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Nutritional status influences socially regulated foraging ontogeny in honey bees

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Summary

In many social insects, including honey bees, worker energy reserve levels are correlated with task performance in the colony. Honey bee nest workers have abundant stored lipid and protein while foragers are depleted of these reserves; this depletion precedes the shift from nest work to foraging. The first objective of this study was to test the hypothesis that lipid depletion has a causal effect on the age at onset of foraging in honey bees (*Apis mellifera* L.). We found that bees treated with a fatty acid synthesis inhibitor (TOFA) were more likely to forage precociously. The second objective of this study was to determine whether there is a relationship between social interactions, nutritional state and behavioral maturation. Since older bees are known to inhibit the development of young bees into foragers, we asked whether this effect is mediated nutritionally *via* the passage of food from old to young bees. We found that bees reared in social isolation have low lipid stores, but social inhibition occurs in colonies in the field, whether young bees are starved or fed. These results indicate that although social interactions affect the nutritional status of young bees, social and nutritional factors act independently to influence age at onset of foraging. Our findings suggest that mechanisms linking internal nutritional physiology to foraging in solitary insects have been co-opted to regulate altruistic foraging in a social context.

Key words: *Apis mellifera*, division of labor, foraging, honey bee, lipid, nutrition, social inhibition.

Introduction

An animal's 'decision' to seek and consume food involves the integration of signals related to internal nutritional status and external environmental cues. Many signaling pathways are known in vertebrates that communicate nutritional information from the gut and adipose tissue to the brain (Saper et al., 2002). Cues from the environment can also have a strong impact on foraging behavior (Carlson, 2000), one of the most important being social conditions (Ben-David et al., 2004; Harwood et al., 2003; Whiteman and Cote, 2004). However, there are few studies investigating how internal nutritional and social cues interact to influence foraging behavior for animals living in complex social groups (Ben-David et al., 2004).

In insect societies, the behavior of individual colony members has been selected to promote colony growth and reproduction (Wilson, 1971). Most worker behaviors, including foraging, benefit the colony and can be considered altruistic. Not only is foraging an activity that carries a high risk of individual mortality (Jeanne, 1986), but food collected by foragers is mostly given to the colony. For example, honey bee pollen foragers deposit pollen loads directly into the comb for storage, and nectar foragers regurgitate their loads to receiver bees that pass the nectar to other bees or place it in the comb for storage (Winston, 1987). By and large, an individual social insect forager does not seek food in the environment for individual sustenance, but instead contributes the food it collects to colony energy reserves. It is thus not surprising that there are strong social influences on the initiation of foraging in social insects (Robinson, 2002). Nonetheless, it is also possible that social insect workers are induced to forage in response to signals of their own nutritional physiology, as in solitary animals. If so, this would suggest that mechanisms that activate foraging in solitary ancestors of social insect species were employed during social evolution to sculpt a more altruistic form of foraging.

In colonies of most social insect species, young workers engage in nest work and old workers perform foraging. For example, honey bees typically perform tasks inside the nest such as brood care ('nursing') during the first 2–3 weeks of adult life and then become foragers. Despite this well-established pattern, the age at which specific tasks are performed is extremely flexible, as bees are able to accelerate, delay or reverse their pattern of behavioral development (Robinson, 1992). The transition to foraging in honey bees is accompanied by changes in diet (Crailsheim et al., 1992), reduced lipid stores (Toth and Robinson, 2005), and reduced blood proteins (Crailsheim, 1986) including the lipoprotein vitellogenin (Fluri et al., 1982). The correlation between reduced internal nutrient stores and foraging has been observed

in numerous species of ants, bees and wasps (Blanchard et al., 2000; Toth and Robinson, 2005).

Recent work suggests the association between nutrition and foraging may go beyond correlation. Schulz et al. (1998) found that starvation of honey bee colonies causes precocious foraging, although effects on individual nutritional physiology were not examined. Schulz et al. (1998) ruled out the possibility that foraging behavior was activated by the presence of empty food comb, and instead implied that individual nutritional state may be a more important factor. Toth and Robinson (2005) found that abdominal lipid stores in honey bees decline prior to the onset of foraging, suggesting this change in nutritional status itself could activate foraging. These results suggest that despite the fact that foraging is altruistic and socially regulated in honey bees, individual nutritional physiology may also play a role. The first goal of our study was to test the hypothesis that lipid depletion in honey bees leads to early initiation of foraging behavior.

A better understanding of how social and nutritional factors interact is essential to developing a more complete picture of how the regulation of altruistic foraging behavior occurs in animal societies. In a honey bee colony, young bees are inhibited from becoming foragers by direct contact with older bees (Huang and Robinson, 1992, 1996, 1999; Leoncini et al., 2004a,b). Recently, a pheromone mediating this process was chemically identified (Leoncini et al., 2004b), but this does not rule out the possibility that social inhibition could also involve a nutritional component. As suggested by Blanchard et al. (2000), it is possible that food transferred from older bees *via* trophallaxis (exchange of regurgitated liquid food) could lead to enhanced nutrient reserves in young bees, delaying the onset of foraging. Therefore, the second goal of our study was to explore how social interactions might interact with nutritional factors to influence foraging ontogeny in honey bees.

Materials and methods

Bees

General methods

Bees were obtained from colonies maintained with standard beekeeping practices at the University of Illinois Bee Research Facility in Urbana, Illinois, USA. Colonies consisted of a typical North American mix of *Apis mellifera* L. European subspecies. One-day-old bees were obtained by collecting frames of emerging brood from typical field colonies, placing them in a 34°C incubator overnight, and collecting all newly emerged bees the following day. Three field experiments (designated Experiments 1–3) were performed in August, 2002 (Experiment 3) and June–September, 2003 (Experiments 1 and 2).

Measurements of abdominal lipid stores

Methods to analyze lipid stores were as in Toth and Robinson (2005). Bee abdomens were detached and all internal organs removed, leaving the cuticle with adhered fat body tissue. Each sample was then individually homogenized in a glass tissue grinder in 2:1 chloroform:methanol using the Folch extraction method (Perkins, 1975), and allowed to extract overnight. The samples were filtered through glass wool and adjusted to a constant volume of 2 ml.

Lipid quantification in sample extracts was accomplished using colorimetric assays. Samples from Experiment 3 were examined using a 0.25% potassium dichromate in 85% sulfuric acid as described previously (Toth and Robinson, 2005). An improvement on this technique using 0.6% vanillin in 85% phosphoric acid (Van Handel, 1985) helped reduce variance between the samples, and this method was used for samples from Experiments 1 and 2. A 100 μl sub-sample of each lipid extract was dried completely, 0.2 ml concentrated sulfuric acid was added, and samples were heated in boiling water for 10 min. Then 2.0 ml vanillin reagent was added to each sample, which was vortexed and dark-incubated for 15 min to allow pink color formation. Absorbance at 525 nm was measured for each sample using a Molecular Devices Spectra Max 190 multi-well spectrophotometer (Sunnyville, CA, USA). A standard curve using known amounts of pure cholesterol was used to calculate lipid amounts. Each lipid sample was analyzed twice in independent assays and average values were used for subsequent analysis.

Effect of TOFA on fatty acid synthesis

To experimentally reduce lipid stores, 1-day-old honey bees were fed a diet containing TOFA (5-tetradecyloxy-2-furanocarboxylic acid, kindly provided by Merck, Inc., Whitehouse Station, NJ, USA). TOFA interferes with acetyl coA carboxylase, the enzyme catalyzing the rate-limiting step in fatty acid synthesis (Halvorson and McCune, 1984; McCune and Harris, 1979). TOFA has previously been shown to interfere with lipid deposition in another insect species (Popham and Chippendale, 1996), but specific effects on fatty acid biosynthesis have been shown only for vertebrates (Parker et al., 1977). 1% TOFA in sucrose solution was determined to be a lethal dose for honey bees (data not shown), so we tested a dose of 0.1% TOFA. One hundred 1-day-old bees were collected, placed in plastic cages, and fed either 50% sucrose, or 50% sucrose with 0.1% TOFA. Food was replaced daily and the amount of sucrose eaten recorded to determine whether TOFA affected food consumption. Cages were kept in a 34°C incubator. Four separate trials were performed, and results was pooled from all trials. When bees were 5 days old, a subset (N=5) of both groups (with and without TOFA treatment) was removed from cages, anaesthetized on ice, and injected with 20 μl 13.5% [^{14}C]acetate (1.4 GBq mmol^{-1}) in bee saline, which amounted to an estimated activity of 6×10^5 d.p.m. per bee. Bees were replaced in the 34°C incubator in cages with sucrose and pollen for 5 h to allow incorporation of ^{14}C *via* fatty acid synthesis. They were then freeze-killed on dry ice and lipid stores extracted in 2:1 chloroform:methanol as described above. The solvent was allowed to evaporate overnight, 5 ml of Fisher ScintiVerse (Fair Lawn, NJ, USA) scintillation fluid was added to each sample, and the direct c.p.m. of each

sample measured using a Packard Tri-carb 2100TR Liquid Scintillation Analyzer (Ramsey, MN, USA).

Experiment 1: Effects of dietary manipulations on lipid stores and behavioral maturation

To test whether experimental lipid depletion can accelerate foraging ontogeny in honey bees, we performed an experiment in which we manipulated lipid stores with treatments combining a lipid-free diet and TOFA. Three trials were performed. A dose of 0.1% TOFA suspended in a diluted honey solution (5:1 honey:water) was used for all field experiments, because this dose was shown to effectively reduce the rate of fatty acid synthesis in the laboratory (see Results). Treatments were administered to separate experimental 'single-cohort colonies'. Each single-cohort colony contained a mated, caged queen, 1200 paint-marked 1-day-old bees, one empty frame of honeycomb, and one honeycomb frame in which dietary treatments were added (and replenished daily). Because the queen was caged no brood was produced, which eliminated any potential differences in brood production and brood pheromone between treatments. Some bees initiate foraging in single-cohort colonies when they are about 1 week old, about 2 weeks earlier than usual, due to the lack of an existing foraging force (Leoncini et al., 2004a). Single-cohort colonies thus provide an efficient method for measuring the effect of various colony manipulations on age at onset of foraging and are used extensively for this purpose (Ben-Shahar et al., 2002; Schulz and Robinson, 2001; Sullivan et al., 2000). The same mix of genotypic backgrounds was used for all single-cohort colonies within each trial.

The following four treatments were used: (a) honey solution without TOFA and an excess of pollen (typical diet), (b) honey solution without TOFA and no pollen (pollen-deprived), (c) honey solution containing TOFA and no pollen (TOFA), (d) honey solution containing TOFA and an excess of pollen (TOFA+pollen). Because pollen is the only external source of dietary lipid, bees in pollen-deprived colonies had a lipid-free diet and could only accumulate lipids by *de novo* synthesis from dietary carbohydrates. Bees in TOFA-treated colonies were predicted to have lower rates of *de novo* lipid synthesis, and therefore reduced lipid stores. Bees in the colonies fed TOFA+pollen, however, could accumulate lipid stores directly from dietary lipids, and were therefore predicted to have partially reduced or normal lipid stores. The TOFA+pollen group was included only in Trial 3. In each trial, a subset of bees from each colony was collected on day 4–5 (prior to the onset of foraging) for lipid analysis. Lipid measurements thus reflect the young bees' lipid stores after dietary treatments, but before foraging began.

Observations were conducted to determine the proportion of bees from each colony that foraged precociously. All colonies within each trial were monitored simultaneously by two observers for 3–4 days (when bees were 5–9 days of age), for 1 h in the morning and 1 h in the afternoon or early evening. Foragers were identified as bees returning to the hive entrance with pollen loads in their corbiculae or with abdomens distended, most likely with nectar (Huang et al., 1994). Foragers were paint-marked on the abdomen (Testor's enamel paint) so that individuals were only counted once. After foraging observations were completed, we calculated the proportion foraging from each treatment group based on the cumulative number observed foraging and the number present at the end of the experiment (Schulz and Robinson, 2001).

Table 1. *Design of Experiment 2*

Social manipulation \ Nutritional treatment	Starved	Fed
Transplant of foragers (SI+)	Starved/SI+ *Unknown*	Fed/SI+ *Social inhibition effect: few foragers* (Huang and Robinson 1992)
Transplant of young bees (SI–)	Starved/SI– *Starvation effect: many foragers* (Schulz et al. 1998)	Fed/SI– *Control: typical number of foragers* (Huang and Robinson 1992, Schulz et al. 1998)

Four colonies were used for each trial and subjected to a combination of treatments: a nutritional treatment (starved or fed) and a social manipulation (SI+, a transplant of foragers which results in social inhibition, or SI–, a transplant of young bees).

Previous studies (Huang and Robinson 1992; Schulz et al., 1998) enable predictions for three of the four combinations: starved/SI–, fed/SI– and fed/SI+. The starved/SI+ group allowed us to determine whether social inhibition operates under starved conditions.

Experiment 2: Effects of nutrition and social inhibition on behavioral maturation

We determined whether the previously established process of social inhibition of foraging depends on nutritional factors. Each trial (of four trials in total) involved four single-cohort colonies consisting of 1000–1200 1-day-old bees. Each colony was given a combination of two treatments (Table 1), both used individually in earlier studies, but never before combined. The nutritional treatment – colony starvation– has been shown to cause precocious foraging (Schulz et al., 1998). The social inhibition treatment (SI) consisted of transplanting a group of foragers into a hive of young bees; this has been shown to inhibit precocious foraging (Huang and Robinson, 1992). The four combined treatments were (1) starved SI+, (2) starved SI–, (3) fed SI+ and (4) fed SI–. Since trophallaxis is already implicated in the process of social inhibition in connection with exchange of an inhibitor pheromone (Leoncini et al., 2004b), this experiment focused specifically on whether nutritional changes that occur as a result of food exchange are necessary for social inhibition. We hypothesized that if foragers inhibit behavioral development *via* a nutritional mechanism involving passage of food during trophallaxis, we would observe no effect of social inhibition under starved conditions. Conversely, if social inhibition can inhibit precocious foraging in starved young bees, this would suggest that this form of social inhibition does not involve nutritional influences.

For the nutritional manipulation, each colony was either fed

(given full access to an excess of pollen and honey) or starved (given 50 ml honey in a honeycomb frame for 3 days, after which any remaining food was removed and replaced with an empty honeycomb frame). The social inhibition manipulation involved adding either foragers (forager transplant) or 1-day-old bees (control transplant) on day 2–3 of the experiment. Returning pollen and nectar foragers were collected from another field colony, chilled so they could be tagged (see below), and then added to each SI+ colony. Because of the high mortality of transplanted foragers, dead foragers were removed at night and replaced the following day to maintain a high and constant level of social inhibition (a minimum of 200 live foragers). Fewer control transplant bees were added (approximately 200 total, no replacement) due to the substantially lower mortality of this group of bees.

Transplanted bees were confined to their hive to prevent them from foraging, bringing food back to the hive, and negating the starvation treatment. This was accomplished using the 'big-back' technique (Withers et al., 1995). A plastic tag was glued to the dorsal surface of the thorax of each transplanted bee (for both forager and control), and a screen with openings just big enough for untagged workers to fit through was placed over the hive entrance. Each colony was provided with a Bee Boost^{TM} queen pheromone strip (Phero Tech, Delta, BC, Canada) instead of a real queen. A strip was used to prevent queen mortality, sometimes seen in starved colonies (A.L.T., personal observation), and to prevent brood production in the colonies. This ensured that our results would not be affected by brood cannibalism (Weiss, 1984) or influences of brood on food consumption (Hrassnigg and Crailsheim, 1998).

Foraging observations were made when focal bees were 5–8 days old, and the proportion of bees foraging from each group was determined as described above (Experiment 1). In Trial 1, a subset of bees from each colony was collected on day 5 (prior to the onset of foraging) for lipid analysis. These measurements were made to ensure the social inhibition treatment had no unforeseen effect on lipid stores.

Upon introduction, transplanted bees may have carried small amounts of food in their crops that could have been shared with starved colony members. This was unlikely to have affected the starvation treatment, for two reasons. First, they were added on day 2–3 of the experiment, at which point starved bees still had access to the honey added at the beginning of the experiment. Second, the amount of sugar carried in the crops of transplanted foragers was unlikely to have been large enough to affect the starvation treatment. Returning nectar foragers have an average of 4 mg sugar per load (Fewell and Winston, 1996). Thus, 200 foragers would have supplied a maximum of 1 g sugar, which is only enough to sustain 1000 bees for a few hours (A.L.T., personal observation).

For logistical reasons, control transplanted bees were added on day 2–3 of the experiment and were 1 day younger than focal bees. Adding 1-day-old bees to a group of slightly older bees (differing in age by only a few days) is known to lead to a slight increase in foraging by the 'older' cohort (Jassim et al., 2000). However, this effect has been observed only when the younger cohort makes up a substantial proportion of the colony (50–97%) and differs in age by several days (Jassim et al., 2000; Page et al., 1992). In our control transplant colonies, we added a much smaller cohort of younger bees (10–20% of the colony) and they were only 1 day younger than focal bees.

Experiment 3: Effects of social isolation on lipid stores and behavioral maturation

To further explore the relationship between social interactions and nutrition, we investigated whether social contact with other bees is necessary for the accumulation of normal fat stores in young adult bees. Bees reared in social isolation (caged to prevent all physical contact, including antennation and food exchange) are known to exhibit precocious foraging (Huang and Robinson, 1992). If social contact is important for the accumulation of lipid stores, it is possible that the early onset of foraging previously observed in isolated bees is partially attributable to nutritional depletion resulting from the isolation.

We tested whether bees reared in social isolation have reduced lipid stores relative to control bees reared in typical colonies. We collected 1-day-old bees and paint-marked each on the thorax, according to treatment group (isolated or colony-reared). We then placed each isolated bee ($N\approx250$) in an individual wooden cage (with one side screened) with bee candy (powdered sugar and honey mixture) only (Trial 1), or bee candy and pollen (Trial 2). Bees were given bee candy only in Trial 1 to mimic the experimental protocol of earlier studies (Huang and Robinson, 1992). Obtaining the same result as Huang and Robinson (1992) in Trial 1 (see Fig. 5), pollen was added in Trial 2 in order to rule out additive effects of pollen depletion during isolation on lipid and foraging measures. We placed the cages containing individual workers in a wooden frame modified to hold them, placed the frame in a screened frame box, and then placed the frame box in a typical colony (Huang et al., 1998). The screened frame box prevented bees in the host colony from having direct interactions with isolated bees, but did expose isolated bees to typical colony odors and hive temperature conditions. Isolated bees were given fresh water daily and checked for mortality; survival was 67.1% (167/249 in Trial 1) and 95.2% (240/252 in Trial 2). Control bees were 1-day-old bees collected on the same day and from the same mix of source colonies used for the isolated bees. One thousand paint-marked control bees were introduced to the same typical field colony used to house the isolated bees, and allowed to move about freely.

We collected isolated and colony-reared control bees after 7 days, following the methods of Huang and Robinson (1992). A sub-sample of 30 bees from each group was freeze-killed and stored in a –80°C freezer for lipid analysis. An equal number of the remaining colony and isolated bees (137 in Trial 1, 172 in Trial 2) were placed in a single-cohort colony that consisted of a naturally mated queen, one honey and one pollen frame, and 1000 additional unmarked 1-day-old bees. Experiments 1 and 2 involved colony nutritional manipulations, so caged

queens (Experiment 1) or queen pheromone strips (Experiment 2) were used. We were able to use an uncaged queen in Experiment 3 because both focal groups were placed in the same colony. In addition, no effect on brood production was expected as this colony was under a normal feeding regime. Foraging observations were conducted as described in Experiment 1, but extended to 5 days. This was to account for the presence of fewer focal bees in each colony resulting from this experimental design (Ben-Shahar et al., 2002).

Statistical analyses

All statistical analyses were performed using the SAS program (SAS Institute 2000). Results of the effects of isolation on lipid stores were analyzed using two-tailed, unpaired *t*-tests assuming equal variance. Results of the fatty acid synthesis assay were pooled for all trials and analyzed using a mixed model analysis of variance (ANOVA) to account for the effect of trial (SAS PROC MIXED). Analyses of lipid levels for each trial of Experiments 1, 2, and 3 were conducted using ANOVA (SAS PROC GLM). All lipid measurements were log-transformed to normalize the data, and extreme outliers were removed on the basis of Studentized residual values. Analyses of age at onset of foraging for each trial were conducted using χ^2 tests (SAS PROC FREQ). For experiment-wide analyses of all trials, a mixed model ANOVA was used with trial as the random term (SAS PROC MIXED). *Post-hoc* comparisons were adjusted for multiple comparisons using a Tukey's adjustment.

Results

Effect of TOFA on fatty acid synthesis

A pooled ANOVA with four trials showed that the incorporation of radioactive acetate into lipid stores was significantly lower in TOFA-fed (14312±1641.8 c.p.m.) than control bees (20586.7±2017.6 c.p.m.; mean ± S.E.M., ANOVA: $F_{1,5.06}$=12.6, P=0.016). Although there was a significant difference between trials (ANOVA: $F_{1,3}$=18.97, P=0.019), there was no trial×TOFA interaction ($F_{1,50}$=0.4, P=0.75). Because there was no interaction and the effect of TOFA was consistent across trials, we report pooled results (Jenkins, 2002). For these same 4 trials, a pooled ANOVA also revealed there was no difference in daily consumption of sucrose solution by bees in cages fed with TOFA (0.64±0.08 ml) and without TOFA (0.61±0.06 ml, ANOVA: $F_{1,3}$=0.19, P=0.69). This assay verified that TOFA feeding treatment reduces rates of fatty acid synthesis in an insect, which had only been shown previously in vertebrates (Parker et al., 1977).

Experiment 1: Effects of dietary manipulations on lipid stores and behavioral maturation

In all three trials, there was a significant effect of dietary treatment on abdominal lipid amounts (ANOVA, Trial 1: $F_{2,37}$=6.92, P=0.003; Trial 2: $F_{2,42}$=5.63, P=0.007; Trial 3: $F_{3,56}$=4.72, P=0.005; Fig. 1A). A pooled analysis of all three trials showed the same result (ANOVA, $F_{3,140}$=12.06, P<0.0001; Fig. 1B). *Post-hoc* tests showed that lipid levels differed significantly between all treatments: bees fed a typical diet had the highest lipid levels, bees deprived of pollen were intermediate, and bees fed TOFA (without pollen) had the lowest. Lipid levels for bees fed a typical diet were similar to those typically found in nurses, and those of TOFA-treated bees were similar to those typically found in foragers (Toth and Robinson, 2005).

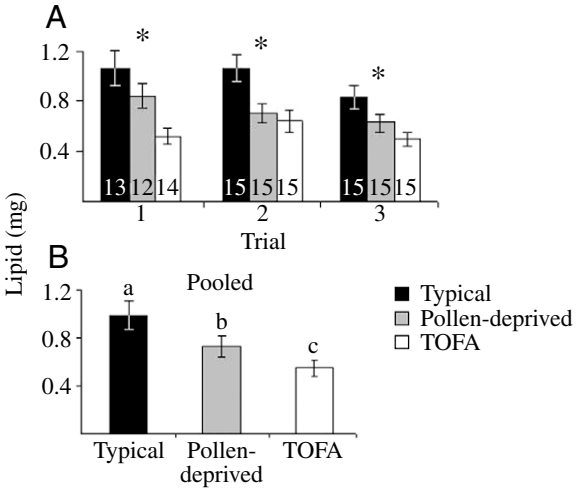

Fig. 1. Effect of dietary manipulation (Experiment 1) on abdominal lipid amounts in 5-day-old bees collected prior to colony onset of foraging. Bees in single-cohort-colonies were fed either a typical diet (honey and pollen; black bars), a pollen-deprived diet (honey only; grey bars), or a diet containing TOFA and honey (no pollen; white bars). (A) Trial by trial results. Values are means ± S.E.M.; numbers within bars represent the number of bees analyzed per group. *P<0.05, ANOVA within trials. (B) Pooled results for three trials (overall ANOVA, P<0.0001); groups that differed significantly are designated with different letters (a,b,c).

In the one trial in which there was also a TOFA+pollen group (Trial 3), these bees had lipid levels that were not significantly different from bees fed a typical diet (0.834±0.104 mg, Tukey-adjusted *post-hoc* test, P=0.86). Although the TOFA+pollen treatment was tested in just one trial, these results are consistent with the prediction that TOFA depletes lipid stores only when bees have no access to dietary sources of fat.

In all three trials, the proportion of bees foraging differed significantly between the treatment groups (χ^2 test; Trial 1: χ^2_2=94.23, P<0.0001; Trial 2: χ^2_2=90.34, P<0.0001; Trial 3: χ^2_3=80.46, P<0.0001; Fig. 2A). In a pooled analysis, there was also a significant effect of dietary treatment on the proportion of bees foraging (ANOVA, $F_{2,4}$=34.66, P=0.003; Fig. 2B). *Post-hoc* contrasts showed that the colonies fed TOFA had a significantly higher proportion of foragers than bees fed typical or pollen-deprived diets, (these latter two groups did not differ). In Trial 3, approximately 4.5% (33 out of 731) of bees in the TOFA+pollen group foraged (data not shown), a value which is similar to the mean percent foraging for bees fed a typical diet (mean across three trials=4.8%). The results of Trial 3 suggest that the TOFA+pollen treatment had no effect

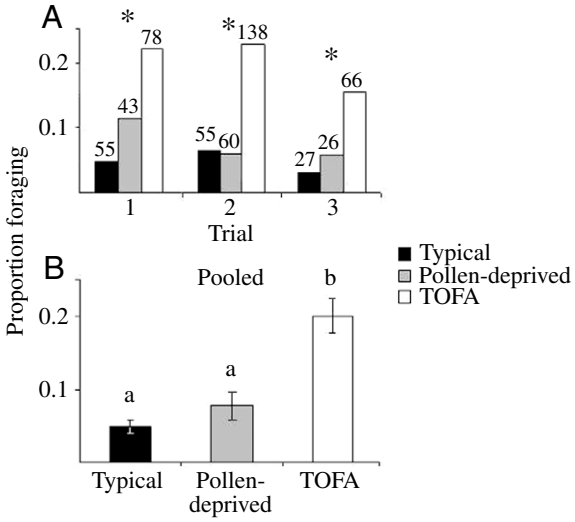

Fig. 2. Effect of dietary manipulation (Experiment 1) on the development of foraging behavior. (A) Trial by trial results. Numbers above bars represent the number of bees observed foraging per group. *P<0.05, χ^2 tests within trials. (B) Pooled results for three trials. Values are means ± S.E.M.; overall ANOVA, P<0.0001; groups that differed significantly are designated with different letters (a,b). Dietary manipulations as in Fig. 1.

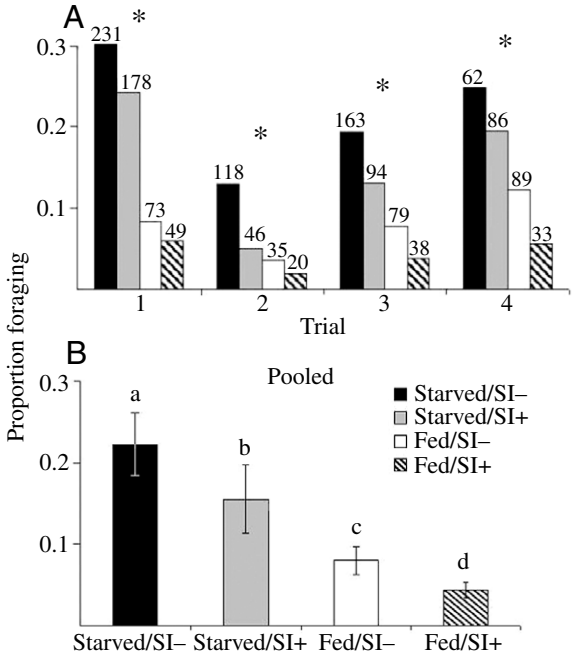

Fig. 3. Effect of nutrition and social inhibition (Experiment 2) on the development of foraging behavior. Bees were placed in single-cohort colonies under a combination of starved/fed and social inhibition/no inhibition treatments. (A) Trial by trial results; numbers above bars represent number of bees observed foraging per group. (B) Pooled results; groups that differed significantly are designated with different letters (a,b). Values are means ± S.E.M.; overall ANOVA: starvation, P<0.001; social inhibition, P<0.001; starvation×social inhibition (SI), not significant. SI+/–, with/without social inhibition treatment (forager transplant).

on foraging behavior, consistent with the lipid results. It is therefore unlikely that the behavioral effects of TOFA were due to non-target side effects.

Experiment 2: Effects of nutrition and social inhibition on behavioral maturation

In all four trials, the proportion of bees foraging differed significantly among the four treatment groups shown in Table 1 (χ^2 tests, Trial 1: χ^2_3=255.58, P<0.0001; Trial 2: χ^2_3=125.62, P<0.0001; Trial 3: χ^2_3=134.23, P<0.0001; Trial 4: χ^2_3=57.14, P<0.0001; Fig. 3A). A pooled analysis also showed significant effects of starvation (ANOVA: $F_{1,9}$=154.9, P<0.0001) and social inhibition ($F_{1,9}$=33.76, P= 0.0003), but no significant starvation×social inhibition interaction ($F_{1,9}$=0.64, P=0.4439; Fig. 3B). *Post-hoc* analyses showed that bees in the starved/SI– group showed the highest amount of foraging, followed by starved/SI+ bees, then fed/SI– bees and then fed/SI+ bees.

Consistent with the behavioral results, there was no influence of social inhibition on lipid stores (Trial 1, ANOVA: $F_{1,56}$=1.81, P=0.19, data not shown). Bees in the starved treatment groups had lower lipid stores than those in the fed groups (ANOVA: $F_{1,56}$=18.37, P<0.0001), but there was no significant starvation×social inhibition interaction (ANOVA: $F_{1,56}$=0.04, P=0.83). This confirmed that the social inhibition treatment had no effect on young bees' lipid stores, and also showed that starvation treatment did result in reduced lipid stores.

Experiment 3: Effects of social isolation on lipid stores and behavioral maturation

Lipid stores were significantly lower in isolated bees as compared to colony-reared bees in both trials (unpaired two-tailed t-tests: Trial 1: t_{18}=4.59, P=0.0002; Trial 2: t_{36}=5.63, P=0.023; Fig. 4). Similar results were obtained with isolated bees given either bee candy only (Trial 1) or bee candy and pollen (Trial 2), suggesting that isolation results in reduced lipid stores even if bees have a full complement of food during isolation. Lipid results from this experiment, although from 7-day-old bees (compared to 5-day-old bees in Experiments 1 and 2), are comparable to those of the other experiments; e.g. lipid levels for both the typical group of Experiment 1 and the colony-reared group of Experiment 3 ranged between approximately 0.9 and 1.2 mg. Therefore, lipid levels of typical 5- to 7-day-old young bees are likely to be in this range.

Chi-squared tests revealed that a significantly higher proportion of isolated bees foraged precociously as compared to colony-reared bees (χ^2 tests: Trial 1: χ^2_1=36.34, P<0.0001, Trial 2: χ^2_1=11.73, P=0.0006; Fig. 5), as expected from a previous study (Huang and Robinson, 1992). Although the data suggest a slightly higher proportion of isolated bees foraging from Trial 2 (fed bee candy with pollen) compared to Trial 1 (bee candy only), when the data are viewed relative to colony-reared bees in each trial, this trend does not hold. Because the baseline number of bees foraging within a trial can vary widely depending on nectar flow and weather conditions, the most important comparisons are made within trials. Viewed in this

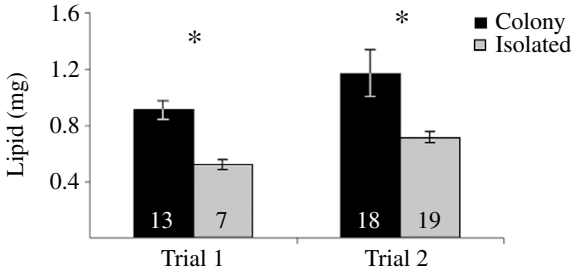

Fig. 4. Effect of social isolation (Experiment 3) on abdominal lipid amounts of 7-day-old bees. Isolated bees were placed in cages within field colonies allowing no social contact; colony-reared bees were allowed to move freely in the same field colony; treatment lasted 7 days. Values are means ± S.E.M. for isolated and colony-reared control bees. The results for two trials are shown. Numbers within bars represent the number of bees analyzed per group. *P<0.05, two-tailed, unpaired t-tests, assuming equal variance.

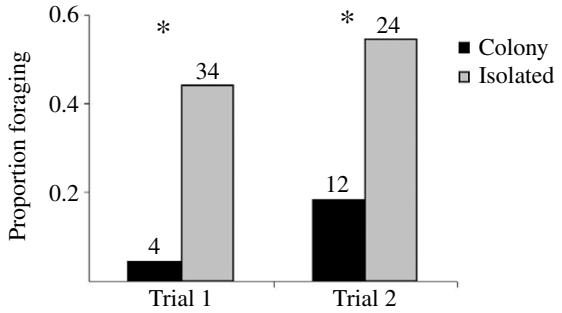

Fig. 5. Effect of social isolation (Experiment 3) on development of foraging behavior (isolated and colony-reared controls). Results for two trials are shown. Numbers above bars represent the number of bees observed foraging per group. *P<0.05, χ^2 tests.

way, Trial 1 shows approximately 3× elevated foraging in isolated compared to colony-reared bees, and approximately 9× in Trial 2; this suggests that foraging was most elevated under isolation treatment in the absence of pollen. This interpretation lends further support to the hypothesis that nutritional deprivation activates foraging.

Discussion

The principal significance of these results is the demonstration that the shift from high to low fat reserves with age, observed in many species of social insects (Blanchard et al., 2000; Toth and Robinson, 2005), reflects a causal relationship between individual nutritional status and the onset of foraging, at least in honey bees. Because food collected by foragers is largely for colony (i.e. non-self) nourishment, this suggests that pathways that translate nutritional stress into food-searching behavior in solitary insects were co-opted during social evolution to yield altruistic foraging in a eusocial insect.

TOFA caused a 40–50% reduction in lipid stores and this resulted in a detectable behavioral change. In contrast, a lower level of lipid depletion, resulting from pollen deprivation, did not affect behavior. This suggests that the relationship between nutrition and foraging ontogeny may be 'well-buffered', such that bees may forage precociously only in response to severe colony food shortage. Nutritional status may be unaffected in a relatively short period of pollen dearth because bees can utilize excess food reserves, as found in the European races of *Apis mellifera* (Winston, 1987). In contrast, nutritionally mediated effects on behavior may be more pronounced in African races of *A. mellifera* living in tropical regions where less honey reserves are stored (Winston, 1987) and colony starvation is more frequent (Schneider and McNally, 1992). Racial or intraspecies differences in physiology can be important life history adaptations for species that are able to occupy widely different environments (Martin et al., 2004), such as the honey bee.

TOFA treatment caused a decrease in fatty acid synthesis, but it is not known whether decreased fatty acid synthesis occurs naturally in bees to cause their striking loss of abominal lipids prior to the onset of foraging (Toth and Robinson, 2005). Pollen consumption decreases after a few weeks of adult life (Crailsheim et al., 1992), but bees continue to receive worker jelly as noted above (Crailsheim, 1991). Other possibilities include an age-related increase in metabolism (Harrison, 1986) and an age-related increase in octopamine levels (Harris and Woodring, 1991; Schulz and Robinson, 1999; Wagener-Hulme et al., 1999), a biogenic amine that plays a role in insect lipid mobilization (Fields and Woodring, 1991). The proximate mechanisms underlying the lipid loss in honey bee fat bodies associated with behavioral development deserve further attention.

We observed an increase of approximately twofold in the proportion of bees foraging in response to TOFA treatment, which is a large change in colony-level task allocation. However, this still amounts to only 20% of the treated bees showing a behavioral effect. Several factors might account for this result. First, perhaps there was unequal distribution of TOFA-containing food or pre-existing differences in lipid stores due to differences in genotype or larval environment; there was considerable variability in lipid levels among TOFA-treated bees (data not shown). Second, it is likely that no single treatment can stimulate all bees in a colony to forage because the effects of any physiological manipulation might be attenuated by social mechanisms that aid in the maintenance of colony division of labor (Beshers and Fewell, 2001; Huang and Robinson, 1999).

Separate pathways appear to be involved in nutritional and social mediation of foraging ontogeny in honey bees. This interpretation is consistent with the recent finding of a pheromone found in foragers that inhibits behavioral development, which appears to be transferred to young bees during food exchange (Leoncini et al., 2004b). The fact that we saw a similar effect of social inhibition in starved and fed colonies suggests that this pheromone acts similarly under both conditions. This agrees with experimental evidence documenting no differences in rate of trophallactic contacts

resulting in food exchange between starved and fed colonies (Schulz et al., 2002). Although we detected separate nutritional and social influences on foraging ontogeny, our results also indicate that social interactions do affect lipid levels in young bees. This is consistent with previous findings showing that nurse bees distribute nutritious 'worker jelly' widely to other adults, including young bees (Crailsheim, 1991).

The storage protein vitellogenin is also synthesized in the fat bodies, and Amdam and Omholt (2003) proposed in a theoretical model that high levels of vitellogenin inhibit the ontogeny of honey bee foraging behavior. The model emphasizes interactions between vitellogenin and juvenile hormone, a hormone that is known to influence the age at onset of foraging (Robinson et al., 1989; Sullivan et al., 2000). Recent empirical work supports this model, showing that injection of vitellogenin dsRNA (which interferes with vitellogenin synthesis) caused precocious foraging (M. Nelson, G. V. Amdam, M. K. Fondrk, and R. E. Page, personal communication). Vitellogenin is a lipoprotein, so the regulation of vitellogenin synthesis might be linked with lipid availability. Our results suggest that future models on the physiological regulation of honey bee behavioral maturation should incorporate other nutritional factors such as stored lipid, in addition to vitellogenin.

Signaling pathways related to individual nutritional status (Garofalo, 2002) may be involved in the regulation of social foraging in other taxa as well. For example, homologs of mammalian neuropeptide Y (NPY) and its receptor have been implicated in gregarious feeding behavior in *C. elegans* and *Drosophila* (de Bono and Bargmann, 1998; Wu et al., 2003). Signaling between adipose tissue and the brain is a burgeoning topic in vertebrates, especially as it relates to obesity in humans (Konturek et al., 2004). Insect homologs to several vertebrate neuropeptides have been reported in *Drosophila* and other insects (Nassel, 2002), and some appear to share similar biological functions (Garofalo, 2002). However, relatively little is known about mechanisms that communicate information about internal nutrient status from the fat body to the brain in insects. Our results suggest that studying nutrient signaling pathways could be important for understanding the evolution of altruistic foraging and worker division of labor in social insects.

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