Naturwissenschaften (2004) 91:575–578
DOI 10.1007/s00114-004-0568-y

SHORT COMMUNICATION

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Pheromone-modulated behavioral suites influence colony growth in the honey bee (*Apis mellifera*)

Received: 10 May 2004 / Accepted: 14 August 2004 / Published online: 25 September 2004

Abstract The success of a species depends on its ability to assess its environment and to decide accordingly which behaviors are most appropriate. Many animal species, from bacteria to mammals, are able to communicate using interspecies chemicals called pheromones. In addition to exerting physiological effects on individuals, for social species, pheromones communicate group social structure. Communication of social structure is important to social insects for the allocation of its working members into coordinated suites of behaviors. We tested effects of long-term treatment with brood pheromone on suites of honey bee brood rearing and foraging behaviors. Pheromone-treated colonies reared significantly greater brood areas and more adults than controls, while amounts of stored pollen and honey remained statistically similar. Brood pheromone increased the number of pollen foragers and the pollen load weights they returned. It appeared that the pheromone-induced increase in pollen intake was directly canalized into more brood rearing. A two-way pheromone priming effect was observed, such that some workers from the same age cohorts showed an increased and extended capacity to rear larvae, while others were recruited at significantly younger ages into pollen-specific foraging. Brood pheromone affected suites of nursing and foraging behaviors allocating worker and pollen resources associated with an important fitness trait, colony growth.

Introduction

Recent studies suggest that populations and species exhibit suites of behaviors (Pankiw 2003; Sih et al. 2004). Patterns of coordinated behavioral organization across situations characterizes a suite of behaviors. Most studies of behavioral suites have concentrated on strains of laboratory organisms (de Bono and Bergmann 1998; Sokolowski 1998), while few have focused on species in the field that are naturally occurring. For social insects, behavioral change is assumed to favor colony-level and therefore individual fitness (Page and Erber 2002). How behavioral suites change in response to environment is rarely addressed. To place behavioral suites in an evolutionary context it is necessary to understand how change in coordinated behaviors contributes to colony growth in a social species. Individual studies of the effects of brood pheromone, fatty acid esters extractable from the surface of larvae, suggest that suites of honey bee brood rearing and foraging behaviors are influenced.

Brood pheromone (BP) has dose-dependent effects on honey bee age of first foraging such that relatively low doses added to colonies decrease and relatively high doses increase foraging age (LeConte et al. 2001). The addition of relatively low doses of BP for 2–4 h increases the number of pollen foragers, pollen load weight returned, and pollen grains extractable from the surface of non-pollen foragers (Pankiw and Page 2001; Pankiw and Rubink 2002; Pankiw 2004). BP affects non-foraging brood-rearing bees called nurses. Hypopharyngeal (HP) glands are paired food producing and processing glands found in the heads of workers. HP gland development is closely linked to worker task performance. In general, glands begin enlarging early in adult life and reach a maximum size by 5–15 days, coinciding with nursing activities (Winston 1987). Nurse bees consume pollen and convert it into proteinaceous glandular secretions progressively provisioned to larvae (Winston 1987). BP effects on gland protein content is dose and pollen diet dependent for bees reared in cages outside the colony (Mohammedi et al. 1996). What is not clear from the above relatively short-term studies is how long-term treatment with BP influences suites of nursing and foraging behaviors, and consequent colony growth.

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Materials and methods

The following experiment was replicated six times. A pair of colonies was established from a common colony and headed by sister queens. At the start of the experiment, colonies contained ~5,000 workers, 0 brood, pollen (1,720 cm^2), honey (3,440 cm^2), and empty space (3,440 cm^2). Empty space was added equally as needed, such that space was never restricted. BP-treated colonies received 2,000 larval equivalents of BP daily for 28 days and the control received a blank (sensu Pankiw 2004). Our rationale for conducting a 28-day experiment was that it allowed us to rear to pupal stage those eggs that were laid in the 3rd week of the experiment, and to continue to measure change in brood area for 4 weeks. The use of comb area for brood, empty space, pollen, and honey was measured using a metered grid every 7 or 8 days (Winston et al. 1991). Brood area summed eggs, larvae, and pupae. Foraging activity was measured by counting pollen and non-pollen foragers entering colonies in a 5-min period every 3rd day, 4 times per day. Every 2nd day, 500 newly emerged bees were added to each colony to compensate for natural mortality. Weekly, 200 newly emerged bees were paint-marked a unique color per cohort and sampled at 7, 14, and 21 days of adult life. Weekly, 15 bees were sampled from each cohort for HP gland protein content measurement. Their HP glands were removed, stored in a buffer solution at −80°C prior to processing for protein quantification using the Bradford assay (Bradford 1976; 500-0202 Quick Start Bradford protein assay kit 2; Bio-Rad Laboratories, Calif.). Remaining bees from the paint-marked cohorts were measured for age of first foraging. Beginning on day 3 to the end of the experiment, colony entrances were blocked with wire-mesh for 15-min intervals separated by at least 30 min for a total of 4 h per day. Foragers were individually captured in cages, immobilized with CO_2 and aged. One pollen pellet was removed from each pollen forager, weighed, and doubled for total pollen weight. Crop contents were expelled into pre-weighed capillary tubes, re-weighed, and net weight recorded. Sucrose concentration of crop contents were measured using a hand-held refractometer. A termination census was conducted on day 29 where all remaining paint-marked bees were collected and counted. Bees that were reared from egg to pupa during the experimental period were emerged as adults in incubators (33°C and 55% RH), and counted.

Results

BP-treated colonies reared significantly more brood area than control colonies (repeated measures (RM) GLM: $F_{1,10}$=11, P=0.008; Fig. 1) except in week 2 (P>0.05)

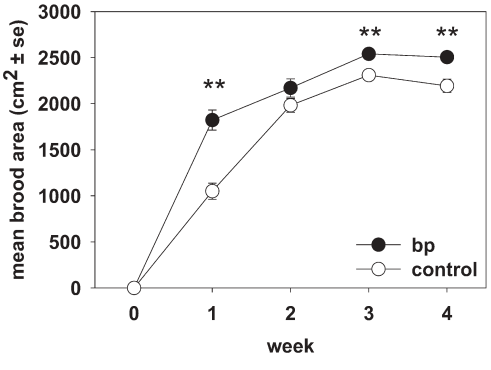

Fig. 1 Brood pheromone (*BP*)-treated colonies (*black circles*) reared significantly more brood area (eggs, larvae, and pupae) than control colonies (*white circles*) over a 28-day period. *Asterisks* (*) indicate significant differences, P<0.01; repeated measures GLM

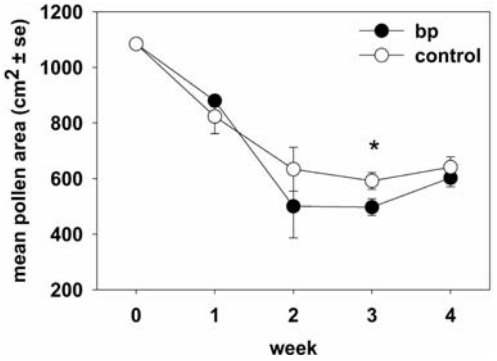

Fig. 2 Brood pheromone (*BP*)-treated (*black circles*) and control (*white circles*) colonies maintained similar amounts of stored pollen. Concurrently, BP-treated colonies had significantly more pollen foragers that returned significantly heavier pollen loads compared with controls. We conclude that BP-induced increased pollen intake was directly canalized into rearing more brood (see Fig. 1). *Asterisks* (*) indicate significant differences, P<0.05; repeated measures GLM

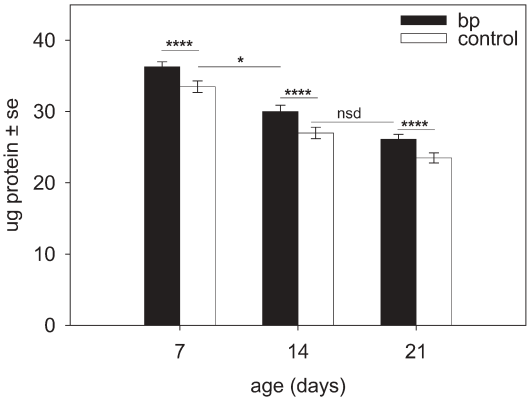

Fig. 3 Mean protein content extracted from hypopharyngeal glands of bees captured from the brood nest areas of brood pheromone (*BP*)-treated (*black columns*) and control (*white columns*) colonies. *nsd* P>0.05, *P<0.05, ****P<0.001

when areas were not significantly different. There was no significant week × treatment interaction for amount of brood area (RM GLM: $F_{3,30}$=2.1, P=0.1) meaning that the effect of the BP on brood area was unidirectional. There was no significant difference in the amount of stored pollen between treatments (RM GLM: $F_{1,10}$=0.2, P=0.6; Fig. 2), except in week 3 when controls contained significantly more stored pollen (GLM: $F_{1,5}$=1.7, P=0.2). Areas of honey and empty space were not different between treatments (RM GLM honey: $F_{1,10}$=1.7, P=0.2; empty space: $F_{1,10}$=0.6, P=0.5).

Age of first foraging was significantly lower in BP-treated colonies (Cox regression X^2=4.9, 1 *df*, P=0.03). There was a significant effect of colony pair on foraging age (X^2=623.2, 5 *df*, P<0.0001; Fig. 3), meaning that in all pairs BP-treated colonies foraged at a significantly younger age than controls. Overall, BP-treated foragers returned with pollen load weights that were significantly heavier ($F_{1,617}$=5.3, P=0.02; BP = 16.3±1.3 mg ; control =

6.9±0.3). There was a significant effect of colony pair ($F_{5,617}$=3.9, P=0.02) on pollen load weight, but no significant interaction of treatment × pair ($F_{5,617}$=2.2, P=0.05). The ratio of paint-marked pollen to non-pollen foragers captured at colony entrances was significantly greater in BP-treated colonies (X^2=80.0, P<0.0001; BP 1.9±0.015: control 0.53±0.01). Nectar quantity ($F_{1,670}$=1.3, P=0.03) and quality (Mann-Whitney U=3,428, P=0.4) expelled from the crops of returning foragers were not significantly different between treatments. Overall, the ratio of pollen to non-pollen foragers counted entering colonies was significantly greater in BP-treated colonies during the 28 days (2×2 X^2=6.3, 1 *df*, P=0.01; BP 1.1±0.1 SE and control 0.6±0.06 SE).

The total number of bees that were reared during the experimental period and emerged as adults from BP-treated colonies was significantly greater than for control colonies; ANOVA, $F_{1,5}$=13.5, P=0.01. There was a significant effect of colony pair on total number of bees that emerged ($F_{5,5}$=106.3, P<0.0001; SAS 2000). An interaction term of treatment × pair was not generated because all variation in the model was explained by the main factors (SAS 2000). On average, 995.2±95.2 SE more bees emerged from BP-treated than control colonies

Overall, greater amounts of protein were extracted from the HP glands of bees reared in BP-treated versus control colonies ($F_{1,5}$=11.5, P=0.02; Fig. 3). A full-factor ANOVA indicated significant effects of colony pair ($F_{5,5}$=38.7, P=0.0005) and age ($F_{2,2}$=23.4, P=0.04) but, no significant interactions of treatment × pair ($F_{5,704}$=1.9, P=0.09) and treatment × age ($F_{2,704}$=2.3, P=0.1; SAS 2000).

Discussion

We found that BP modulated suites of brood rearing and foraging behaviors that coordinate colony growth. HP gland protein content was significantly greater in brood nest bees of BP-treated colonies, indicating an enriched nutritional environment. Pollen intake was increased by a higher ratio of pollen to non-pollen foragers, greater pollen load weight, and decreased foraging age for pollen-specific foragers. Long-term BP treatment sustained long-term high pollen foraging activity. All previous studies examining the effect of BP on colony-level forage choice patterns were conducted over 2–4 h rather than days (Pankiw et al. 1998; Pankiw and Page 2001; Pankiw and Rubink 2002; Pankiw 2004). Brood area naturally increased over time in both the BP-treated and control colonies. Apparently, long-term use of BP did not reduce the BP cue to "background noise" for pollen-specific foraging behavior and was sufficient to sustain increased pollen foraging within a dynamic intra-colony stimulus environment. An increase in amount of brood area is preceded by an increase in queen egg-laying rate. BP clearly induced an increase in queen egg-laying rate within week 1. Egg-laying rate increases with the rate at which queens are fed and the duration of individual feeding bouts (Chauvin 1956; Allen 1960). It remains to be tested whether BP regulates egg-laying through the modulation of worker–queen interactions and increased nutritional environment.

Increased brood rearing, in part, resulted as a consequence of increased pollen intake as opposed to increased consumption of stored pollen. We conclude that BP-induced increase in pollen intake was directly canalized into raising more bees. Colonies provisioned with additional amounts of stored pollen consume stored pollen to pre-manipulation levels and also rear additional brood (Fewell and Winston 1992; Eckert et al. 1994). Schmid-Hempel et al. (1993) introduced the concept of a "preferred state" model, which if adopted by the colony at a particular time, results in maximum expected lifetime reproductive success. This concept is such that colonies work to achieve a homeostatic set point for amounts of stored pollen. The mechanisms through which a "preferred state" is attained, such as replenishing depleted pollen, is accomplished by modulating suites of behaviors associated with colony growth (Schmid-Hempel et al. 1993). In our study, amounts of stored pollen in were similar except in week 3, when the control colonies contained significantly more stored pollen. The results of this study and those of Fewell and Winston (1992) and Eckert et al. (1994) support the "preferred state" model such that when colonies are subject to manipulations as diverse as increasing amounts of BP, stored pollen, and larvae, colonies adjust the amount of brood reared while maintaining a homeostatic set point for stored pollen. What is not clear from the model or empirical study is whether the stored pollen homoeostatic set point changes with colony size.

The amount of BP used here concurrently decreased the age of first foraging and increased the amount of extractable protein from HP glands in bees as old as 21 days. This suggests a complex interaction of BP on worker development. We propose two-way priming of BP on behavioral and physiological development in the experimental cohort whereby one subset was accelerated into foraging and another was delayed as nurses with high protein content HP glands. The two-way priming effect of BP on nurses and foragers is consistent with a view that individuals have different response thresholds for BP stimuli that prime and release behavioral and physiological pathways associated with nursing and pollen foraging behaviors, acting on coordinated suites of nursing and foraging behaviors and thus influencing colony growth.

Acknowledgements This work was supported by grants from the USDA and Texas Legislative Honey Bee Initiative to T. Pankiw.

References

Allen MD (1960) The honeybee queen and her attendants. Anim Behav 8:201–208

Bono M de, Bergmann CI (1998) Natural variation in a neuropeptide Y receptor homology modifies social behavior and feeding response in *C. elegans*. Cell 94:679–689

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Chauvin R (1956) Les facteurs qui gouverment la ponte chez la reine des abeilles. Insectes Soc 3:499–504

Eckert CD, Winston ML, Ydenberg RC (1994) The relationship between population size, amount of brood, and individual foraging behaviour in the honey bee, *Apis mellifera* L. Oecologia 97:248–255

Fewell JH, Winston ML (1992) Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. Behav Ecol Sociobiol 30:387–393

LeConte Y, Mohammedi A, Robinson GE (2001) Primer effects of a brood pheromone on honeybee behavioural development. Proc R Soc Lond B 268:1–6

Mohammedi A, Crauser D, Paris A, Le Conte Y (1996) Effect of a brood pheromone on honeybee hypopharyngeal glands. C R Acad Sci Paris 319:769–772

Page RE, Erber J (2002) Levels of behavioral organization and the evolution of division of labor. Naturwissenschaften 89:91–106

Pankiw T (2003) Directional change in a suite of foraging behaviors in tropical and temperate evolved honey bees (*Apis mellifera* L.). Behav Ecol Sociobiol 54:458–464

Pankiw T (2004) Brood pheromone regulates foraging activity of honey bees (Hymenoptera: Apidae). J Econ Entomol 97:748–751

Pankiw T, Page RE (2001) Brood pheromone modulates sucrose response thresholds in honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 49:206–213

Pankiw T, Rubink WL (2002) Pollen foraging response to brood pheromone by Africanized and European honey bees (*Apis mellifera* L.). Ann Entomol Soc Am 95:761–767

Pankiw T, Page RE, Fondrk MK (1998) Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). Behav Ecol Sociobiol 44:193–198

SAS (2000) The SAS system, version 8.01. SAS Institute, Cary, N.C.

Schmid-Hempel P, Winston ML, Ydenberg RC (1993) Foraging of individual workers in relation to colony state in the social hymenoptera. Can Entomol 125:129–160

Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19:372–378

Sokolowski MB (1998) Genes for normal behavioral variation: recent clues from flies and worms. Neuron 21:463–466

Winston ML (1987) The biology of the honey bee. Harvard University Press, Cambridge, Mass.

Winston ML, Higo HA, Colley SJ, Pankiw T (1991) The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera* L.) reproductive swarming. J Insect Behav 4:649–660