

# Social influences on body size and developmental time in the bumblebee *Bombus terrestris*

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**Abstract** In many social insects, including bumblebees, the division of labor between workers relates to body size, but little is known about the factors influencing larval development and final size. We confirmed and extend the evidence that in the bumblebee *Bombus terrestris* the adult bee body size is positively correlated with colony age. We next performed cross-fostering experiments in which eggs were switched between incipient (before worker emergence) and later stage colonies with workers. The introduced eggs developed into adults similar in size to their unrelated nestmates and not to their same-age full sisters developing in their mother colony. Detailed observations revealed that brood tending by the queen decreases, but does not cease, in young colonies with workers. We next showed that both worker number and the queen presence influenced the final size of the developing brood, but only the queen influence was mediated by shortening developmental time. In colonies separated by a queen excluder, brood developmental time

was shorter in the queenright compartment. These findings suggest that differences in body size are regulated by the brood interactions with the queen and workers, and not by factors inside the eggs that could vary along with colony development. Finally, we developed a model showing that the typical increase in worker number and the decrease in brood contact with the queen can account for the typical increase in body size. Similar self-organized social regulation of brood development may contribute to the optimization of growth and reproduction in additional social insects.

**Keywords** Bumblebee · *Bombus terrestris* · Size determination · Division of labor · Caste determination · Alloethism

## Introduction

Division of labor among workers is one of the organization principles of insect societies. Studies on the mechanisms that influence task performance are therefore critical for understanding both the function and the evolution of insect societies. In many social insects, division of labor is based on profound variation in worker body size, a system that is termed “alloethism” (Wilson 1971). Alloethism is common in social insects such as ants (Wilson 1978), bees (Goulson et al. 2005), and termites (Miura and Matsumoto 1995). Large workers typically specialize in activities outside the nest such as foraging and nest defense whereas small workers are more likely to perform activities inside the nest including brood care and nest cleaning (Wilson 1971). By contrast to the mechanisms of age-related division of labor which have been extensively studied in honey bees (e.g., Robinson 1992; Beshers and Fewell 2001; Smith et al. 2008; Calderone and Page 1991; Seeley and Kolmes 1991), little is known about the mechanisms regulating size-related division of labor.

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Insect body size is influenced by internal and external factors, and their interactions (Nijhout 2003; Davidowitz et al. 2004). Internal factors influencing size polymorphism include genetic variation between individuals, epigenetic markers, and hormones or resources deposited in the egg. For example, in the fruit fly *Drosophila melanogaster* genetic dissections have linked specific genes and molecular pathways such as *MAP4K3*, *Hippo*, and the insulin/insulin-like signaling pathway to the developmental processes determining adult body size (Edgar 2006; Pan 2007; Bryk et al. 2010). Mutant fruit flies developing with no prothoracicotropic hormone (PTTH) show prolonged larval growth and an increase in ultimate adult mass (McBrayer et al. 2007). In honey bees, specific areas in the genome (QTLs—quantitative trait loci) are associated with body size differences between European and African subspecies (Hunt et al. 1998). In the moth *Manduca sexta*, lack of Juvenile Hormone (JH) in the last larva instar resulted in early pupation and miniature adults (Nijhout and Williams 1974); in the ant *Pheidole bicarinata*, elevated JH levels during the last larval instar induced the larvae to develop to soldier workers that are significantly larger than typical workers (Wheeler and Nijhout 1981). In some harvester (Genus *Pogonomyrmex*) and *Pheidole pallidula* ants, ecdysteroids deposited in the egg by the queen influence the development and ultimate body size, as well as the caste of the larvae (Suzzoni et al. 1980; Schwander et al. 2008). In the termite *Reticulitermes speratus*, the queen adjusts the egg size according to colony development (Matsuura and Kobayashi 2010).

Many external factors also influence insect body size. For example, the ambient temperature was shown to influence the development of several species including *M. sexta* (Davidowitz et al. 2003, 2004) and the solitary bee *Osmia bicornis* (Radmacher and Strohm 2010). Food amount and composition can also have major impacts on insect body size (Davidowitz et al. 2004; Sutcliffe and Plowright 1990; Roulston and Cane 2000, 2002; Tasei and Aupinel 2008; Radmacher and Strohm 2010; Quezada-Euan et al. 2011). The diet fed to female larvae determines caste in honey bees (Patel et al. 2007; Smith et al. 2008).

The goal of our study was to identify factors that influence body size in the bumblebee *Bombus terrestris*. Bumblebees (tribe Bombini) are an important and large taxon of social insects showing size-related division of labor. Bumblebees are commonly considered “primitively eusocial” since there are no morphological differences between queens and workers other than size (Michener 1974; Cnaani and Hefetz 2001; Goulson 2003), and there is less specialization in the division of labor relative to highly eusocial species such as honey bees and ants (Michener 1974; Goulson 2003; Jandt et al. 2009). In temperate regions, bumblebee colonies are typically founded during the spring by a single mated overwintered queen and reach a population of up to few hundred workers. The worker

population shows profound size polymorphism with up to tenfold difference in body mass (Michener 1974; Alford 1975). Worker body size is associated with task performance. Smaller bees typically perform more in-nest activities such as brood care, whereas larger bees are more likely to forage outside the nest (Cumber 1949; Brian 1952; Free 1955; Michener 1974; Alford 1975; Yerushalmi et al. 2006). Larger bees are more efficient in bringing pollen and nectar back to the colony (Goulson et al. 2002; Spaethe and Weidenmuller 2002). They appear to be better suited for foraging activities as they have better visual discrimination, odor sensitivity, learning abilities, and stronger circadian rhythms and phototactic response compared with their smaller full-sister bees (Spaethe and Chittka 2003; Worden et al. 2005; Yerushalmi et al. 2006; Kapustjanskij et al. 2007; Spaethe et al. 2007; Merling 2008). These differences in behavior are associated with relevant size-related variation in morphology and neuroanatomy. Larger workers have more ommatidia with wider facets in their compound eyes, elevated density of olfactory sensilla on the antennae, and additional brain neurons that are immunostained with antiserum against the circadian neuropeptide Pigment Dispersing Factor (PDF) compared with their smaller sisters (Spaethe and Chittka 2003; Spaethe et al. 2007; Weiss et al. 2009). Additionally, variation in metabolic and stress response protein levels in the brain and abdomen between small and large sister workers has been recently reported (Wolschin et al. 2012).

We hypothesized that both internal factors in the eggs, such as hormones or nutritional factors, and environmental factors, such as the number of workers in the colony and the presence or absence of the queen, may influence larval development and adult body size. Previous studies suggest that genetic variation does not account for the profound size polymorphism in bumblebees. Full-sister workers, which are very similar genetically, still differ profoundly in body size (Schmid-Hempel and Schmid-Hempel 2000); sib-mating, which further reduces genetic variation, does not influence the variation in body size of workers or drones (Gerloff et al. 2003).

We first recorded the body size of bumblebee workers emerging from colonies in the laboratory. This experiment was performed to confirm and expand on earlier results demonstrating that worker body size increases with colony age. We next performed a cross-fostering experiment allowing us to decouple the influence of factors in the eggs from environmental determinants of body size. Given the support to the hypothesis that the bee ultimate body size is under strong environmental influence, we then performed additional experiments to explore the influence of specific social factors including queen presence and worker number. We then conducted detailed observations of queen behavior relating to larval care to further elucidate her maternal care role and the specific effects of queen–brood interactions. Lastly, we used a split-colony design by queen excluder to

demonstrate that the queen influence is at least partially mediated by close distance interactions with the larvae.

## Materials and methods

### Bumblebees

*B. terrestris* founding queens and incipient colonies were obtained from Polyam Pollination Services, Kibbutz Yad-Mordechai, Israel. Incipient colonies contained a queen, 5–10 workers, and brood in various developmental stages. Founding queens were obtained after diapause and before they started to lay eggs. Each colony or founding queen was housed in a wooden nesting box (21 × 21 × 12 cm). The nesting boxes included a front wall and a top cover made of transparent Plexiglas®, enabling detailed observations of colony development, comb structure, and bee behavior. The nesting boxes were placed in an environmental chamber [29 ± 1 °C; 45 ± 5 % relative humidity (RH)] in constant darkness at the Bee Research Facility at the Edmond J. Safra campus of the Hebrew University of Jerusalem. The experiments were conducted between January 2010 and July 2011. The colonies were fed ad libitum with commercial sugar syrup obtained from Polyam Pollination Services and “pollen cakes” made of fresh pollen (collected by honey bees) mixed with sugar syrup. All observations and treatments were made under dim red light. As an index for body size, we measured (under a dissection microscope, ×10 magnification) the length of the front wing marginal cell. The length of the marginal cell is highly correlated with wing length and other indices for body size, can be precisely measured, and does not change with age or flight intensity (Knee and Medler 1965; Owen 1988; Yerushalmi et al. 2006).

**Experiment 1:** the influence of colony stage on the body size of emerging bees

The aim of this experiment was to record the body size of bees emerging in typical colonies in the laboratory. We housed two incipient bumblebee colonies in nesting boxes as described above. We collected all newly emerging bees (0–48 h post-emergence) during the first 50 days following the emergence of the first worker and tagged each bee with a colored number tag (Graze, Weinstadt, Germany). After 50 days, we froze the colony (−20 °C) and measured the length of the marginal wing cell for all tagged bees. Given that in *B. terrestris* there is no overlap between the body size of queens and workers (Goulson 2003; see also Cnaani and Hefetz 2001), we assumed that significantly larger (marginal cell >4.2 mm) female bees emerging at the latest stages of colony development are queens (in this study, marginal cell size range for workers was 2.05–3.6 mm,  $n=713$ , and for queens 4.2–4.8 mm,  $n=51$ ). We

analyzed the results using a linear regression model, with day of emergence as the independent variable and the length of the marginal cell as the dependent variable (SPSS 17.0 software package, IBM, was used for all statistical analysis in this study).

**Experiment 2:** the influence of internal factors in the egg and colony stage on worker body size

We used a cross-fostering experimental design in which we switched batches of eggs between founding colonies (“Incipient”) and young colonies at a more advanced stage of development (“Established”). The incipient colonies contained only the mother queen and her first batches of eggs. The established colonies were 5–8 days after the emergence of the first worker and contained a queen, 5–10 workers, and brood at all stages of development. To transfer eggs between colonies, we first removed all bees from both the incipient and the established colonies. We then collected 6–10 eggs from each colony and transplanted them into an empty egg cup in the paired colony of the alternate maturity from which we had already removed the local eggs. Transferred eggs were 2–3 days old. We used a standard beekeeping grafting tool to collect and transfer the eggs between the colonies. After placing the eggs gently inside the recipient egg cup of the host colony, we carefully sealed the cup with wax from the host colony (as in Bloch 1999). Following cup sealing, we first returned the queen and shortly afterwards the workers back to the nest box. In each colony, we also had sham handled egg cups of a similar age that were similarly opened and sealed, but without removing the eggs. The sham treatment served as a control for the interference associated with opening and resealing the egg cups. The whole procedure lasted ~15 min. We observed the colony for 3 h following the manipulation to assure that the queen did not replace the introduced eggs with her own eggs. To track the position and development of the cross-fostered and control brood, we photographed the wax comb every 1–2 days. When the focal larvae had pupated, we transferred them into a small box (8 × 12 × 5 cm) in which they emerged. We then froze the bees emerging from these pupae and measured their size as described above. We used a one-way ANOVA followed by Fisher’s least significant difference (LSD) post hoc test to analyze the results. We repeated this experiment three times (using new colonies for each replicate).

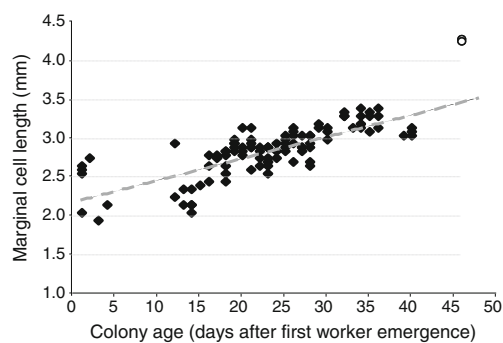
**Experiment 3:** the contribution of the queen and the workers to larval nursing during early stages of colony development

In order to quantify brood nursing by the queen and workers, we performed detailed observations in which we recorded larvae feeding in colonies at early stages of development. We observed incipient colonies with the founding queen and her first batch of eggs. A second set of observations was performed after the second batch of eggs was laid in these colonies, which

at this point contained at least 10 workers. The second set of observations was performed 23–27 days after the first batch of eggs was laid. In each set of observations, we observed the focal brood cells for 1 h each day for five successive days. During each observation, we recorded all feeding events by the queen and the workers. A feeding event was recorded when a bee was seen opening a larval cup, inserting her head into the cup, and contracting her abdomen (Ribeiro 1999). The age of the larvae was recorded relative to the clearly identified day of pupation. This experiment was repeated with four different colonies. We used Student's *t* test to compare the average total number of feeding events between the two colony stages for the four replicates. The number of focal larvae fed during the observation session was estimated based on counting the number of adult bees developing from the focal larvae cups. In addition, at each observation day we counted the number of larvae in individual cells. During the early stages of development, bumblebee larvae are clumped together in a common cell, but later at about 5 days before pupation each larva has its own individual wax cover and can be clearly identified. The number of emerging adults was very similar to the number of counted larvae suggesting that there was no significant mortality between the larva and adult stages. The colonies were healthy, developed normally, and there were no indication for significant larval death in our focal groups during the last 5 days prior to pupation when most of our data was collected. Finally, we analyzed the results of each batch separately using a linear regression model, with age of the larvae as the independent variable and the average number of feeding as dependent variable. We used Wilcoxon signed rank test to compare feeding rate in the first and second batch for each of the colonies.

**Experiment 4: the influences of queen presence and worker number on brood developmental time and final body size**

In this experiment, we compared the offspring of a single queen developing in four different social environments (treatments): (1) 10 workers without a queen ( $10w$ ), (2) a single worker ( $w$ ), (3) a single queen ( $Q$ ), and (4) a queen with 10 or more workers ( $Q, 10+w$ ) (see Supplementary Fig. 1 for flow chart for this experiment). We placed a young colony founding queen together with 12 foreign (not her offspring) 1-day-old workers in a small wooden cage ( $8 \times 12 \times 5$  cm) and left her for 2–3 days to lay her first batch of eggs. We then transferred the queen together with two workers into a new cage, leaving the eggs to develop with the remaining 10 workers ( $10w$ , treatment 1). We left the queen and the two workers for 1–3 days in the new cage to lay a second batch of eggs, and then removed the queen together with one of the workers and placed them in a new cage; the eggs of the second batch were left to develop with the remaining single worker ( $1w$ , treatment 2). After 1–2 days when the queen laid in the new cage, we removed the worker



**Fig. 1** The body size of newly emerging bees is positively correlated with colony age. Each *filled diamond* represents a single worker bee ( $n=101$ ); each *open circle* represents a gyne ( $n=4$ ). Day 1 is the day of first worker emergence. There is a significant positive correlation between worker body size and colony age (linear regression  $R^2=0.59$ ,  $p<0.001$ ). A similar analysis for an additional colony is presented in Supplementary Fig. 3

and left the queen to rear her brood by herself ( $Q$ , treatment 3; a treatment that most closely resembles a natural colony at the founding stage). We color marked all the workers that developed from the brood of treatment 3. When 10 workers were present, we moved the entire colony into a larger nesting box ( $21 \times 21 \times 20$  cm). The queen laid additional eggs in the new nesting box within 1 day. We tracked this batch of eggs, which were tended by the full-sister workers and by the queen ( $Q, 10+w$ , treatment 4). The queens calmed down quite rapidly after each transferred and laid again; at the day following transfer, they seemed to behave normally. We followed the development of all four brood batches and recorded the days in which they emerged as adults. When all adult bees had emerged from the focal batches, we froze them and later measured their size as described above. All the cages with the brood experiencing the four different treatments were housed in the same environmental chamber ( $29 \pm 1$  °C;  $45 \pm 5$  % RH). We repeated the experiment simultaneously with three queens. We used a one-way ANOVA with LSD post hoc tests to compare body size and development duration for bees that developed in the four social environments (a flow chart for the experiment is provided in Supplementary Fig. 1).

**Experiment 5: the influence of close distance interactions with the queen on brood size and developmental time**

We compared two brood batches from the same colony separated by a queen excluder mesh (workers, but not the queen, were able to pass through the mesh). We split a young colony (3–7 days after first worker emergence) that was housed in a nesting box ( $21 \times 21 \times 20$  cm) into two parts using a queen excluder mesh ( $8 \times 8$ -mm holes; herein referred to as “queen excluder colonies”). There were egg cells with known day of oviposition on both sides of the queen excluder. To motivate the workers to move across the queen excluder, we placed the

pollen on the queenright side and the sugar syrup on the queenless side of the colony. We tracked the development of the brood on both sides of the mesh by daily photographing the nest. When the focal larvae had pupated, we transferred them into a small box (8×12×5 cm) in which they emerged. We then froze the bees emerging from these pupae and measured their size as described above. In control queenright colonies, we conducted the same procedure but used a mesh allowing a free queen passage (10×10-mm holes + 2 holes of 20×20 mm; herein referred to as “queenright colonies”). A second control colony was as described above but without a queen in the nest. The separating mesh allowed free worker passage (10×10 mm; herein referred to as “queenless colonies”). We repeated this experiment three times. We used Student’s *t* tests to compare the body size and developmental time between the two sides of each colony and nested ANOVA with Scheffé’s post hoc tests to compare between the different colony types.

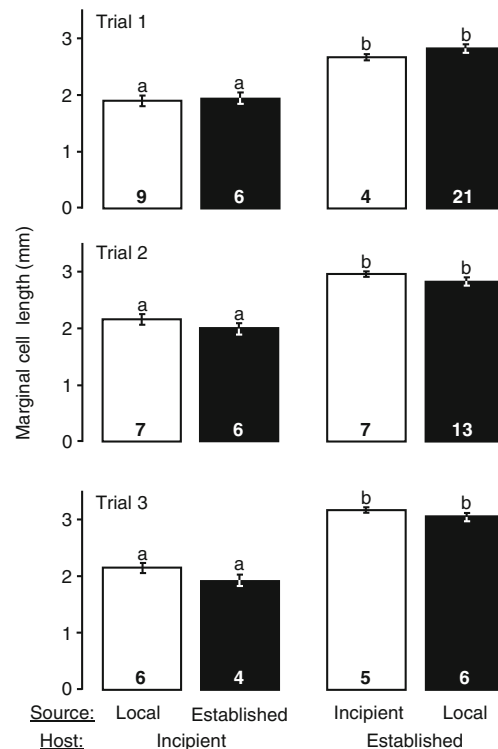
## Results

Experiment 1: the influence of colony stage on the body size of emerging bees

Bee body size was positively correlated with colony stage, measured as days after the emergence of the first worker (Fig. 1;  $R^2=0.59$ ,  $p<0.001$ ). The workers that emerged during early stages of colony development were smaller compared with their later emerging full sisters. The average length of the front wing marginal cell in the first brood was  $2.34\pm0.13$  mm ( $n=7$ ) compared with  $3.15\pm0.05$  mm ( $n=10$ ) in the last batch of workers emerging just before the emergence of the first gynes (virgin queens; two-tailed Student’s *t* test,  $p<0.001$ ; similar size differences for bees from additional four colonies from experiment 3 are summarized in Supplementary Fig. 2). In a second colony that we monitored, both males and females began emerging at the onset of colony development. This pattern is atypical as haploid males normally only emerge during the later stages of colony development (Alford 1975; Goulson 2003). Early emergence of males as observed in this colony typically indicates that males are developing from diploid eggs that are produced when a queen mates with a male with a similar allele to hers at the sex determination locus (Duchateau et al. 1994). Nevertheless, this colony also showed a similar positive correlation between worker body size and colony age. The slope of the regression line for the males was similar to that of the workers, though males were significantly larger at all stages (males =  $3.24\pm0.05$  mm,  $n=46$ ; workers =  $2.84\pm0.04$  mm,  $n=56$ ; two-tailed Student’s *t* test,  $p<0.001$ ; Supplementary Fig. 3). This study presents the most detailed correlation between body size and colony age in bumblebees. The results confirm and extend previous studies with bumblebees (Knee and Medler 1965; Plowright and Jay 1968).

Experiment 2: the influence of internal factors in the egg and colony stage on worker body size

The increase in body size with colony development in the first experiment could stem from differences in the eggs laid by the queen at different colony stages (e.g., genotype, epigenetic markers, or hormonal differences), from changes in the colony environment (e.g., worker or brood number), or from both. To uncouple these two sources of variation, we used a cross-fostering experimental design in which we exchanged eggs between an incipient colony (queen and her first batch of eggs) and an established colony (a queen with ~10 workers and brood in all stages of development). In all three trials of this experiment, the introduced eggs developed into bees with a body size similar to that of nestmates developing in the foster colony at the same time (Fig. 2, one-way ANOVA,  $p<0.001$ ). The body size of bees emerging from introduced eggs was significantly different from that of their full sisters



**Fig. 2** The colony environment rather than factors in the eggs influences the increase in body size with colony development. We used a cross-fostering experimental design, switching eggs between a colony with only a queen (*Incipient*) and a colony at a later stage containing a queen, 10 workers, and brood in all stages (*Established*). Egg cells of similar age in the host colony (*Local*) served as the control. *Source* indicates the source colony in which the eggs were laid and *Host* indicates the foster colony in which the brood emerging from these eggs developed. The *Local* egg cups were sham handled. Values are mean±SE; sample size is given inside the bars. Body size of emerging brood differed between treatment groups in all three repetitions (one-way ANOVA,  $p<0.001$ ); bars with different letters are significantly different (LSD post hoc test,  $p<0.05$ ). Bars with the same filled color in each trial indicate that the bees were full sisters

who remained in the source (donor) colony (Fig. 2). In all three trials, the bees (local or introduced) that developed in an incipient colony were smaller than those from an established colony (local or introduced). These results indicate that the nest environment rather than internal factors in the egg determines the final body size of the developing brood.

**Experiment 3: the contribution of the queen and the workers to larva nursing during early stages of colony development**

To study brood care during colony development, we observed the number of feeding events per larva in colonies containing the first or second brood batches. During the founding stage, only the queen cared for the brood whereas in colonies with a second batch of brood, both the queen and the workers cared for the brood. Our observations indicated that the queen continued to feed the larvae of the second batch mostly at the early stages of brood development (7–5 days before pupation). The percentage of feeding events by the queen was about 7 % during the first 3 days of the observations and later decreased to 0 % during the two last observation days (Fig. 3). In all four trials, the bees emerging from the second batch were significantly larger than bees emerging from the first batch (Student's *t* test,  $p < 0.01$ ; Supplementary Fig. 2). In both batches, larvae growth was associated with a progressive increase in the number of feeding events per hour (Fig. 3; first batch—linear regression model  $R^2 = 0.7$ ,  $p = 0.075$ ; second batch— $R^2 = 0.84$ ,  $p = 0.01$ ). The total number of larval feeding events per hour throughout the entire observation period was similar for the first and second batches (Wilcoxon signed rank test— $p = 0.222$ ,  $n = 4$  colonies, 4 days). Our sample size was too small to compare larvae at specific ages developing during the early and later



**Fig. 3** The queen continues to feed larvae in established colonies with nurse workers. The first batch of brood is the brood from which the first workers in the colony emerged. The second batch was laid when the colony contained a queen and about 10 workers. The numbers below the bars show the age of the larvae relative to the date of pupation (0=day of pupation); the lower x-axis shows the age of the colony relative to the emergence of the first worker (marked with a 0). Data is based on direct observations of brood-tending behavior. Values are mean±SE based on data from four different colonies

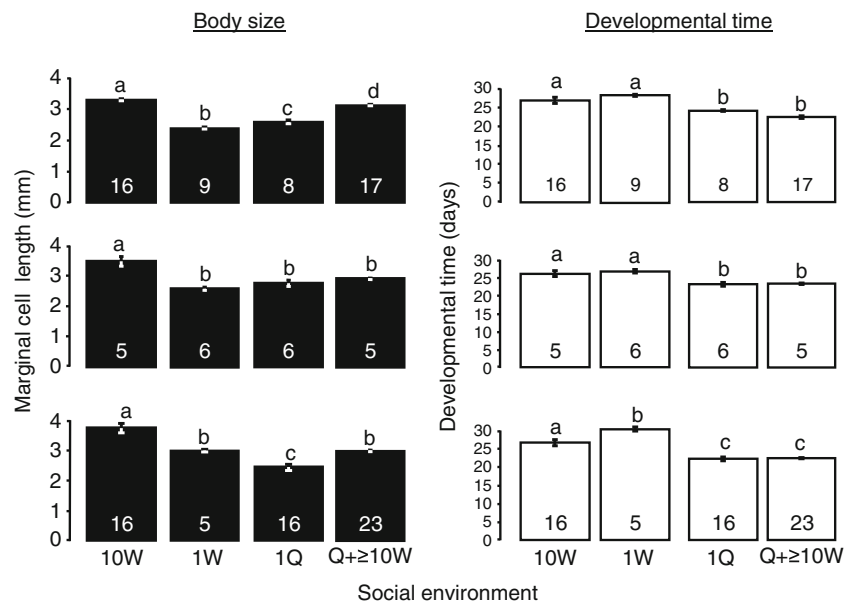
stages of colony development; we therefore cannot exclude the possibility that the rate of feeding of larvae at certain ages differs between the first and second batches. These observations show that the second batch of brood is fed mostly by the workers, but the queen still tends the brood and feeds larvae, primarily at early stages of development.

**Experiment 4: the influence of queen presence and the number of workers on brood developmental time and final body size**

Given that our observations (Fig. 3) suggest that the frequency of brood contact with the queen and workers changes during colony development, we next tested the influence of the queen and the workers on larval developmental time and ultimate body size in a tightly controlled laboratory setup. Bees that developed in different social environments (“treatments”, Supplementary Fig. 1) differed both in body size and in development duration in all three trials of this experiment (Fig. 4; one-way ANOVA,  $p < 0.001$ ). Bees that were cared for by 10 workers without a queen were significantly larger than bees cared for by 10 workers (or more) with a queen (LSD post hoc test,  $p < 0.05$ ); some of these larvae developed into gynes (trial 1—0/16, trial 2—1/5, trial 3—7/16). In trials 1 and 3, the brood raised by a queen with 10+ workers were significantly larger than those reared by a queen alone (LSD post hoc test,  $p < 0.05$ ), a similar but statistically insignificant trend was seen in trial 2 ( $p = 0.075$ ). There was no consistent trend across trials for the comparison of body size for brood reared by a single queen or a single worker. In all three trials, the duration of development from egg to adult was shorter when a queen was present compared to queenless conditions (Fig. 4; one-way ANOVA,  $p < 0.001$ ; LSD post hoc test,  $p < 0.05$ ). Developmental time was similar for brood in cages with only a queen or with a queen and 10+ workers (LSD post hoc test,  $p > 0.05$ ). The duration was also similar for brood in cages with a single worker or 10+ workers in trials 1 and 2 (LSD post hoc test,  $p > 0.05$ ), and was longer for brood attended by a single worker in trial 3 (LSD post hoc test,  $p < 0.05$ ). These results suggests that both the presence of the queen and the total number of nursing bees affect body size, but of the factors tested, only the presence of the queen affected brood developmental time.

**Experiment 5: the influence of close distance interactions with the queen on brood size and developmental time**

Given our findings on the impact of the queen on brood developmental time and body size in experiment 4, and our observations of brood nursing in experiment 3, we tested whether the queen influence is mediated through close distance interactions with the larvae. Bees developing in the queenless side of the queen excluder in the “queen excluder colonies”, and that did not have close distance interactions



**Fig. 4** The presence of the queen and the number of workers affect final body size and brood developmental time. *Left column*: worker body size; *right column*: egg to adult developmental time. *10W*: 10 workers without a queen, *1W*: a single worker, *1Q*: a single egg-laying queen, *Q+≥10W*: an egg-laying queen with 10 or more workers and brood in all stages. Each *row* depicts a different repetition of the

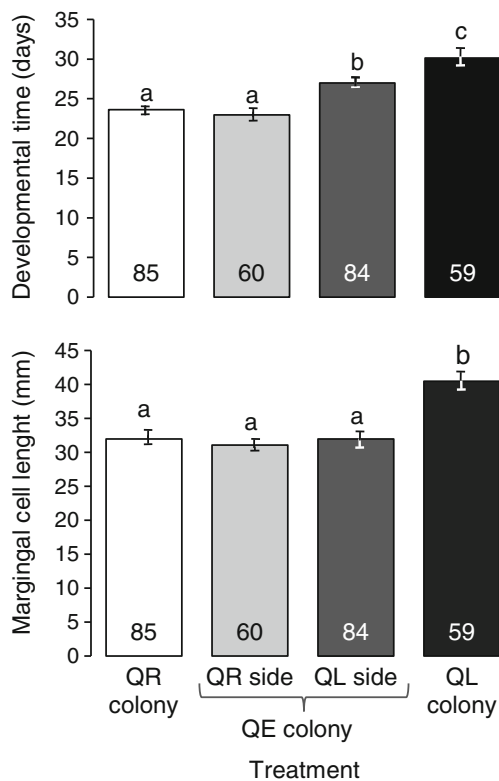
experiment. Values are mean±SE; sample size is given inside the *bars*. Body size of emerging brood and developmental time differed between treatment groups in all three repetitions (one-way ANOVA,  $p<0.001$ ); groups with different *letters* are significantly different (LSD post hoc test,  $p<0.05$ ). The same queen laid all of the eggs for the four experimental groups in each trial

with the queen, developed for an additional 2–3 days compared with their sisters developing on the queenright side of the colony (Student's  $t$  test,  $p<0.001$ ); the results were similar across the three trials of this experiment. The size of the bees from the two sides was similar in two repetitions and was larger in the queenless side for the third repetition (Student's  $t$  test,  $p=0.02$ ); none of the bees in these colonies developed into gynes (Supplementary Fig. 4). In the queenright colonies, the developmental time and the size of the bees was the same on both sides of the mesh in all three trials (Supplementary Fig. 4). In the queenless control colonies, the size of the bees was the same on the two sides of the mesh in all three trials; the developmental time was similar in two colonies and longer in the pollen side in one colony (Student's  $t$  test,  $p<0.01$ ). Most of the bees (39 out of 59) in the queenless colonies developed into gynes. When comparing the bees from the different treatments, we found that the average size of the bees from the queenright colonies, the queenright side of the queen excluder colonies, and the queenless side of the queen excluder colonies was the same and significantly smaller from the bees developing in the queenless colonies (nested ANOVA,  $p<0.001$ ; Fig. 5, lower panel). Developmental time was similar for bees developing in the queenright colonies, and in the queenright side of the queen excluder colonies, and was significantly shorter than for bees developing on the queenless side of the queen excluder colonies and in the queenless colonies. The developmental time of the brood in the queenless side of the queen

excluder colonies was shorter compared with bees developing in the queenless colonies (nested ANOVA,  $p<0.001$ ; Fig. 5, upper panel). This experiment suggests that the queen influences brood developmental time, ultimate body size, and caste differentiation. Close distance interaction (probably direct contact) with the queen influenced larval developmental time.

## Discussion

Size polymorphism is a hallmark of the division of labor in many social insects, including bumblebees, but little is known about the factors determining the body size of social insects. Our results show that the typical increase in the body size of emerging *B. terrestris* workers along with colony development is influenced by changes in colony environment over time rather than by differences in the eggs laid by the queen during different stages of colony growth. The presence of a queen and the number of workers caring for the brood influenced ultimate body size. The influence of the queen was mediated by shortening larval developmental duration, an effect that at least in part required close distance interactions (probably direct contact) between the queen and the larvae. To the best of our knowledge, this is the first evidence that the queen influence on larval development in bees is mediated by close distance interactions. These social influences on brood development can in turn influence



**Fig. 5** The queen influences on larval developmental time and ultimate body size are mediated by both long and close distance signals. Colonies were split into two equal parts using a queen excluder (*QE colony*); larval developmental time and size for the queenright side (*QR side*) and the queenless side (*QL side*) were tracked. Control queenright colonies (*QR colonies*) were separated by mesh enabling the queen to visit all parts of the nest. Control queenless colonies (*QL colony*) had a mesh allowing the worker to pass between sides but did not have a queen. The data presented in the plots were pooled from three independent repetitions. Detailed data for each repetition is presented in Supplementary Fig. 3. Values are mean $\pm$ SE; sample size is given inside the bars. Both body size (*lower panel*) and developmental time (*upper panel*) differed between treatments (nested ANOVA,  $p < 0.001$ ). Treatments with different letters were significantly different (Scheffé post hoc test,  $p < 0.05$ )

worker division of labor and the social organization of the whole colony.

The positive correlation between body size and colony age was very consistent in our experiments, and corresponds with previous measurements in field and laboratory colonies (Knee and Medler 1965; Plowright and Jay 1968; Goulson 2003). However, it was important to precisely quantify bee body size over the entire period of worker production as a similar increase was not found in all studies (Duchateau 1989; Couvillon et al. 2010). It is not clear whether this apparent inconsistency stems from species-specific variation, differences in data collection (e.g., in some of the studies size was measured only for bees emerging during the later stages of colony development), or resulted from stress associated with manipulating colonies in some of these studies. For example, Knee and Medler (1965) showed that

colonies that were transferred from the field to the laboratory or were infected with parasites reared small workers and did not show the typical increase in average body size with colony development.

The cross-fostering experiment (experiment 2, Fig. 2) showed that the typical increase in body size can be explained by variation in the colony environment, but not by differences between the eggs laid by the queen at early versus later stages of colony development. All the colonies in the cross fostering experiment were housed in the same environmental chamber and therefore experienced similar temperature, humidity, and illumination, and were provided with the same food ad libitum. Therefore, it is likely that social rather than physical factors differing between the incipient and established colonies accounted for the observed influence of the environments on worker body size. We focused on two key social factors, the number of workers present and larval interactions with the queen. Both appear to be important. Notably, however, the queen and worker influences on larval growth appear to be mediated by different mechanisms since only the queen presence reduced brood developmental duration. We found that brood reared by 10 workers with or without a queen were larger than sister brood cared for by a single worker or a queen (Fig. 4). These results are consistent with the premise that the number of workers or the worker/larva ratio influence brood development and ultimate body size in bumblebees (Plowright and Jay 1968). However, the body size of the nursing workers does not seem to be an important factor as brood nursed by large or small worker bees in colonies at the same developmental stage developed into adult workers of a similar size (Cnaani and Hefetz 1994).

The importance of interactions with the queen for brood development and final size was evident in experiment 4 in which larger bees developed when reared by 10 workers without a queen compared to 10 or more workers with a queen (Fig. 4). The adult/larva ratio in these two groups appeared similar (but this was not precisely quantified), and we therefore assume that the presence of the queen was the most important factor varying between these treatment groups. The presence of a queen also inhibits the development of female larvae into new queens, which are not only larger than workers but also differ in their physiology (Cnaani et al. 2000a, b). Experiment 5 further showed that the influence of the queen on larvae development is mediated at least in part by close distance interactions, probably direct contact. The developmental time (and in one colony also body size) differed between the queenless and queenright sides of the excluder in the queen excluder colonies. In a broader evolutionary perspective, the finding that the queen inhibits larval growth is consistent with predictions of the Parental Manipulation Hypothesis for the evolution of eusociality (Alexander 1974).



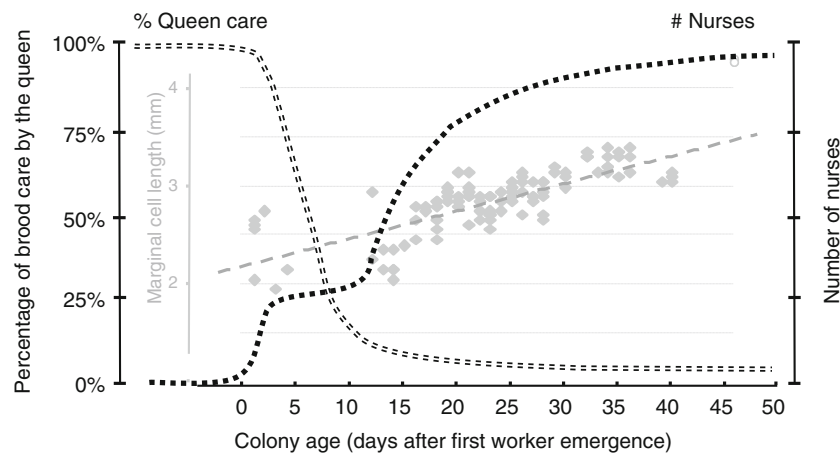
Our findings suggest that there is a “critical period” during larval growth in which it is most sensitive to inhibitory signals from the queen. Critical periods during early developmental stages with profound influence on later developmental programs have been described in many animal species (Smith et al. 2008; Schwander et al. 2010). In *B. terrestris*, it has been shown that the first 5 days during larval development are the critical period for caste determination (Cnaani et al. 2000b) and may also be critical for the developmental program determining body size. Additional factors that we did not test such as the amount or quality of food provided to the larvae may also influence larval growth and final body size. Differences in diet, including in the amount of Major Royal Jelly proteins, are important regulators of honey bee development (e.g., Kamakura 2011). However, their importance in bumblebees has been questioned because no differences were found in the composition of the food provided by the workers to gyne and worker destined larvae (Pereboom 2000), and the single *B. terrestris* member of the Major Royal Jelly protein family does not appear to have a nutritive function (Kupke et al. 2012). Although we did not find significant differences in feeding rates between incipient and later stage colony, we do not argue that the amount of food provided to the larvae does not change during colony development. Ribeiro et al. (1999) reported, based on detailed video recording and an extended observation period, that larval feeding rate was significantly higher in colonies with workers and a queen relative to colonies with only the founding queen. In addition, differences in feeding frequency have been shown to affect the size and the weight of hand-reared larvae (Pereboom et al. 2003). Perhaps, we did not detect a statistically significant increase in the amount of feeding events per larva in later stage colonies because we observed the colonies for a relatively short period and in colonies with a small number of workers (Fig. 3).

Our results show that the queen can also influence development without close distance interactions with the brood; gynes were developing in the queenless colonies but not in the queenless part of the colony in the queen excluder colonies (experiment 5). These findings suggest that in the later colonies the queen influenced the development of brood also in the side of the queen excluder that she could not reach. Röseler (1970) similarly showed that larvae separated from the queen by a queen excluder did not develop into gynes. The differences between the queenless colonies and the queenless side of the queen excluder colonies in experiment 5 (Fig. 5) could be mediated by volatile pheromones or by contact pheromones passing from the queen to the workers and to the larva developing in the queenless compartment. It is also possible that the queen influenced the behavior of workers with which she interacted in the queenright side of the colony and who cared for the brood in the queenless side of the colony. Lopez-Vaamonde et al. (2007) split *B. terrestris* colonies with a

single or double mesh that did not allow queen or worker passage and reported that under these conditions, the queen did not inhibit queen production on the queenless compartment. Transferring workers or wax once a day from the queenright to the queenless compartment did not improve the inhibition of gyne production in the queenless side. The findings of Lopez-Vaamonde et al. (2007) are not consistent with the hypotheses that volatile queen pheromones, contact queen pheromones transferred by the worker, or queen-induced changes in workers’ behavior mediate the inhibitory influence of the queen on gyne production. Alaux et al. (2004) showed that the queen inhibits workers’ reproduction in colonies split by a queen excluder, but only if the workers were in continuous close exposure to the queen; the inhibition was weaker compared to queenright workers. Thus, perhaps the queen influence is mediated by altering workers’ behavior, and this affect requires repeated exposure to the queen that was not available in the worker transfer experiment of Lopez-Vaamonde et al. (2007).

Brood contact with the queen decreases during the typical development of the annual bumblebee colony. Our detailed observations (Fig. 3) revealed that the queen continued to feed larvae in the presence of workers, but at a reduced rate compared with incipient colonies in which the queen is the sole brood tender. These observations that focused on the brood are consistent with complementary observations focusing on the queen and showing that in the presence of workers (emerged naturally or supplemented experimentally), the queen decreases her brood feeding activity compared to when caring for the brood alone (in un-manipulated incipient colonies or in young colonies from which all workers were removed; Woodard et al. 2013).

Based on our studies and the literature, we propose a self-organization model to explain how the natural increase in worker number and variation in brood contact with the queen may produce a gradual increase in worker body size along with colony development (Fig. 6). The first batch of larvae in the colony (leftmost part of the plot in Fig. 6) develops into small bees because they are cared for by a single brood tender (the queen), which is suboptimal (e.g., compare single and multiple brood tender in Fig. 4), and because direct contact with the queen appears to decrease larval developmental duration (experiments 4 and 5). When workers emerge, they contribute to brood nursing, and the increase in their number may improve the overall care for brood, an improvement that is reflected in larger final body size. The influence of worker number on brood size was exemplified by the consistent larger size of bees reared by 10 queenless workers compared to a single worker in experiment 4 (Fig. 4, left column). Pereboom et al. (2003) hand-reared larvae and showed that larvae that are fed at higher rates attain larger final size, and an increase in feeding rate in later stage colonies was reported by Ribeiro et al. (1999).



**Fig. 6** Schematic self-organization model for the influence of brood care by the queen and worker number on the body size of developing brood throughout colony development. *Double dashed line*—the relative contribution of queen to brood care. *Dotted line*—the number of workers nursing the brood. The number of brood tending workers stops increasing around the time when new workers stop to emerge (around

day 40 in the colony presented in Fig. 1). The *gray diamonds* and the *dashed regression line* were copied from Fig. 1 to show the typical increase in body size with colony age. The model assumes that the contact with the queen and worker number determines the body size of the developing brood

The emergence of the first workers in the colony is associated with a sharp decrease in the queen brood feeding rate (Woodard et al. 2013), and her relative contribution to brood care diminishes (Fig. 3). These two processes together lead to a decrease in brood contact with the queen and enable the development of larger brood. The combined effects of worker number and queen contact can explain why in experiment 5 there was a consistent reduction in developmental time but not in body size in the queenright side of the queen excluder colonies; the large number of workers in these colonies could provide more care and compensate for the inhibitory influence of the queen. This premise is also consistent with the findings of experiment 4 in which bees developing with a queen and 10 or more workers were larger than their sisters developing with only a queen, but developmental time was similar for the two groups (Fig. 4). Although our experiments were not designed to explicitly test the social influence on caste determination, our model suggests that the decrease in queen influence on larva development and the increase in worker number may culminate in large brood that develop into new gynes. Consistent with this premise are the findings that an artificial increase in worker number enhances new queen production (Pomeroy and Plowright 1982; Bloch 1999).

Our study focused on factors affecting the increase in worker body size during colony growth. However, it should be noted that there is also significant size variation between brood developing at the same colony stage (e.g., Fig. 1 and Supplementary Fig. 3) and even between larvae developing together in the same cell. In several species, including *Bombus impatiens*, there is evidence suggesting that some of this variation can be explained by the brood location. It has been suggested that brood developing in the periphery of the comb are “neglected”, receiving less food, and are therefore smaller

than brood located at the center of the comb (Plowright and Jay 1968; Couvillon and Dornhaus 2009). It would be interesting to test whether brood location affects the contact with the queen and the feeding by the workers, or if this variation is generated by factors that are not included in our model.

An increase in worker size along with colony development has also been reported in several other social insects including paper wasps (Jeanne and Suryanarayanan 2011; Miyano 1998; Kudo 2003), fire ants (Tschinkel 1988), and leaf-cutting ants (Wilson 1983). Shortening larval developmental time by direct contact with the queen may be functionally significant in newly founded colonies of social Hymenoptera. Specifically, the rapid and economic production of the first batch of workers is crucial for the survival of newly founded colonies in the spring. A model with a similar logic to ours was suggested to explain size and caste determination in social paper wasps. The two principal factors in this model were feeding frequency which is influenced by the number of workers tending the brood and a growth inhibitory vibration signal to which the larva is exposed. The vibrations are high early in colony development and are thought to indicate the presence of a vital egg-laying female (Brillet et al. 1999; Jeanne and Suryanarayanan 2011; Suryanarayanan et al. 2011). It is interesting to note that a different situation was reported for some hemimetabola termites in which egg size is correlated with adult body size, and the first nymphs (juveniles, comparable in life stage to larvae in holometabola insects) are larger when compared with their siblings emerging at later stages of colony development (Matsuura and Kobayashi 2010). Perhaps the differences between the social Hymenoptera and the termites relate to the fact that termite nymphs are part of the working force of the colony whereas in the holometabolous hymenoptera the offspring workers contribute to the work force only as

adults. In the social Hymenoptera, rapid development of small workers might be the most efficient use of the limited resources of the incipient colony whereas in termites larger nymphs may have higher survival and can rapidly provide significant contribution to the growth of the colony during the early and critical founding stage. During later stages of colony development in social Hymenoptera, when there are more workers to collect food, regulate the colony environment, and care for the brood, it may be beneficial to invest in producing larger bees that are better foragers (Goulson et al. 2002). The larger and more effective foragers enable the colony to collect the large amount of food needed for the production of drones and gynes.

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## References

- Aiaux C, Jaisson P, Hefetz A (2004) Queen influence on worker reproduction in bumblebees (*Bombus terrestris*) colonies. *Insect Soc* 51:287–293
- Alexander RD (1974) The evolution of social behavior. *Annu Rev Ecol Syst* 5:325–383
- Alford DV (1975) Bumblebees. Davis-Poynter, London
- Beshers SN, Fewell JH (2001) Models of division of labor in social insects. *Annu Rev Entomol* 46:413–440
- Bloch G (1999) Regulation of queen–worker conflict in bumble-bee (*Bombus terrestris*) colonies. *Proc R Soc B* 266:2465–2469
- Brian AD (1952) Division of labour and foraging in *Bombus agrorum* Fabricius. *J Anim Ecol* 21:223–240
- Brillet C, Tian-Chansky SS, Le Conte Y (1999) Abdominal waggings and variation of their rate of occurrence in the social wasp, *Polistes dominulus* Christ. I. Quantitative analysis. *J Insect Behav* 12:665–686
- Bryk B, Hahn K, Cohen SM, Teleman AA (2010) MAP4K3 regulates body size and metabolism in *Drosophila*. *Dev Biol* 344:150–157
- Calderone NW, Page RE (1991) Evolutionary genetics of division-of-labor in colonies of the honey-bee (*Apis mellifera*). *Am Nat* 138:69–92
- Cnaani J, Hefetz A (1994) The effect of workers size frequency distribution on colony development in *Bombus terrestris*. *Insect Soc* 41:301–307
- Cnaani J, Robinson GE, Bloch G, Borst D, Hefetz A (2000a) The effect of queen–worker conflict on caste determination in the bumblebee *Bombus terrestris*. *Behav Ecol Sociobiol* 47:346–352
- Cnaani J, Robinson GE, Hefetz A (2000b) The critical period for caste determination in *Bombus terrestris* and its juvenile hormone correlates. *J Comp Physiol A* 186:1089–1094
- Cnaani J, Hefetz A (2001) Are queen *Bombus terrestris* giant workers or are workers dwarf queens? Solving the ‘chicken and egg’ problem in a bumblebee species. *Naturwissenschaften* 88:85–87
- Couvillon MJ, Dornhaus A (2009) Location, location, location: larvae position inside the nest is correlated with adult body size in worker bumblebees (*Bombus impatiens*). *Proc Biol Sci* 276:2411–2418
- Couvillon MJ, Jandt JM, Duong N, Dornhaus A (2010) Ontogeny of worker body size distribution in bumblebee (*Bombus impatiens*) colonies. *Ecol Entomol* 35:424–435
- Cumber RA (1949) The biology of humble-bees, with special reference to the production of the worker caste. *Trans Roy Ent Soc Lond* 100:1–45
- Davidowitz G, D’Amico LJ, Nijhout HF (2003) Critical weight in the development of insect body size. *Evol Dev* 5:188–197
- Davidowitz G, D’Amico LJ, Nijhout HF (2004) The effects of environmental variation on a mechanism that controls insect body size. *Evol Ecol Res* 6:49–62
- Duchateau MJ (1989) Agonistic behaviors in colonies of the bumblebee *Bombus terrestris*. *J Ethol* 7:141–151
- Duchateau MJ, Hoshihara H, Velthuis HHW (1994) Diploid males in the bumblebee *Bombus terrestris* sex determination, sex alleles and viability. *Entomol Exp Appl* 71:263–269
- Edgar BA (2006) How flies get their size: genetics meets physiology. *Nat Rev Genet* 7:907–916
- Free JB (1955) The division of labour within bumblebee colonies. *Insect soc* 2:195–212
- Gerloff CU, Ottmer BK, Schmid-Hempel P (2003) Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. *Funct Ecol* 17:582–589
- Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH (2002) Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim Behav* 64:123–130
- Goulson D (2003) Bumblebees, their behaviour and ecology. Oxford University Press, New York
- Goulson D, Derwent LC, Peat J (2005) Evidence for alloethism in stingless bees (Meliponinae). *Apidologie* 36:411–412
- Hunt GJ, Guzman-Novoa E, Fondrk MK, Page RE (1998) Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* 148:1203–1213
- Jandt JM, Huang E, Dornhaus A (2009) Weak specialization of workers inside a bumble bee (*Bombus impatiens*) nest. *Behav Ecol Sociobiol* 63:1829–1836
- Jeanne RL, Suryanarayanan S (2011) A new model for caste development in social wasps. *Commun Integr Biol* 4:373–377
- Kamakura M (2011) Royalactin induces queen differentiation in honey bees. *Nature* 473:478–483
- Kapustjanskij A, Streinzer M, Paulus HF, Spaethe J (2007) Bigger is better: implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. *Funct Ecol* 21:1130–1136
- Knee WJ, Medler JT (1965) Seasonal size increase of bumblebee workers (Hymenoptera—*Bombus*). *Can Entomol* 97:1149–1155
- Kudo K (2003) Growth rate and body weight of foundress-reared offspring in a paper wasp, *Polistes chinensis* (Hymenoptera, Vespidae): no influence of food quantity on the first offspring. *Insect Soc* 50:77–81
- Kupke J, Spaethe J, Mueller MJ, Roessler W, Albert S (2012) Molecular and biochemical characterization of the major royal jelly protein in bumblebees suggest a non-nutritive function. *Insect Biochem Molec* 42:647–654
- Lopez-Vaamonde C, Brown RM, Lucas ER, Pereboom JJM, Jordan WC, Bourke AFG (2007) Effect of the queen on worker reproduction and new queen production in the bumblebee *Bombus terrestris*. *Apidologie* 38:171–180
- Matsuura K, Kobayashi N (2010) Termite queens adjust egg size according to colony development. *Behav Ecol* 21:1018–1023
- McBrayer Z, Ono H, Shimell M, Parvy JP, Beckstead RB, Warren JT, Thummel CS, Dauphin-Villemant C, Gilbert LI, O’Connor MB (2007) Prothoracicotropic hormone regulates developmental timing and body size in *Drosophila*. *Dev Cell* 13:857–871
- Merling M (2008) The influence of task, size and age differences on the phototactic response in the bumblebee, *Bombus terrestris* (L.). Dissertation, The Hebrew University of Jerusalem
- Michener CD (1974) The social behavior of bees: a comparative study. Belknap, Cambridge

- Miura T, Matsumoto T (1995) Worker polymorphism and division of labor in the foraging behavior of the black marching termite *Hospitalitermes medioflavus*, on Borneo Island. *Naturwissenschaften* 82:564–567
- Miyano S (1998) Amount of flesh food influences the number, larval duration, and body size of first brood workers, in a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). *Entomol Sci* 1:545–549
- Nijhout HF, Williams CM (1974) Control of moulting and metamorphosis in tobacco hornworm, *Manduca sexta* (L.)—cessation of juvenile hormone secretion as a trigger for pupation. *J Exp Biol* 61:493–501
- Nijhout H (2003) The control of body size in insects. *Dev Biol* 261:1–9
- Owen RE (1988) Body size variation and optimal body size of bumblebee queens (Hymenoptera, Apidae). *Can Entomol* 120:19–27
- Pan DJ (2007) Hippo signaling in organ size control. *Gene Dev* 21:886–897
- Patel A, Fondrk MK, Kaftanoglu O, Emore C, Hunt G, Frederick K, Amdam GV (2007) The making of a queen: TOR pathway is a key player in diphenic caste development. *PLoS One* 2:e509
- Pereboom JJM (2000) The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*. *Insect Soc* 47:11–20
- Pereboom JJM, Velthuis HHW, Duchateau MJ (2003) The organization of larval feeding in bumblebees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insect Soc* 50:127–133
- Plowright RC, Jay SC (1968) Caste differentiation in bumblebees (*Bombus* LTAR.: HYM.) 1.—the determination of female size. *Insect Soc* 15:171–192
- Pomeroy N, Plowright RC (1982) The relation between worker numbers and the production of males and queens in the bumblebee *Bombus perplexus*. *Can J Zool* 60:954–957
- Quezada-Euan JGG, Lopez-Velasco A, Perez-Balam J, Moo-Valle H, Velazquez-Madrado A, Paxton RJ (2011) Body size differs in workers produced across time and is associated with variation in the quantity and composition of larval food in *Nannotrigona perilampoides* (Hymenoptera, Meliponini). *Insect Soc* 58:31–38
- Radmacher S, Strohm E (2010) Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie* 41:169–177
- Ribeiro MF (1999) Long-duration feedings and caste differentiation in *Bombus terrestris* larvae. *Insect Soc* 46:315–322
- Ribeiro MF, Velthuis HHW, Duchateau MJ, van der Tweel I (1999) Feeding frequency and caste differentiation in *Bombus terrestris* larvae. *Insect Soc* 46:306–314
- Robinson GE (1992) Regulation of division of labor in insect societies. *Annu Rev Entomol* 37:637–665
- Röseler PF (1970) Differences in caste determination between bumblebee species *Bombus hypnorum* and *Bombus terrestris*. *Z Naturforsch Pt B* 25:543–548
- Roulston TH, Cane JH (2000) The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). *J Kansas Entomol Soc* 73:129–142
- Roulston TH, Cane JH (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evol Ecol* 16:49–65
- Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp. from Central Europe. *Insect Soc* 47:36–41
- Schwander T, Humbert JY, Brent CS, Cahan SH, Chapuis L, Renai E, Keller L (2008) Maternal effect on female caste determination in a social insect. *Curr Biol* 18:265–269
- Schwander T, Lo N, Beekman M, Oldroyd BP, Keller L (2010) Nature versus nurture in social insect caste differentiation. *Trends Ecol Evol* 25:275–282
- Seeley TD, Kolmes SA (1991) Age polyethism for hive duties in honey bees - illusion or reality? *Ethology* 87:284–297
- Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. *Nat Rev Genet* 9:735–748
- Spaethe J, Weidenmüller A (2002) Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insect Soc* 49:142–146
- Spaethe J, Chittka L (2003) Interindividual variation of eye optics and single object resolution in bumblebees. *J Exp Biol* 206:3447–3453
- Spaethe J, Brockmann A, Halbig C, Tautz J (2007) Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften* 94:733–739
- Suryanarayanan S, Hantschel AE, Torres CG, Jeanne RL (2011) Changes in the temporal pattern of antennal drumming behavior across the *Polistes fuscatus* colony cycle (Hymenoptera, Vespidae). *Insect Soc* 58:97–106
- Sutcliffe GH, Plowright RC (1990) The effects of pollen availability on development time in the bumblebee *Bombus terricola* K. (Hymenoptera, Apidae). *Can J Zool* 68:1120–1123
- Suzzoni JP, Passera L, Strambi A (1980) Ecdysteroid titer and caste determination in the ant, *Pheidole pallidula* (NYL) (Hymenoptera, Formicidae). *Experientia* 36:1228–1229
- Tasei JN, Aupinel P (2008) Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie* 39:397–409
- Tschinkel WR (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. *Behav Ecol Sociobiol* 22:103–115
- Weiss R, Dov A, Fahrbach SE, Bloch G (2009) Body size-related variation in Pigment Dispersing Factor-immunoreactivity in the brain of the bumblebee *Bombus terrestris* (Hymenoptera, Apidae). *J Insect Physiol* 55:479–487
- Wheeler DE, Nijhout HF (1981) Soldier determination in ants—new role for juvenile hormone. *Science* 213:361–363
- Wilson EO (1971) *The insect societies*. Belknap, Cambridge
- Wilson EO (1978) Division of labor in fire ants based on physical castes (Hymenoptera: Formicidae: *Solenopsis*). *J Kansas Entomol Soc* 51:615–636
- Wilson EO (1983) Caste and division of labor in leaf-cutter ants (Hymenoptera, Formicidae, *Atta*). 4. Colony ontogeny of *Atta cephalotes*. *Behav Ecol Sociobiol* 14:55–60
- Wolschin F, Shpigler H, Amdam GV, Bloch G (2012) Size-related variation in protein abundance in the brain and abdominal tissue of bumble bee workers. *Insect Mol Biol* 21:319–325
- Woodard HS, Bloch G, Band M, Robinson GE (2013) Social regulation of maternal traits in nest-founding bumble bee (*Bombus terrestris*) queens. *J Exp Biol* (in press).
- Worden BD, Skemp AK, Papaj DR (2005) Learning in two contexts: the effects of interference and body size in bumblebees. *J Exp Biol* 208:2045–2053
- Yerushalmi S, Bodenheimer S, Bloch G (2006) Developmentally determined attenuation in circadian rhythms links chronobiology to social organization in bees. *J Exp Biol* 209:1044–1051