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RESEARCH ARTICLE

Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.)

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ABSTRACT

Most organisms are constantly faced with environmental changes and stressors. In diverse organisms, there is an anticipatory mechanism during development that can program adult phenotypes. The adult phenotype would be adapted to the predicted environment that occurred during organism maturation. However, whether this anticipatory mechanism is present in eusocial species is questionable because eusocial organisms are largely shielded from exogenous conditions by their stable nest environment. In this study, we tested whether food deprivation during development of the honey bee (*Apis mellifera*), a eusocial insect model, can shift adult phenotypes to better cope with nutritional stress. After subjecting fifth instar worker larvae to short-term starvation, we measured nutrition-related morphology, starvation resistance, physiology, endocrinology and behavior in the adults. We found that the larval starvation caused adult honey bees to become more resilient toward starvation. Moreover, the adult bees were characterized by reduced ovary size, elevated glycogen stores and juvenile hormone (JH) titers, and decreased sugar sensitivity. These changes, in general, can help adult insects survive and reproduce in food-poor environments. Overall, we found for the first time support for an anticipatory mechanism in a eusocial species, the honey bee. Our results suggest that this mechanism may play a role in honey bee queen–worker differentiation and worker division of labor, both of which are related to the responses to nutritional stress.

KEY WORDS: Nutrition, Eusocial species, Anticipatory mechanism, Stress response, Adaptive response, Division of labor, Glucose, Trehalose, Glycogen, Lipid

INTRODUCTION

Most organisms are faced with variable and often stressful environmental conditions (McMillen and Robinson, 2005). Studies in several model organisms have suggested that one strategy to overcome such challenges is to have a mechanism that tailors development to produce an adult phenotype adapted to the conditions that the organism encountered during maturation in anticipation of that condition occurring during its adult stage (Krause et al., 2009; Mitchell et al., 2009; Kuzawa et al., 2010; Lee and Zucker, 1988; Simpson et al., 2001; van den Heuvel et al., 2013). For example, desert locust embryos (*Schistocerca gregaria*) respond to the crowding conditions that their mothers experience (Simpson et al., 2001) by maturing into one of two adult phenotypes that differ in morphology, physiology and behavior (Applebaum and Heifetz, 1999). Each form is adapted to either high- or low-density environments, exhibiting enhanced survival so long as that predicted condition persists (reviewed by Simpson et al., 1999). Similarly, the butterfly *Bicyclus anynana* can develop a phenotype adapted to food scarcity if it experiences food restriction during development (Saastamoinen et al., 2010). In addition, molecular evidence for an advantageous response to environmental anticipation of stresses has been found in metabolic and respiratory networks in bacteria (*E. coli*) and yeast (*S. cerevisiae*) (Mitchell et al., 2009). However, this anticipatory mechanism may be limited in eusocial organisms, which typically experience uniform nest conditions during development. For example, honey bee (*Apis mellifera*) colonies contain thousands of individuals that live in an enclosed nest, where worker bees maintain a stable temperature, humidity and food supply for their nest mates. Any disruptions to this homeostasis, such as nest damage or temperature fluctuations, are handled within minutes to hours (Free, 1967; Fewell and Winston, 1992; Camazine et al., 2001). When food is abundant, surplus is stored and brood rearing increases. In contrast, when food is scarce, the colonies limit food consumption by cannibalizing young brood, ostracizing males and restricting egg production (Atkins et al., 1975). The result is a shielded nesting environment for developing brood that enhances a colony's ability to survive and reproduce. Similar protective mechanisms are present in other eusocial species (Rickard and Lummaa, 2007; Wells, 2007), and may have resulted in a reduced capacity for a developing individual to respond to deleterious environmental conditions in a lasting way.

To determine whether the larvae of a eusocial species are still capable of producing a phenotypic response to developmental stress, which can enhance adult survival under stressful conditions, we examined how maturing honey bees respond to starvation, a common stress in diverse organisms (Scheiner, 1993; Lind and Johansson, 2009; Moczek, 2010), if they experienced it during the larval stage. It has been recognized that early environmental factors have long-term effects on adult honey bees. One recent study reported that larvae brought up in an environment with high levels of aggression exhibited heightened aggression as adults as well as enhanced immune resistance (Rittschof et al., 2015). And it is well known that diet influences caste development in honey bees; third instar larvae fed copious amounts of high-quality food develop a queen phenotype, while those provided a more restrictive feeding diet develop a worker (female helper) phenotype (Haydak, 1970). Moreover, nutritional manipulation of worker-destined fifth instar larvae influences adult worker traits such as body mass and number of ovarioles (ovarian filaments) in the ovary (Hoover et al., 2006; Wang et al., 2014). However, two recent studies showed that seasonal pollen fluctuation and poor nutrition in beekeeping management appear to negatively affect honey bee health and foraging performance (Hoover et al., 2006; Scofield and Mattila,

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2015). Thus, it is still unclear whether honey bees have also retained the capacity to produce an adaptive response to nutritional stress like other solitary animals, after experiencing similar stress during development.

Here, we used an established food-restriction technique (Wang et al., 2014) to test whether briefly starved larvae develop into adult workers more capable of dealing with food scarcity. We first measured adult survival rate during starvation, comparing bees that had been starved versus fed normally (control) during larval development. In addition, we studied adult morphological, physiological and molecular markers potentially connected to stress resilience and energy metabolism (Amdam and Omholt, 2002; Seehuus et al., 2006; Amdam, 2011; Amdam et al., 2012; Wang et al., 2012a). The variables included body mass, ovary size, hemolymph (blood) glucose and trehalose (sugars), circulating juvenile hormone (JH) titers, the expression of central genes in metabolic pathways, food-related behavior (gustatory perception of sugar), and energy reserves, such as glycogen and lipids, in the fat body. The fat body is a metabolic center in insects that is functionally homologous to the mammalian liver and white adipose tissue (Wu and Brown, 2006).

We found evidence of increased starvation resilience in adult bees that experienced starvation during the fifth larval instar. This group of worker bees was characterized by reduced body mass and ovary size, elevated JH titers, increased glycogen in the fat body and a reduced gustatory response to sugar. Overall, this phenotypic shift appears to improve the starvation resilience of the individual bees.

MATERIALS AND METHODS

Bee preparation

Bees were maintained at the Honey Bee Research Laboratory at the Arizona State University Polytechnic Campus (Mesa, AZ, USA). Three wild-type colonies were used as donors for the experiments. Each queen was caged on a wax comb and allowed to lay eggs for 24 h, after which the comb was placed in a separate foster colony. At 180 h after oviposition, half the resulting fifth instar larvae were isolated with two layers of pushing cages for 10 h to prevent them from being fed by nurse bees. The combs were collected just prior to adult emergence (20 days after oviposition), and placed in an incubator set at 34°C and 80% relative humidity where the bees emerged. Exclusion cages were used to prevent starved and untreated bees from intermingling as they emerged as adults from the combs.

From each experimental group (starved, control), half of the <24 h old bees were collected immediately for sample collection. The remaining half were marked on their thoraces with a spot of paint and placed into one of three foster colonies. Six days later, the marked bees were collected from the foster colonies for further experiments and sampling in the laboratory.

Validation of early-starvation effects on body mass and ovariole number

We demonstrated previously that short-term starvation of honey bee larvae at fifth instar produces adults with lower body mass and smaller ovaries than non-starved controls (Wang et al., 2014). In this study, we confirmed that the previous results were replicable.

For each age group (<24 h and 7 day old adult bees) and for each experimental group (starved, control), 20 bees were chilled to immobility before being weighed on a digital scale (VWR). Subsequently, the <24 h old bees were dissected under a stereomicroscope to count the number of ovarioles in both ovaries. Ovariole number was only recorded in <24 h old bees because this trait is established during larval development and does not change during adult life.

Starvation resistance

To test our hypothesis that an advantageous phenotype can be produced in response to the rearing environment, we tracked the survival of mature adult bees (7 day olds) under starvation stress. Adult starvation resistance was tested with a previously published protocol (Wang et al., 2012a). The tested 7 day old bees were kept in small holders that restricted their movement and they were placed in an incubator at 34°C and 80% humidity. Bees were left unfed and survivorship was noted every 3 h for 72 h.

Hemolymph glucose and trehalose titers

Separate sets of <24 h and 7 day old bees were collected from each of the three foster colonies to quantify two major circulating carbohydrates (glucose and trehalose) (Wang et al., 2012a). Hemolymph glucose is a major energy fuel for honey bees, and hemolymph trehalose is a major carbohydrate storage molecule in insects (Wang et al., 2012a).

All bees were anesthetized by chilling before the procedures. A 1 µl hemolymph sample was collected from each individual bee. Glucose content was measured by adding 400 µl glucose reagents that include hexokinase and glucose 6-phosphate dehydrogenase (Sigma-Aldrich). The mix was incubated for 15 min at 37°C. Using a spectrophotometer (Bio-Rad xMARK Microplate spectrophotometer), absorbance at 340 nm (A_{340}) was measured to determine total glucose in each sample. After measuring glucose titer, the same samples were used to measure trehalose (Broughton et al., 2005) by adding the enzyme trehalase (Sigma-Aldrich) to a final concentration of 0.05 U ml^{-1}. The resulting solution was incubated at 37°C overnight followed by a second reading of A_{340}. The amount of glucose produced from trehalose was calculated by subtracting the amount in the first reading from that in the final reading. The amount of trehalose was estimated using the equation: trehalose (µg)=glucose (µg)×342.3/(180.2×2) (Wang et al., 2012a; Hartfelder et al., 2013). Three replicates were tested for each sample. The final concentrations were determined by reference to standard curves (Hartfelder et al., 2013).

Glycogen and lipid reserves in fat bodies

After hemolymph was collected for the carbohydrate analyses, the fat body (eviscerated abdomen, with intestine, ovaries and sting apparatus removed, leaving cuticle and attached fat bodies) was dissected out of each individual bee (either <24 h or 7 days old), then flash-frozen in liquid nitrogen. The tissue was used for measuring glycogen and triglycerides. Glycogen is the major carbohydrate reserve and triglycerides constitute the primary (~90%) form of stored lipids in the fat body (Arrese and Wells, 1997). Glycogen and triglycerides in the fat body can be enzymatically broken down into free glucose, glycerol and fatty acids, which can be determined using modified versions of previously described methods (Arrese and Wells, 1997; Iijima et al., 2009; Palanker et al., 2009) in which the total protein content is used to normalize the glycogen and triglyceride levels.

Each fat body sample was homogenized in 200 µl of a glycogen assay buffer containing 10 mmol l^{-1} KH_2PO_4 and 1 mmol l^{-1} EDTA (pH 7.4). The homogenates were heated at 75°C for 5 min to degrade general enzymes before being centrifuged at 2000 rpm at 4°C for 10 min. The supernatants were transferred into another set of tubes. Total protein level was measured using the Quick-Start Bradford Reagent (Sigma-Aldrich), and BSA was used as a standard (Bio-Rad). The A_{540} was read using a spectrometer (Bio-Rad).

For measuring glycogen, we adopted and slightly revised a widely used method in *Drosophila* (Iijima et al., 2009). Two sets of

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5 µl of the homogenate from each fat body were used: one was incubated with 400 µl of glucose reagents (Sigma-Aldrich) at 37°C and the other was incubated with 1 U amyloglucosidase and 400 µl of glucose reagents at 37°C. After 30 min of incubation, we measured the A_{540}. Each homogenate reaction had three replicates on a microplate. Final concentrations of glycogen plus glucose, and glucose, were determined from standard curves using the average absorbance from three replicates. The amount of glycogen was calculated by subtracting the amount of glucose in the first set of homogenates from the glycogen plus glucose of the second set, and was normalized to the amount of protein from the same sample.

For measuring triglycerides, we also adopted and slightly revised a well-established method from *Drosophila* research (Palanker et al., 2009). Two sets of 10 µl homogenate from each fat body were used as for glycogen measurement: the homogenate from one set was incubated with 25 µl PBS at 37°C and the homogenate from another set was incubated with 25 µl triglyceride reagent (Sigma-Aldrich) for 30 min at 37°C. Each sample was then incubated with 200 µl Free Glycerol Reagent (Sigma-Aldrich) for 5 min at 37°C. The A_{540} was recorded as above. The triglyceride level was determined by subtracting the amount of free glycerol in the PBS-treated samples from the total glycerol from triglycerides and free glycerol in the samples treated with triglyceride reagent, then normalized to the amount of protein.

Total lipid content

We also measured the total lipid level of the fat body because we previously found it to be correlated with honey bee metabolism and behavioral physiology (Wang et al., 2012a). A separate set of fat body samples was collected from both <24 h and 7 day old bees for measuring the total lipid content of the fat body. All bees were anesthetized by chilling before the procedure. Each fat body sample was freeze-dried, homogenized in a 2:1 chloroform:methanol solution and dried down to a final volume 200 µl. A lipid assay was carried out using 100 µl of each sample following the protocol of Toth and Robinson (2005). Three replicates were performed for each sample. A_{525} was measured and the average absorbance reading of each sample was converted to mg using a curve generated from a cholesterol standard mix.

JH measurement

JH is a central regulator of insect development and reproduction (Sheng et al., 2011; Jindra et al., 2013). In honey bees, JH has classical functions in larval and pupal development and has specific functions in ovary size differentiation between queens and workers (Capella and Hartfelder, 2002). In addition, JH influences social behavior (Huang et al., 1991) and affects energy metabolism in adult worker bees (Wang et al., 2012a).

JH was titered using a highly specific and sensitive GC-MS method (Bergot et al., 1981; Shu et al., 1997) previously used in honey bees (Amdam et al., 2010). Prior to hemolymph collection, bees were anesthetized on ice for 5 min and placed in a harness on a beeswax-covered plate under a dissecting scope. Hemolymph was collected with calibrated 1 µl micropipettes after puncturing the abdomen between the third and the fourth tergite with a sterile needle, avoiding sample contamination by tissue fragments and foregut content. For each sample, a total volume of 1 µl of hemolymph pooled from three adult bees was collected into individual glass vials containing 200 µl 50% acetonitrile (HPLC grade) and then stored at −80°C until analysis. The samples were extracted three times with 500 µl hexane (HPLC grade). The hexane fractions were recombined in a clean vial and dried down by vacuum centrifugation. The residue was washed out of the vials with three rinses of 300 µl hexane and added to glass columns filled with aluminium oxide for purification. Filtered samples were derivatized by incubation at 60°C in 5% trifluoroacetic acid/95% methyl-d_3 alcohol-d. Derivatized JH was then analyzed using a 7890A Series GC (Agilent Technologies) equipped with a 30 m×0.25 mm Zebron ZB-WAX column (Phenomenex) coupled to a 5975C inert mass selective detector. Helium was used as a carrier gas. Samples were analyzed using the MS SIM mode monitoring at *m*/*z* 76 and 225 to ensure specificity for the d_3-methoxyhydrin derivative of JHIII, the known JH isomer for *A. mellifera* (Hagenguth and Rembold, 1978). Total abundance was quantified against a standard curve of derivatized JH III. The detection limit of the assay is approximately 1 pg.

Gene expression analysis

Our observations of changed starvation resilience, glycogen stores and JH titers led us to investigate genes that could contribute to the phenotypic shift. Insulin/insulin-like peptides (AmILP1 and AmILP2) and their putative receptors (InR1 and InR2) can be involved in the regulation of glucose metabolism in adult worker bees (Ament et al., 2008; Wang et al., 2012a). Adipokinetic hormone (AKH) and its receptor (AKHR) play central roles in lipid metabolism in insects and they may also be involved in glucose metabolism in honey bee workers (Ament et al., 2008). Vitellogenin (Vg) is a nutrient-sensitive protein involved in honey bee reproduction, somatic maintenance and feeding behaviors, and levels of *vitellogenin* (*vg*) mRNA are correlated with adult nutrition (Di Pasquale et al., 2013). Tyramine is a neurotransmitter (biogenic amine) that can regulate glycogen metabolism via its receptors (TYR1) in insects (Roeder, 2005). The expression of these genes (*AmILP1*, *AmILP2*, *InR1*, *InR2*, *AKH*, *AKHR*, *vg* and *TYR1*) was measured in the adult fat body, contrasting individuals starved and not starved as larvae.

Another set of abdominal samples (fat bodies) without internal organs from <24 h and 7 day old bees were flash-frozen and stored in −80°C. After thawing and homogenization in TRIzol reagent (Invitrogen), RNA was extracted following the TRIzol manufacturer's instructions. The quality and quantity of RNA was determined by spectrophotometry (GE Healthcare). DNase (DNA-free Kit, Applied Biosystems, Bedford, MA, USA) was added to total RNA to remove trace DNA contaminants and 1 µg of treated RNA was used for reverse transcription following an established method (Wang et al., 2009) using TaqMan® Reverse Transcription Reagents (Applied Biosystems).

First-strand cDNA was used for real-time quantitative PCR (RT-qPCR) assays. Before performing the RT-qPCR, PCR amplicons from each gene were sequenced to validate the specificity of the primers (Table S2). A dilution series of cDNA was used to establish standard curves for each gene and amplification efficiencies were calculated based on an established method (Livak and Schmittgen, 2001; Pfaffl, 2001). After verifying that candidate genes and actin primers had similar amplification efficiencies, 15 samples were randomly picked from each treatment group for expression analysis. Each biological sample was run in technical triplicate on an ABI Prism 7500 Real-Time PCR system (Applied Biosystems) for measuring candidate gene transcript levels, in comparison with those of the reference gene *actin* by means of the ΔΔCt method (Livak and Schmittgen, 2001). Studies have shown that *actin* is stably expressed during honey bee development (Lourenco et al., 2008; Reim et al., 2012) as well as in adults (Cameron et al., 2013). Therefore, *actin* is commonly used as a reference gene in studies of honey bee gene expression (Scharlaken et al., 2008; de Azevedo and

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Hartfelder, 2008; Lourenco et al., 2008; Wang et al., 2009). RT-qPCR conditions were used as described in other studies (Wang et al., 2009, 2010a,b). By monitoring negative control samples (without reverse transcriptase) and using a melting curve analysis, we verified that the RT-qPCR assays were not confounded by DNA contamination or primer dimers (Vandesompele et al., 2002).

Gustatory response to sugar

Lastly, we measured gustatory perception of sugar (Wang et al., 2010b, 2012a, 2014). We used an established proboscis extension response (PER) test (Wang et al., 2013a,b). We collected <24 h and 7 day old adult bees in the morning and placed them individually in cylindrical mesh cages. Each bee was chilled until it showed the first signs of immobility. It was then mounted in a metal holder and fixed with two strips of adhesive tape between the head and thorax and over the abdomen (Bitterman et al., 1983). After 1 h, gustatory responsiveness was tested using the established PER method (Scheiner, 2004; Wang et al., 2010b, 2012a, 2013a,b, 2014). The bee was tested by touching both antennae with a droplet of water followed by a concentration series of 0.1, 0.3, 1, 3, 10 and 30% sucrose. The recorder was blind to the treatment identity of the bees. The inter-stimulus interval was 5–7 min, varying with the number of individuals tested at one time. Usually, 40–60 bees were tested per trial run. A bee was noted as 'responding' whenever it fully extended its proboscis after antennal contact with water or sucrose solution. Gustatory response scores ranged between 0 (no response to water or any of the sucrose solutions) and 7 (response to all solutions). The sum of the responses to the entire solution series was used to calculate the gustatory response score (GRS) (Scheiner, 2004). After all sucrose solutions had been tried, each bee was exposed to a droplet of honey. If it did not respond to honey, the GRS results from that bee were excluded.

Statistics

Gene expression data were log transformed to approximate normality (Wang et al., 2009) as verified by Bartlett and Levene's homogeneity test. Except for survival scores and the GRS data, the datasets of our study conformed to assumptions of normality without transformation. Student's *t*-tests were applied to the normally distributed data in the comparisons between starvation and control groups. We used a one-tailed test for *TYR1*. We expected *a priori TYR1* down-regulation because of a consistent correlation between ovariole number and *TYR1* mRNA levels in other studies (Wang et al., 2012b; Y.W., G.V.A. and R.E.P., unpublished data).

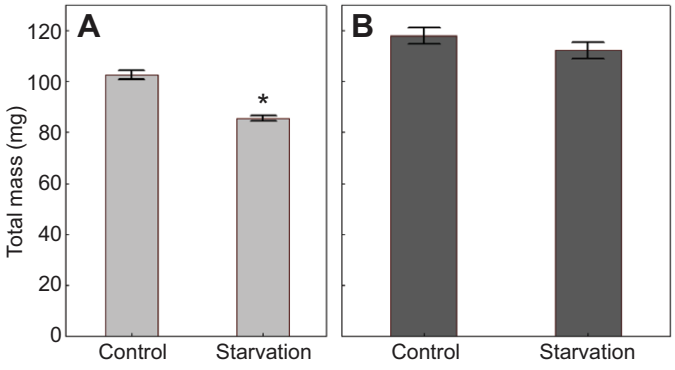

Fig. 1. Effect of larval starvation on the body mass of honey bees. (A) Newly emerged workers (<24 h old). (B) Mature (7 day old) workers. The asterisk indicates a statistically significant difference between groups (P<0.05). Bars represent means±s.e.

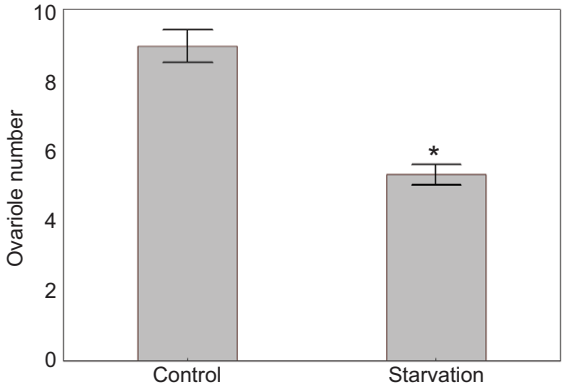

Fig. 2. Effect of larval starvation on ovariole number in newly emerged (<24 h) adult worker bees. The asterisk indicates a statistically significant difference between groups (P<0.05). Bars represent means±s.e.

For the survival analysis, comparisons of life spans between the bees that experienced early starvation and controls were conducted with Cox's *F*-test. The GRS data were analyzed with the non-parametric Mann–Whitney *U*-test. Analyses were performed using STATISTICA 10.0 (StatSoft).

RESULTS

Validation of methodology

We first confirmed that larval starvation reduced adult body mass when measured in adults <24 h old (Student's *t*-test: t=8.4430, N=40, P<0.0001; Fig. 1A). Furthermore, we documented for the first time that this difference later disappears: no significant difference was found in the body mass of 7 day old bees (Student's *t*-test: t=0.3014, N=51, P=0.1961; Fig. 1B). This change might be due to compensatory mechanisms at the level of behavior and/or physiology. Second, we verified that starvation treatment led to a significant reduction in ovary size, as determined by the ovariole number (Student's *t*-test: t=6.6472, N=66, P<0.0001; Fig. 2). Although worker bees usually do not reproduce, their ovaries and ovariole number influence metabolic biology and food-related behavior (Wang et al., 2009, 2010a, 2012b).

Effect of early food deprivation on starvation resistance

We compared the starvation resistance of bees that had experienced food deprivation during the fifth instar with that of controls

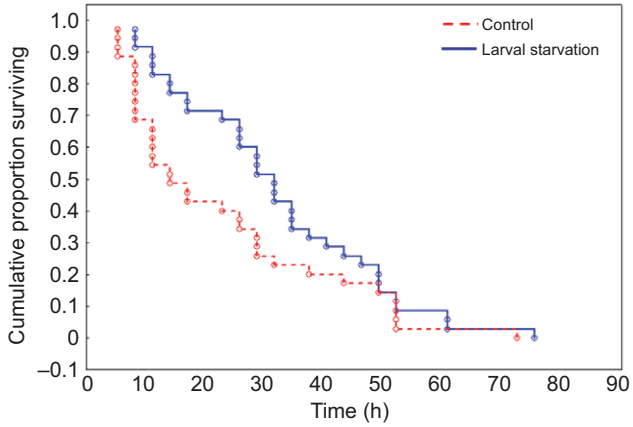

Fig. 3. Effect of larval starvation on the survivorship of 7 day old adult worker bees. The adult bees were subjected to food deprivation during the survival experiment.

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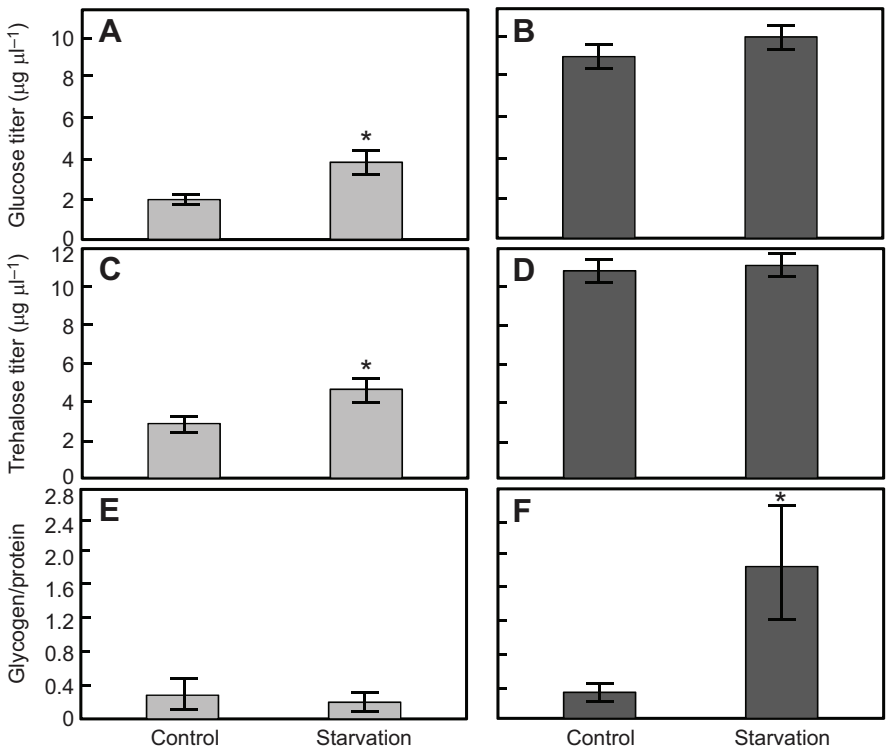

Fig. 4. Effect of larval starvation on glucose and trehalose titers in the hemolymph and glycogen level in the fat body in honey bees. (A,C,E) Newly emerged (<24 h old) worker bees. (B,D,F) Mature (7 day old) worker bees. The amount of glycogen was normalized to the total protein content of each sample. The asterisk indicates a statistically significant difference between groups (P<0.05). Bars represent means±s.e.

that received normal nutrition. We found that those adult individuals that experienced starvation during development were more resilient to starvation compared with the controls (Cox's F-test: N=35, P=0.0441; Fig. 3).

Effect of early food deprivation on metabolic physiology

Hemolymph glucose and trehalose titers

Starvation during development was associated with elevated glucose levels (Student's t-test: t=3.2550, N=36, P=0.0064; Fig. 4A) as well as increased trehalose titer in <24 h old adults (Student's t-test: t=4.4778, N=36, P=0.0096; Fig. 4C). This difference in sugar titers between controls and treated bees disappeared during adult maturation, so that no significant differences could be measured in 7 day old bees (Student's t-test: glucose, t=−1.0478, N=30, P=0.2991; trehalose, t=−0.0434, N=30, P=0.9655; Fig. 4B,D). These results suggest a metabolic impact of larval starvation that could be compensated for with adult nutrition.

Fat body glycogen and lipid levels

In adult bees <24 h old, we found no difference between the starved and control groups in the levels of glycogen (Student's t-test: t=1.0166, N=12, P=0.3195; Fig. 4E), glycerol (Student's t-test: t=0.3945, N=12, P=0.7042; Fig. S1A), triglycerides (Student's t-test: t=4.3631, N=12, P=0.1861; Fig. S1C) and total lipids (Student's t-test: t=0.5223, N=12, P=0.6066; Fig. S1E). There were similarly no differences among mature 7 day old bees in the levels of glycerol (Student's t-test: t=0.2901, N=13, P=0.7835; Fig. S1B), triglycerides (Student's t-test: t=1.0614, N=13, P=0.3032; Fig. S1D) or total lipids (Student's t-test: t=1.5534, N=16, P=0.1327; Fig. S1F). However, larval starvation led to significantly higher levels of glycogen in the 7 day old adult bees (one-tailed Student's t-test: t=−1.6812, N=16, P=0.0503; Fig. 4F). These elevated glycogen levels may provide one possible explanation for the starvation resilience of the adults starved as larvae.

Effect of early food deprivation on hemolymph JH titer

JH titers were significantly increased in adult bees that were starved during the fifth larval instar. This difference was maintained in <24 h old (Student's t-test: t=−3.93, N=12, P=0.0010; Fig. 5A) and 7 day old adult bees (Student's t-test: t=−8.4661, N=9, P<0.0001; Fig. 5B). These data show that starvation during the last larval instar had significant impacts on adult endocrine physiology that could not be mitigated during adult maturation.

Fat body gene expression associated with early food deprivation

The insulin/insulin-like pathway genes, the AKH pathway genes and *vg* were not differently expressed at <24 h and 7 days (Figs S2–S5 and Table S1). Yet, the bees that experienced starvation during development had significantly lower *TYR1* levels than the controls when measured at <24 h (one-tailed Student's t-test, t=−1.80,

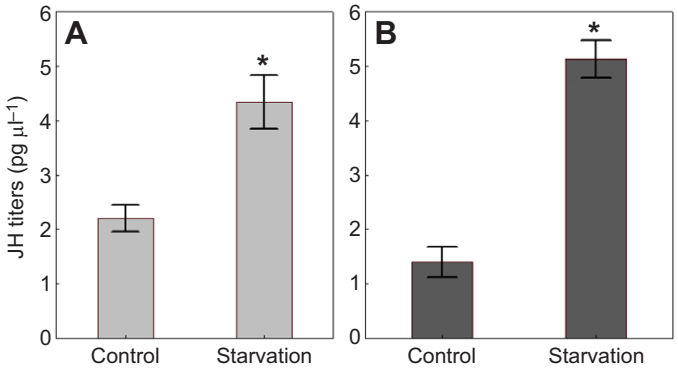

Fig. 5. Effect of larval starvation on juvenile hormone (JH) titers in the hemolymph of honey bees. (A) Newly emerged (<24 h old) workers. (B) Mature (7 day old) workers. The asterisk indicates a statistically significant difference between groups (P<0.05). Bars represent means±s.e.

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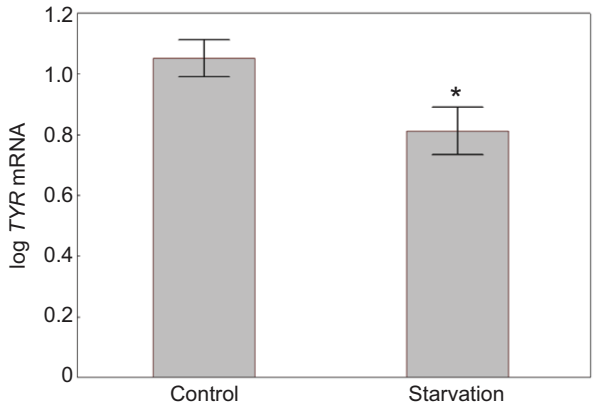

Fig. 6. Effect of larval starvation on the expression of the *tyramine receptor 1* (*TYR1*) gene in newly emerged (<24 h) adult worker bees. The fold change in mRNA was measured by real-time quantitative PCR (RT-qPCR). The asterisk indicates a statistically significant difference between groups (P<0.05). Bars represent means±s.e.

N=16, P=0.0412; Fig. 6). Here, we were able to use a one-tailed test for *TYR1* because we had *a priori* expectations of a reduction in *TYR1* expression in the bees that experienced starvation during development. Previously, we found that the bees with fewer ovarioles had less *TYR1* mRNA (Wang et al., 2012b; Y.W., G.V.A. and R.E.P., unpublished data). This transient difference in *TYR1* expression coincided with a difference between the groups in hemolymph glucose and trehalose titers at <24 h (see above).

Effect of early food deprivation on gustatory response

At the adult age of <24 h, there was no significant difference in gustatory perception of sugar between bees that were starved during development and controls (Mann–Whitney U-test: U=1944, N=65–67, P=0.2890; Fig. 7A). However, at 7 days of age, the treated bees showed reduced sugar sensitivity (Mann–Whitney U-test: U=474, N=38–41, P=0.0028; Fig. 7B). This result suggests that the mature feeding behavior of honey bee workers can be affected by developmental food restriction.

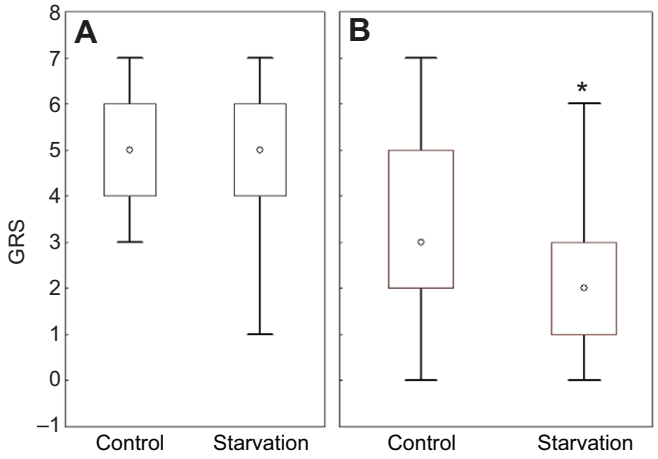

Fig. 7. Effect of larval starvation on gustatory perception of honey bees. (A) Newly emerged (<24 h old) workers. (B) Mature (7 day old) workers. Gustatory response score (GRS) indicates the level of gustatory perception of adult workers. The asterisk indicates a statistically significant difference between groups (P<0.05). The central rectangle spans 25–75% quantiles. The circle inside the rectangle indicates the median and the bars above and below the box show the non-outlier range. The asterisk indicates a statistically significant difference between groups (P<0.05).

DISCUSSION

Adaptive responses to an anticipatory starvation

Our objective was to determine whether and how members of a eusocial species, in this study the honey bee, can produce an adult phenotype that is adapted to survive better under adverse conditions to which it was exposed during its developmental stage. We exposed fifth instar larvae to short-term food restriction and found that those individuals survived longer than controls during subsequent starvation stress as adults. Additionally, differences in morphology, metabolism, endocrinology, gene expression and sucrose responsiveness between adults that were reared in stressed and non-stressed environments suggest that adaptive phenotypic changes are induced at the physiological, molecular and behavioral levels.

Consistent with our previous results (Wang et al., 2014), our study verified that food restriction during the last larval stage reduced the body mass of <24 h old bees. In addition, the bees that experienced starvation during development gained more mass during the first days of adult life, attaining the same mass as controls at 7 days. Their ‘catch-up’ growth might be explained by greater rates of food consumption or reduced metabolic rates. Correspondingly, prenatal malnutrition in mammals leads to the birth of underweight offspring, followed by catch-up growth in subsequent development. Human infants that are exposed to this scenario have increased risk of metabolic disorder in adulthood (Ozanne and Hales, 2004; Cameron et al., 2005; Ibanez et al., 2009, 2010).

We have repeatedly found that workers starved as fifth instar larvae developed fewer ovarioles (Wang et al., 2014; this paper). In mammals, under-nutrition or over-nutrition during the prenatal or postnatal period can impact adult reproductive capability (reviewed by Gardner et al., 2009), which may reflect an adaptive adjustment of reproductive strategy (Rae et al., 2002; Rhind, 2004; Jasienska et al., 2006; Kuzawa et al., 2010). In general, a strategy of reducing reproductive investment while increasing somatic maintenance is a typical survival mechanism in insects (Elkin and Reid, 2005; García-Roger et al., 2006).

Our studies of metabolic physiology also revealed that 7 day old bees store more glycogen in the fat body if they experienced starvation as larvae. In insects, glycogen and lipid stores in the fat body satisfy regular biological demands and are utilized under stress such as flight, starvation and temperature changes (Suarez et al., 1996; Harrison and Roberts, 2000; Arrese and Soulages, 2010). In honey bees, glycogen is a major fuel during flight and other stressful activities (Suarez et al., 1996, 2005; Harrison and Roberts, 2000). Therefore, we speculate that the increased glycogen stores of the 7 day old bees that experienced starvation as larvae enabled these individuals to survive longer when starved as adults.

Starvation of fifth instar larvae also changed the endocrine physiology of the adult bees, as shown by a lasting effect on worker JH titer. JH commonly increases when insects are exposed to diverse stressors such as injury, starvation, temperature changes, and chemical and mechanical challenges (reviewed by Janković-Hladni, 1991), although this response is usually transient. Studies in the fruit fly *Drosophila melanogaster* indicate that elevated JH levels can enhance stress resistance, at least in the short term (Gruntenko et al., 1996, 2003, 2010). The role of JH in the stress response of honey bees is less clear (Even et al., 2012), but various stressors (temperature changes, experimental handling and

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transitioning to activities outside the protective nest) induce or are associated with increased JH levels (Sullivan et al., 2000; Lin et al., 2004; Page, 2013). It remains to be tested whether the changes in JH that we observed in this study can contribute to starvation resilience.

We investigated metabolic gene pathways to reveal possible mechanisms to explain the post-starvation changes to the adult phenotype that we observed. In solitary insects, insulin/insulin-like signaling and AKH pathways regulate carbohydrate and lipid metabolism (Oldham et al., 2000; Brogiolo et al., 2001). In honey bees, insulin/insulin-like signaling and AKH may regulate carbohydrate metabolism (Wang et al., 2012a). Surprisingly, the expression of candidate genes in these two pathways was not altered in adults exposed to starvation during the larval stage. Instead, a neurotransmitter (biogenic amine) receptor, *TYR1*, was down-regulated in the fat body. Tyramine (TA) is a biological precursor of octopamine (OA), and both these biogenic amines cooperatively and also independently affect glycogenolysis, behavior, reproduction and stress resistance in various insect species (Candy, 1978; Downer, 1979; Mentel et al., 2003; Roeder, 2005). In honey bees, OA and TA levels in the brain appear to influence locomotory behavior (Fussnecker et al., 2006), gustatory perception (Scheiner et al., 2014a) and foraging preference (Scheiner et al., 2014b). We have recently uncovered associations between *TYR1* expression in the fat body, ovariole number (Wang et al., 2012b), gustatory perception and foraging behavior (Y.W., G.V.A. and R.E.P., unpublished data). Furthermore, some evidence suggests that regulation of stress responses in honey bees is influenced by relationships between OA, TA and JH (Even et al., 2012), and that OA mediates the effect of stress on worker bee social behavior (Schultz and Robinson, 1999; Barron et al., 2002; Page and Erber, 2002; Spivak et al., 2003; Barron et al., 2007; Giray et al., 2007). Summarizing these data, we speculate that the changed adult expression level of *TYR1*, which we observed after larval starvation, takes part in a regulatory response that involves reproductive signaling systems, endocrine hormones, energy metabolism and stress responses, thereby converging on our primary outcome variable: starvation stress resilience.

The cumulative data, furthermore, suggest that the regulatory response that confers phenotypic plasticity to starvation involves the foraging behavior of the adult worker bees. Indeed, we found that larval starvation impacted adult feeding behavior, measured as the gustatory perception of sugar. Gustatory perception is critical for animal survival, perhaps especially in a low-nutrient environment (Linford et al., 2015), as it helps an organism locate energy-rich food sources in order to maintain adequate nutrition (Drewnowski, 2000; Rohwedder et al., 2012). In our study, we observed reduced gustatory perception in the adult bees that experienced starvation as larvae. This altered perception implies that they preferentially consume richer sugar syrups than controls. In a related manner, adult rats that experience food restriction during development exhibit hyperphagia (Vickers et al., 2000) and prefer high-fat food (Bellinger et al., 2004), which promotes rapid mass gain and excess storage of endogenous resources. Perhaps similarly, the bees that experienced larval starvation in our experiments developed into adults that preferred rich sugar syrups, gained more mass during the first days of adult life and stored more glycogen. Overall, these results suggest that many effects of developmental nutritional stress are shared between phylogenetically diverse species.

Although our study did not investigate foraging behavior, a recent study showed that bees with poor larval nutrition foraged earlier in life than controls (Scofield and Mattila, 2015). In general, early foraging is an acute adaptive response of the colony as a social unit to deal with different stressors (Perry et al., 2015) such as a loss of foragers (Sekiguchi and Sakagami, 1966; Robinson et al., 1994; Huang and Robinson, 1999), an increase in food demand, colony starvation (Schulz et al., 1998; Janmaat and Winston, 2000; Toth and Robinson, 2005), a reduction of foraging resources in the field (Atkins et al., 1975; Leimar et al., 2012; Free, 1961), wax deprivation (Fergusson and Winston, 1988) and disease (Janmaat and Winston, 2000; Higes et al., 2008; Woyciechowski and Moron, 2009; Goblirsch et al., 2013). By expediting the transition to foraging, the colony can rapidly compensate for any losses to the foraging forces to enhance food collection (Winston, 1987; Giray et al., 2007; Perry et al., 2015). This behavioral response may serve as evidence for an adaptive response at the colony level to nutritional deprivation during larval development.

Nutritional stress in larvae and worker performance

Honey bees are crucial pollinators in US agriculture and their recently declining population is a growing public concern. There is a consensus that poor nutrition synergistically acts with other environmental stressors, such as pesticides, global weather changes, parasites and pathogens, to undermine colony performance (Huang, 2012; van Dooremalen et al., 2013). Therefore, understanding how honey bees respond to nutritional stress is of great importance in enhancing honey bee health and performance. A recent study suggested that larval nutritional deprivation caused foraging deficits in workers (Scofield and Mattila, 2015), an apparent contradiction to our results. However, this study did not control for adult nutritional condition. A hypothesis called the 'predictive adaptive response' (PAR) points out that a phenotypic response to developmental stressors or perturbation can be adaptive if the adult environment matches the developmental environment; otherwise, the response is maladaptive (Gluckman et al., 2005, 2007). In further support of this anticipatory mechanism in honey bees, a recent study suggests that the physiology and performance of adult honey bees are affected by the interaction between larval nutrition and adult nutrition (Hoover et al., 2006). Our study further demonstrates the importance of considering both developmental and adult environments when we study stress responses.

Stress response and division of labor in honey bees

Our study shows a link between larval stress and adaptive adult morphology, physiology and behavior. Similar responses to developmental food deprivation have been found throughout the animal kingdom (Lee and Zucker, 1988; Applebaum and Heifetz, 1999; Simpson et al., 1999; Krause et al., 2009; Mitchell et al., 2009; Kuzawa et al., 2010; van den Heuvel et al., 2013). Our study also suggests that mechanisms involved in the responses to larval nutritional stress imposed by nurse bees (young adult workers that feed the larvae) play a key role in honey bee division of labor (Page, 2013).

In honey bees, the quality and quantity of food ingested by female larvae is controlled by nurse bees. Food intake results in the differential development of adult queens or workers (Atkins et al., 1975; Page, 2013). When a female larva is fed *ad libitum* with higher quality food throughout development, she becomes a queen with about 200 ovarioles and produces eggs throughout her adult life. When a female larva receives a restricted diet of lower quality, she becomes a worker that usually has fewer than 20 ovarioles, is facultatively sterile, and is responsible for feeding brood, nest construction and maintenance, colony defense and foraging for food for the colony. In addition, workers exhibit an age-related division

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of labor (Atkins et al., 1975; Page, 2013) in which younger workers (nurses) feed larvae in the nest, while older workers (foragers) forage outside and have a bias for collecting nectar or pollen. Nurse bees control the amount of food delivered to worker-destined larvae. Enhancing food availability increases the number of ovarioles comprising the ovaries (Page, 2013), and workers with more ovarioles exhibit higher gustatory responsiveness (Tsuruda et al., 2008). Our findings that larval food restriction reduced worker ovariole number and gustatory response are consistent with the associations found in the previous studies (Page, 2013; Tsuruda et al., 2008), suggesting that the linkage between developmental nutritional stress, responses of ovarian function, and behavioral physiology may have been co-opted during the evolution of sociality and used to affect division of labor.

Furthermore, although honey bee queens and workers have the same genetic makeup, they experience divergent nutritional environments during both development and adulthood. These differences ultimately determine their phenotype and social role (Winston, 1987; Page, 2013). Queen-destined larvae are unrestrictedly fed with a high-quality diet through their whole larval stage. In contrast, worker-destined larvae are fed a less nutritious diet that is restricted during early development, and then they are starved during the last larval stage (Atkins et al., 1975; Page, 2013). As adults, a queen is fed protein-rich royal jelly *ad libitum* by nurses, but the major food for a forager is nectar or honey, and a worker bee is under nutritional stress when foraging outside (Atkins et al., 1975; Page, 2013). Larval nutrition (Page, 2013) and the insulin and the target of rapamycin (TOR) nutritional pathways play central roles in queen–worker differentiation (Patel et al., 2007; Mutti et al., 2011; Wang et al., 2013a,b). Adult nutritional status (Toth et al., 2005; Toth and Robinson, 2005), insulin pathway and AKH pathway play significant parts in determining the social roles of queens and workers and the resultant division of labor (Corona et al., 2007; Ament et al., 2008, 2010, 2011a,b; Wang et al., 2010a,b, 2012a,b). The overlap between these regulatory mechanisms further supports the potential for an anticipatory mechanism that regulates development to adapt to a variable nutritional environment being co-opted to produce the behavioral and physical castes that make honey bees more adaptive to the environment at the colony level.

Long-term effect of larval starvation on worker behavioral physiology

Vg is a yolk protein precursor and its level in the hemolymph is correlated with ovariole number (Amdam et al., 2004): workers with more ovarioles generally have a higher Vg titer than workers with fewer ovarioles. Vg is in a feedback loop with JH, regulating worker division of labor (Amdam and Omholt, 2003; Amdam et al., 2004, 2006a): nurse bees have a high level of Vg and a low level of JH in the hemolymph, and then Vg rapidly reduces and JH rises, which initiates worker foraging. Vg knockdown amplifies JH titer (Nelson et al., 2007; Wang et al., 2012a,b), resulting in an increase in glucose responsiveness (Amdam et al., 2006b; Wang et al., 2012a), early onset of foraging and a foraging bias for nectar (Nelson et al., 2007). Topical application of a JH analog, methoprene, to young workers increases sucrose responsiveness (Pankiw and Page, 2003), and JH paces behavioral development in workers, as is evident by its ability to induce an earlier onset of foraging (Jaycox et al., 1974; Jaycox, 1976; Fahrbach and Robinson, 1996; Sullivan et al., 2000, 2003; Chang et al., 2015). In our study, the bees that experienced larval starvation had fewer ovarioles, higher hemolymph JH titer and lower sucrose responsiveness, but had no change in Vg mRNA. These results confirm the occurrence of a previously identified disconnection between Vg and JH (Ihle et al., 2010), and suggest that the link between JH and sucrose responsiveness may also be broken. The correlation between ovariole number and sucrose responsiveness still held, which emphasizes the critical role of ovaries in determining worker social behavior (Amdam et al., 2006a; Wang et al., 2010a). Our results also make it clear that understanding the full complexity in the relationships between ovary development, Vg, JH and sucrose responsiveness in workers will require a thorough study of the changes to these factors during the course of development and adult maturation.

Conclusions

We found for the first time support for an anticipatory mechanism in a eusocial species, the honey bee. An adaptive response was revealed after nutritional deprivation during a late larval stage, resulting in complex changes in the adult phenotype and culminating in increased starvation resilience. Our findings extend knowledge of the mechanisms underlying long-term responses to early environmental conditions. Moreover, our study suggests that predictive stress responses may play a role in division of labor in honey bees.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Y.W. developed the general concept and approaches in this study. G.V.A. and R.E.P. provided helpful comments. O.K. prepared honey bee colonies and performed the starvation treatment on larvae. C.S.B. measured JH titers and Y.W. performed all other tests and assays. Y.W. analyzed data and prepared the paper which C.S.B., R.E.P. and G.V.A. edited prior to submission.

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Supplementary information

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References

Amdam, G. V. (2011). Social context, stress, and plasticity of aging. *Aging Cell* **10**, 18-27.

Amdam, G. V. and Omholt, S. W. (2002). The regulatory anatomy of honeybee lifespan. *J. Theor. Biol.* **216**, 209-228.

Amdam, G. V. and Omholt, S. W. (2003). The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. *J. Theor. Biol.* **223**, 451-464.

Amdam, G. V., Norberg, K., Fondrk, M. K. and Page, R. E., Jr. (2004). Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. USA* **101**, 11350-11355.

Amdam, G. V., Csondes, A., Fondrk, M. K. and Page, R. E., Jr. (2006a). Complex social behaviour derived from maternal reproductive traits. *Nature* **439**, 76-78.

Amdam, G. V., Norberg, K., Page, R. E., Jr, Erber, J. and Scheiner, R. (2006b). Downregulation of vitellogenin gene activity increases the gustatory responsiveness of honey bee workers (*Apis mellifera*). *Behav. Brain Res.* **169**, 201-205.

Amdam, G. V., Page, R. E., Jr, Fondrk, M. K. and Brent, C. S. (2010). Hormone response to bidirectional selection on social behavior. *Evol. Dev.* **12**, 428-436.

Amdam, G. V., Fennern, E. and Hauvukainen, H. (2012). Vitellogenin in honey bee behavior and lifespan. In *Honeybee Neurobiology and Behavior*, Chapter 12 (ed. C. G. Galizia, D, Eisenhardt and M. Giurfa), pp. 17-30. Dordrecht: Springer.

Ament, S. A., Corona, M., Pollock, H. S. and Robinson, G. E. (2008). Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proc. Natl. Acad. Sci. USA* **105**, 4226-4231.
Ament, S. A., Wang, Y. and Robinson, G. E. (2010). Nutritional regulation of division of labor in honey bees: toward a systems biology perspective. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2**, 566-576.
Ament, S. A., Velarde, R. A., Kolodkin, M. H., Moyse, D. and Robinson, G. E. (2011a). Neuropeptide Y-like signalling and nutritionally mediated gene expression and behaviour in the honey bee. *Insect Mol. Biol.* **20**, 335-345.
Ament, S. A., Chan, Q. W., Wheeler, M. M., Nixon, S. E., Johnson, S. P., Rodriguez-Zas, S. L., Foster, L. J. and Robinson, G. E. (2011b). Mechanisms of stable lipid loss in a social insect. *J. Exp. Biol.* **214**, 3808-3821.
Applebaum, S. W. and Heifetz, Y. (1999). Density-dependent physiological phase in insects. *Annu. Rev. Entomol.* **44**, 317-341.
Arrese, E. L. and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* **55**, 207-225.
Arrese, E. L. and Wells, M. A. (1997). Adipokinetic hormone-induced lipolysis in the fat body of an insect, Manduca sexta: synthesis of sn-1,2-diacylglycerols. *J. Lipid Res.* **38**, 68-76.
Atkins, E. L., Banker, R., Butler, C. G., Cale, G. H., Cale, G. H., Jr, Crane, E. and Dadant, C. C. (1975). *The Hive and the Honey Bee*. Hamilton, IL: Datant & Sons.
Barron, A., Schulz, D. and Robinson, G. (2002). Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **188**, 603-610.
Barron, A. B., Maleszka, R., Vander Meer, R. K. and Robinson, G. E. (2007). Octopamine modulates honey bee dance behavior. *Proc. Natl. Acad. Sci. USA* **104**, 1703-1707.
Bellinger, L., Lilley, C. and Langley-Evans, S. C. (2004). Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br. J. Nutr.* **92**, 513-520.
Bergot, B. J., Schooley, D. A. and de Kort, C. A. D. (1981). Identification of JH III as the principal juvenile hormone in Locusta migratoria. *Experientia* **37**, 909-910.
Bitterman, M. E., Menzel, R., Fietz, A. and Schafer, S. (1983). Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J. Comp. Psychol.* **97**, 107-119.
Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R. and Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* **11**, 213-221.
Broughton, S. J., Piper, M. D. W., Ikeya, T., Bass, T. M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D. J., Leevers, S. J. et al. (2005). Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA* **102**, 3105-3110.
Camazine, S., Deneubourg, J. L., Franks, N. R., Sneyd, J., Theraulaz, G. and Bonabeau, E. (2001). *Self-Organization in Biological Systems*. Princeton, NJ: Princeton University Press.
Cameron, N., Preece, M. A. and Cole, T. J. (2005). Catch-up growth or regression to the mean? Recovery from stunting revisited. *Am. J. Hum. Biol.* **17**, 412-417.
Cameron, R. C., Duncan, E. J. and Dearden, P. K. (2013). Stable reference genes for the measurement of transcript abundance during larval caste development in the honeybee. *Apidologie* **44**, 357-366.
Candy, D. J. (1978). The regulation of locust flight muscle metabolism by octopamine and other compounds. *Insect Biochem.* **8**, 177-181.
Capella, I. C. S. and Hartfelder, K. (2002). Juvenile-hormone-dependent interaction of actin and spectrin is crucial for polymorphic differentiation of the larval honey bee ovary. *Cell Tissue Res.* **307**, 265-272.
Chang, L.-H., Barron, A. B. and Cheng, K. (2015). Effects of the juvenile hormone analogue methoprene on rate of behavioural development, foraging performance and navigation in honey bees (*Apis mellifera*). *J. Exp. Biol.* **218**, 1715-1724.
Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A. and Robinson, G. E. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* **104**, 7128-7133.
de Azevedo, S. V. and Hartfelder, K. (2008). The insulin signaling pathway in honey bee (*Apis mellifera*) caste development - differential expression of insulin-like peptides and insulin receptors in queen and worker larvae. *J. Insect Physiol.* **54**, 1064-1071.
Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L. and Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS ONE* **8**, e72016.
Downer, R. G. H. (1979). Trehalose production in isolated fat body of the American cockroach, Periplaneta americana. *Comp. Biochem. Physiol. C Comp. Physiol.* **62**, 31-34.
Drewnowski, A. (2000). Sensory control of energy density at different life stages. *Proc. Nutr. Soc.* **59**, 239-244.
Elkin, C. M. and Reid, M. L. (2005). Low energy reserves and energy allocation decisions affect reproduction by Mountain Pine Beetles, Dendroctonus ponderosae. *Funct. Ecol.* **19**, 102-109.
Even, N., Devaud, J.-M. and Barron, A. B. (2012). General stress responses in the honey bee. *Insects* **3**, 1271-1298.
Fahrbach, S. E. and Robinson, G. E. (1996). Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Dev. Neurosci.* **18**, 102-114.
Fergusson, L. A. and Winston, M. L. (1988). The influence of wax deprivation on temporal polyethism in honey bee (*Apis mellifera* L) colonies. *Can. J. Zool.* **66**, 1997-2001.
Fewell, J. H. and Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **30**, 387-393.
Free, J. B. (1961). Hypopharyngeal gland development and division of labor in honey-bee (*Apis mellifera* L.) colonies. *Proc. R. Entomol. Soc. Lond. Ser. A Gen. Entomol.* **36**, 5-8.
Free, J. B. (1967). Factors determining the collection of pollen by honeybee foragers. *Anim. Behav.* **15**, 134-144.
Fussnecker, B. L., Smith, B. H. and Mustard, J. A. (2006). Octopamine and tyramine influence the behavioral profile of locomotor activity in the honey bee (*Apis mellifera*). *J. Insect Physiol.* **52**, 1083-1092.
García-Roger, E. M., Martínez, A. and Serra, M. (2006). Starvation tolerance of rotifers produced from parthenogenetic eggs and from diapausing eggs: a life table approach. *J. Plankton Res.* **28**, 257-265.
Gardner, D. S., Ozanne, S. E. and Sinclair, K. D. (2009). Effect of the early-life nutritional environment on fecundity and fertility of mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 3419-3427.
Giray, T., Galindo-Cardona, A. and Oskay, D. (2007). Octopamine influences honey bee foraging preference. *J. Insect Physiol.* **53**, 691-698.
Gluckman, P. D., Hanson, M. A., Spencer, H. G. and Bateson, P. (2005). Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. *Proc. R. Soc. B Biol. Sci.* **272**, 671-677.
Gluckman, P. D., Hanson, M. A. and Beedle, A. S. (2007). Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am. J. Hum. Biol.* **19**, 1-19.
Goblirsch, M., Huang, Z. Y. and Spivak, M. (2013). Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by Nosema ceranae infection. *PLoS ONE* **8**, e58165.
Gruntenko, N. E., Khlebodarova, T. M., Mazurov, M. M., Grenbek, L. G., Sukhanova, M., Zakharov, I. K., Khemmok, B. D. and Raushenbakh, I. (1996). Role of juvenile hormone metabolism in the adaptation of *Drosophila* populations to stressful environmental conditions. *Genetika* **32**, 1191-1198.
Gruntenko, N. E., Chentsova, N. A., Andreenkova, E. V., Bownes, M., Segal, D., Adonyeva, N. V. and Rauschenbach, I. Y. (2003). Stress response in a juvenile hormone-deficient *Drosophila melanogaster* mutant apterous56f. *Insect Mol. Biol.* **12**, 353-363.
Gruntenko, N. E., Wen, D., Karpova, E. K., Adonyeva, N. V., Liu, Y., He, Q., Faddeeva, N. V., Fomin, A. S., Li, S. and Rauschenbach, I. Y. (2010). Altered juvenile hormone metabolism, reproduction and stress response in *Drosophila* adults with genetic ablation of the corpus allatum cells. *Insect Biochem. Mol. Biol.* **40**, 891-897.
Hagenguth, H. and Rembold, H. (1978). Identification of Juvenile Hormone-3 as the only JH homolog in all developmental stages of the honey bee. *Z Naturforschung* **33C**, 847-850.
Harrison, J. F. and Roberts, S. P. (2000). Flight respiration and energetics. *Annu. Rev. Physiol.* **62**, 179-205.
Hartfelder, K., Bitondi, G. M., Brent, C. S., Guidugli-Lazzarini, K. R., Simoes, Z. L., Stabentheiner, A., Tanaka, E. D. and Wang, Y. (2013). Standard methods for physiology and biochemistry research in *Apis mellifera*. *J. Apic Res.* **51**. doi:10.3896/IBRA.1.52.1.06
Haydak, M. H. (1970). Honey bee nutrition. *Annu. Rev. Entomol.* **15**, 143-156.
Higes, M., Martin-Hernandez, R., Botias, C., Bailon, E. G., Gonzalez-Porto, A. V., Barrios, L., del Nozal, M. J., Bernal, J. L., Jimenez, J. J., Palencia, P. G. et al. (2008). How natural infection by Nosema ceranae causes honeybee colony collapse. *Environ. Microbiol.* **10**, 2659-2669.
Hoover, S. E. R., Higo, H. A. and Winston, M. L. (2006). Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *J. Comp. Physiol. B* **176**, 55-63.
Huang, Z. (2012). Pollen nutrition affects honey bee stress resistance. *Terr. Arthropod. Rev.* **5**, 175-189.
Huang, Z. Y. and Robinson, G. E. (1999). Social control of division of labor in honey bee colonies. In *Information Processing in Social Insects* (ed. C. Detrain, J. L. Deneubourg and J. M. Pasteels), pp. 165-187. Basel: Birkhauser.
Huang, Z.-Y., Robinson, G. E., Tobe, S. S., Yagi, K. J., Strambi, C., Strambi, A. and Stay, B. (1991). Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *J. Insect Physiol.* **37**, 733-741.
Ibanez, L., Lopez-Bermejo, A., Diaz, M., Marcos, M. V., Casano, P. and de Zegher, F. (2009). Abdominal fat partitioning and high-molecular-weight adiponectin in short children born small for gestational age. *J. Clin. Endocrinol. Metab.* **94**, 1049-1052.
Ibanez, L., Lopez-Bermejo, A., Diaz, M., Jaramillo, A., Marin, S. and de Zegher, F. (2010). Growth hormone therapy in short children born small for gestational age: effects on abdominal fat partitioning and circulating follistatin and high-molecular-weight adiponectin. *J. Clin. Endocrinol. Metab.* **95**, 2234-2239.

Ihle, K. E., Page, R. E., Frederick, K., Fondrk, M. K. and Amdam, G. V. (2010). Genotype effect on regulation of behaviour by vitellogenin supports reproductive origin of honeybee foraging bias. *Anim. Behav.* **79**, 1001-1006.
Iijima, K., Zhao, L., Shenton, C. and Iijima-Ando, K. (2009). Regulation of energy stores and feeding by neuronal and peripheral CREB activity in *Drosophila*. *PLoS ONE* **4**, e8498.
Janković-Hladni, M. (1991). Hormones and metabolism in insect stress (historical survey). In *Hormones and Metabolism in Insect Stress* (ed. J. Ivanović and M. Janković-Hladni), pp. 5-26. Boca Raton, FL: CRC Press.
Janmaat, A. F. and Winston, M. L. (2000). The influence of pollen storage area and *Varroa jacobsoni* Oudemans parasitism on temporal caste structure in honey bees (*Apis mellifera* L.). *Insectes. Soc.* **47**, 177-182.
Jasienska, G., Thune, I. and Ellison, P. T. (2006). Fatness at birth predicts adult susceptibility to ovarian suppression: an empirical test of the Predictive Adaptive Response hypothesis. *Proc. Natl. Acad. Sci. USA* **103**, 12759-12762.
Jaycox, E. R. (1976). Behavioral changes in worker honey bees (*Apis mellifera* L.) after injection with synthetic juvenile hormone (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* **49**, 165-170.
Jaycox, E. R., Skowronek, W. and Guynn, G. (1974). Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of a juvenile hormone mimic. *Ann. Entomol. Soc. Am.* **67**, 529-534.
Jindra, M., Palli, S. R. and Riddiford, L. M. (2013). The juvenile hormone signaling pathway in insect development. *Annu. Rev. Entomol.* **58**, 181-204.
Krause, E. T., Honarmand, M., Wetzel, J. and Naguib, M. (2009). Early fasting is long lasting: differences in early nutritional conditions reappear under stressful conditions in adult female zebra finches. *PLoS ONE* **4**, e5015.
Kuzawa, C. W., McDade, T. W., Adair, L. S. and Lee, N. (2010). Rapid weight gain after birth predicts life history and reproductive strategy in Filipino males. *Proc. Natl. Acad. Sci USA* **107**, 16800-16805.
Lee, T. M. and Zucker, I. (1988). Vole infant development is influenced perinatally by maternal photoperiodic history. *Am. J. Physiol.* **255**, R831-R838.
Leimar, O., Hartfelder, K., Laubichler, M. D. and Page, R. E., Jr. (2012). Development and evolution of caste dimorphism in honeybees – a modeling approach. *Ecol. Evol.* **2**, 3098-3109.
Lin, H., Dusset, C. and Huang, Z. Y. (2004). Short-term changes in juvenile hormone titers in honey bee workers due to stress. *Apidologie* **35**, 319-327.
Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog Rana temporaria under divergent selection pressures. *Evolution* **63**, 1508-1518.
Linford, N. J., Ro, J., Chung, B. Y. and Pletcher, S. D. (2015). Gustatory and metabolic perception of nutrient stress in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **112**, 2587-2592.
Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods* **25**, 402-408.
Lourenco, A. P., Mackert, A., dos Santos Cristino, A. and Simoes, Z. L. P. (2008). Validation of reference genes for gene expression studies in the honey bee, *Apis mellifera*, by quantitative real-time RT-PCR. *Apidologie* **39**, 372-385.
McMillen, I. C. and Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol. Rev.* **85**, 571-633.
Mentel, T., Duch, C., Stypa, H., Wegener, G., Muller, U. and Pfluger, H. J. (2003). Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J. Neurosci.* **23**, 1109-1113.
Mitchell, A., Romano, G. H., Groisman, B., Yona, A., Dekel, E., Kupiec, M., Dahan, O. and Pilpel, Y. (2009). Adaptive prediction of environmental changes by microorganisms. *Nature* **460**, 220-224.
Moczek, A. P. (2010). Phenotypic plasticity and diversity in insects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**, 593-603.
Mutti, N. S., Dolezal, A. G., Wolschin, F., Mutti, J. S., Gill, K. S. and Amdam, G. V. (2011). IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. *J. Exp. Biol.* **214**, 3977-3984.
Nelson, C. M., Ihle, K. E., Fondrk, M. K., Page, R. E., Jr and Amdam, G. V.(2007). The gene *vitellogenin* has multiple coordinating effects on social organization. *PLoS Biol.* **5**, 673-677.
Oldham, S., Bohni, R., Stocker, H., Brogiolo, W. and Hafen, E. (2000). Genetic control of size in *Drosophila*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 945-952.
Ozanne, S. E. and Hales, C. N. (2004). Lifespan: catch-up growth and obesity in male mice. *Nature* **427**, 411-412.
Page, R. E. (2013). *The Spirit of the Hive: the Mechanisms of Social Evolution*. London, England: Harvard University Press.
Page, R. E. and Erber, J. (2002). Levels of behavioral organization and the evolution of division of labor. *Naturwissenschaften* **89**, 91-106.
Palanker, L., Tennessen, J. M., Lam, G. and Thummel, C. S. (2009). *Drosophila* HNF4 regulates lipid mobilization and beta-oxidation. *Cell Metab.* **9**, 228-239.
Pankiw, T. and Page, R. E., Jr. (2003). Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera L.*). *J. Comp. Physiol. A Sens. Neural. Behav. Physiol.* **189**, 675-684.
Patel, A., Fondrk, M. K., Kaftanoglu, O., Emore, C., Hunt, G., Frederick, K. and Amdam, G. V. (2007). The making of a queen: TOR pathway governs diphenic caste development. *PLoS ONE* **2**, e509.
Perry, C. J., Sovik, E., Myerscough, M. R. and Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proc. Natl. Acad. Sci. USA* **112**, 3427-3432.
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic. Acids Res.* **29**, e45.
Rae, M. T., Kyle, C. E., Miller, D. W., Hammond, A. J., Brooks, A. N. and Rhind, S. M. (2002). The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. *Anim. Reprod. Sci.* **72**, 63-71.
Reim, T., Thamm, M., Rolke, D., Blenau, W. and Scheiner, R. (2012). Suitability of three common reference genes for quantitative real-time PCR in honey bees. *Apidologie* **39**, 372-385.
Rhind, S. M. (2004). Effects of maternal nutrition on fetal and neonatal reproductive development and function. *Anim. Reprod. Sci.* **82-83**, 169-181.
Rickard, I. J. and Lummaa, V. (2007). The predictive adaptive response and metabolic syndrome: challenges for the hypothesis. *Trends Endocrinol. Metab.* **18**, 94-99.
Rittschof, C. C., Coombs, C. B., Frazier, M., Grozinger, C. M. and Robinson, G. E. (2015). Early-life experience affects honey bee aggression and resilience to immune challenge. *Sci. Rep.* **5**, 15572.
Robinson, G. E., Page, R. E. and Huang, Z.-Y. (1994). Temporal polyethism in social insects is a developmental process. *Anim. Behav.* **48**, 467-469.
Roeder, T. (2005). Tyramine and octopamine: ruling behavior and metabolism. *Annu. Rev. Entomol.* **50**, 447-477.
Rohwedder, A., Pfitzenmaier, J. E., Ramsperger, N., Apostolopoulou, A. A., Widmann, A. and Thum, A. S. (2012). Nutritional value-dependent and nutritional value-independent effects on *Drosophila melanogaster* larval behavior. *Chem. Senses* **37**, 711-721.
Saastamoinen, M., van der Sterren, D., Vastenhout, N., Zwaan, B. J. and Brakefield, P. M. (2010). Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana*. *Am. Nat.* **176**, 686-698.
Scharlaken, B. de Graaf, D. C., Goossens, K., Brunain, M., Peelman, L. J. and Jacobs, F. J. (2008). Reference gene selection for insect expression studies using quantitative real-time PCR: the head of the honeybee, *Apis mellifera*, after a bacterial challenge. *J. Insect Sci.* **8**, 1-10.
Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Evol. Syst.* **24**, 35-68.
Scheiner, R. (2004). Responsiveness to sucrose and habituation of the proboscis extension response in honey bees. *J. Comp. Physiol. A* **190**, 727-733.
Scheiner, R., Kulikovskaja, L. and Thamm, M. (2014a). The honey bee tyramine receptor AmTYR1 and division of foraging labour. *J. Exp. Biol.* **217**, 1215-1217.
Scheiner, R., Toteva, A., Reim, T., Sovik, E. and Barron, A. B. (2014b). Differences in the phototaxis of pollen and nectar foraging honey bees are related to their octopamine brain titers. *Front. Physiol.* **5**, 116.
Schulz, D. J., Huang, Z.-Y. and Robinson, G. E. (1998). Effects of colony food shortage on behavioral development in honey bees. *Behav. Ecol. Sociobiol.* **42**, 295-303.
Schultz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol. A* **184**, 481-488.
Scofield, H. N. and Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PloS ONE* **10**, e0121731.
Seehuus, S.-C., Krekling, T. and Amdam, G. V. (2006). Cellular senescence in honey bee brain is largely independent of chronological age. *Exp. Gerontol.* **41**, 1117-1125.
Sekiguchi, K. and Sakagami, S. F. (1966). Structure of foraging population and related problems in the honeybee, with considerations on division of labour in bee colonies. *Hakkaido Nat. Agric. Exp. Stat. Rep. No.* **69**, 1-58.
Sheng, Z., Xu, J., Bai, H., Zhu, F. and Palli, S. R. (2011). Juvenile hormone regulates vitellogenin gene expression through insulin-like peptide signaling pathway in the red flour beetle, Tribolium castaneum. *J. Biol. Chem.* **286**, 41924-41936.
Shu, S., Park, Y. I., Ramaswamy, S. B. and Srinivasan, A. (1997). Hemolymph juvenile hormone titers in pupal and adult stages of southwestern corn borer [Diatraea grandiosella (Pyralidae)] and relationship with egg development. *J. Insect Physiol.* **43**, 719-726.
Simpson, S. J., McCaffery, A. R. and Hagele, B. F. (1999). A behavioural analysis of phase change in the desert locust. *Biol. Rev.* **74**, 461-480.
Simpson, S. J., Despland, E., Hägele, B. F. and Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *Proc. Natl. Acad. Sci. USA* **98**, 3895-3897.
Spivak, M., Masterman, R., Ross, R. and Mesce, K. A. (2003). Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *J. Neurobiol.* **55**, 341-354.

Journal of Experimental Biology

Suarez, R. K., Lighton, J. R., Joos, B., Roberts, S. P. and Harrison, J. F. (1996). Energy metabolism, enzymatic flux capacities, and metabolic flux rates in flying honeybees. *Proc. Natl. Acad. Sci. USA* **93**, 12616-12620.

Suarez, R. K., Darveau, C.-A., Welch, K. C., Jr, O'Brien, D. M., Roubik, D. W. and Hochachka, P. W. (2005). Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J. Exp. Biol.* **208**, 3573-3579.

Sullivan, J. P., Jassim, O., Fahrbach, S. E. and Robinson, G. E. (2000). Juvenile hormone paces behavioral development in the adult worker honey bee. *Hormon. Behav.* **37**, 1-14.

Sullivan, J. P., Fahrbach, S. E., Harrison, J. F., Capaldi, E. A., Fewell, J. H. and Robinson, G. E. (2003). Juvenile hormone and division of labor in honey bee colonies: effects of allatectomy on flight behavior and metabolism. *J. Exp. Biol.* **206**, 2287-2296.

Toth, A. L. and Robinson, G. E. (2005). Worker nutrition and division of labour in honeybees. *Anim. Behav.* **69**, 427-435.

Toth, A. L., Kantarovich, S., Meisel, A. F. and Robinson, G. E. (2005). Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* **208**, 4641-4649.

Tsuruda, J. M., Amdam, G. V. and Page, R. E., Jr. (2008). Sensory response system of social behavior tied to female reproductive traits. *PLoS ONE* **3**, e3397.

van den Heuvel, J., Saastamoinen, M., Brakefield, P. M., Kirkwood, T. B. L., Zwaan, B. J. and Shanley, D. P. (2013). The predictive adaptive response: modeling the life-history evolution of the butterfly Bicyclus anynana in seasonal environments. *Am. Nat.* **181**, E28-E42.

van Dooremalen, C., Stam, E., Gerritsen, L., Cornelissen, B., van der Steen, J., van Langevelde, F. and Blacquiere, T. (2013). Interactive effect of reduced pollen availability and Varroa destructor infestation limits growth and protein content of young honey bees. *J. Insect Physiol.* **59**, 487-493.

Vandesompele, J., De Paepe, A. and Speleman, F. (2002). Elimination of primer-dimer artifacts and genomic coamplification using a two-step SYBR green I real-time RT-PCR. *Anal. Biochem.* **303**, 95-98.

Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L. and Gluckman, P. D. (2000). Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am. J. Physiol. Endocrinol. Metab.* **279**, E83-E87.

Wang, Y., Amdam, G. V., Rueppell, O., Wallrichs, M. A., Fondrk, M. K., Kaftanoglu, O. and Page, R. E., Jr. (2009). PDK1 and HR46 gene homologs tie social behavior to ovary signals. *PLoS ONE* **4**, e4899.

Wang, Y., Kaftanoglua, O., Siegela, A. J., Page, R. E., Jr and Amdama, G. V. (2010a). Surgically increased ovarian mass in the honey bee confirms link between reproductive physiology and worker behavior. *J. Insect Physiol.* **56**, 1816-1824.

Wang, Y., Mutti, N. S., Ihle, K. E., Siegel, A., Dolezal, A. G., Kaftanoglu, O. and Amdam, G. V. (2010b). Down-regulation of honey bee IRS gene biases behavior toward food rich in protein. *PLoS Genet.* **6**, e1000896.

Wang, Y., Brent, C. S., Fennern, E. and Amdam, G. V. (2012a). Gustatory perception and fat body energy metabolism are jointly affected by vitellogenin and juvenile hormone in honey bees. *PLoS Genet.* **8**, e1002779.

Wang, Y., Kocher, S. D., Linksvayer, T. A., Grozinger, C. M., Page, R. E. and Amdam, G. V. (2012b). Regulation of behaviorally associated gene networks in worker honey bee ovaries. *J. Exp. Biol.* **215**, 124-134.

Wang, Y., Baker, N. and Amdam, G. V. (2013a). RNAi-mediated double gene knockdown and gustatory perception measurement in honey bees (*Apis mellifera*). *J. Vis. Exp.* **25**, e50446.

Wang, Y., Azevedo, S. V., Hartfelder, K. and Amdam, G. V. (2013b). Insulin-like peptides (AmILP1 and AmILP2) differentially affect female caste development in the honey bee (*Apis mellifera L.). J. Exp. Biol.* **216**, 4347-4357.

Wang, Y., Kaftanoglu, O., Fondrk, M. K. and Page, R. E. (2014). Nurse bee behaviour manipulates worker honeybee (*Apis mellifera* L.) reproductive development. *Anim. Behav.* **92**, 253-261.

Wells, J. C. K. (2007). Flaws in the theory of predictive adaptive responses. *Trends Endocrinol. Metab.* **18**, 331-337.

Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.

Woyciechowski, M. and Moron, D. (2009). Life expectancy and onset of foraging in the honeybee (*Apis mellifera*). *Insect Soc.* **56**, 193-201.

Wu, Q. and Brown, M. R. (2006). Signaling and function of insulin-like peptides in insects. *Annu. Rev. Entomol.* **51**, 1-24.

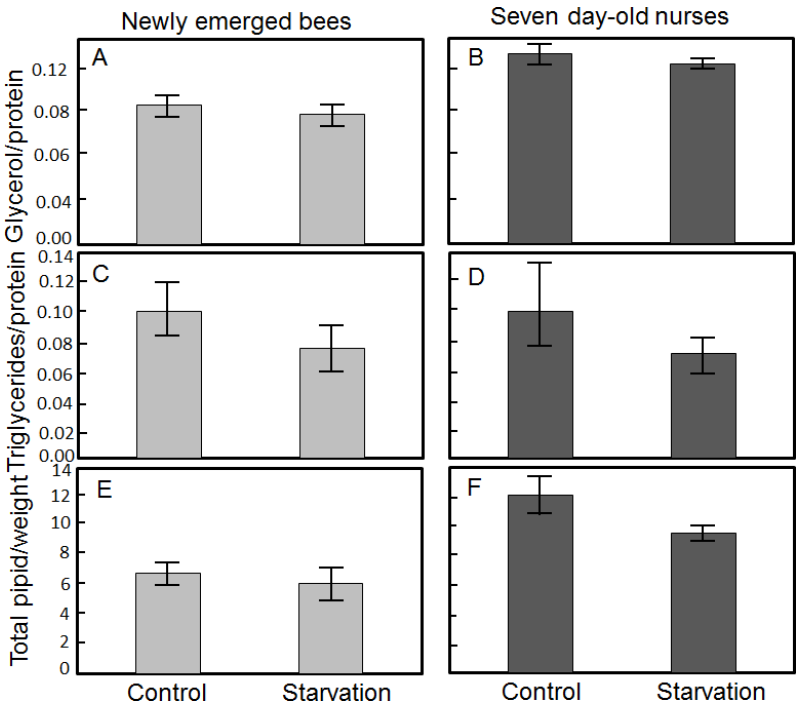

Fig. S1. Effect of larval starvation on fat body glycerol, triglycerides and total lipid of < 24h bees and 7-day-old nurses. The values presented are means ± se. Statistical significance was analyzed by the two-tailed t test.

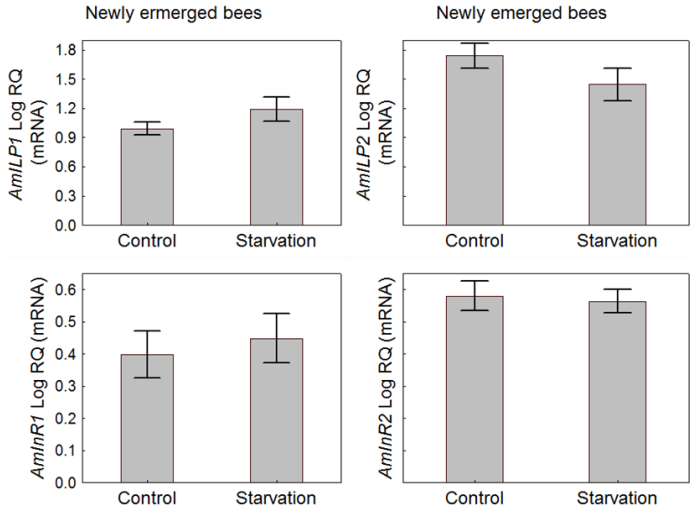

Fig. S2. Effect of larval starvation on gene expression of *AmIlp1*, *AmIlp2*, *AmInR1* and *AmInR2* of < 24h bees. The values presented are means ± se. Statistical significance was analyzed by the two-tailed t test.

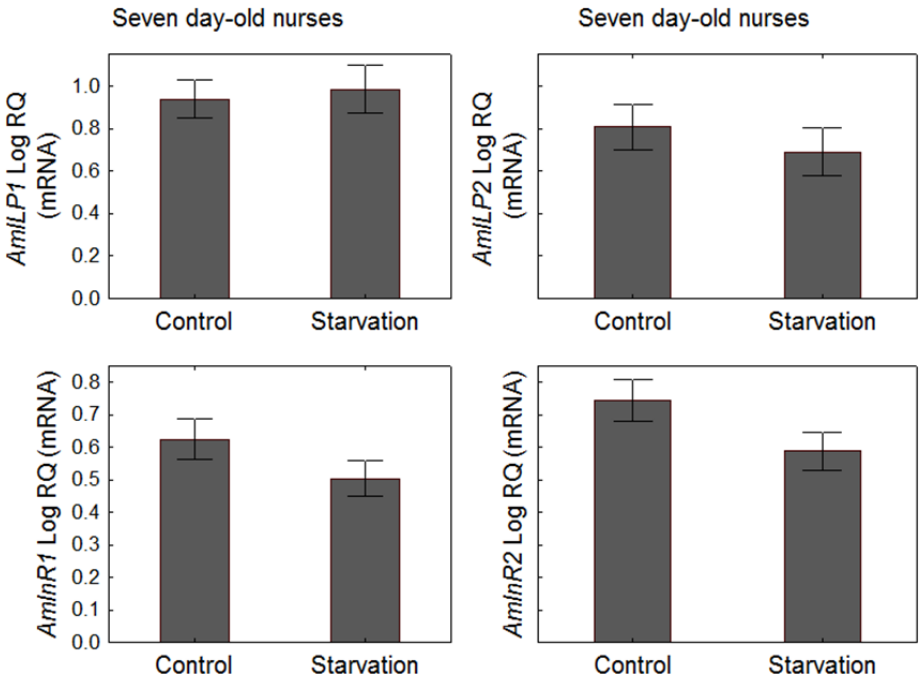

Fig. S3. Effect of larval starvation on gene expression of *AmIlp1*, *AmIlp2*, *AmInR1* and *AmInR2* of 7-day-old nurses. The values presented are means ± se. Statistical significance was analyzed by the two-tailed t test.

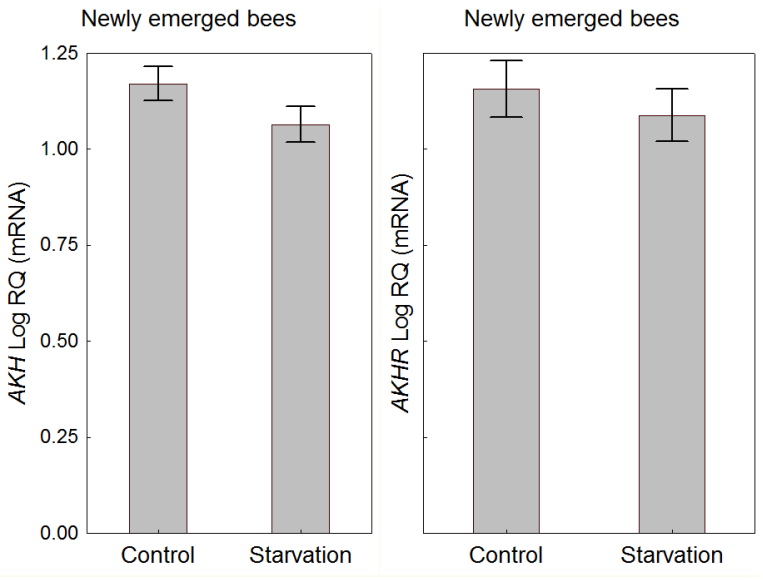

Fig. S4. Effect of larval starvation on gene expression of *AKH* and *AKHR* of < 24h bees. The values presented are the mean ± se. Statistical significance was analyzed by the two-tailed t test.

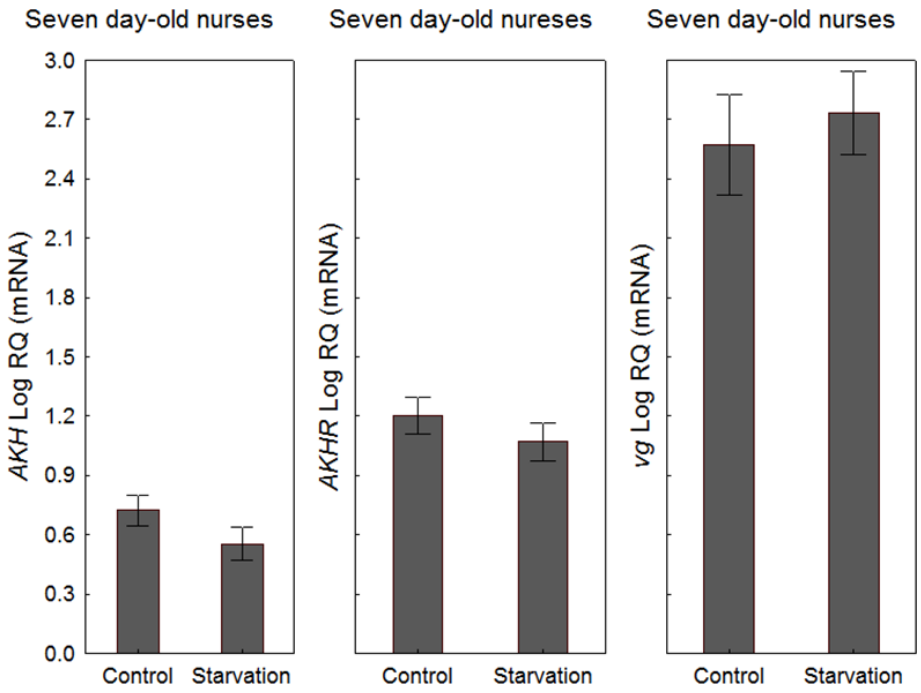

Fig. S5. Effect of larval starvation on gene expression of *AKH*, *AKHR* and *vg* of 7-day-old nurses. The values presented are mean ± se. Statistical significance was analyzed by the two-tailed t test.

Table S1: Statistical results of candidate gene expression

Student's t-test	< 24 h			7 d old		
	Sample size (n)	t	p	Sample size (n)	t	p
Ilp1	16	-1.420	0.166	16	-0.334	0.740
Ilp2	16	1.432	0.164	16	0.767	0.450
InR1	16	-0.470	0.642	16	1.454	0.156
InR2	16	0.283	0.780	16	1.796	0.08
AKH	16	1.623	0.115	16	1.452	0.157
AKHR	16	0.690	0.496	16	0.987	0.331
vg	N/A	N/A	N/A	16	-0.429	0.671

Table S2: Candidate gene primers

Gene Names	Forward Primers	Reverse Primers
ILP1	TCAAAACGGCCATGTTCAG	TGAGACTTTTCGAGCACAGC
ILP2	TTCCAGAAATGGAGATGGATG	TAGGAGCGCAACTCCTCTGT
InR1	GGATCTGGTGTGGGACAGTT	ATCCCCACGTCGAGTATCTG
InR2	GGGAAGAACATCGTGAAGGA	CATCACGAGCAGCGTGTACT
AKH	CGTAAGCTTCGACCAAGTTTTT	CATTCGACAACTCCGATCCT
AKHR	ATAATCACCACCACGGGATT	GACCTTCGTTGAATCGCATA
vg	GTTGGAGAGCAACATGCAGA	TCGATCCATTCCTTGATGGT
TYR1	GTTCGTCGTATGCTGGTTGC	GTAGATGAGCGGGTTGAGGG