

The nurse's load: Early-life exposure to brood-rearing affects behavior and lifespan in honey bees (*Apis mellifera*)

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Abstract:

Long-lived honey bees (*Apis mellifera*) develop in fall. This pattern may be explained by reduced nurse loads. When the amount of brood in colonies declines as a function of adverse foraging conditions, adult bees build up surplus nutrient stores that include vitellogenin, a behavioral effector protein that also can increase lifespan. Although the seasonal reduction in exposure to nursing tasks predictably results in vitellogenin accumulation, the assumption that long-lived adults thereby develop is confounded by a concomitant decline in foraging effort. Foraging activity reduces lifespan, and is influenced by colony resource consumption, brood pheromones, availability of nectar and pollen, and weather. Here, we perform the first controlled experiment where the nursing environment of pre-foraging sister bees was set to vary, while their foraging environment later was set to be the same. We measure vitellogenin, age at foraging onset and lifespan. We establish that reduced brood-rearing increases vitellogenin levels, and delays foraging onset and death. Longevity is largely explained by the effect of nursing on the onset of foraging behavior, but is also influenced by the level of brood-rearing independent of behavioral change. Our findings are consistent with the roles of vitellogenin in regulation of honey bee behavior and lifespan.

Keywords: Longevity Social behavior, Nursing, Foraging, Vitellogenin, Life-history regulation

Article:

1. Introduction

In temperate climates, honey bee colonies are characterized by a summer population of short-lived bees (summer bees) and a winter population of long-lived bees (winter-, or diutinus bees) ([Maurizio, 1950](#); [Mattila et al., 2001](#)). After emerging as adults, summer bees first perform tasks in the nest such as nursing brood ([Seeley, 1982](#)). About 2–3 weeks later, they go through a distinct behavioral transition to begin foraging for nectar and pollen in the field. Nectar (carbohydrate) and pollen (protein) are resources for colony growth, reproduction and survival ([Winston, 1987](#)). Colonies rear some hundreds to a few thousand males, tens to hundreds of thousands of female workers, and zero to tens of queens ([Page, 1980](#)).

Workers are essentially sterile. They conduct all the nursing and foraging tasks and participate in reproductive swarms, each headed by a single queen ([Winston, 1987](#)). Colony brood-rearing, foraging and reproduction, however, decline in late summer and fall ([Sekiguchi and Sakagami, 1966](#); [Free and Racey, 1968](#); [Sakagami and Fukuda, 1968](#); [Winston, 1987](#)). The queen and workers survive the unfavorable season inside the protected nest on stored resources, while remaining males are killed ([Maeterlinck, 1901](#)). This life-history results in a bimodal lifespan-distribution of worker cohorts, with a summer bee lifespan of 3–8 weeks, and a diutinus lifespan of up to 280 days ([Maurizio, 1950](#); [Amdam and Omholt, 2002](#)).

Efforts to explain this bimodality have centered on effects of brood-rearing on adult physiology ([Haydak, 1963](#); [Fluri et al., 1977](#)). It was suggested that, as exposure to nursing tasks declines, the young “nurse bees” can build up surplus nutrient stores that include vitellogenin ([Amdam and Omholt, 2002](#); [Omholt and Amdam, 2004](#)).

Vitellogenin is a conserved yolk precursor protein that has evolved a function in honey bee brood-rearing (Amdam et al., 2003). Although worker bees rarely lay eggs, they synthesize vitellogenin at elevated rates during early life. When the colony is active in brood-rearing, vitellogenin constituents are transferred by mouth to larvae, callow workers and the queen, and the turn-over of the protein is at balance or exceeds the production rate of mature nurse bees (Amdam et al., 2003; Amdam and Page, 2005). Conversely, when brood-rearing ceases the vitellogenin titer can increase 5- to 10-fold, reaching concentrations of 100 µg/µl in hemolymph (blood) (Amdam et al., 2005). This physiology is characteristic of diutinus bees (Amdam and Omholt, 2002).

Vitellogenin can influence worker longevity in several ways. It is a nutrient-providing storage protein (Engels et al., 1990), and by zinc-binding it may affect immune cell integrity and stress resistance (Amdam et al. 2004; Seehuus et al., 2006). Amdam et al. (2004) established a correlative relationship between the hemolymph titers of zinc and vitellogenin in worker bees, and a causal association between the levels of zinc and hemocyte (immune cell) survival in a culture system. Seehuus et al. (2006) demonstrated that vitellogenin is preferentially carbonylated (oxidatively modified) after injection of the oxidative stress-inducing agent paraquat to workers, and that RNA interference RNAi-mediated suppression of vitellogenin causes increased susceptibility to paraquat. Further RNAi knockdown experiments have shown that vitellogenin suppresses foraging onset by interplay with the systemic juvenile hormone (JH) (Nelson et al., 2007; Marco Antonio et al., 2008). Age at foraging onset is a predictor of worker life-span (Rueppell et al., 2007; Rueppell et al., 2008). This is because the pre-foraging life-phase can vary greatly in length while the duration of the foraging life-phase typically is less variable, seldom extending beyond 7–10 days (Neukirch, 1982; Visscher and Dukas, 1997).

Foraging efforts are affected by colony resource consumption and pheromones from brood (demand) (Pankiw et al., 1998), as well as the amount of nectar and pollen in the field and weather (opportunity). These factors change seasonally, and thereby, foraging activity is constrained in fall. However, demands on foraging also are affected in colonies where brood-rearing is experimentally changed (Eischen et al., 1984). Therefore, although insights so far suggest that nursing can influence lifespan and that an explanation for this is that nurse loads affect vitellogenin levels, it is unclear if nursing has an independent effect or if longevity patterns are explained by the reduced demands on foraging. To address this question, we studied the life-history of sister workers that were subjected to different levels of brood-rearing until 12 days old and, thereafter, initiated foraging in a common environment.

2. Materials and methods

2.1. Brood-rearing phase and observation hive setup

Four pairs of colonies (=8 colonies total) were established in five-frame nucleus colonies, each with a frame (wax comb) of honey and about 645 cm² of pollen. Two nurse load treatments were prepared: ‘High Brood’ and ‘No Brood’. High Brood received an average of 832 cm² of brood every four days (eggs from a queen that was caged for 3 days). No Brood was given empty comb. Brood was replaced three times over 12 days in the High Brood treatment for a total of 2495 cm² brood per colony. All queens were kept in excluder cages so only experimentally provided brood was present. About 500 newly emerged, number-tagged sister bees, 100 newly emerged paint-marked sister bees and 1200 unmarked medium-aged unrelated bees were used to build the worker population of each nucleus colony. Sisters were reared in common host colonies.

One pair of colonies (one High Brood and one No Brood) was set up on each of four consecutive days. On Day 12, 20–30 paint-marked bees were collected from two colony pairs for quantification of vitellogenin. Thereafter, each pair was placed in front of the entrance of a single observation hive, shaken into a swarm, and hived together on four frames (one with open brood, one with emerging brood, one with pollen and one with honey). A queen from a separate source was given to each combined colony, which was placed into the observation hive the following day.

2.2. Relative quantification of vitellogenin titer in pre-foragers

Drummond micropipettes were used to extract 1 µl hemolymph from each paint-marked bee. Hemolymph was dissolved in 50 µl Tris-buffer (20 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, pH 7.5, 1 mM

phenylmethylsulfonyl fluoride, 5 mM benzamidin, 0.7 μ M pepstatin, 8 μ M chymostatin, 10 μ M leupeptin and 0.8 μ M aprotinin, Sigma–Aldrich). After randomization, samples were separated by 7.5% SDS–PAGE (Laemmli, 1970). Densitometrically semi-quantification followed the method of Lin et al., where vitellogenin is detected as one band of 180 kDa (Wheeler and Kawooya, 1990; Lin et al., 1999). Quantity One imaging software (Bio-Rad) was used for the analysis after staining with Commassie Brilliant Blue (Sigma–Aldrich). Gel-to-gel variation was controlled by background correction as before (Amdam et al., 2006).

2.3. Foraging phase

Beginning on day 13, daily evening or early morning surveys (while the bees were not active in foraging) were conducted for all four observation hives; an observer slowly scanned each hive twice and recorded all numbered tags into a laptop running the voice recognition program of Microsoft Word. Beginning on day 14, hive entrances were observed for two 40 min observation periods daily (during non-orientation flight periods) and all arriving tagged bees were recorded.

2.4. Statistical analysis

Vitellogenin quantities were analyzed using ANOVA. Age at for-aging onset and survivorship were examined with Cox Proportional Hazard regression analysis using observation hive as stratifying variable. A bee's age at foraging onset was her age when first observed to arrive at the hive entrance during a non-orientation flight time. A bee's total lifespan was the number of days alive (last day alive = last day observed). A multiple regression model was used to separate the effect of 'nurse load' from 'observation hive' and 'age at foraging onset'.

3. Results

3.1. The effect of brood-rearing on the vitellogenin level

By measuring the relative vitellogenin titer of 78 bees, we verified a physiological effect of the High Brood and No Brood treatments before the 12-day-old bees were placed into common colonies. As expected (Amdam et al., 2005), the reduced exposure to nursing tasks (No Brood treatment) significantly increased the hemolymph vitellogenin concentration of the bees (ANOVA: $F_{1,75} = 31.0$, $P < 0.000001$). On average, the 12-day-old No Brood workers had about twice the vitellogenin amount of the same-aged High Brood bees (Fig. 1). When the level of brood-rearing was controlled for, the nucleus colonies per se did not affect the vitellogenin titer (ANOVA: $F_{3,75} = 1.9$, $P = 0.16$).

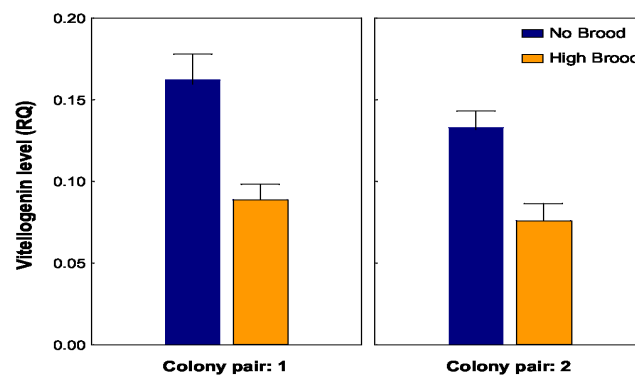


Fig. 1. Vitellogenin protein level (relative quantity, RQ) in worker bees from two randomly chosen colony pairs. In both replicates, the No Brood treatment resulted in increased vitellogenin titers compared to the High brood treatment. Bars are means with standard errors.

3.2. Foraging onset and longevity

We next recorded the foraging onset of 1708 bees across the four observation hives. We established that in these common environments, the preceding No Brood treatment caused workers to initiate foraging significantly later in life compared to the High Brood treatment (Cox Proportional Hazard: Hazard ratio = 1.27 days (95% CI = 1.16–1.4 days); Likelihood Ratio Test= 24.2, $df = 1$, $p < 0.0000009$; Cox Proportional Hazard Assumption met, $\rho = 0.008$, ns; Fig. 2). Mean age of foraging onset ranged from 22.2 to 25.8 days old (range for the observation hive means) for bees from the No Brood treatment and 21.3–24.1 days old for bees from the High Brood treatment.

Thereafter, we determined the lifespan of the same 1708 workers. We found that the No Brood treatment significantly increased worker lifespan (Cox Proportional Hazard: Hazard ratio = 1.26 days (95% CI = 1.15–1.39 days); Likelihood Ratio Test = 23.0, df = 1, $p < 0.000002$; Cox Proportional Hazard Assumption met, $\rho = -0.029$, ns). The mean longevity ranged from 33.0 to 38.4 days old (range for the four observation hive means) for bees from the No Brood treatment and 32.1–37.1 days old for bees from the High Brood treatment (Fig. 3).

Across the four observation hives, age at foraging onset and life-span were significantly correlated (Pearson Correlation, $r = 0.63$, $P < 0.001$, $N = 1708$). This association was established for honey bees before (Rueppell et al., 2007; Rueppell et al., 2008), and is expected since the pre-foraging life-phase is a major variance component of total longevity (Neukirch, 1982; Visscher and Dukas, 1997). When taking this relationship into account by controlling for age at foraging onset in our analysis of lifespan, we found that the No Brood and High Brood treatments also affected longevity directly, i.e., independent from the treatment effect on worker foraging behavior (Multiple Regression: observation hive $F=87.4$, $P < 0.0000000001$; age at foraging onset, $F = 1105.1$, $P < 0.0000000001$; nurse load, $F = 7.618$, $P < 0.006$).

4. Discussion

We show that the life history of worker honey bees is influenced by brood-rearing. This question has been studied before (e.g. Haydak, 1963; Eischen et al., 1984; Le Conte et al. 2001), but our experiment controlled for several confounding factors for the first time. By using newly-emerged sister bees from common host colonies, we controlled for genotype and larval-to-adult rearing environment. Subsequently, after 12 days of exposure to different levels of brood-rearing (No Brood vs. High Brood), we controlled for foraging environment by placing sister bees back together in observation hive where they experienced the same demands and opportunities for foraging. Our four replicate pairings of colonies, furthermore, add strength to the results. The treatment groups were similarly affected among the observation hives, although the demographic trajectories of the separate sets of worker cohorts were not identical (Figs. 2 and 3).

The finding that reduced brood-rearing increases vitellogenin titers, and delays foraging onset and worker death, is consistent with previous results. It was shown before that vitellogenin titers increase when brood is removed from colonies (Amdam et al., 2005), and, when vitellogenin titers are reduced by gene silencing, workers forage precociously and live shorter lives (Nelson et al., 2007; Marco Antonio et al., 2008). The magnitude of the treatment effects in our study, although seemingly small, also is in accordance with previous analyses. Vitellogenin gene silencing by RNAi reduces vitellogenin protein levels 2- to 5-fold and lowers the age at foraging onset and longevity with 1.43 days and 1.29 days, respectively. Correspondingly, our No Brood group, which experienced a 2-fold increase in vitellogenin levels, postponed foraging onset with 1.16–1.4 days and lived 1.15–1.39 days longer than the High Brood group.

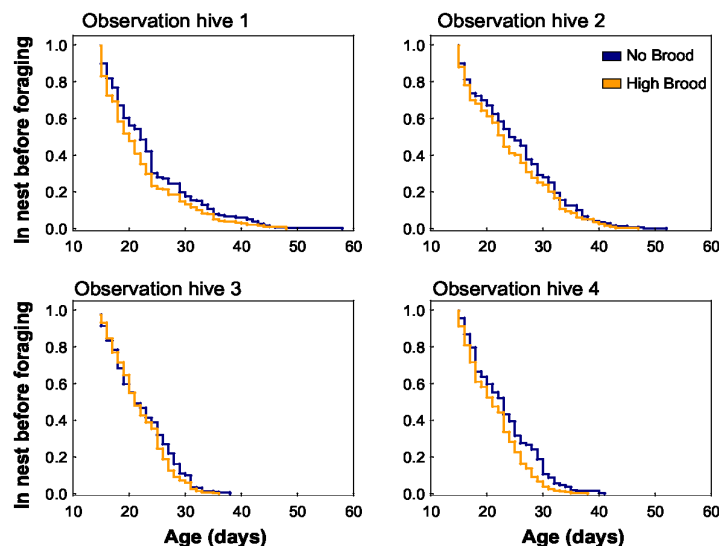


Fig. 2. Foraging onset shown as the cumulative proportion of bees that has not yet foraged. Reduced brood-rearing (No Brood) resulted in delayed foraging onset. The panels show data from all tagged workers that were observed to forage at least twice.

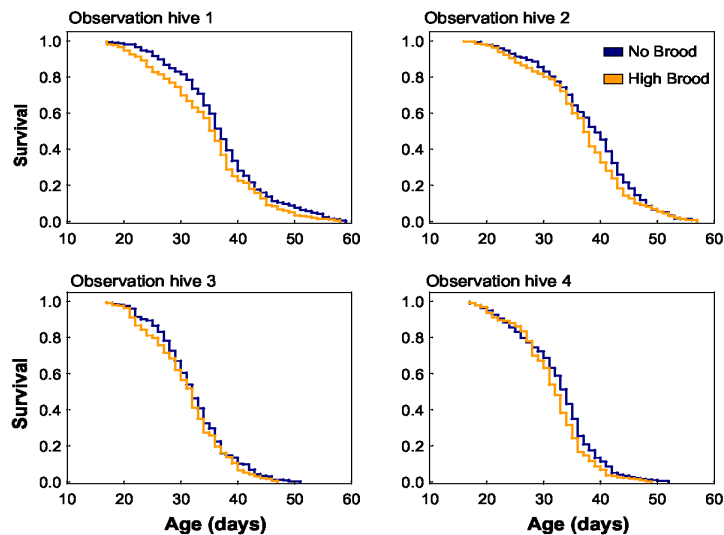


Fig. 3. Worker longevity shown as the cumulative proportion of marked bees that is still alive. Reduced brood-rearing (No Brood) extended the lifespan of workers. The panels show data from all tagged workers that were observed alive during the study.

Exposure to brood and brood pheromones can have many effects on the social behavior and endocrine physiology of mature worker bees, including changes in the titer of JH (Le Conte et al., 2001). JH is a life-shortening hormone in *Drosophila melanogaster* that in honey bees is suppressed by high vitellogenin levels. Yet, when released from repression, JH feeds back to inhibit vitellogenin synthesis, and this mutually suppressive relationship between vitellogenin and JH appears to be a central integrator of honey bee social behavior (reviewed by Amdam and Omholt, 2003).

Our study confirms a well-established correlation between age at foraging onset and lifespan, but also documents a negative effect of nursing on longevity that is independent of foraging behavior. The finding that brood-rearing shortens life by effects on foraging onset and longevity that are partly separate, is consistent with the negative influence of nursing on the vitellogenin titer of the workers (Fig. 1). Vitellogenin suppresses JH and onset of foraging behavior, but also, by increasing the level of oxidative stress resistance vitellogenin may extend life independent of worker behavior (Seehuus et al., 2006; Nelson et al., 2007). Thus, although we cannot fully exclude that these relationships are caused by unknown common factors, we conclude that our results support the hypothesis that vitellogenin has central functions in the regulation of honey bee physiology and behavior.

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