Food manipulation in honeybees induces physiological responses at the individual and colony level

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Original article

Food manipulation in honeybees induces physiological responses at the individual and colony level

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Abstract – *Apis mellifera* experiences large population declines in the USA and honeybee health is affected by many, potentially interacting factors that need to be addressed through a variety of approaches. In this context, we evaluated the impact of nutritional manipulations on worker physiology and colony demography. Specifically, we manipulated protein availability by feeding colonies on royal jelly, low-quality pollen, or regular pollen stores. After acclimation to these treatments, experimental cohorts of workers were introduced and later assessed with regards to life expectancy, protein content, and intestinal stem cell proliferation. We also monitored their hives for the amount of workers, brood, and pollen trapped in front of the hive entrances. Workers that fed on royal jelly showed a reduced rate of intestinal stem cell proliferation at nurse bee age. Total soluble protein content of individuals and adult worker lifespan were not systematically affected. However, we cannot exclude an auxiliary role of poor nutrition to declining bee health by weakening the intestinal epithelium. In contrast to the weak experimental effects on individual variables, the brood production differed drastically among the experimental hives. Although not yet replicated, this observation might indicate that hive demographic plasticity rather than individual plasticity is important for acclimation to different food regimes.

intestinal stem cells / pollen / nutrition / demographic plasticity / mortality

1. INTRODUCTION

The western honeybee, *Apis mellifera* (L), is vital to modern agriculture in North America because it provides essential pollination services for a wide variety of crops (Vinson 1985; Morse and Calderone 2000). Declines in feral and managed honeybee populations (Ellis and Munn 2005) and in particular the recent, dramatic disappearances of adult bee populations from their hives (vanEngelsdorp et al. 2008), have resulted in a surge of studies addressing honeybee health. The honeybee gut is one of the primary interfaces of honeybees with their environment. It is of particular interest with regards to the multiple factors that could contribute to declining bee health (Johnson et al. 2009a; vanEngelsdorp et al. 2009) because it is responsible for the uptake of food, toxins, and pathogens. As a result of their continuous digestive activity and environmental insults, the cells of the gut epithelium need to be continuously replaced by intestinal stem cells (ISCs; Ohlstein and Spradling 2006). These ISCs are the only major population of replicative somatic cells in adult honeybees (Ward et al. 2008). Their active cell cycle may make the ISCs particularly sensitive physiological indicators for intestinal health (Davila et al. 2004). In honeybees, their proliferative activity co-varies with the nutritive demands on the individual among age classes and castes (Ward et al. 2008), but the

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ISCs have not yet been studied with regards to honeybee health. The only study that has specifically investigated the honeybee gut with respect to bee health focused on a global assessment of gene expression patterns (Johnson et al. 2009a). Ribosomal RNA fragments were overrepresented in health-compromised samples (Johnson et al. 2009a), which could be a sign of viral infection (Johnson et al. 2009b) but also might indicate starvation (Zundel et al. 2009). This project sought to extend our previous studies of the ISCs (Ward et al. 2008) to the health-related context of nutrition by comparing the ISC replication pattern between three different diet treatments.

Bees derive almost all protein from pollen, even though it is difficult to digest (Crailsheim 1990b). Thus, pollen availability translates directly into different individual titers of protein (Bitondi and Simoes 1996). Protein availability, and specifically the protein concentration of pollen, is essential for brood rearing (Maurizio 1950; Crailsheim 1990b; Crailsheim et al. 1992), and plays a role in the longevity, functioning, and health of individuals and colonies (Haydak 1970; Amdam and Omholt 2002; Sagili et al. 2005; Mattila and Otis 2006a, b; Sagili and Pankiw 2007). Specifically, bees restrict brood rearing to seasons when pollen is available and become cannibalistic when pollen is scarce (Schmickl and Crailsheim 2001). Pollen-deprived adult bees have been reported to act normally but die at significantly younger ages (Haydak 1937) but the mortality affect seems to be context dependent (Mattila and Otis 2006b). Thus, we compared honeybee workers under different food regimes with regards to their life expectancy, soluble protein content, and ISC replication rate. In addition, we monitored hive brood production and pollen collection. The observed treatment effects revealed sub-lethal effects on ISC proliferation but not on soluble protein content. Overall, the results suggested that individual, physiological responses to nutritional differences may not be very pronounced in honeybees, which might be facilitated by demographic plasticity at the colony level.

2. METHODS AND MATERIALS

2.1 Demographic assay and colony variables

Three four-frame observation hives were established, each with 3,000 workers and one queen of mixed European background. Two weeks prior to the onset of the experiment, all pollen and brood was removed from the hives and two hives were equipped with a pollen trap that stripped returning foragers of any collected pollen before entering the hive. To increase the effectiveness of pollen removal, this trap was custom-built into the entrance of the observation hives with two sequential passages through 45º angled double-screen queen excluder (#5 mesh hardware cloth). Visual inspections confirmed that this trap removed most of the pollen from incoming foragers and resulted in a >95% reduction of natural pollen stores in the manipulated hives. One of these hives received a 1:1 mixture of finely-ground, dried pollen, and cellulose (# 310687, Sigma, MO, USA) as a low quality food treatment (Waddington et al. 1998), referred to henceforth as “low”. Although this pollen was consumed, such a dilution is perceived by the workers (Waddington et al. 1998) and presumably leads to a higher digestive demand relative to the nutrients obtained. The second hive was fed daily with 20 ml of fresh royal jelly (GloryBee Foods, Eugene, OR, USA) as a nutritionally augmented treatment, referred to henceforth as “high”. The majority of the royal jelly was eaten promptly by the workers and we assume that this food is richer and more easily digested than pollen (Winston 1987). The third, control hive remained undisturbed after the initial set up procedure without a pollen trap.

Frames with emerging young bees were removed from eight unrelated hives of European decent and transferred to an incubator (34°C, 60% relative humidity) overnight. The newly emerged workers were individually tagged with plastic colored number tags (BeeWorks, Canada) and introduced into the observation hives. Each hive received a focal cohort of 500 randomly assigned bees. Tagged individuals were monitored at night for 36 consecutive days in order to measure individual survival, with the assumption that workers died 1 day after they were

last recorded in the hive (Rueppell et al. 2005, 2007, 2008). In addition, we recorded for 60 min per hive each morning when marked bees were returning from foraging trips with or without pollen in order to calculate their age of first foraging (AFF) and pollen specialization (measured as the fraction of foraging records that included pollen foraging). The foraging observations were also included in the survival estimates in cases where workers were not recorded during the nightly census but were observed foraging. Bees that died during the first 5 days of the experiment were excluded from the analysis to account for handling and other experimental effects.

At the colony level, we estimated total worker population size because colony size influences worker life expectancy (Rueppell et al. 2009) and brood production, which in turn influences worker life expectancy (Amdam et al. 2009). We also determined the dry weight of pollen trapped in the pollen traps of the "low" and "high" hive over 30 days. Worker population size in each hive was determined by averaging two independent counts of all workers present in the hive at night by two observers at the beginning and at the end of the experiment. Brood production was determined by estimating the comb area covered by open and capped brood on day 15 of the experiment. Each frame was inspected on both sides and the area of cells with brood measured with a measuring tape to calculate the fraction of each side that contained open or capped brood. Open brood represents young larvae that are not yet completely grown and of varying body mass. It is impossible to quantify the exact biomass non-invasively, and therefore the open brood area was divided by two account for the smaller biomass of open brood relative to mature brood (=capped brood cells). The full frames used were assumed to contain 10,000 cells to calculate the absolute number of brood items produced. Before any brood could emerge, frames were removed from the colony and replaced with empty comb throughout the experiment. The brood frames were placed into the incubator and newly emerging bees were used for protein and ISC proliferation measurements (see below). The pollen collected from the pollen traps over the 30 days was dried in aliquots at 65°C and weighed on a Mettler PL202-S scale until weight constancy was achieved, indicating complete dryness.

2.2 ISC proliferation assay

In addition to the individually marked cohorts used for the demographic study, further workers from the same group of emerging bees were color-marked and introduced simultaneously to serve as samples for the comparison of the proliferative activity of intestinal stem cells and protein quantification (see below) under the different food treatments. At 8 and 36 days of age, 10 bees were randomly sampled from each cohort in the "low" and "high" treatment groups. These individuals were assayed for the quantity of cell proliferation in the central midgut with immuno-histological detection of the thymidine-analog 5′-bromo-2′-deoxyuridine (BrdU), which is incorporated into newly synthesized DNA, following (Ward et al. 2008) with slight modifications.

The collected bees were starved in an incubator at 34°C and 60% relative humidity for 30 min before being harnessed in a plastic drinking straw for feeding. Each bee was fed 5 μl of 25% sucrose solution mixed with 5 mg/ml BrdU (Sigma, MO, USA). Bees that consumed the entire amount of the BrdU solution were selected and placed back in the incubator overnight. Subsequently, the abdomen of each bee was removed with a razor blade and placed in bee saline for dissection. The removed midgut was fixed in Carnoy's fixative for 1 h before being dehydrated in three consecutive 15-min treatments with 100% ethanol. This procedure was followed by 15-min incubations in each of the following mixtures: 1:1 ethanol and xylene, 100% xylene, 1:1 xylene and Paraplast (Thermo Fisher Scientific, MA, USA), and 100% Paraplast. Tissues were placed into glass vials containing fresh Paraplast for storage.

Individual midgut samples were removed from their glass vials by melting the Paraplast at 60°C in an incubator and embedded in fresh Paraplast. Sections were cut on a microtome at 10 μm in thickness. After sectioning, the paraffin was removed from the slides by soaking in three 100% xylene baths for 5 min each. The slides were then rehydrated in 100% ethanol (twice), 95% ethanol, and 70% ethanol, each for 5 min. This was followed by two 1× PBS-T rinses for 5 min and 1-h incubation in 1× PBS-T with 2 N HCl added. The slides were then rinsed in two washes of PBS-T for 5 min each. Blocking was performed with 0.5% normal goat serum (Biomeda, CA, USA) and 0.2 g/l BSA

(Sigma, MO, USA) in PBS-T for 1 h. The tissue was then covered with 1:500 anti-BrdU mouse antibody in PBS-T and incubated overnight in a humid chamber at 4°C.

After incubation, the slides were washed with PBS-T for 10 min three times and then treated with 1:40 goat anti-mouse in PBS-T in a humidity chamber for 2 h at 4°C., followed by three PBS-T and two 1× PBS rinses for 10 min each. SigmaFast DAB (Sigma, MO, USA) was used to stain the tissue until the tissue color was visibly changed in approximately 5–10 min. Stained slides were rinsed in three consecutive 1-min distilled water washes and then dehydrated by soaking for 5 min in 70%, 95%, and 100% ethanol. Finally, the slides were placed in three 5 min baths of citrus clearing solution (VWR, PA, USA) and mounted with Permount (Thermo Fisher Scientific, MA, USA). The stained cells in three crypts were counted and added to one individual data point per bee. Staining and counting was randomized and conducted blindly with respect to treatment group.

2.3 Protein assay

Ten workers of the original cohort were collected from each treatment at 25 and 36 days of age for assessing nutritional status of the bees. In addition, we sampled emerging workers that had been raised under the three experimental conditions. We measured the total quantity of soluble protein in head, thorax, and abdomen with a Coomassie Blue spectrophotometric assay (Coomassie (Bradford) Protein Assay Kit, Thermo Scientific, Rockford, IL, USA). Individuals were sampled at random, divided into head, thorax, and abdomen with a razor blade, and frozen immediately at −20°C. Subsequently, samples were transferred into liquid nitrogen and homogenized in individual micro-centrifuge tubes with a Teflon pestle. To each tube, 500 μL of 1× TBE buffer was added and the mixture was vortexed at high speed for 3–5 min. The tubes were then centrifuged for 5 min at 10,000 rpm. Two microliters of the clear supernatant was then mixed with 8 μL of distilled water and 10 μL of Coomassie blue solution (Thermo Scientific, IL, USA). Following the manufacturers recommendations, protein concentration of this mixture was measured on a NanoDrop™ spectrophotometer (NanoDrop, DE, USA) by comparison to BSA standards (Pierce). Total protein content of the sample was then calculated by multiplying the concentration by 10 (to account for the dilution) and by the ratio of the total supernatant volume to the 2 μL examined.

2.4 Statistical analyses

Following earlier studies (Rueppell et al. 2007, 2009), worker survival was compared with a simple Mantel–Cox log rank test and with Cox regressions using the AFF and the proportion of foraging trips devoted to pollen foraging (pollen proportion) as covariates. Sample sizes for ISC proliferation counts were small and therefore non-parametric tests were used. However, protein content data met parametric assumptions after removal of extreme outliers (6/267 data points were excluded because they were >3 quartiles removed from the sample median) and consequently were assessed using ANOVA with Dunnett's T3 post hoc tests. Throughout the study, a significance threshold of $p<0.05$ was used with Bonferroni correction when appropriate.

3. RESULTS

3.1 Hive observations

Overall, the three experimental hives were different in size: The "high" hive was largest at the beginning and the end of the observation period (2,400 and 2,650 workers, respectively), while the "low" hive was smallest (1,675 and 1,500 workers) and the control hive intermediate (1,850 and 2,000 workers). The "high" hive also produced most brood items in absolute terms (26,250) and per initial worker (10.9), followed by the control hive (17,333 and 9.4, respectively), and the "low" hive (10,417 and 6.2). Large quantities of pollen were trapped in front of the "low" and the "high" hive (464.6 g and 1,218.0 g, respectively).

3.2 Worker demography

In total, 1,109 workers were included in the survival analysis. There was no significant

overall effect of treatment on survival (Figure 1; χ^2=5.7, *df*=2, *P*=0.058) with similar average life expectancies in the control hive (mean: 19.7 days, 95% confidence interval 18.9–20.5, *n*=395), the "low" hive (20.7, 95%CI: 19.8–21.6, *n*=370), and the "high" hive (19.3, 95%CI: 18.4−20.1, *n*=344). However, the data displayed a mortality cross-over from lower initial survival to higher survival after 13 days in the "low" hive relative to both other hives (Figure 1).

The multi-factorial Cox regression revealed a significant effect of AFF (hazard rate=0.93, 95% confidence interval: 0.92−0.95, *P*<0.001) but not of pollen proportion (hazard ratio=1.14, 95%CI: 0.92−1.42, *P*=0.223) or treatment (*P*=0.067). The effect of AFF was significant and consistent in all treatment groups varying from a hazard rate of 0.92 (95%CI: 0.89−0.94, χ^2=36.9, *df*=1, *P*<0.001) in the "high" hive to 0.93 (0.91−0.95, χ^2=44.0, *df*=1, *P*<0.001) in the control hive and 0.96 (0.93−0.98, χ^2=11.8, *df*=1, *P*=0.001) in the "low" hive. Concomitantly, the regression of remaining lifespan (lifespan after foraging initiation=foraging lifespan) on AFF was strongest in the "low" hive (β=−0.53, r^2=0.29, *n*=199, *P*<0.001), followed by the control hive (β=−0.39, r^2=0.15, *n*=258, *P*<0.001) and the "high" hive (β=−0.32, r^2=0.10, *n*=207, *P*<0.001). The AFF itself was significantly different among the three treatment groups (χ^2=34.8, *df*=2, *P*<0.001), with bees in the "low" treatment group starting to forage significantly later (18.7 days, 95%CI: 18.0−19.4), than bees in the "high" treatment (15.6, 14.8−16.3) or controls (14.5, 13.8−15.3). No significant differences existed among treatment groups in the proportion of pollen trips observed (χ^2=1.9, *df*=2, *P*=0.38).

3.3 ISC proliferation

Counts of labeled cells indicating cell proliferation (Figure 2) showed no significant overall effect of age (Mann–Whitney *Z*=1.9, *n*=60, *P*=0.051). Treatment affected intestinal stem cell proliferation counts in 8-day-old bees (Kruskal–Wallis *H*=7.5, *df*=2, *P*<0.023) but not in 36 day olds (*H*=0.9, *df*=2, *P*<0.637). Post hoc tests revealed that the former effect was due to a significantly lower count in bees from the "high" treatment than the "low" treatment (*P*<0.05). Post hoc testing revealed no other significant differences, but simple pairwise testing also found a significant difference between the "control" and the "high" group (*Z*=2.2, *n*=20, *P*=0.023).

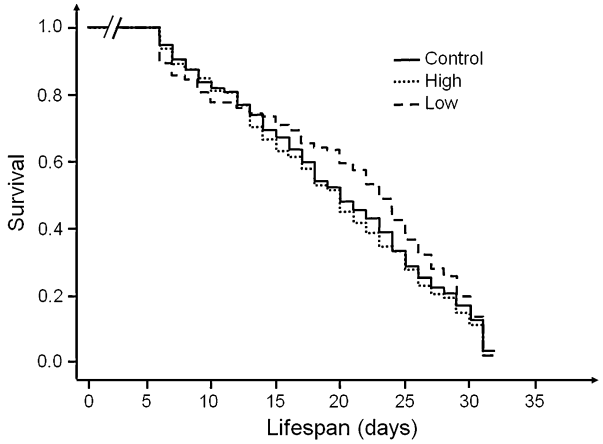

Figure 1. Overall, life expectancy of worker honeybees was not significantly affected by three different nutritional regimes, ranging from a cellulose-diluted pollen ("low") to an unmanipulated ("control") and a fresh royal jelly ("high") diet at the colony level. Individual mortality risk was best explained by the age at the onset of foraging in all groups. Worker mortality during the first 5 days was disregarded to eliminate experimental artifacts.

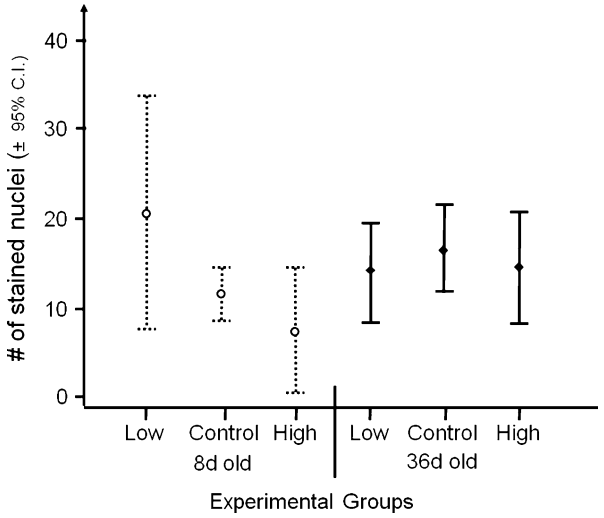

Figure 2. Amount of BrdU-labeled cells in the intestinal epithelium of young and old workers honeybees under three different nutritional regimes, ranging from a cellulose-diluted pollen ("low") to an unmanipulated ("control") and a fresh royal jelly ("high") diet at the colony level. The labeling, indicative of the rate of cell replication by intestinal stem cells, was significantly lower in young bees of the "high" than of the "low" treatment, with the "control" bees intermediate. No significant treatment effect was measured at older ages.

3.4 Protein content

Among the workers from the original cohort, a three-factorial ANOVA indicated that the measurements of soluble protein (Figure 3) were significantly affected by age and body part, but not treatment (Table I). On average, protein measurements were higher in 36-day-old workers (849.0 ng±302.1; mean ± SD) than in 25-day-old workers (768.9 ng±151.7). Across both ages, the thorax scored highest (1,009.2 ng±299.2), followed by the abdomen (766.5 ng±87.0), and then head (656.3 ng±121.1). However, all two-way interaction effects were significant (Table I), requiring more detailed analyses. The interaction between treatment and age was caused by an increase in average protein measurements in the 25-day-old workers from the low treatment (722.7 ng±134.3) to the control (781.9 ng±132.0) and high (800.8 ng±177.4) treatments but not in the 36-day-old workers (low, 895.0 ng±296.1; control, 771.7 ng±151.3; high, 875 ng±395.1). The interaction between age and body part was due to a significant increase with age in soluble protein in the thorax (25 days, 892.1 ng±122.1; 36 days 1,118.5 ng±369.4; $F_{(1,56)}=9.5$, $P=0.003$) but not in the head (25 days, 647.3 ng±119.4; 36 days, 665.4 ng±124.2) or abdomen (25 days, 775.4 ng±104.5; 36 days, 757.1 ng±63.8). The interaction between body part and treatment arose due to an increase in abdominal protein measurements from low (740.8 ng±96.3) to control (774.3 ng±79.8) to high treatment (785.4 ng±81.1), but different patterns in the head (low, 688.9 ng±141.1; control, 663 ng±106.4; high, 616 ng±107.1) and thorax (low, 1,011.5 ng±313.6; control, 898.5 ng±117.1; high, 1,112.2 ng±372.7). Among the newborn workers raised under the experimental regimes, soluble protein content was unaffected by treatment (low, 805.9 ng±90.9; control, 812.0 ng±139.7; high, 824.6 ng±111.2; $F_{(2,76)}=0.2$, $P=0.860$) but significantly different among body parts (head, 797.1 ng±87.9; thorax, 894.3 ng±75.5; abdomen, 756.0 ng±129.6; $F_{(2,78)}=12.8$, $P<0.001$), with thorax significantly higher than both head ($P<0.001$) and abdomen ($P<0.001$) but no difference between head and abdomen ($P=0.412$).

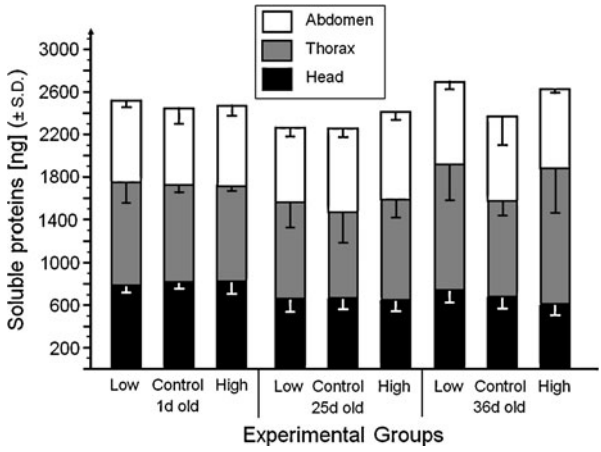

Figure 3. Total soluble protein in the three principal body parts of worker honeybees under three different nutritional regimes, ranging from a cellulose-diluted pollen ("low") to an unmanipulated ("control") and a fresh royal jelly ("high") diet at the colony level. The two older cohorts experienced these conditions throughout their adult life, while the 1-day-old workers were reared from eggs under these conditions. Protein measurements varied relatively homogeneous and unaffected by treatment although significant effects of body part and age group, as well as two-way interactions existed (see main text for details).

4. DISCUSSION

The experimental manipulations did not significantly affect worker life expectancy and all treatment hives retained adults throughout our study and beyond. Nevertheless, we cannot rule out nutritional stress as a contributor to declining bee health. Nutritional stress may interact with pesticides or pathogens (Mayack and Naug 2009; Naug and Gibbs 2009). Specifically, nutritional stress could deplete ISC proliferative capacity by requiring more cell replacement (Ward et al. 2008) and thus compromise the long-term turn-over of epithelial cells in the intestine. The turn-over of epithelial cells is important for maintaining a functional gut and preventing the spread of pathogens and toxins to other tissues. Thus, the intestinal health is a likely key contributor to honeybee health (Johnson et al. 2009a) and our results suggest that it may be affected by overall dietary quality.

Average life expectancy of worker bees did not differ between any treatment groups although their AFF did. The "low" quality pollen diet resulted in a significant delay of AFF, contrary to the effect of carbohydrate starvation (Schulz et al. 1998). These opposing results are not surprising because foraging for protein (pollen) and foraging for carbohydrates (nectar) are independently regulated through different mechanisms (Fewell and Winston 1996; Page and Erber 2002). As in previous studies (DeGrandi-Hoffman et al. 2008), protein deprivation in our "low" colony was associated with a severe reduction in brood, which in turn may have decreased overall foraging activity and recruitment of new foragers

Table I. ANOVA results of the effects of age, body part, and treatment on total soluble protein content.

Factor	Stats	Significance (*p*)
Treatment	$F_{(2,158)}=1.8$	0.166
Age	$F_{(1,158)}=8.6$	0.004
Body part	$F_{(2,158)}=63.7$	<0.001
Treatment × age	$F_{(2,158)}=4.2$	0.016
Treatment × body part	$F_{(4,158)}=3.5$	0.009
Body part × age	$F_{(2,158)}=8.8$	<0.001
Treatment × age × body part	$F_{(4,158)}=2.1$	0.083

(Maurizio 1950; Rueppell et al. 2007). Interestingly, pollen foraging seemed not to be down-regulated by the ample availability of protein in form of royal jelly in the hive, which supports the view of a direct feedback between the colony pollen stores and pollen collection (Fewell and Winston 1992). These colony level responses were strong, but we need to caution here that they only rely on singular observations.

A delayed AFF is usually associated with a longer lifespan (Rueppell et al. 2007, 2008, 2009) because the initiation of foraging increases extrinsic and intrinsic mortality factors (Amdam and Omholt 2002; Amdam et al. 2005; Rueppell et al. 2007). On an individual basis, AFF is also in this study the strongest predictor of lifespan. The AFF effect is consistent across and within treatment groups and its effect size is comparable to previous studies (Rueppell et al. 2007, 2008, 2009). However, workers in the "low" treatment group did not live significantly longer than in the control or "high" treatment group even though they delayed their initiation of foraging. This can be explained by the initially higher mortality in the "low" group before the onset of foraging is a major factor. Mortality in the "low" group was significantly lower after 12 days (Figure 1; χ^2=9.4, *df*=2, *P*<0.009) when foraging initiation commenced. In sum, the relatively low life expectancy of the "low" workers relative to their AFF may be due to the direct effects of the nutritional stress.

In non-manipulated honeybees in a common hive environment, a lower protein status in workers translates into an earlier AFF (Nelson et al. 2007). Thus, the later AFF in the "low" group prompted a prediction of relatively high protein titers, contrary to our original prediction that the protein titers would be highest in the "high" group, followed by control and then "low" group bees (Haydak 1970; Crailsheim 1990b). Neither was the case; although some significant differences in soluble protein concentration existed among the experimental groups, the differences were subtle with complex interaction between age and body compartment. It is noteworthy that the strongest effects were observed in the thorax, where the flight musculature plays a critical role in survival and performance of older, foraging age bees (Schippers et al. 2006). However, given different brood and foraging levels and the possibility of demographic selection, it is impossible to establish a clear cause and effect argument. Moreover, the lowest protein titers were measured in the control bees, not in the "low" group. Therefore, our results suggest that soluble protein measures may not be a good indicator of colony nutrition or individual honeybee health (vanEngelsdorp et al. 2009).

Our experimental manipulations also failed to affect the protein status of newly emerging workers. These workers were raised during the experimental period and experienced the effect of the experimental manipulations. We predicted that a shortage of readily available protein would particularly affect growing individuals. Body size effects had been previously reported (Eischen et al. 1982; Crailsheim 1990b). However, our results do not indicate a major effect on soluble protein quantities that persist into adulthood (Mattila and Otis 2006b). Instead, our tentative conclusion is that the colonies seem to adjust the quantity of brood, favoring demographic plasticity over individual physiological plasticity (Allen and Jeffree 1956; Blaschon et al. 1999; Schmickl and Crailsheim 2001; Rueppell et al. 2008). It is important to note that the pollen trapping did not prevent 100% of the natural pollen intake but restricted it quantitatively. Thus, any observed responses are due to a quantitative effect of the experimental manipulations, not the absolute absence of pollen.

At the individual level, our most sensitive indicator of nutrition was the measure of ISC proliferation of 8-day-old workers. With very few exceptions workers of this age are nurse bees, which imposes a high demand for digestive activity (Ward et al. 2008) because this temporal caste is primarily responsible for digestion of the pollen and redistribution of proteinaceous feeding secretions to developing and adult nestmates (Crailsheim 1990a, b). The relatively low ISC proliferation observed in the "high" treatment bees as compared to control and "low" conditions could be attributed to the royal jelly diet. This "predigested" food source

potentially reduces the digestive demand on the intestine, thus reducing the rate of cellular death and need for ISC proliferation (Ward et al. 2008). In contrast, the increased mechanical stress caused by the cellulose in the "low" pollen quality may contribute to higher rates of cell death, and thus increased ISC proliferation, observed as an increased number of BrdU-stained nuclei. The localization and characteristic pattern of the labeled cells adjacent to the basement membrane (see also Ward et al. 2008) indicates that the labeling is not due to endoreplication of pre-existing cells.

Our results corroborate the hypothesis that the ISC replication rate responds to digestive demands (Ward et al. 2008) or other factors that stress the intestinal epithelium and cause epithelial cell death, such as pathogens or pesticides. This plasticity and its relation to cell death remains to be further evaluated because it may represent a quantitative, easy, and standardized indicator to assess honeybee health in natural colonies. ISC replication rate is an integrative measure in a central tissue that is well suited to monitor multi-causal health problems. Furthermore, it remains to be studied whether high proliferation rates at young age limits the proliferative capacity of the ISCs and thus the intestinal tissue repair at older ages. This might be the case if ISC proliferation potential is limited (Rando 2006) and could proximately explain the nutritional depletion of older foragers. Thus, honeybee health research needs to address the dual role of ISCs, as disease indicators and as effectors.

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La manipulation des sources de nourriture chez les abeilles provoque des réponses physiologiques au niveau de l'individu et de la colonie.

cellule souche intestinale / pollen/ nutrition / plasticité démographique / mortalité

Zusammenfassung – Physiologische Reaktionen auf Manipulationen des Nahrungsangebots von Individuen und Völkern. Die aktuellen Gesundheitsprobleme der Honigbienen sind vermutlich auf mehrere Faktoren zurückzuführen und daher werden vielfältige Ansätze verfolgt. In diesem Zusammenhang wurden Bienen in dieser Studie am Polleneintrag in den Stock gehindert und stattdessen entweder mit minderwertigen Pollen oder mit frischem Futtersaft (Gelee Royale) gefüttert. Nach einer Eingewöhnungsphase, wurden die zu untersuchenden, jungen Bienen in die Stöcke gegeben und später auf Proteingehalt und Replikation der Stammzellen im Darm untersucht. Zudem wurden die Koloniegrössen, Brutmengen, und die Masse des abgefangenen Pollens der Kolonien aufgezeichnet. Die experimentelle Manipulation der Ernährung im Stock wirkte sich auf individueller und Stock-Ebene aus. Die individuelle Mortalität von Arbeiterinnen und deren interne Proteintiter wurden allerdings kaum beeinträchtigt, aber die Futtersaftdiät führte im Ammenstadium zu einer signifikanten Reduzierung der Zellteilungsraten von Stammzellen in der Darmwand. Die Ernährungsqualität scheint die Darmwand zu beeinflussen. Am Ausgeprägtesten waren jedoch die Unterschiede bei der Brutmenge zwischen den drei Völkern. Dies könnte darauf hinweisen, dass die demografische Plastizität eines Bienenvolkes stärker ist als die individuelle physiologische Plastizität.

Intestinale Stammzellen / Pollen / Ernährung / Demographische Plastizität / Mortalität

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