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The effects of colony-level selection on the social organization of honey bee (Apis mellifera L.) colonies: colony-level components of pollen hoarding

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Abstract Two-way selection for quantities of stored pollen resulted in the production of high and low pollen hoarding strains of honey bees (*Apis mellifera* L.). Strains differed in areas of stored pollen after a single generation of selection and, by the third generation, the high strain colonies stored an average 6 times more pollen than low strain colonies. Colony-level organizational components that potentially affect pollen stores were identified that varied genetically within and between these strains. Changes occurred in several of these components, in addition to changes in the selected trait. High strain colonies had a significantly higher proportion of foragers returning with loads of pollen, however, high and low strain colonies had equal total numbers of foragers. Colony rates of intake of pollen and nectar were not independent. Selection resulted in an increase in the number of pollen collectors and a decrease in the number of nectar collectors in high strain colonies, while the reciprocal relationship occurred in the low strain. High and low strain colonies also demonstrated different diurnal foraging patterns as measured by the changing proportions of returning pollen foragers. High strain colonies of generation 3 contained significantly less brood than did low strain colonies, a consequence of a constraint on colony growth resulting from a fixed nest volume and large quantities of stored pollen.These components represent selectable colony-level traits on which natural selection can act and shape the social organization of honey bee colonies.

Key words *Apis mellifera* · Colony-level selection Pollen storage · Foraging · Colony growth

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Introduction

How does colony organization evolve in social insects? Genetic information, the ultimate unit of change in evolutionary processes, resides within the nuclei of cells of individual members of insect colonies; there is no "colony level" genome to orchestrate colony development and activities. Therefore, natural selection must act on phenotypic variability arising from variability in the genotypic composition of queens, workers, and males within colonies. Colony organizational patterns can "emerge" from the collection of individuals through their activities and interactions, or be a direct consequence of a centralized colony informational structure, such as organizational queen pheromones (Seeley 1989). Most colony-level behavioral patterns, however, are probably not due to centralized information but due to self-organizing processes that affect individual worker behavior (Page and Mitchell 1991; Page and Robinson 1991; Huang and Robinson 1992). Varying patterns of social organization within and among colonies are caused by varying environmental stimuli and phenotypic distributions of worker response thresholds (Robinson and Page 1989).

Studies of insect colony organization have traditionally focused on the relationships between colony size and development, and organizational patterns associated with specific environments. However, in order to understand how colony organizational patterns evolve we need to identify the organizational structure of colonies, identify those features (components) that demonstrate heritable variation, then determine the relationships among component parts. One way to do this is to select for colony-level traits and determine how selection affects colony organization and the behavior of individuals within colonies. Hellmich et al. (1985) conducted such a study when they selected strains of honey bees for the amount of pollen they stored in combs. Their two-way selection

was based on colony-level evaluations and they successfully separated high and low strains in a single generation. Their study, however, only demonstrated a response to selection of the same phenotypic trait they selected (amount of stored pollen); they did not study the effects of their selection on individual worker behavior or other potential components of colony organization.

Calderone and Page (1988, 1991, 1992) studied individual worker behavior in the same strains selected by Hellmich et al. (1985) and demonstrated two selectable components of division of labor that affect colony organization: the likelihood of performing a specific task, and the age of initiation of foraging behavior. Workers of the high strain were more likely to forage for pollen, even when raised in the same environments as low strain workers. Workers of the high strain also initiated foraging behavior at a younger average age. Combined, these traits should result in greater numbers of pollen foragers in high strain than in low strain colonies resulting in a potentially higher rate of pollen intake.

Although it is likely that these observed behavioral differences are consequences of the colony-level selection for stored pollen, they could also be a result of chance. Quantities of stored pollen are regulated by colonies (Fewell and Winston 1992) and the mechanisms of regulation could be unrelated to individual foraging behavior. The observed behavioral differences could have occurred, instead, as a consequence of random fixation of genetically-variable traits within the small breeding populations (three queens of each strain) constituting each strain. Other plausible mechanisms that may be responsible for changes in quantities of stored pollen are presented in the discussion.

For this study, we repeated the selection program of Hellmich et al. (1985). Our study differs from theirs in that we used larger breeding populations and identified and quantified eight colony organizational traits that may affect quantities of stored pollen. We conducted three generations of two way colony-level selection for high and low pollen hoarding and identified those traits that varied genetically (are selectable) and those that actually changed as a consequence of selection. We then examined the relationships between component parts to understand how colony-level selection changes components of social organization that result in changes in a complex colony phenotype.

Methods

Colony evaluations were standard throughout the selection program. First, we present the common features of our evaluation methods. Then specifics of methodology are given for each generation.

Evaluations

Colony size

For the initial commercial population, the number of workers was estimated in colonies by opening each hive and determining the number of wax combs (frames) covered by workers. Evaluations were performed in the morning, before active foraging flight began. Estimates were made to the nearest half frame of bees. A frame of bees contains approximately 1900 workers (Kauffeld 1975).

Estimates of colony strength were made for each subsequent generation at the same time as brood and pollen stores evaluations. The number of frames covered with workers was estimated to the nearest tenth of a frame.

Pollen stores

Honey bees tend to store pollen in contiguous areas surrounding the brood located in the center of the nest. All combs of each colony were examined individually. A 6.45-cm^2 wire grid was placed over the areas containing pollen and individual grid squares lying over pollen were counted.

Brood area

Eggs, larvae, and pupae are maintained and raised in individual wax cells located in the center of the nest. Each comb of each colony was inspected and the area of brood was estimated to one-tenth of a frame for each side of each comb. Estimates were then transformed from units of frames to square centimeters by multiplying the estimated number of frame sides by 768 cm^2, the area of one frame side.

Foraging

Foragers returning with and without loads of pollen were counted at the entrances of colonies. We made foraging observations in the morning when orientation flights were not likely. Observations were made at the entrances of each colony for 4 or 5 min, depending on the generation. Four to six rounds of observations were made over 1–3 days for each generation. Each of four or five observers were blindly and randomly assigned a set of colonies to evaluate during a given round of observations. Any given round required no more than 60 min to complete observations on all colonies. Sets of colonies and observers were reassigned for each round.

Initial stocks

Approximately 400 commercial colonies were inspected during late February and early March 1990. Colonies were distributed throughout five almond orchards within 35 km of the Bee Biology Facility, University of California Davis. Colonies were owned by four different beekeepers, providing us with a diverse sample of available commercial genotypes from which to initiate our selection program. Colonies were evaluated when the orchards were estimated to be at about 60% bloom.

Within each orchard, colonies were selected for evaluation that varied by no more than two frames of bees, based on strength estimates for that orchard. All selected colonies in all orchards contained six to ten frames of bees. Selected colonies were marked, recorded, and evaluated within 2 days for areas of stored pollen. The highest and lowest performing colonies were then selected from each orchard to produce virgin queens and drones to constitute the foundation sublines of our two-way selection program. Ten selected high-pollen-stores colonies were designated H1-H10, and 10 low-pollen colonies were designated L1-L10. Each generation, virgin queens were raised using standard methods (Laidlaw 1979) and were

instrumentally inseminated with semen from single drones (Laidlaw 1977).

Five maternal sublines were maintained within the high and low strain populations throughout the selection program. Each generation, the superior (high or low pollen stores) performing daughter colony of each subline was selected to produce virgin queens and drones. Eight to ten daughter colonies were produced from each subline, each generation. Colonies that showed signs of low brood viability resulting from homozygosity at the sex locus (Woyke 1986), or that had severe disease problems, were eliminated from the study. Each generation 49–57 surviving colonies were subsequently tested. Crosses were made between sublines that minimized inbreeding. Between-subline crosses were rotated each generation (Fig. 1b).

Generation 1

Initial crosses were performed (Fig. 1a) to constitute low and high sublines A-E and Q-U, respectively. Queens were produced and instrumentally inseminated in late March and early April 1990. They were introduced into single-story Langstroth hives with 1-kg packages of workers (approximately 7000–8000 bees) during 7–11 April. Most queens were laying eggs by 20 April. All colonies were managed equally and blindly, for all generations. Colonies were fed supplemental sugar syrup as needed while they expanded their worker populations. Colonies were moved to the University of California Davis Arboretum for evaluation.

After sufficient time had passed for all of the workers within colonies to be the progeny of our high and low queens, 7, 8, 3, 6, and 5 colonies were evaluated for low strain sublines A–E, respectively. For the high strain, we evaluated 5, 4, 4, 9, and 4 colonies of sublines Q–U, respectively. Estimates of colony strength, brood, and pollen areas were performed on 30 June. Two rounds of 5-min foraging observations were made for each colony on 3 July, beginning at approximately 0920 and 1100 hours.

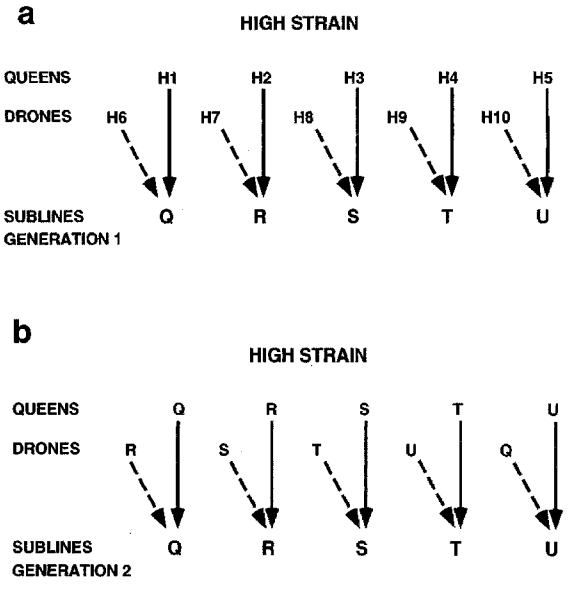

Fig. 1 Mating design for the high strain of the two-way selection program. The upper figure **a** shows the initial crosses made of virgin queens and drones derived from commercial colonies that generated the 5 high sublines. The lower figure **b** shows the rotational mating scheme for generation 1 queens and drones. *Solid lines* represent egg gametes; *broken lines*, sperm. Low strain sublines were intiated and crossed using the same design

Generation 2

The following high and low subline crosses were made to produce colonies with generation 2 workers (Fig. 1b). The first letter in each cross designates the source of the virgin queen, the second letter is the subline source of the single drone used to instrumentally inseminate the queen. The number of colonies evaluated from each cross are shown in parentheses. Low strain crosses were: A × D (4), B × E (7), C × B (2), D × A (7), and E × C (7). High strain crosses were: Q × R (6), R × S (6), S × T (4), T × U (3), and U × Q (3). Queens were produced in mid-July 1990, inseminated on 1 August, and placed into single-story Langstroth hives containing 1.5 kg of workers and three frames of brood. Colonies developed, overwintered, and were moved into an almond orchard located near Davis, California in mid-February 1991.

Colony strength evaluations and estimates of brood and pollen areas were performed on 7 March. Two rounds of 4-min foraging observations were conducted during the morning on each of 3 days: 25–26 February and 6 March. Evaluations taken on the 3rd day followed 7 days of rain that greatly reduced the almond bloom and depleted resouces available in the orchards.

Generation 3

The following high and low subline crosses were made to produce colonies with generation 3 workers. Low strain crosses were: A × B (6), B × D (6), C × E (5), D × C (8), and E × A (5). High strain crosses were: Q × T (5), R × U (6), S × Q (3), T × R (7), and U × S (6). Queens were produced in late March, instrumentally inseminated in early to mid April, then placed into single-story Langstroth hives with 1-kg packages of workers on 14 and 23 April 1991.

Four evaluations were made of pollen area in order to determine the "development" of pollen stores in newly founded colonies. The first evaluation took place at the time the first generation 3 workers began emerging in the colonies, 30 days following the introduction of the queens, 14 and 23 May for the two sets of introductions, respectively. [During introduction, queens usually remain caged in colonies for about 3 days before they are released and free to lay eggs. It then often takes a few days before they initiate egg laying. Worker honey bees require about 21 days to develop from egg to adult (see Winston 1987)]. Following the second evaluation of 30 May and 3 June, colonies were moved from the Bee Biology Facility into the University of California Davis Arboretum. Two additional evaluations of pollen stores occurred: 14 and 17 June, for the two sets of inseminations, respectively, and 5 July for all colonies combined.

Only pollen area was estimated for the first evaluation; pollen, brood, and colony strength were estimated for evaluations 2–4. Two rounds of 4 min foraging observations were conducted during the mornings of 2 and 3 July.

Crop loads of foragers

Returning workers were classified as pollen or nonpollen foragers during our foraging evaluations. One result of our selection could have been to select workers in our low strain that foraged unsuccessfully. To test this, we determined the relative frequencies at which pollen and nonpollen foragers of generation 3 low and high strains returned with loads of nectar, water, or were empty. Five high and five low line colonies were selected for sampling. Each colony was one of the top two high or low performers from each of the five high and five low sublines.

Two kinds of samples were taken, individual samples at the colony entrance, and vacuum samples of all returning foragers that were detained by a screen placed over the entrance.

Individual samples

Hive entrances were covered with 8-mesh hardware screen for approximately 30 s prior to collecting bees. The screen prevented returning foragers from entering. Foragers accumulated on the screen and were individually collected into wire screen cages and immediately placed into a container of dry ice and were frozen. Collecting took place only for a few minutes and only as long as food sharing was not occurring on the entrance. Samples were collected during the morning of 11 July and morning and afternoon on 12 July. Collection continued until at least 50 pollen and 50 nonpollen bees were collected from each colony.

Vacuum samples

Colony entrances were screened for 30 s then all returning foragers were collected into a screen cage using a vacuum device (Gary and Lorenzen 1990) until at least 50 pollen and 50 nonpollen foragers were collected. Colony entrances remained screened for a maximum of 5 min to prevent food sharing by detained foragers. Bees were disrupted by the airflow through the vacuum device and, as a consequence, food sharing was not observed during this 5 min operation. Collections were made between 1300 and 1400 hours on 11 July.

Determination of crop content

Frozen bees were thawed briefly before expressing the content of their crop into a 75 m capillary tube. The crop contents were then measured with a millimeter rule to determine volume. The sugar concentration of the crop contents was then determined using a hand-held refractometer. Workers carrying less than 5 µl were considered empty (Gary and Lorenzen 1976), those that contained less than 10% sugar were classified as water collectors.

Statistics

Selective breeding itself affects the population distribution of characters that are selected or correlated with selected characters. Therefore, some evaluated traits did not meet the assumptions of ANOVA. As a consequence, we used nonparametric Kruskal-Wallis, Mann-Whitney *U*-tests, and Spearman rank correlation tests on most of the data. In addition to the nonparametric tests, we conducted repeated measures ANOVA on arcsine transformed proportions of foraging data and two-way ANOVA on pollen areas for evaluation 1, generation 3. *G*-tests of heterogeneity were performed on crop contents data (Sokal and Rohlf 1981).

Results

The 127 commercial colonies selected for pollen evaluation had an average worker population of 7.48 ± 1.289 (SDs are presented throughout) frames of bees and an average quantity of stored pollen of 1210 ± 482.8 cm^2. From these we selected ten colonies with an average of 2051 ± 472.7 cm^2 and ten colonies with an average of 647 ± 183.5 cm^2 stored pollen to produce our generation 1 high and low strain parents, respectively.

Worker population

The number of workers in colonies did not differ between colonies of the high and low strains for any generation (Table 1). However, population did vary among sublines of low strains in generations 1 and 3, suggesting that it is a selectable trait (Table 2). Population correlated significantly with five other traits that are discussed below (Table 3).

Table 1 Mean values for high and low strain colonies for three generations

Variable	Strain	Generation 1	2	3
Population (frames)	high	5.62[a]	3.37	4.92
	low	5.91	3.22	5.24
Pollen (cm^2)	high	223**	339**	647***
	low	97	79	108
Brood (frames)	high	3.12	2.20	3.00***
	low	3.44	2.43	3.87
Total foragers	high	543	590	907
	low	555	542	950
Pollen foragers	high	146	289	490**
	low	124	228	358
Non-pollen foragers	high	397	301	417**
	low	431	314	592
Proportion pollen foragers	high	0.268*	0.490**	0.548***
	low	0.223	0.424	0.375

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

[a]High and low strains were compared using the Mann-Whitney *U* test

Table 2 Results of Kruskal-Wallis tests for variability among sublines within high and low strains and among all sublines combined

Variable	Strain	Generation 1	2	3
Population	high	NS	NS	NS
	low	*	NS	*
	combined	NS	NS	**
Pollen area	high	NS	NS	*
	low	NS	NS	*
	combined	*	*	***
Brood area	high	NS	NS	NS
	low	NS	NS	*
	combined	NS	NS	**
Total foragers	high	NS	NS	*
	low	NS	NS	NS
	combined	NS	NS	*
Pollen foragers	high	NS	NS	*
	low	NS	NS	NS
	combined	NS	NS	**
Non-pollen foragers	high	NS	NS	*
	low	*	NS	NS
	combined	*	NS	***
Proportion	high	NS	NS	NS
	low	NS	NS	NS
	combined	*	NS	***

NS $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 3 Spearman rank correlations between variables for generation 3

Comparison[a]	Strain		
	High	Low	Combined
Population : pollen area	0.080	−0.459	−0.219
Population : brood area	0.607**	0.815***	0.707***
Population : total foragers	0.642**	0.581**	0.628***
Population : pollen foragers	0.392*	0.199	0.236
Population : nonpollen foragers	0.732***	0.540**	0.652***
Population : proportion pollen foragers	−0.514**	−0.150	0.315*
Brood : pollen area	0.060	−0.640***	−0.522***
Brood : total foragers	0.175	0.432*	0.348*
Brood : nonpollen foragers	0.148	0.206	0.211
Brood : pollen foragers	0.555**	0.212	0.176
Brood : proportion pollen foragers	−0.152	−0.099	−0.372**
Pollen area : total foragers	0.460*	−0.011	0.059
Pollen area : pollen foragers	0.497*	0.189	0.466***
Pollen area : nonpollen foragers	0.452*	0.019	−0.295*
Pollen area : prop. pollen foragers	−0.131	0.160	0.530***
Tot. foragers : prop. pollen foragers	−0.186	0.082	−0.091

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

[a]55 of 57 colonies were evaluated for foraging behavior

Stored pollen

The area of stored pollen varied significantly between strains after a single generation of selection. In generation 1 the high strain colonies contained an average 2.3 times more pollen than low strain colonies (Table 1). The difference increased to 6 times for the fourth evaluation of generation 3. Only the high strain responded to selection after the first generation. Significant variability in pollen stores remained after three generations of selection among sublines within both strains, suggesting that genetic variability still existed within both populations.

High- and low-strain generation 3 colonies did not differ in quantities of stored pollen during their first evaluation. During the first evaluation, all resident adult workers were wild-type while the queen and brood were of the high and low strains. A total of 37 low and 28 high-strain colonies were evaluated. Data met the assumptions of ANOVA and were analyzed using a two-way mixed effects model. Effects of strain (2 levels) were Model I while insemination rounds (2 levels) were analyzed as Model II. Round and interaction effects were not significant ($F=2.91$, $df=1$ and 62 for round effects; $F=1.05$, $df=1$ and 61 for the strain × round interaction). Strain effects also were not significant ($F=3.44$, $df=1,61$; $P>0.05$). Areas of pollen in the high and low strains diverged following the emergence of low and high strain adult workers as observed in the second evaluation (Fig. 2).

Stored pollen correlated significantly with five other variables for generation 3. Pollen area was positively correlated with the numbers of pollen foragers, negatively correlated with nonpollen foragers, and correlated positively with the proportion of foragers that foraged for pollen. This is expected because the numbers of pollen foragers and nonpollen foragers were not independent: selection increased the numbers of pollen foragers in the high strain and decreased them in the low strain, but did not affect total numbers of foragers. In the high strain alone, pollen area correlated positively with pollen foragers, nonpollen foragers, and total foragers, a consequence of the strong positive correlation between population and numbers of foragers.

Brood area

The area of the nest containing brood was significantly smaller in generation 3 high strain colonies. This difference was not manifested until the fourth evaluation period. There were no between-strain or subline differences in brood area in generations 1 and 2.

Population had the strongest correlative relationship with brood area. It correlated positively within both strains and when strains were combined (Table 3). Brood area also correlated negatively with pollen area

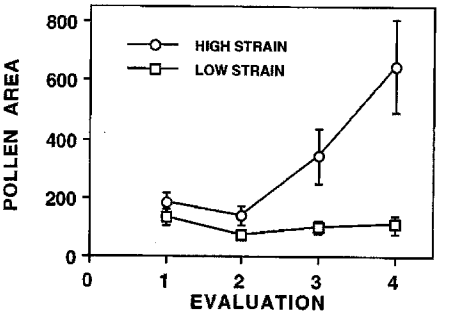

Fig. 2 Area of pollen (cm^2) for high and low strain colonies for the 4 evaluations of generation 3. Means and 95% confidence intervals are presented for 29, 30, 30, and 30 low strain colonies and 23, 27, 27, and 27 high strain colonies for evaluations 1–4 respectively

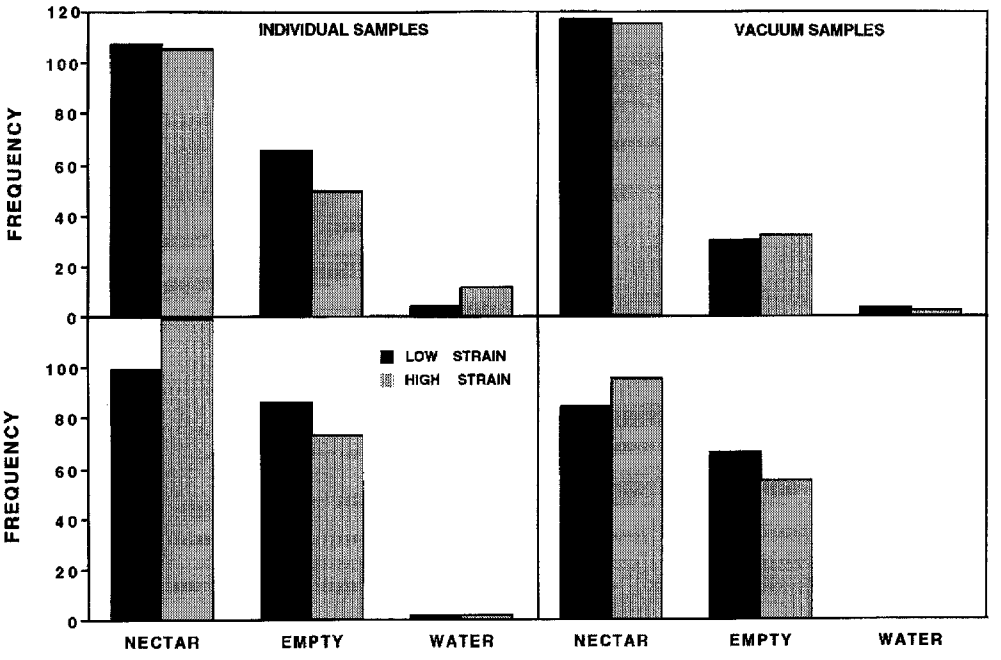

Fig. 3 Frequencies of returning foragers without pollen (*upper*) and with pollen (*lower*) that carried loads of nectar, were empty, or carried water for individually collected samples and vacuum samples

and positively for numbers of foragers for evaluation 4, generation 3. The positive correlation with foragers is confounded with the positive correlation with population which also correlates with numbers of foragers. Within the high strain, brood area correlated positively with numbers of pollen foragers, however, this relationship did not hold in the low strain colonies or when high and low strains were combined. Brood area did correlate significantly with the proportion of pollen foragers, however, the correlation was negative. This negative correlation resulted from combining for analyses high strain colonies with large areas of pollen and small areas of brood, with low strain colonies with small pollen and large brood areas.

Foragers

Generation 3 high strain colonies had 37% more pollen foragers and 16% fewer nonpollen foragers than the low strain. The proportion of pollen collectors in high and low strain colonies varied among observational rounds. Proportions changed from the early to late rounds each observational day. However, high and low strain colonies changed differentially ($X_{high}=0.872$ and 0.769 for observation periods 1 and 2, day 1; $X_{low}=0.661$ and 0.674 for day 1; $X_{high}=0.906$ and 0.844 for periods 1 and 2, respectively, for day 2; $X_{low}=0.580$ and 0.629 for day 2), generating a significant genotype × sample interaction effect ($F=8.66$, $df=3$ and 159, $P<0.0001$). High strain colonies decreased pollen collecting while low strain colonies increased pollen collecting between the early and late morning counts.

The proportion of pollen foragers varied between strains after a single generation of selection. There were no differences between strains for total foragers in any generation. After three generations of selection, the high strain still demonstrated significant between-subline variation for pollen and nonpollen foragers. This was probably a consequence of between-subline variation in total foragers.

Nectar loads

Returning high and low strain pollen and nonpollen foragers, respectively, were equally likely to carry nectar, water, or to be empty. Water foragers were rare (see Fig. 3) and, therefore, were removed from the analyses. For the sample of individually collected bees, 54 and 62% of pollen-collecting foragers of the low and high strains, respectively, returned with nectar loads ($G=2.77$, $1df$, $P>0.05$). Vacuum samples were equivalent ($G=0.23$, $1df$, $P>0.05$, based on totals of high and low strain foragers) with 56 and 63% ($G=1.68$, $1df$, $P>0.05$). Of individually collected low and high strain nonpollen foragers 62 and 68% carried loads of nectar ($G=1.28$, $1df$, $P>0.05$) compared with 80 and 78% for the low and high strain foragers collected with the vacuum device ($G=0.07$, $1df$, $P>0.05$).

Nonpollen foragers were more likely to return with nectar than were pollen foragers ($G=3.83$, $1df$, $P\simeq0.05$; $G=26.42$, $1df$, $P<0.0001$; for individual and vacuum samples). Pollen foragers of the low and high strains, combined, carried nectar 58% and 60% of the time for the individual and vacuum samples, compared with 65% and 79% of the time for the nonpollen foragers. Individual and vacuum samples differed between nonpollen forager samples ($G=15.04$, $1df$, $P<0.0001$). This is not surprising because the different types of samples were taken at different days, under presumably different foraging conditions.

Table 4 Colony-level components of division of labor associated with pollen hoarding[a]

Component	Effect	Selected?	Selectable?	Reference[b]
Queen cues	increase foraging stimulus	no	?	Page & Fondrk, unpublished data
Brood cues	increase foraging stimulus	no	?	Page & Fondrk, unpublished data
Brood quantity	increase foraging stimulus	yes	yes	
Population	increase foragers and pollen intake	no	yes	Page et al. 1993
Total foragers	increase pollen foragers and intake	no	yes	Page et al., unpublished manuscript
Number of pollen foragers	increase pollen intake	yes	yes	
Proportion of pollen foragers	increase pollen intake	yes	yes	Guzmán-Novoa & Gary 1993 Guzman et al. 1994 Calderone & Page 1988, 1991, 1992
Diurnal foraging pattern	increase pollen intake	yes	yes	

[a] These components are not necessarily independent.
[b] Studies that support the evidence in this table.

Discussion

This study identified a total of six selectable components that define interactive pathways on which colony-level selection can potentially act to change colony phenotypes (Table 4). We selected for a single trait, the amount of pollen stored in combs. We measured pollen stores and evaluated colonies during relatively early stages of colony growth and development, either soon after colony initiation, or during early stages of spring colony growth following the winter decline (Nolan 1925). Our end result of high strain colonies containing more stored pollen could have been achieved by several hypothetical, organizational paths containing components that demonstrate genetic variability (these organizational paths are not necessarily independent of each other):

H1: There was more stored pollen in high strain colonies because queens and/or brood produced qualitatively or quantitatively different cues

We did not find evidence for queen or brood effects. Differences in pollen areas between colonies with high and low-strain queens and brood did not vary significantly for the first evaluation of generation 3. All pollen was collected and stored during this period by wild-type workers, therefore, the first evaluation tested the effects of the queen and her brood independent of low and high strain workers. Subsequent studies (R.E. Page and M.K. Fondrk unpublished data) have also failed to demonstrate this effect when new colonies were initiated with wild type workers and mature high and low strain queens that had been ovipositing for several months (see also Calderone and Page 1992).

H2: There was more stored pollen in high strain colonies because they had more brood and, therefore, a greater stimulus for pollen

This hypothesis cannot explain the results because in generation 3 the high strain colonies had significantly smaller quantities of brood.

H3: There was more stored pollen in high strain colonies because they raised less brood and, therefore, consumed less pollen

High strain colonies did have less brood after three generations of selection. However, this only occurred during the fourth evaluation and probably represents a "developmental constraint" imposed on colonies by the size of the nest cavity. Pollen is stored in the central areas of the nest surrounding the brood. As the colonies grew in worker population and occupied larger volumes of the nest cavity, they used greater areas of wax comb for stored pollen and constricted the space that was available for brood rearing.

We tested generation 4 colonies under commercial conditions where they were housed in two-story (84-l) hives (R.E. Page and M.K. Fondrk, unpublished data), containing twice the volume of the hives in generations 1–3. Under these conditions, high and low strain colonies contained different quantities of pollen but equal quantities of brood, supporting our argument that reduced brood rearing is a consequence of a space constraint. Volumes and comb areas of natural nests are probably closer to those obtained in our 42-l single-story hives of generation 3 (Seeley and Morse 1976), suggesting that space imposes constraints on developing, natural colonies.

H4: There was more stored pollen in high strain colonies because they had larger worker populations

This hypothesis is not supported by our results. Sublines varied with respect to colony population (Table 2) demonstrating genetic variability for the trait, but selection did not result in measurable differences between colonies of the high and low strains. In addition, there was no significant correlation between population and stored pollen. We reject H4.

H5: There was more stored pollen in high strain colonies because they had more foragers and, therefore, more pollen foragers

We found differences among sublines in the relative numbers of foragers, demonstrating genetic variability for the trait. However, we reject H5 because high and low strain colonies did not differ from each other. Even though genetic variability existed for the trait, selection did not measurably segregate the variability into the high and low strains. In addition, pollen area was not significantly correlated with total number of foragers when data from low and high strains were combined.

H6: There was more stored pollen in high strain colonies because they had a higher proportion of their foragers collecting pollen

The total number of foragers did not vary between high and low strain colonies. However, the high strain colonies had significantly more pollen foragers and low strain colonies had significantly more nonpollen foragers. This was a consequence of colonies having a "fixed" forager population and a constraint on individual foragers: they either foraged for pollen or they did not. Selection for increased pollen stores increased pollen collecting in high strain colonies while it concurrently decreased nectar foragers, resulting in an increase in the proportion of pollen foragers. The reciprocal relationships occurred in low strain colonies. These results were consistent with those of Calderone and Page (1988, 1991, 1992) using high and low strain workers derived from Hellmich et al. (1985).

The underlying mechanisms that led to an increase in the proportion of pollen collectors could involve variable foraging success of individuals, or variability among workers of different strains to perceive or respond to levels of stimuli that induce pollen foraging. High and low strain pollen and nonpollen foragers did not differ in nectar foraging success (as measured by the proportion of foragers returning with nectar loads), therefore, differential foraging success is not a plausible explanation. The response of workers to pollen and nectar foraging stimuli is an individual trait for which genotypic variability already has been demonstrated. Calderone and Page (1992) showed that cofostered high and low strain workers responded differently to changes in foraging stimuli. Fewell and Page (1993) showed differences in pollen and nectar collecting behavior among workers derived from three different genetic sources. Workers emerged in an incubator and were cofostered in a colony containing wild-type workers. The colony was maintained in a cage and was offered fixed, controlled foraging resources. Changes in quantities of stored pollen and brood resulted in changes in individual choices to forage for pollen and nectar. Workers from the different sources varied in their behavioral plasticity as measured by changes in foraging behavior. Likewise, changes in sugar concentration at artificial nectar feeders and changes in pollen quality at artificial pollen feeders resulted in differential changes in foraging behavior.

Phenotypic variance and naturally mated queens

A significant proportion of the observed phenotypic variance among colonies containing naturally mated queens is a consequence of differences in genotypic composition of the colonies. We selected our high and low strain parents for the first generation of workers from commercial colonies with naturally mated queens that presumably had mated with a large number of males (see Page 1986 for review). In generation 1 we had significantly separated the means of the two strains, a consequence of high heritability (here defined in the colony-level sense of Hellmich et al. 1985) of pollen hoarding among the commercial colonies.

Pollen and nectar intake covary

Selection for the amount of pollen stored in the nest affected the nectar intake of colonies. Pollen and nectar collecting covary at the colony level. Selection for more stored pollen resulted in more pollen and fewer nonpollen collectors. Pollen collectors returned with nectar loads less frequently than did nonpollen collectors (see also Calderone and Page 1992). Additional studies have also shown (R.E. Page and M.K. Fondrk, unpublished data) that nectar loads are smaller for pollen collectors. This lack of independence of pollen and nectar intake should be considered in foraging models and empirical studies.

It is also possible that differences in nectar collecting occurred between strains due to chance alone. This could occur if nectar collecting is a heritable trait and we selected, by chance, colonies that had high stores of pollen and low individual nectar collecting behavior for the high strain, and colonies with low pollen stores and high nectar collecting individual workers for the low strain. Low and high strain colonies varied significantly in the proportion of pollen foragers in all generations, including generation 1. Generation 1 con-

sisted of crossing queens and drones derived from 20 different colonies with naturally mated queens. The large number of parent colonies initially selected to constitute the strains, and the rapid, increasing change in proportion of pollen and nonpollen foragers between strains, makes this an unlikely explanation. In addition, these results are consistent with those of Calderone and Page (1988, 1991, 1992) based on strains derived from the independent selection program of Hellmich et al. (1985).

Constraints on selection

Constraints could exist among component pathways. The components that changed may have had the most additive genetic variance and/or been those that were least constrained by genetic and phenotypic correlations with other traits. For example, Page et al. (1992) showed a negative correlation between the construction of wax comb containing drone and worker sized cells by newly founded honey bee colonies. Colonies derived from different drone lineages did not vary in the total amount of comb constructed during a 3-week interval, but did vary in their allocation of wax for constructing drone and worker comb. In that case, they were constrained by total comb area; selection to increase one type of comb would necessarily decrease the other.

High pollen hoarding strain colonies had significantly larger numbers of pollen collectors, fewer nectar collectors, but did not vary with respect to total foragers, demonstrating a constraint on nectar foragers based on the total numbers of foragers. Total foragers, however, did vary among sublines, but not between strains, suggesting it is under separate genetic control (no genetic constraint) from pollen and nectar collecting. Selection, however, did not result in more foragers, which would have resulted in more pollen and nectar foragers.

Conclusions

High and low strain populations responded rapidly to colony-level selection demonstrating that significant levels of genetic variability existed in the initial commercial population from which our strains were derived. What maintains this variability under the conditions of commercial colony selection? Do the same conditions apply to feral, noncommercial colonies? These are important questions for determining how results from studying commercially derived honey bees can be applied to understanding evolutionary processes under natural conditions. Hypotheses have been proposed for the adaptive significance of genetic variability for social insects (see Crozier and Page 1985; Sherman et al. 1988; Kolmes et al. 1989; Oldroyd et al. 1992). This paper does not directly address those issues, though they remain important.

Genetic variability is essential for evolution by natural selection regardless of the adaptive significance, if any, of within-colony genotypic variability. It is likely that commercial and feral populations vary only in degree of genetic variation, not in kind. Therefore, demonstrations of colony-level selection on commercial populations provide us with "probes" into the kinds of colony and individual level traits that exist, vary, and are subject to selection. They also clarify integrative relationships between social organizational components that build colony-level traits from individual organisms and should be applicable to social insects in general. To truly understand the evolution of the structure of insect societies we must also know the phenotypic components of individual workers. We must examine the relationships between genotypes, individuals, and colony phenotypes. In this paper, we have dealt with colony-level organizational components. In subsequent papers we will examine the mechanisms of inheritance underlying individual and colony phenotypes.

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