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RESEARCH ARTICLE

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Hungry for the queen: Honeybee nutritional environment affects worker pheromone response in a lifestage-dependent manner

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Abstract

1. Animal nutritional state can profoundly affect behaviour, including an individual's tendency to cooperate with others. We investigated how nutritional restriction at different life stages affects cooperative behaviour in a highly social species, *Apis mellifera* honeybees.
2. We found that nutritional restriction affects a worker's queen pheromone response, a behavioural indicator of investment in group vs. individual reproduction. Nutritional restriction at the larval stage led to reduced ovary size and increased queen pheromone response, whereas nutritional restriction at the adult stage led to reduced lipid stores and reduced queen pheromone response.
3. We argue that these differences depend upon the extent of reproductive plasticity at these life stages and that individual worker honeybees may adjust their behavioural and physiological traits in response to nutritional stress to invest nutritional resources in either their own or their colony's reproduction.
4. These results support the role of nutritional stress in the maintenance of cooperative behaviour, and we suggest that historical nutritional scarcity may be an important contributor to the evolution of extreme forms of cooperation.

KEYWORDS
diet restriction, early-life stress, ovary, queen mandibular pheromone, social behaviour

1 | INTRODUCTION

Nutritional regulation of behaviour via deeply conserved pathways may reflect the conditions that led to the origin and evolutionary maintenance of cooperation. When nutritional resources are scarce, studies from several systems suggest cooperative behaviours may be pronounced. This trend has been observed across many animal lineages, from blood-meal sharing in vampire bats (Wilkinson, 1984) to social foraging in tadpoles (Sontag, Wilson, & Wilcox, 2006), to the multicellular aggregations of otherwise solitary *Dictyostelium* amoebae (Kessin, 2001). However, resource limitation in some species, for example, baboons and other primates, may also lead to increased competition and aggression (Vitousek, Manke, Gray, & Vitousek, 2004). The decision to invest in cooperative behaviour vs. self under nutritional duress may depend on reproductive options available to an individual, but we lack a solid understanding of how these trade-offs are mediated within a species. The social insects, a pinnacle of cooperative evolution, are an ideal system to study how nutrition can regulate social behaviour. Not only is there variation in cooperative behaviour between species, but also between different castes (e.g., queens vs. workers) as well as between individuals of the same caste.

In social insects, nutritional differences organize social life as the major determinant of the reproductive division of labour. In many social Hymenoptera (ants, social bees and social wasps), early-life nutrition of a female has a drastic effect on adult phenotype. The honeybee *Apis mellifera* serves as an illustrative model of how these early-life differences in nutrition have permanent effects on an

adult's behaviour, morphology and physiology. Honeybees live in a colony of several thousand sterile workers, and one reproductive: the queen. Whether a developing larva will become a queen or worker depends on the diet she receives (Winston, 1987). Additionally, adult nutritional state can affect behaviour. A worker's nutritional state acts in part to regulate behavioural caste, in that nurses tend to have higher lipid stores than foragers (Toth & Robinson, 2005) and reduced nutritional state causes early, and more frequent foraging (Mattila & Otis, 2006; Schulz, Huang, & Robinson, 1998; Toth, Kantarovich, Meisel, & Robinson, 2005). In other social insects, differential nutrition during larval development can also lead to differences in size and behaviour, contributing to a division of labour among the workforce, such as in the bumblebee *Bombus impatiens* (Couvillon & Dornhaus, 2009). As in other social insects, consistent behavioural differences between same-aged honeybee workers within a colony do exist (Walton & Toth, 2016), but the mechanisms that mediate these differences are not yet fully known. In this study, we explore whether differential nutrition may be a factor in the regulation of inter-individual differences in cooperative behaviour.

Nutritional regulation of cooperative behaviour may be especially important in social insects and the balance between "me" and "we" modes of reproduction. If nutrient availability is high, investment in "me" (one's own) reproduction is favourable, even in a highly social species with limited (but non-zero) personal reproductive opportunities. But, if nutritional resources are scarce, investment in "we" (a group of relatives) reproduction may be the best option, especially when personal reproductive probabilities approach zero (Hunt, 1991; Rossi & Hunt, 1988; Wheeler, 1986). Thus, in environments where nutrition is limited, cooperation may offer a selective advantage. It has been suggested that historical nutritional scarcity could have contributed to the evolution of extreme forms of cooperation, such as insect eusociality (Hunt & Nalepa, 1994). If the molecular and physiological pathways that contributed to these behavioural options continue to modulate behavioural differences in honeybees, we expect workers that receive a high nutrition diet should shunt investment to their own ovaries and behave less cooperatively. Conversely, a nutritionally restricted worker should be unable to invest in her own ovaries and behave more cooperatively.

One potential regulatory link between nutritional state and behaviour in worker honeybees is the ovaries. Although under normal colony conditions honeybee worker's ovaries are inactive, natural variation in the size of worker ovaries (the number of ovarioles that make up each ovary) does exist. The ovary is uncoupled from direct reproduction in workers in queenright colonies, yet the ovary and conserved reproductive pathways may regulate aspects of worker behaviour such as nursing and pollen foraging, as proposed by the ground plan hypotheses of West-Eberhard, Amdam and colleagues (Amdam, Csondes, Fondrk, & Page, 2006; Amdam, Norberg, Fondrk, & Page, 2004; Amdam & Page, 2010; West-Eberhard, 1987). These hypotheses are supported by evidence that variation in ovariole number contributes to honeybee behavioural maturation and the division of labour (Wang, Kaftanoglu, Siegel, Page, & Amdam, 2010; Wang et al., 2012). Although worker ovariole number is affected by genotype (Makert, Paxton, & Hartfelder, 2006; Robinson, Page, & Fondrk, 1990), ovariole number is also highly affected by environmental factors (Backx, Guzman-Novoa, & Thompson, 2012). For example, seasonal variation in nutritional availability influences ovariole number; workers that develop during periods of high pollen availability have higher ovariole number than those during pollen dearth (Hoover, Higo, & Winston, 2006). Thus, ovaries are likely targets for reduced allocation during nutritional stress, which in turn may affect behaviour in the long term. This is especially true in honeybee workers because, although they do not normally reproduce, variation in worker ovary size determines which workers will lay unfertilized eggs if a colony becomes queenless (Ratnieks, 1993). Because of the potentially important role of the ovaries as a site of nutritional and reproductive trade-offs, in this study we integrated information about ovariole number and lipid stores with an indicator cooperative behaviour, response to queen pheromone.

Social insect queens can enforce worker cooperation and sterility in several ways, including physical aggression (Reeve, 1991) and chemical communication (Kocher & Grozinger, 2011; Slessor, Winston, & Le Conte, 2005). In the honeybee, the queen utilizes queen mandibular pheromone (QMP), which prevents worker ovarian activation (Slessor et al., 2005). QMP also elicits a "retinue response" from workers, in which they face the queen, and antennate and tend her (Slessor, Kaminski, King, Borden, & Winston, 1988). The task of queen tending (feeding, examining and grooming the queen) is a form of worker–queen cooperation necessary to colony function. The queen is singly occupied by the task of laying eggs, so the workers must feed and maintain her. Thus, the workers' response to the queen is of key importance to colony health. Natural variation in response to the queen exists among the workers of a honeybee colony (Kocher, Ayroles, Stone, & Grozinger, 2010; Walton & Toth, 2016). This variation in response may contribute to the colony's division of labour (specific individuals are more likely to respond to, and thus care for, the queen).

In this study, we assayed individual variation in QMP response to test the hypothesis that nutritional restriction enhances cooperation. We manipulated the nutritional environment of honeybee workers in two separate ways: adult pollen deprivation (Experiment 1 and Experiment 2) and acute larval starvation (Experiment 2). We predicted that nutritionally stressed larvae would exhibit a higher response to QMP as adults. We predicted that the effect of adult diet would follow the same pattern: Pollen-supplemented adults would be less responsive to QMP than adults deprived of pollen. If nutrition mediates cooperative behaviour via reproductive physiology, we predict bees that experienced high nutrition to invest these resources in their own reproductive potential and thus have larger ovaries and higher lipid stores. We found evidence that nutritional stress during larval development does lead to enhanced QMP response and smaller ovaries, suggesting nutritional stress leads bees to divest their own reproduction and invest in their colonies. Interestingly, we found the opposite pattern in adults, suggesting different strategies for dealing with nutritional stress depending on life stage and level of reproductive plasticity.

2 | MATERIAL S AND METHODS

2.1 | Bees

Honeybee (*Apis mellifera* L.) colonies were maintained at the Iowa State University Horticulture Research Station in Ames, Iowa, during the summers of 2015, 2016 and 2017. Adult bees were transferred to rearing facilities at Iowa State University, and all observational data were collected there.

2.1.1 | Experiment 1: Adult restriction: Pollen deprivation

Brood frames containing pre-eclosion workers were removed from six un-manipulated hives at the Iowa State University Horticulture Research Station apiary and placed in a 33°C incubator overnight to emerge. Upon emergence, adult bees were divided into cages, 30 bees per cage (see *Cage Assays* below). These cages were subdivided into pollen-fed (49 cages) or pollen-deprived treatments (55 cages). In the pollen-fed treatment, cages were fed 1 gram of bee-collected chestnut (Pollenergie, France) pollen daily for the course of the experiment (seven days).

2.1.2 | Experiment 2: Larval and adult restriction: Acute larval starvation and adult pollen deprivation

Four queens in four different colonies were caged over a frame of empty drawn comb with a push-in cage and allowed to lay eggs for 48 hr, after which the cage was removed and the comb placed in a separate colony. At 180 hr after eggs were laid, a starvation procedure was performed (Wang, Kaftanoglu, Brent, Page, & Amdam, 2016; Wang, Kaftanoglu, Fondrk, & Page, 2014; Wang, Campbell, et al., 2016). Nurse bees were removed from the frame, and then, a wire push-in cage was placed over half of the larvae, preventing nurses from feeding or in any way caring for them. The other half of the larvae were left uncovered, so nurses could feed and care for them, and placed back in the colonies they were removed from. This process took approximately 2 min per treatment replicate. The cages were removed after 10 hr, just before larvae initiate spinning behaviour and terminate feeding (Jay, 1963), and the larvae allowed to pupate normally. When pupae reached the pharate stage, these frames were removed and placed in a 33°C incubator overnight. Importantly, the method of larval starvation was designed so that larvae would not receive compensatory feeding when wire mesh cages were removed. Worker larvae generally begin spinning behaviour; that is, they are no longer feeding, at the beginning of the 9th day of development at 192 hr after laying (reviewed in Jay, 1963). This leaves little to no time for compensatory feeding after the starvation event and provides a justification for why we performed the starvation assay at this particular time in honeybee development, as in previous studies employing this method (Wang et al., 2014).

When adults emerged, they were divided into cages. These cages were further divided into pollen-fed or pollen-deprived treatments. In the pollen-fed treatment, cages were fed 1 gram of bee-collected pollen daily for the course of the experiment (seven days). The pollen used in these experiments was from a single homogenous stock of pollen gathered by honeybees at an earlier date and stored in a −20°C freezer. Thus, in this experiment there were two possible larval treatments (starved vs. not starved) and two following adult treatments (pollen-fed vs. pollen-deprived) resulting in a total of four possible cage-level treatments (starved larvae + pollen-deprived, starved larvae + pollen-fed, not starved larvae + pollen-deprived and not starved larvae + pollen-fed). Different food restriction treatment regimes were used for adults and larvae by necessity, because adults and larvae have different dietary needs and forms of feeding (e.g., larvae must be directly fed by nurse bees, whereas adult bees feed themselves from pollen stores). We intentionally chose diet restrictions that had been previously demonstrated to have known physiological effects on larvae and adults, respectively (Di Pasquale et al., 2013; Wang, Campbell, et al., 2016; Wang, Kaftanoglu, et al., 2016; Wang et al., 2014). The larval starvation treatment we used was previously shown to have effects on mass and ovarian development (Wang et al., 2014), whereas the adult pollen deprivation treatment we used was previously demonstrated to have effects on hypopharyngeal gland development and gene expression (Di Pasquale et al., 2013).

2.2 | Cage assays

When adult bees from each experiment emerged, groups of 30 day-old bees were placed in acrylic cages (dimensions: 10.6 × 10.16 × 7.62 cm) and kept in an incubator at 33°C and 50% relative humidity and fed 50% sucrose solution ad libitum. Each day, any dead bees were removed and a glass microscope slide containing synthetic QMP (Pherotech International, Delta, British Colombia) was inserted. QMP was diluted with 1% water/isopropanol to 0.01 queen equivalents, which has been shown to elicit a strong queen response (Pankiw, Winston, & Slessor, 1994). A queen equivalent is equal to the average amount of pheromone in the mandibular glands of a laying queen (Slessor et al., 1988). When the bees were 7 days old, response to the QMP slide was recorded. The number of individuals contacting the slide was recorded every 5 min for 30 min. This assay has been shown to elicit natural queen response and has been well established in the literature (Kocher et al., 2010; Slessor et al., 1988; Pankiw, Winston, Fondrk, & Slessor, 2000; Hoover, Keeling, Winston, & Slessor, 2003). We confirmed the efficacy of this assay in our experimental set-up and confirmed that 0.01 queen equivalents of QMP elicits a strong retinue response from young worker bees (see Supporting Information Figure S1). Although QMP response is only one of many possible cooperative behaviours performed by honeybee workers (e.g., trophallaxis, allogrooming), we chose to focus on this specific behaviour because QMP response is an aspect of queen care behaviour and thus provides a window into a worker's level of investment in colony reproduction.

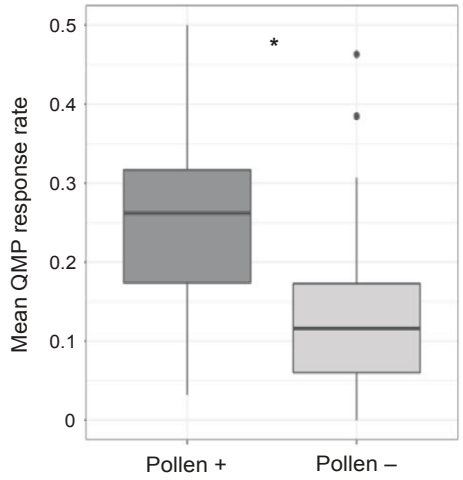

FIGURE 1 Effect of adult pollen deprivation. Bees fed pollen as adults showed a higher response to QMP than bees deprived of pollen (GLMM: z-value = 7.69, p-value = <0.0001, n = 49 pollen-fed cages and 55 pollen-deprived cages). Boxplots display median, interquartile range and full range of the data

2.3 | Physiological measurements

Newly emerged bees were collected on dry ice. We removed the gut to prevent lipid contamination from any food stored in the gut, and we measured the mass of each abdomen. Bees were processed for lipid quantification using a phospho-vanillin spectrophotometric assay (Toth & Robinson, 2005). Abdomens were placed in 5 ml of 2:1 chloroform:methanol, homogenized with a glass pestle and allowed to extract overnight. The extract was filtered through glass wool and adjusted to a constant volume. A subsample of 300 μl extract was dried, combined with 200 μl sulphuric acid, and then placed in a boiling water bath for 10 min. Next, 2 ml of the phospho-vanillin reagent (6 mg vanillin per ml of water to 4 ml 85% phosphoric acid) was added. Samples were agitated then removed from light to allow the reaction to occur for 15 min. Two hundred microlitres of each undiluted sample was pipetted into a 90-well spectrophotometry plate, and absorbance at 525 nm was measured using a Spectra Max 190 multi-well spectrophotometer. Absorbance measurements were converted to milligrams of lipid using a cholesterol standard curve. Lipid concentrations from 15 bees per treatment were compared. We also dissected out the ovaries of newly emerged bees from larval diet manipulation experiments. The total number of ovarioles in both ovaries was recorded.

2.4 | Statistics

Statistical analyses were performed using R version 3.3.1 (R Core Team, 2016). The QMP response rate per cage was calculated as the number of individuals responding to the QMP microscope slide divided by the number of bees in the cage, which was different in each cage, due to mortality. However, mortality did not differ significantly between diet treatments (linear model: F-statistic = 2.237, df = 3, 46, p-value = 0.10, n = 14 starved larvae + pollen-deprived, 11 starved larvae + pollen-fed, 14 not starved larvae + pollen-deprived and 11 not starved larvae + pollen-fed cages). For each cage, the QMP response rate was averaged across the six observation periods.

To analyse the effect of diet treatment on queen response, we used a generalized linear mixed-effects model with a binomial error structure using the function "glmer" in the R package "lme4" (Bates, Maechler, Bolker, & Walker, 2015), controlling for hive source and trial. For analyses of queen response in Experiment 3, post hoc contrasts between treatment groups were performed using the function "lsmeans" in the R package "lsmeans" (Lenth, 2016).

3 | RESULTS

3.1 | Experiment 1: Adult pollen deprivation effects on behaviour

Bees fed pollen as adults showed a higher response to QMP than adults deprived of pollen (GLMM: z-ratio = 7.69, p-value <0.0001, n = 49 pollen-fed cages and 55 pollen-restricted cages) (Figure 1).

3.2 | Experiment 2: Acute larval starvation and adult pollen deprivation effects on behaviour

Adult bees that had been restricted from contact with nurses as larvae exhibited a higher response to QMP than those that were never restricted (generalized linear mixed model: z-ratio = −5.35, p-value <0.0001, n = 25 cages per treatment; larval diet contrast results averaged over adult diet treatment) (Figure 2, Supporting Information Table S1). Adult bees fed supplemental pollen showed a higher response to QMP than adult bees not supplemented with pollen (generalized linear mixed mode: z-ratio = −8.28, p-value <0.0001, n = 25 cages per treatment; adult diet contrast results averaged over larval diet treatment) (Figure 2; Supporting Information Table S1). There was no interaction effect of larval and adult diet treatments on QMP response (generalized linear mixed model: z-value = 0.83, p-value = 0.40).

3.3 | Experiment 2: Acute larval starvation and adult pollen deprivation effects on physiology

Bees fed pollen as adults had higher per cent lipid content than bees deprived of pollen (linear mode: t-ratio = −3.72, p-value = 0.0005, n = 29 pollen-fed bees and 30 pollen-restricted bees; adult diet contrast results averaged over larval diet treatment) (Figure 3a), and pollen-fed adults had a higher average mass than bees deprived of pollen (linear model: t-ratio = −4.35, p-value = 0.0001, n = 29 pollen-fed bees and 30 pollen-restricted bees; adult diet contrast results averaged over larval diet treatment) (Figure 3a). Per cent lipid content was not affected by acute larval starvation (linear model: t-ratio = −0.45, p-value = 0.66, n = 30 restricted diet bees and 29 unrestricted diet bees; larval diet contrast results averaged over adult diet treatment) (Figure 3a), nor did acute larval starvation affect mass (linear model: t-ratio = −1.59, p-value = 0.16, n = 30 low-larval-diet

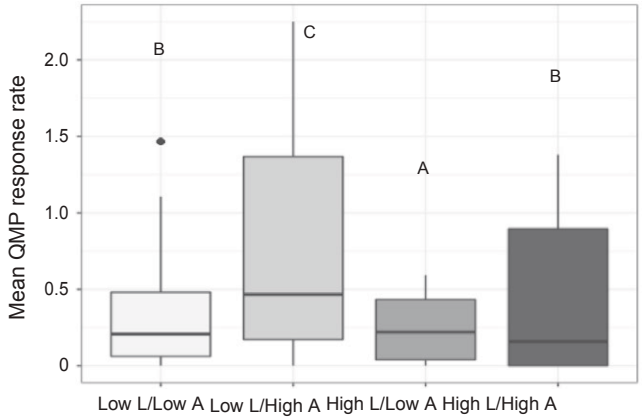

FIGURE 2 Effects of acute larval starvation and adult pollen deprivation on QMP response. Bees from low larval quantity diet treatments (Low L) exhibited a higher response to QMP than bees from high larval quantity diet treatment (High L). Letters denote significant differences (GLMM: z-ratio = −5.349, p-value < 0.0001, n = 25 cages per treatment, larval diet contrast results averaged over adult diet treatment). Adult bees fed supplemental pollen (High A) showed a higher response to QMP than adult bees not supplemented with pollen (Low A) (GLMM: z-ratio = −8.283, p-value < 0.0001, n = 25 cages per treatment, adult diet contrast results averaged over larval diet treatment). There was no interaction effect of larval and adult diet treatments on QMP response (z-value = 0.833, p-value = 0.4046). Boxplots display median, interquartile range and full range of the data

bees and 29 high-larval-diet bees; larval diet contrast results averaged over adult diet treatment) (Figure 3a). Bees from the starved larval treatment had fewer ovarioles than those from the unstarved larval treatment (t test: p-value = 0.0005, n = 55 unrestricted bees and 65 restricted bees) (Figure 3b), replicating the findings of Wang, Campbell, et al., 2016; Wang, Kaftanoglu, et al., 2016; Wang et al., 2014 and confirming the efficacy of our treatment regime.

4 | DISCUSSION

Early-life environments have the potential to affect an animal's life-history strategy through adaptive adjustments in plastic phenotypic traits (Monaghan, 2008). In this study, we provide evidence that individual worker honeybees may adaptively adjust their behavioural and physiological traits in response to nutritional stress. Specifically, we found a relationship between the nutritional environment a honeybee worker experiences and her likeliness to respond to queen pheromone, an indicator of investment in colony reproduction. When developing larvae experience a period of acute starvation, they become more responsive to queen pheromone later in life no matter their adult diet. Interestingly, adult nutritional stress had the opposite effect on behaviour. Adult bees deprived of pollen had a lower response to queen pheromone than adult bees fed pollen. Together, these data suggest nutritional stress at different life stages can have differential effects on bees' investment in colony reproduction.

The fact that larval nutritional stress also influences ovary development suggests possible connections between individual and colony reproductive trade-offs in worker bees. In concurrence with previous studies (Linksvayer et al., 2011; Wang, Kaftanoglu, et al., 2016), we found that diet quantity deprivation (restricted access to nurse bees) during the fifth instar of larval development resulted in decreased ovariole number. This manipulation of larval diet supports the hypothesis that, in honeybee workers, nutritional stress leads to divestment in ovarian development and an increase in cooperative behaviour.

Diet stress had strikingly opposite effects on behaviour and physiology of larval and adult honeybees. We hypothesized that cooperative behaviour would be promoted by nutritional stress, and therefore, we predicted increased response to queen pheromone from bees that experienced diet restriction, both as larvae and as

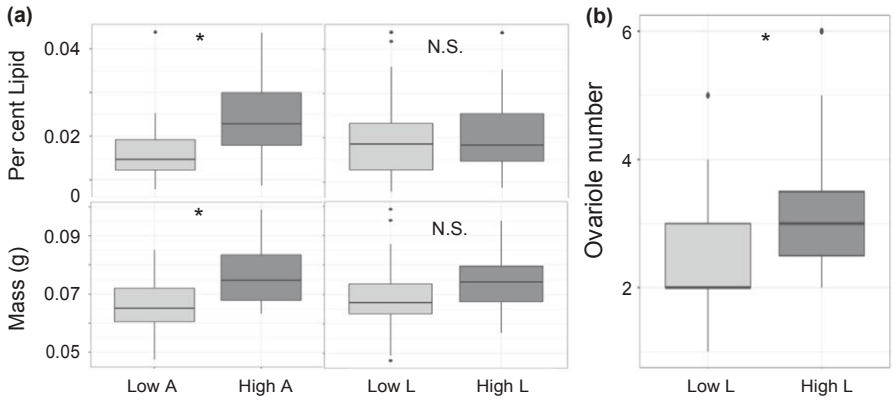

FIGURE 3 Physiological effects of acute larval starvation and adult pollen deprivation. (a) Bees fed pollen as adults (High A) had higher per cent lipid content than bees not fed pollen (Low A) (lm: t-ratio = −3.715, p-value = 0.0005, n = 30 Low A and 29 High A bees; adult diet contrast results averaged over larval diet treatment) and greater mass (lm: t-ratio = −4.35, p-value = 0.0001, n = 30 Low A and 29 High A bees; adult diet contrast results averaged over larval diet treatment). Per cent lipid content was not affected by larval quantity diet treatment (lm: t-ratio = −0.445, p-value = 0.6578, n = 30 Low L and 29 High L bees; larval diet contrast results averaged over adult diet treatment) nor was mass (lm: t-ratio = −1.59, p-value = 0.16, n = 30 Low L and 29 High L bees; larval diet contrast results averaged over adult diet treatment). (b) Bees from low larval quantity diets treatment had fewer ovarioles than those from the high larval quantity (t-test: p-value = 0.0005, n = 55 High L and 65 Low L). Boxplots display median, interquartile range and full range of the data

adults. However, this relationship was only evident in bees that experienced diet restriction as larvae, and was accompanied by decreased ovary development. The exact opposite effect occurred in honeybees that experienced diet restriction as adults. In addition, while larvae invested nutritional resources in their ovaries, adults invested nutritional resources in their abdominal fat stores. Adult fat stores are likely to be metabolized for fuelling colony-level activities such as wax production and brood food production (Hepburn et al., 1991; Toth & Robinson, 2005). Thus, how nutrition mediates cooperative behaviour differs greatly depending on the life stage at which individuals experience a nutritional environment.

We suggest this life stage-dependent effect of nutrition may be, in part, due to the different degree of developmental plasticity honeybees have at these different life stages (Figure 4).

Female honeybee larvae are reproductively totipotent (they can develop into either a queen or a worker) for their first 3–4 days of age (Weaver, 1957). After this point, worker-destined larvae can no longer develop into viable queens (Winston, 1987). However, their reproductive potential is not yet entirely fixed, as worker ovaries (the number of ovarioles) only begin to reduce via programmed cell death in the fifth larval instar (Hartfelder & Steinbrück, 1997). Diet restriction appears to mediate ovariole programmed cell death, as nurse bees can control the food quantity developing larvae receive at this sensitive stage (Wang et al., 2014). Thus, workers retain developmental plasticity through the fifth larval instar, in the form of variable numbers of ovarioles. This correlates with adult reproductive potential, as workers with more ovarioles are more likely

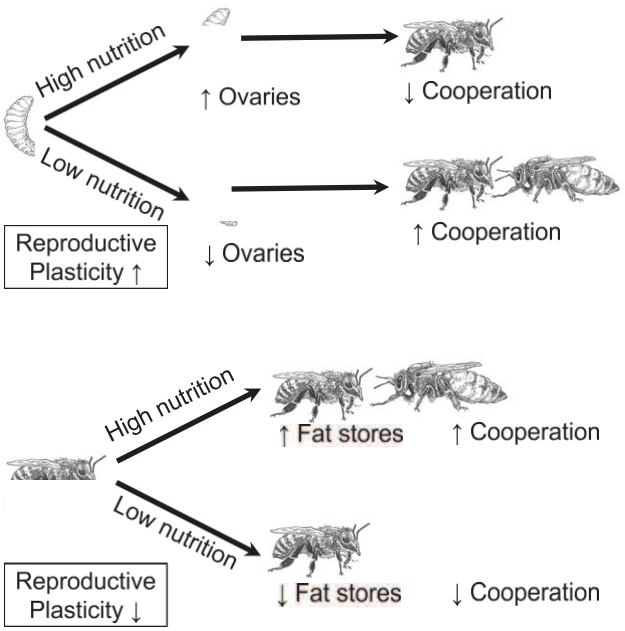

FIGURE 4 Hypothetical idea for different strategies for investment of nutritional resources, depending on reproductive plasticity. When reproductive potential is plastic, as in larvae, a worker invests nutritional resources in her ovaries and exhibits low cooperation. When reproductive potential is fixed, as in adults, a worker invests nutritional resources in lipid stores and exhibits high cooperation

to lay eggs of their own (Makert et al., 2006). As an adult, however, a worker's ovariole number is fixed, and diet can no longer influence this aspect of her reproductive physiology (Hartfelder & Steinbrück, 1997). Although adult worker bees do retain some level of reproductive plasticity in the form of activating their ovaries and laying unfertilized eggs, this behaviour is not typically seen under normal queenright conditions. Thus, reproductive traits remain somewhat plastic as larvae, but are predominantly fixed by adulthood.

Consequently, if as we hypothesize, nutritional resource availability mediates cooperative behaviour *via* reproductive pathways, then nutrition's effect on cooperative behaviour may depend on the degree of reproductive plasticity present. Therefore, we hypothesize that nutritional stress promotes cooperation, but this effect is limited to situations in which individuals have greater plasticity in reproductive potential. In other words, if an individual is unable to shunt adequate nutritional resources towards sustaining reproductive development, cooperation with others may be the best option to increase their fitness. We predict that when an individual's reproductive potential is plastic (as in larval honeybees), nutritional resource availability will negatively correlate with cooperative behaviour. In such situations, resources may be shunted to an individual's own reproductive development (favouring "me" instead of "we"), as in the case of increased ovariole number in larval honeybee workers. Higher ovariole number will correlate with a reduction in cooperative behaviours as an adult, such as reduced response to the queen (Kocher et al., 2010). In addition, we predict that when an individual's reproductive potential is fixed (as in adult honeybees with generally low reproductive potential), nutritional resource availability will positively correlate with cooperative behaviour. Because energy obtained from nutritional resources can no longer be used to bolster the individual's own reproductive development, these resources should be invested in the group (favoring "we" instead of "me") (Wheeler, 1986). We observed that adult worker honeybees invested nutritional resources in increased queen responsiveness and lipid stores, which are likely metabolized to fuel cooperative activities such as brood rearing, queen rearing and wax production (Hepburn et al., 1991; Svoboda, Thompson, Herbert, Shortino, & Szczepanik-Vanleeuwen, 1982).

Although our data are consistent with the argument that nutritional stress leads to adaptive changes in physiological and behavioural life-history strategies in honeybees, there are other possible explanations. The observed connection between larval nutritional stress and increased queen pheromone response could instead be a form of worker emergency response. Perhaps experiencing nutritional stress as larvae cues workers to exhibit higher queen care, protecting the queen when the hive is in dire condition. Further experimentation with other potential colony "emergency" status cues (i.e., high pest pressure, heat stress, toxin exposure, disease) could help elucidate whether developing larvae can sense colony stressors and adjust their behaviour adaptively upon eclosion.

The results of this study support the hypothesis that nutritional stress can affect cooperation, but further research on cooperative

behaviours other than queen pheromone response could further cement this idea. Honeybees exhibit many cooperative and selfish behaviours (Walton & Toth, 2016), and testing whether these behaviours are also influenced by nutrition could further clarify the nutritional environment's role in cooperative behaviour. Additionally, comparative studies can illuminate how universal this connection may be, and enhance understanding of how plasticity of reproductive potential affects how nutrition mediates cooperation. Experiments examining the effects of nutritional stress on cooperation would be especially informative across species with gradients in reproductive plasticity, especially on other eusocial insects with higher levels of reproductive plasticity that persist through adulthood (e.g., *Polistes* wasps: Reeve, 1991). The general principle that nutritional stress fuels cooperation has been observed much more broadly than in social insects, for example, in some vertebrates and slime moulds (Kessin, 2001; Sontag et al., 2006), but studies from other systems also suggest the opposite trend to occur (Vitousek et al., 2004). We hypothesize that nutritional stress should fuel cooperation in kin groups with limited reproductive opportunities, whereas it should dampen cooperation in other species or situations with ample opportunities for individual reproductive success. In the future, broad-scale comparative studies can address whether the patterns recorded in this study persist across different levels of reproductive plasticity and across lineages through evolutionary time.

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AUTHORS' CONTRIBUTIONS

A.W., A.G.D. and A.L.T. conceived the ideas and designed methodology; A.W. and M.A.B. collected the data; A.W. analysed the data; A.W. and A.L.T. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILIT Y

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2rh22m7 (Walton, Dolezal, Bakken, & Toth, 2018).

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