged young (2–4 days from the brood nest) and forager bees (14 days or older) were collected for pheromone extraction and for the age at foraging onset bioassay (see below). Young worker (YWP) and foraging worker (FWP) pheromone extracts were prepared by rinsing 100 nurse and 100 forager bees with 100 ml of HPLC grade *n*-hexane (Fisher Scientific) for 1 min at 20°C. The extracts were decanted into glass vials and stored at −20°C prior to use.

Experiment 1: Age at foraging onset bioassay

The focal cohort comprised 50 bees, ≤24 h post-emergence, marked on the dorsal side of the thorax with a unique paint color. Focal cohorts were fostered in "triple-cohort" colonies (Huang and Robinson 1996). Six pairs of triple-cohort colonies were established, each comprising 500 bees ≤24 h post-emergence, 500 nurse-aged bees, and 500 foragers. Queens were confined to a comb area of 400 cm^2 using queen-excluding material that allowed workers to freely pass. Every third day the queen was re-confined to fresh empty space. The comb containing eggs was removed from the colony to control for confounding effects of larval cues on age at foraging onset (LeConte et al. 2001). Trials were conducted over a 2-year period.

Three colonies were randomly selected for daily treatment with 2,000 BEq YWP and paired with a control (hexane rinse). Three colonies were randomly selected for daily treatment with 2,000 BEq FWP and paired with a control. The pheromones and the controls were delivered to the center of the colony on a glass plate (20×10 cm), after the hexane evaporated. Total volume of pheromone and control solutions was 200 µl. One hour after daily treatment, colony entrances were blocked with wire mesh twice per hour for 15 min intervals for at least 4 h per colony per day. Focal forager bees that gathered at the entrance were captured without replacement and counted. Pheromone treatment continued until 50% of the focal cohort per colony was collected as a forager. Age at foraging onset was estimated by the number of days from emergence to capture.

Experiment 2: Adult worker pheromones modulate sucrose response thresholds

Pairs of treatment and control plexiglass/wire-mesh cages (15×8×5 cm) were stocked with newly emerged adult bees from multiple colony sources; 300 bees per cage; and 30% sucrose solution and water were available ad libitum. Control and treatment cages were reared in separate incubators to ensure odor isolation. Incubators were maintained at 30°C and 55 RH. Daily for 5 days, the pheromone treated bees received one of four treatments delivered on glass plates. QMP treatment consisted of one queen equivalent per day as Bee Boost (PheroTech; Delta, B.C., Canada). Three hundred larval equivalents of brood pheromone components (Sigma Chemicals, St. Louis, Mo.) were presented daily, as described by Pankiw and Page (2003). At least 60 bees per replicate per treatment were tested in the PER (proboscis extension response) assay.

One hundred newly emerged bees were placed in cages (as above). Daily for 5 days one pair of cages received 500 bee equivalents (BEq) of YWP (ratio of 5 BEq YWP to 1 bee). The control received a blank glass plate rinsed with hexane. Another pair of cages received 500 BEq of FWP (ratio of 5 FWP to 1 bee) and a control. At least 48 bees per treatment were tested on the 6th day. This experiment was replicated in three trials. The sucrose response assay was conducted as described by Pankiw and Page (2003). The antennae of each bee were sequentially touched with the following ascending sucrose concentrations; 0.1%, 0.3%, 1%, 3%, 10%, and 30% (w/v). Sucrose response threshold is estimated from the sum of proboscis extensions in response to sucrose. A relatively high proboscis response sum corresponds to a low sucrose response threshold, and a low proboscis response corresponds to a high response threshold.

Results

Hexane-extractable compounds washed from the surface of young and forager workers regulated foraging ontogeny. Age at foraging onset data were normally distributed (YWP: Levene F=2.9, df_1=1, df_2=148, P=0.9; FWP: Levene F=2.7, df_1=1, df_2=148, P=0.1). A full-factor ANOVA analysis showed that treatment with forager extract increased age at foraging onset (FWP: $F_{1, 144}$ = 75.4, P=0.013; Fig. 1a). Treatment with YWP signifi-

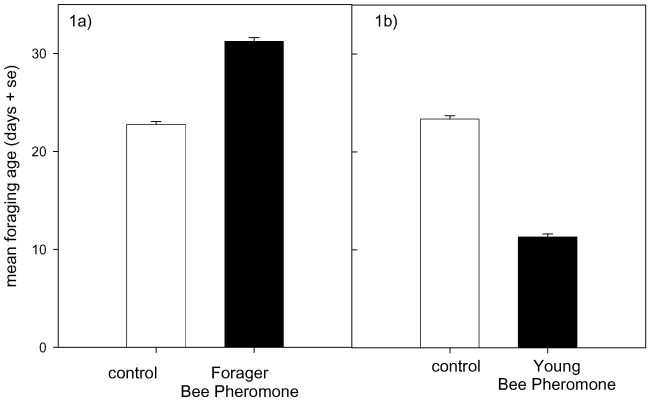

Fig. 1 Hexane-extractable substances of **a** forager bees (FWP) and **b** young pre-foraging bees (YWP) regulated age at foraging onset in colonies of honey bees

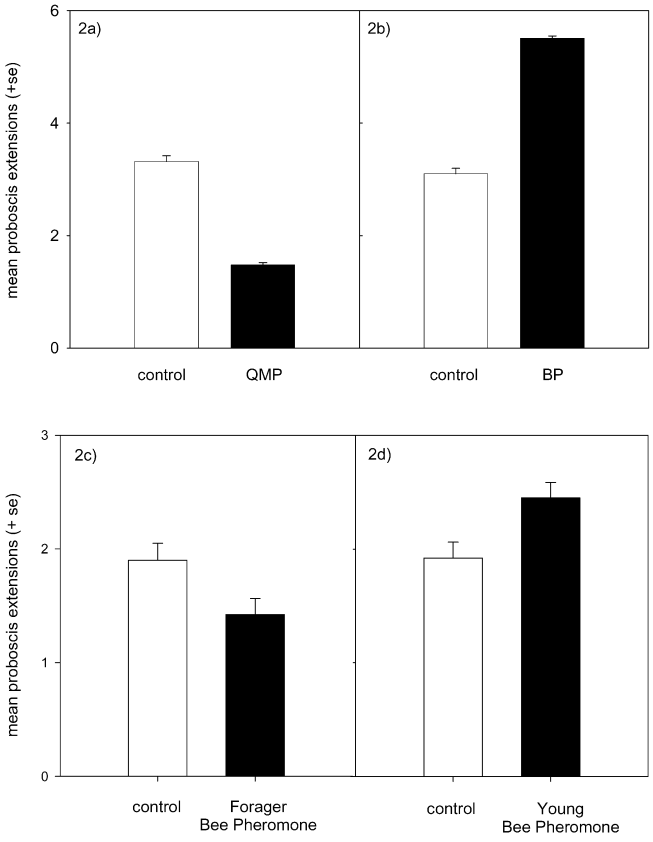

Fig. 2a–d Direction of primer pheromone induced modulation of proboscis extension responses to sucrose predicts regulation of age at foraging onset. **a** Queen mandibular pheromone (QMP) decreased proboscis responses to sucrose and is associated with delayed foraging ontogeny, **b** brood pheromone (BP) increased sucrose response and is associated with accelerated foraging ontogeny. **c** Worker pheromone extracts of forager bees (FWP) decreased proboscis extensions and delayed foraging ontogeny (see Fig. 1a), and **d** extracts of young pre-forager bees (YWP) increased proboscis extensions and accelerated foraging ontogeny (see Fig. 1b)

cantly decreased foraging age ($F_{1, 144}$ =99.8, P=0.010; Fig. 1b). There was no significant effect of trial (FWP: $F_{2, 144}$=5.7, P=0.15; YWP: $F_{2, 144}$=1.13, P=0.47).

The known primer pheromones QMP and BP significantly-changed mean proboscis extensions in response to sucrose compared with controls. QMP: Mann-Whitney $n1$,$n2$=300, U=14,475, P<0.001; BP: $n1$,$n2$=300, U= 11,772, P<0.001, (Fig. 2a, b). Adult bee extracts also significantly modulated PER scores of caged bees compared with controls. There was no significant effect of trial in the FWP experiment (χ^2=1.3, df=2, P=0.5) or in YWP experiment (Kruskal-Wallis χ^2=3.2, df=2, P=0.2). For the purposes of further analyses and visual displays, trials of FWP and YWP were pooled. Bees treated with FWB had significantly higher mean proboscis extensions in response to sucrose (low sucrose response threshold) than control bees ($n1$,$n2$ =144, U=8,887.0, P=0.005; Fig. 2c). Bees treated with YWP had significantly lower mean extensions (high sucrose response threshold) versus controls ($n1$,$n2$=144, U=8,433.0, P=0.04; Fig. 2d).

Discussion

This is the first known direct demonstration of primer pheromone activity derived from extracts of adult worker bees. The direction of foraging ontogeny regulation with young pre-forager and forager crude extracts was similar to that observed in colony manipulations using nurse and forager bees (Huang and Robinson 1996; Huang et al. 1998). Foraging ontogeny response to the addition of young pre-forager extract resulted in accelerated foraging ontogeny, like the addition of nurse bees. The addition of forager extract, delayed foraging ontogeny in a similar way to that observed with the addition of forager bees (Huang and Robinson 1996; Huang et al. 1998). This suggests that the worker bee extracts in this study contained substances that were perceived as age distribution cues. LeConte et al. (2001) concluded that the brood-pheromone-mediated delay in foraging development following treatment with relatively high amounts of brood pheromone lengthened the nursing phase of bees. The populous worker members of the colony, from larva to adult, appear to produce chemical cues that function as regulators on their foraging behavior development.

Workers are using chemical cues, at least in part, to regulate foraging ontogeny. This generates testable hypotheses for adult pheromone characteristics. A continuum of pheromonal cues from larva to adult may exist that change in quantity and ratio, much like the ten larval esters that change in quantity and ratio with sex, caste, and age of individual larvae (Trouiller et al. 1991). A continuum of QMP cues also changes with the reproductive status of young virgin to older mated and egg-laying queens (Pankiw et al. 1996). Equally likely is the possibility that adult workers are synthesizing novel compounds that regulate foraging ontogeny alone or in combination with known primer esters.

Honey bee responsiveness to sucrose appears to represent neurosensitivity to stimuli associated with response probabilities to forage and learn (reviewed by Page and Erber 2002). In this study, the extracts of young and forager bees modulated sucrose response thresholds in directions that were consistent with accelerated and delayed foraging ontogeny, respectively. The proboscis extension response to sucrose assay is currently being used to screen for active fractions of adult extracts and will likely serve as an important pheromone bioassay tool. Pheromonal activity of hexane-extractable compounds suggests that at least some of the compounds were non-polar. However, pheromonal activity of hexane extracts does not imply that all active components will be found in non-polar fractions (Keeling et al. 2003).

This study represents only one of many more steps necessary to identify the adult worker chemicals that regulate foraging ontogeny. If the adult worker honey bee primer pheromones share common characteristics with

the queen and brood primer pheromones then we can expect multi-component, multi-functional, and complex blends that are not easily revealed.

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