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ORIGINAL ARTICLE

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Genotype and colony environment affect honeybee (*Apis mellifera* L.) development and foraging behavior

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Abstract We examined the interaction of genotype and environment on foraging-behavior development and forage choice in honeybees. High- and low-pollen-hoarding strains and unselected wild-type bees were co-fostered in pairs of colonies manipulated to differentially stimulate high and low pollen foraging. The high-pollen-foraging stimulus consisted of high amounts of larvae, a known stimulus for pollen foraging, plus low amounts of pollen, known to induce pollen foraging. The low-pollen-foraging stimulus consisted of low amounts of larvae plus high amounts of pollen. We estimated the median age at which bees initiated foraging, determined forage choice, and the quality and quantity of resources collected. High-strain bees consistently foraged at younger ages than workers from the other sources. High-strain bees appeared to be more sensitive to the pollen-foraging-stimulus treatments, showing greater differences in foraging age and behavior. Three-way interactions of genotype, pollen foraging stimulus, and colony pair (replicate) were statistically significant for most foraging variables measured suggesting that additional, unknown environmental factors also affect foraging behavior. Our results suggest there is a functional relationship between age of first foraging and forage choice with a strong genetic component that is modulated by colony environment.

Keywords Genotype-environment interaction · Foraging behavior · Honeybee ontogeny

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Introduction

Honeybee colonies display a pronounced division of labor where some individuals are reproductive (queens and drones) and others are workers with diminished reproductive capabilities. Labor among workers is further divided such that individuals perform specific tasks repeatedly and non-randomly (e.g., brood tending, foraging for nectar or pollen), facilitating concurrent performance of all the tasks associated with colony growth and maintenance (see Winston 1987 for a review). A division of labor occurs as a consequence of a distribution of response thresholds among individuals in a colony resulting in differential behavioral responses to task-releasing stimuli (Pankiw and Page 2000). Colony environment affects foraging behavior. Increasing the number of larvae and empty comb space in colonies, or the chemical cues larvae produce, increases the number of pollen foragers (Fewell and Winston 1992; Eckert et al. 1994; Pankiw et al. 1998; Dreller et al. 1999; Pankiw and Page 2001). Foraging behavior also changes in response to changes in the foraging environment. Increasing concentrations of sucrose result in increases in nectar-foraging trips per unit time and load size collected (von Frisch 1967; Seeley 1995). Higher concentrations of sucrose elicit higher probabilities that recruitment dances will be performed, and dances are of longer durations (Seeley 1995).

Division of labor among workers is also a result of age-related behavioral changes known as temporal polyethism (Hölldobler and Wilson 1990; Robinson 1992). In general, during the first 2 weeks of life, a honeybee worker performs tasks within the nest. Then, in the 3rd to 4th weeks of life, workers make a transition to foraging outside the nest (Winston 1987; Seeley 1995). Genetic variation for the age of foraging onset has been demonstrated repeatedly (Calderone and Page 1988; Robinson et al. 1989; Calderone and Page 1991; Page et al. 1991; Robinson 1992; Giray and Robinson 1994). The genotype of an individual also affects the probability that she will perform a given task (Winston and Kaatz

1982; Calderone and Page 1988, 1996; Robinson and Page 1988; Calderone and Page 1992; Page et al. 1992; Calderone 1993; Page and Fondrk 1995; Giray et al. 2000; Pankiw et al. 2000). As a consequence, age-related shifts in behavioral development may be organized on several levels according to genotype and environmental stimuli, presumably allowing colonies to be flexible with changes in the colony and foraging environments.

Bees selected for the amount of surplus pollen they store have provided valuable tools for studying foraging behavior (Hellmich et al. 1985; Page and Fondrk 1995). Two-way selection for the amount of stored pollen has resulted in differences between strains for foraging age, the probability of forage choice such as nectar, pollen, and water, the concentration of sucrose returned by nectar foragers, individual's perception of sucrose reward, and performance during associative-learning assays (Calderone and Page 1988, 1996; Page and Fondrk 1995; Page et al. 1998; Pankiw and Page 1999; Scheiner et al. 1999, 2001, in press). Bees of the high-pollen-hoarding strain of Hellmich et al. (1985) initiated foraging at younger ages than did the low-strain bees in a total of seven of nine independent trials (Calderone and Page 1988, 1991). Differences have also been noted (T. Pankiw, R.E. Page, M.K. Fondrk, unpublished data) in foraging onset between the high and low strains of Page and Fondrk (1995). These strains were selected independently from those of Hellmich et al. (1985). Combined, these results suggest a causal association between increased pollen hoarding and foraging onset. Here we examine, for the first time, the effects of genotype, pollen-foraging stimulus environment, and their interaction on age of first foraging, forage choice, and quality of resources collected.

Methods

Newly emerged unselected wild-type, and selected high- and low-pollen-hoarding strains of bees were introduced to six colony pairs. A pair was produced by equally dividing an existing colony into two five-frame colonies headed by super-sister queens. One colony of each pair was maintained with low quantities of brood and high quantities of pollen (low-pollen-foraging-stimulus treatment), and the other colony of each pair was maintained with high quantities of brood and low quantities of pollen (high-pollen-foraging-stimulus treatment). Foraging behavior was measured as described below. The experiment was divided into two rounds with three colony pairs (replicates) per round. Round 1 was conducted from 15 June to 8 July 1999 (23 days); round 2 was conducted from 29 June to 29 July 1999 (28 days).

Colony pairs, queens, and pollen-foraging treatments

Colony pairs were produced by equally dividing existing, established colonies into two equal parts. Each colony in a pair consisted of approximately 10,000 adult workers and a young naturally mated super-sister queen. Colony pairs were established 7 days prior to the introduction of experimental bees and treatments (pollen-foraging-stimulus manipulations). Treatments were maintained during the course of the experiment. Every 3rd day, colony manipulations were performed to maintain the pollen-foraging-stimulus treatments. The high-pollen-foraging treatment consisted of approximately 742 cm^2 of larvae, 45 cm^2 of pollen, one frame of empty space, and two frames of honey. The low-pollen-foraging treatment consisted of approximately 45 cm^2 of larvae, 1,484 cm^2 of pollen, one frame of empty space and two frames of honey.

Bees

We used the high- and low-pollen-hoarding strains of Page and Fondrk (1995). Combs containing the selected strains and unselected wild-type pupae were placed in separate cages in an incubator in the evening (50% relative humidity and 34–35°C). Bees that emerged over a 12-h period were color coded with paint (Testors) applied to the thorax to distinguish genotype. Three hundred wild-type, 300 high-, and 300 low-strain bees were introduced to each colony. We controlled colony pair demographics by daily introductions of 300 newly emerged non-experimental bees. The sources of these bees were the same colonies from which colony pairs were derived.

Foraging-behavior measurements

The foraging age of an individual was determined by the number of days from emergence to capture at the colony entrance as a returning forager. Colony entrances were observed for returning experimental bees for 20 min during the first 4 days after introduction. Beginning on the 5th day to the termination of the experiment, each colony entrance was blocked with wire-mesh for 15-min intervals from 0800–1700 hours for a minimum of 60 min to a maximum of 120 min per day. All colonies were blocked for an equal amount of time per day. Marked foragers were removed from the wire-mesh and destructively sampled (see below). In round 1, entrances were blocked every day from days 5 to 23 with the exception of day 18. In round 2, entrances were blocked every day from day 5 to 28 with the exception of days 16 and 19.

Daily counts of returning pollen and non-pollen foragers were performed for a 3-min period (see Page and Fondrk 1995) prior and subsequent to blocking entrances. This count was conducted to determine the colony's responses to the pollen-foraging-stimulus treatments. Visual observation of returning bees is limited to two forager classes according to whether bees returned with pollen or no pollen (non-pollen) seen on the hind legs. Observers used two hand-held counters to count all pollen foragers with one hand and all non-pollen foragers with the other.

Foragers were individually captured in small cylindrical wire cages. Captured foragers were anesthetized with CO_2. Crop contents were expelled by gently squeezing the abdomen. Contents were collected into pre-weighed capillary tubes, the tubes were re-weighed and the subtotal recorded (Kimax-51; see Gary and Lorenzen 1976). The nectar loads were measured for sugar concentration using a hand-held refractometer (Bausch & Lomb Optical). Pollen load weight was determined by weighing one pollen pellet removed from the corbicula of a hind leg and doubling the value. Workers with trace amounts of pollen (<0.001 g) were assumed to have a total of 0.001 g of pollen.

Termination census

Termination censuses were performed at the end of each round. These censuses were used to control for potential differences in pre-foraging mortality by determining the number of bees of each genotype that were available to become foragers. The data were used to estimate the median foraging ages.

Censuses were conducted between 0500 and 0700 hours (prior to bee flight activity) at the termination of each round (9 and 30 July). Marked bees were collected with forceps off the combs in the hive and placed into a soap-water solution. This solution does not remove Testors paint from the thorax. Bees were strained from the solution, sorted by genotype, and counted.

Table 1 Median age of first foraging in days

Pair	Pollen foraging treatment	Genotype	Quartile Estimates[a]		Linear regression median estimates[b]	Percent foraged
			25%	50%		
1	High	High	11.0	23.0	–	53.4
		Low	21.0	–	27.4	41.3
		Wild	15.0	–	25.8	41.2
	Low	High	11.0	13.0	–	79.2
		Low	20.0	–	28.5	38.1
		Wild	13.0	22.0	–	52.6
2	High	High	14.0	22.0	–	55.6
		Low	23.0	–	35.4	26.8
		Wild	21.0	–	33.9	30.2
	Low	High	11.0	23.0	–	51.0
		Low	–	–	55.4	15.6
		Wild	14.0	–	26.4	35.8
3	High	High	15.0	21.0	–	66.5
		Low	21.0	–	25.3	42.8
		Wild	19.5	–	25.6	42.7
	Low	High	13.0	–	22.6	45.2
		Low	23.0	–	29.6	33.9
		Wild	22.0	–	34.4	30.5
4	High	High	17.0	20.0	–	81.2
		Low	20.0	27.0	–	57.5
		Wild	22.0	–	34.3	37.4
	Low	High	17.0	25.0	–	58.1
		Low	23.0	–	34.0	36.9
		Wild	24.0	–	39.5	32.3
5	High	High	17.0	22.0	–	84.8
		Low	21.0	25.0	–	68.1
		Wild	21.0	27.0	–	67.4
	Low	High	15.0	17.0	–	95.2
		Low	17.0	20.0	–	95.5
		Wild	17.0	20.0	–	92.8
6	High	High	23.0	–	30.6	47.5
		Low	27.0	–	45.3	22.1
		Wild	–	–	60.8	19.2
	Low	High	13.0	20.0	–	73.7
		Low	23.0	–	30.1	45.4
		Wild	15.0	–	25.2	47.3

[a] Quartile estimates within 95% confidence intervals. Where too few individuals foraged to calculate an estimate a *dash* is shown. Only 6 of 36 cohorts reached the 75th quartile, which is therefore not shown
[b] Where less than 50% of the cohort foraged, an estimated median foraging age was calculated based on the linear regression of cumulative foraging rate. A *dash* indicates that 50% or more bees foraged

Statistical analyses

Foraging age, pollen weight, nectar weight, and nectar concentration variables were subjected to standard diagnostics to determine any significant deviations from normality (Sokal and Rohlf 1995). Age of first foraging was not normally distributed, therefore, survival analysis was used to analyze age-of-first-foraging data. The day of initiation of foraging is equivalent to "death" and allows the use of this procedure (Le 1997; SAS 2000). Less than 50% of many of the experimental genotypes did not forage (Table 1). A direct estimate of median foraging age was not possible for these genotypes, therefore we estimated their median foraging ages using linear regression. Experimental genotypes showed increasing hazard (or risk) functions generated by LifeTables (Le 1997; SAS 2000). For small increments of time between observations, as in this case, the risk function approximates the proportion of individuals foraging at a given time (Le 1997). Increasing hazard functions permit the use of first-order linear regression to estimate the median foraging age of each genotype (Kleinbaum 1996; Le 1997; Allison 1998). Cox proportional hazards regression was used to analyze main effects of genotype, pollen-foraging treatment, and colony pair, and their interactions on age of first foraging (PROC PHREG in SAS 2000; Allison 1998). Ties were handled using the EXACT option (PROC PHREG in SAS 2000).

We classified the foragers according to their loads prior to analyzing the quantity and quality of resources collected. All foragers returning with some pollen were classified as bees 'with pollen'; this class includes bees returning with pollen as well as nectar and pollen (see Table 4). Those bees returning with no other resource except pollen were classed as 'pollen-only' foragers. Foragers returning with some nectar were classified as bees 'with nectar,' including those returning with nectar as well as pollen and nectar. Those returning with no other resource except nectar were classed as 'nectar only.' Pollen and nectar weights were normally distributed. ANOVA was used to analyze these variables. The proportion of the total weight of the load that was pollen was calculated for bees 'with pollen.' Arcsine-squareroot transformations were performed on the proportions to meet the assumptions of ANOVA (Sokal and Rohlf 1995). The effects of pollen-foraging treatment and genotype on nectar concentrations were analyzed using Mann-Whitney U-tests. Interaction terms were analyzed with ANOVA because the Mann-Whitney U is not capable of performing interaction analyses; ANOVA, however, is robust against departures from normality (Sokal and Rohlf 1995).

We named forage choice categories as pollen, nectar, both pollen and nectar, empty, water, and water and pollen foragers. Saturated categorical model analyses of variance were performed to determine the effects of genotype, pollen-foraging treatment, colony pair, and interactions on forage choice (CATMOD procedure of SAS 2000; Stokes et al. 1997). Chi-square contingency table analysis was used to determine the effect of pollen-foraging treatment on pollen and non-pollen forager entrance counts on colony pairs (Sokal and Rohlf 1995).

Results

Foraging age

High-strain bees consistently foraged at significantly younger ages than low-strain and wild-type bees (Table 2). Wild-type bees foraged later in life, more like the low strain. Pollen-foraging treatments also significantly affected foraging age, though the effects varied with colony pairs (Tables 1, 3). Genotypes also responded differentially to pollen foraging treatments as indicated by the significant genotype×pollen foraging treatment interaction (Table 2). In general, high-strain bees were the most responsive.

Colony pair also affected foraging age (Table 2). Most interaction terms involving colony pair were significant. We interpret this as a colony environment effect on foraging behavior. Significant colony pair interactions means that there were unknown colony environment factors that differentially affected the foraging behavior of genotypes, and/or responses to pollen-foraging treatments.

To indicate the direction and statistical significance of differential effects of genotype and pollen-foraging treatments on foraging age, we performed Cox proportional hazards regression analyses on individual colony pairs (Table 3). There was a significant pollen-foraging-stimulus treatment effect on the high stain in all six colony pairs (Table 3). High-strain bees foraged significantly earlier in the high-pollen-foraging treatment in four of six pairs. Low-strain and wild-type bees foraged earlier in the high-pollen-foraging treatment in only two and three of six pairs, respectively (Table 3).

The complexity of foraging behavior is further demonstrated by the significant three-way interaction of genotype×pollen foraging treatment×colony pair (Table 2). This indicates significant differential strain responses to pollen-foraging treatments as well as colony pair for foraging age.

Table 2 Cox proportional hazards regression analysis of main effects on age of first foraging

Source	*df*	Wald χ^2	*P*
Genotype	2	204.0	<0.0001
Pollen-foraging treatment	1	45.5	<0.0001
Colony pair	5	614.9	<0.0001
Genotype×pollen-foraging treatment	2	6.2	<0.05
Three-way interaction	10	213.1	<0.0001

Forage choice

High-strain bees were more likely to collect pollen and low-strain bees were more likely to collect nectar (Table 4). Significantly more bees collected pollen in colonies manipulated to stimulate high pollen foraging (Table 4). There was no genotype×pollen-foraging treatment interaction on forage choice (Table 5). This means that the high-pollen-foraging treatment stimulated more pollen foraging in all genotypes. There were significant interactions for all terms, including colony pair, on forage choice (Table 5; see above).

Resources returned

Genotype and pollen-foraging treatment significantly affected the proportion of total load weight that was pollen

Table 3 Cox proportional hazards regression analysis of foraging age on genotype between pollen-foraging treatments. Values in *parentheses* indicates the number of days the high- or low-pollen-foraging treatments significantly increased median age of first foraging

Genotype	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
High strain	*P*<0.0001 (High +10 days)	*P*<0.0001 (High +1 day)	*P*<0.001 (Low +2 days)	*P*<0.0001 (Low +5 days)	*P*<0.0001 (High +5 days)	*P*<0.0001 (High +10 days)
Low strain	*P*>0.05	*P*<0.01 (High +10 days)	*P*>0.05	*P*<0.0001 (Low +7 days)	*P*<0.0001 (High +5 days)	*P*<0.0001 (High +15 days)
Wild type	*P*<0.01 (High +4 days)	*P*>0.05	*P*<0.05 (Low +8 days)	*P*>0.05	*P*<0.0001 (High +7 days)	*P*<0.0001 (High +36 days)

Table 4 Total number of bees and their forage choice

Forager	High-pollen-foraging treatment			Low-pollen-foraging treatment		
	High strain	Low strain	Wild type	High strain	Low strain	Wild type
Pollen	279	95	80	92	21	28
Nectar	129	208	193	335	365	358
Both	256	195	167	206	146	171
Empty	119	104	85	132	98	103
Water[a]	5	2	2	2	0	3
Water and pollen[a]	5	2	2	4	2	3

[a] Too few to include in the analysis

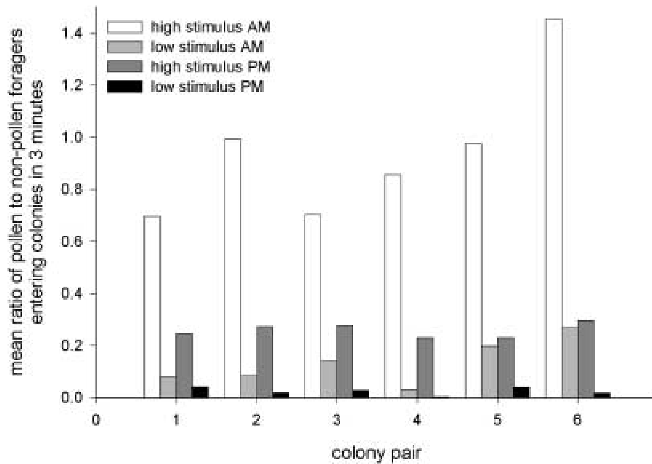

Fig. 1 Forager entrance counts of colony pairs manipulated to stimulate high and low pollen foraging

Table 5 Effects of experimental factors on individual forage choices for pollen, nectar, both pollen and nectar, or empty. There were too few foragers returning with water and water plus pollen to include in the analysis. Saturated categorical model analysis

Source	*df*	χ^2	*P*
Genotype	2	14.5	<0.001
Pollen foraging treatment	1	39.3	<0.0001
Colony pair	5	128.6	<0.0001
Genotype×pollen-foraging treatment	2	0.3	>0.05
Colony pair×genotype	10	26.0	<0.01
Colony pair×pollen foraging treatment	5	26.3	<0.0001
Three-way interaction	10	19.2	<0.05

(Table 6). Pollen load size was significantly affected by colony pair for bees returning with pollen ($F_{5,1264}$=12.9, P<0.0001). Significant interactions for pollen load weight were also observed in bees returning with pollen (colony pair×pollen foraging treatment: $F_{5,1264}$=3.8, P<0.01 and, colony pair×genotype: $F_{5,1264}$=2.3, P<0.01).

In general, nectar loads were heavier in low-strain bees and bees in the low-pollen-foraging treatment (Table 6). High-strain bees returned with more concentrated nectar than the low-strain bees (Table 6). Bees in the high-pollen-foraging treatment returned with significantly less concentrated nectar than bees in the low-pollen foraging-treatment (Table 6). Colony pair affected nectar weight (with nectar: $F_{5,2540}$=31.9, P<0.0001; only nectar: $F_{5,1408}$=15.6, P<0.0001) and nectar concentration (with nectar: Kruskal-Wallis χ^2=10.8, *df*=5, P<0.05; only nectar: Kruskal-Wallis χ^2=22.5, *df*=5, P<0.001).

Colony entrance counts

Colonies manipulated to stimulate high pollen foraging had a significantly higher ratio of pollen to non-pollen foragers than colonies manipulated to stimulate low pollen foraging (morning: contingency table χ^2=1,693.4, 1 df, P<0.0001; afternoon: contingency table χ^2=1,299.4, 1 df, P<0.0001; Fig. 1).

Discussion

High-strain bees consistently foraged at younger ages in both pollen-foraging treatments compared to low-strain and wild-type bees. This cannot be explained by missing low-strain and wild-type bees because they were retrieved from colonies at the termination of the experiment. These results are consistent with those of Calderone and Page (1988, 1991, 1996) and our unpublished data, suggesting a functional relationship between forage choice and foraging ontogeny.

High-strain bees most consistently varied with colony treatment for their median ages of foraging onset. That is, in four of the six colony pairs, the high strain foraged at significantly earlier ages in the high-pollen-foraging-stimulus treatment. We interpret this as high-strain bees having greater sensitivity to colony environments. We attempted to control for colony environment by making each treatment in each pair as identical as possible, except for quantities of stored pollen and young larvae. We also attempted to make treatments across pairs as identical as possible, but from the comparisons of treatment effects between pairs we obviously did not sufficiently control all of the stimuli involved.

Some of the inconsistent results we saw may have resulted from dose-dependent responses of bees to brood pheromones that varied between treatments and possibly among pairs. Le Conte et al. (2001) reported that a blend of ten fatty acid esters extracted from the cuticle of larvae have a dose-dependent effect on foraging ontogeny (LeConte et al. 2001). Young bees in colonies receiving high amounts of pheromone have delayed foraging ontogeny relative to bees in colonies receiving low amounts of pheromone, but those with no brood or pheromone are more delayed that those with low levels (LeConte et al. 2001). Our mixed results were potentially a consequence of uncontrolled differences in brood pheromone doses.

Brood pheromone also has dose-dependent effects on sucrose sensitivity, as measured by the proboscis extension response. Bees extend their proboscis when a droplet of sucrose solution of sufficient concentration is touched to the antennae (Bitterman et al. 1983). Brood pheromone modulates sensitivity to sucrose. Modulation is dose dependent such that bees caged with lower doses of brood pheromone have increased sensitivity to sucrose, while bees caged with higher doses have decreased sensitivity to sucrose (Pankiw and Page 2001). High-strain bees are more sensitive to lower concentrations of sucrose solutions under all test conditions and ages than low-strain bees (Page et al. 1998; Pankiw and Page 1999). Pollen-foraging wild-type bees likewise are more sensitive to sucrose than nectar foragers, demonstrating a functional link between sucrose perception and foraging behavior (Pankiw and Page 2000). Pollen forag-

Table 6 Mean (±SE) measures of resources collected by foraging bees. Means followed by *different letters* are significantly different (Fisher's PLSD, P<0.05). Values in *parentheses* correspond to the number of individuals measured

	High-pollen-foraging treatment			Low-pollen foraging treatment			Genotype		Pollen-foraging treatment		Interaction	
	High strain	Low strain	Wild type	High strain	Low strain	Wild type	Statistic	P	Statistic	P	Statistic	P
Pollen weight (mg)												
With pollen	6.0±3^{b} (372)	6.2±3^{b} (224)	6.0±3^{b} (192)	6.4±5^{ab} (193)	7.2±4^{a} (161)	6.3±4^{ab} (158)	$F_{2,1264}$=0.3	>0.05	$F_{1,1264}$=.003	>0.05	$F_{2,1264}$=0.6	>0.05
Proportion of total load that is pollen												
Proportion with pollen	0.69±002 (372)	0.53±003 (224)	0.54±003 (192)	0.56±003 (193)	0.48±003 (161)	0.47±003 (158)	$F_{2,1264}$=9.2	<0.0001	$F_{1,1264}$=15.6	<0.0001	$F_{2,1264}$=1.0	>0.05
Pollen weight (mg)												
Pollen only	6.2±5^{a} (171)	6.2±7^{a} (45)	7.8±6^{a} (52)	5.4±6^{b} (43)	8.0±1.2^{a} (10)	4.2±1.2^{a} (9)	$F_{2,304}$=0.07	>0.05	$F_{1,304}$=0.5	>0.05	$F_{2,304}$=1.2	>0.05
Nectar weight (mg)												
With nectar	21.1±8^{d} (375)	26.3±8^{b} (390)	23.7±8^{c} (346)	28.1±7^{a} (504)	29.8±7^{a} (469)	28.9±7^{a} (492)	$F_{2,2540}$=12.0	<0.0001	$F_{1,2540}$=94.0	<0.0001	$\mathrm{F}_{2,2540}$=3.7	<0.05
Nectar only	26.6±1.5^{b} (119)	29.7±1.3^{a} (196)	27.2±178^{b} (178)	31.2±9^{a} (299)	31.0±9^{a} (323)	31.1±8^{a} (329)	$F_{2,1408}$=0.4	>0.05	$F_{1,1408}$=18.2	<0.0001	$F_{2,1408}$=0.4	>0.05
Nectar concentration (%)												
With nectar	45.8±8 (632	44.4±7 (379)	45.9±8 (330)	49.4±6 (471)	48.54±6 (457)	46.7±6 (473)	χ^2_2=14.2	<0.001	$U_{1070,1411}$=663,379	<0.0001	$F_{2,2471}$=1.4	>0.05
Nectar only	47.1±1.3 (111)	44.6±1.0 (189)	46.5±1.0 (165)	50.9±8 (271)	48.1±7 (314)	47.0±8 (312)	χ^2_2=17.6	<0.0001	$U_{473,911}$=182,435.5	<0.0001	$F_{2,1361}$=2.3	>0.05

ing is released when colonies are given supplemental doses of brood pheromone (Pankiw et al. 1998; Pankiw and Page 2001). Therefore, brood pheromone is acting as a dose-dependent primer that apparently affects the rate of onset of foraging behavior, modulates sucrose responses that are associated with foraging decisions, and is a releaser of pollen-foraging behavior. The results of this study suggest that high-strain bees are more sensitive to brood pheromone primer effects on foraging ontogeny and releaser effects on pollen foraging. However, the pollen-foraging treatments included amounts of brood and stored pollen; therefore, we cannot rule out the independent effects of amount of stored pollen on foraging behavior.

As non-pollen foragers, high-strain bees generally return with nectar that has lower sucrose concentrations and are more likely to return with water than low-strain foragers (Page et al. 1998; Pankiw and Page 1999). Low-strain non-pollen foragers return with nectar that is more concentrated with sucrose and are more likely than high-strain foragers to return empty (Page et al. 1998; Pankiw and Page 1999). We interpret this as high-strain bees having lower response thresholds to sucrose and being more accepting of lower-quality nectar (and water) than the low-strain bees. In this study, we observed high-strain bees collecting more concentrated nectar than low-strain bees. This was apparently an artifact of the changing foraging environment over time. High-strain bees foraged at significantly earlier ages than the low-strain bees (see above). The average nectar concentration in the first half of the experiment was 47% and in the second half, 34%. High-strain bees composed the greatest proportion of foragers in the first half of the experiment when the nectar collected was more concentrated than in the second half when low-strain and wild-type bees began to forage. The lower-quality nectar returned by bees in the high-pollen-foraging-stimulus environment (Table 6) may suggest that the greater amount of larvae decreased sucrose sensitivity in these bees resulting in the return of lower-quality nectar than that returned by bees in the low-pollen-foraging-stimulus environment (Pankiw and Page 2001).

In this study, we clearly demonstrated that selection for pollen hoarding also selected earlier foraging age. Selection for a colony-level trait, the amount of stored pollen, has revealed a set of behaviors and associated sensory-physiological traits that influence the age that bees initiate foraging, the choice of collecting pollen and nectar, and how bees evaluate the quality of nectar and pollen resources (Page et al. 1998; Waddington et al. 1998; Pankiw and Page 2000). Collectively, these traits are primary mechanisms that organize division of labor within honeybee colonies. Brood pheromone is emerging as an important modulator of foraging behavior acting through primer and releaser functions. Brood pheromone modulates sucrose response thresholds that affect foraging behavior, results in dose-dependent effects on the rate of maturation of sucrose perception, releases pollen-foraging behavior (Pankiw et al. 1998; Pankiw and Page 2001) and appears to have dose dependent effects on the age that bees initiate foraging. The genetic and physiological relationships of brood pheromone primer and releaser effects on foraging behavior are currently being studied.

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