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# Social regulation of ageing by young workers in the honey bee, *Apis mellifera*

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ABSTRACT

Organisms' lifespans are modulated by both genetic and environmental factors. The lifespan of eusocial insects is determined by features of the division of labor, which itself is influenced by social regulatory mechanisms. In the honey bee, *Apis mellifera*, the presence of brood and of old workers carrying out foraging tasks are important social drivers of ageing, but the influence of young adult workers is unknown, as it has not been experimentally teased apart from that of brood. In this study, we test the role of young workers in the ageing of their nestmates. We measured the impact of different social contexts characterized by the absence of brood and/or young adults on the lifespan of worker nestmates in field colonies. To acquire insight into the physiological processes occurring under these contexts, we analyzed the expression of genes known to affect honey bee ageing. The data showed that young workers significantly reduced the lifespan of nestmate workers, similar to the effect of brood on its own. Differential expression of vitellogenin, major royal jelly protein-1, and methylase transferase, but not methyl farneosate epoxidase genes suggests that young workers and brood influence ageing of adult nestmate workers via different physiological pathways. We identify young workers as an essential part of the social regulation of ageing in honey bee colonies.



## 1. Introduction

Honey bees survive the winter in temperate regions as colonial units, in contrast with other hymenoptera, such as wasps and bumblebees, in which only queens survive and independently found a new colony in the spring. Overwintering as a colony allows an early and efficient exploitation of resources toward the production of sexuals and thus increases direct and indirect fitness (Winston, 1987; Seeley, 2014). Several adaptations for survival over a period of up to several winter months are required, when no foraging and hence no brood rearing is possible to renew the worker force. One such adaptation is the hoarding behavior, which consists of the storage of carbohydrates that provide the fuel needed for heat production in the winter cluster (Winston, 1987; Seeley, 1995). In addition, it requires individuals with a long lifespan that can survive until new generations of workers are born. The variations in lifespan that can be observed within the worker caste and between the worker and queen castes make social insects, and honey bees in particular, a good model for studies aimed at understanding the mechanisms of ageing (Amdam and Page, 2005; Jemielity et al., 2005; Keller and Jemielity, 2006; Corona et al., 2007; Rueppell et al., 2009).

In spring and summer, honey bee colonies are composed of so-called "summer bees", which display a lifespan of three to six weeks (Maurizio, 1950; Omholt and Amdam, 2004). During the first one to three weeks, they perform tasks such as nursing and cleaning within the nest and later leave its protection to forage for one to two weeks before dying (Neukirch, 1982; Winston, 1987; Seeley, 1995). In late summer, falling temperatures reduce foraging activity and brood rearing declines. The winter bees then emerge from the last brood reared (Merz et al., 1979; Mattila et al., 2001; Amdam and Omholt, 2003; Smedal et al., 2009; Fluri, 2012). In contrast to summer bees, winter bees have a life expectancy of five to eight months (Maurizio, 1950; Fluri et al., 1982). Their tasks consist of maintaining the nest at temperatures that ensure the survival of the winter cluster and resuming brood rearing the following spring, before beginning to forage.

Experimental manipulations of colonies have shown that the worker lifespan plasticity required to ensure colony functions and adaptation to a variable environment is affected by a variety of external factors, such as season (temperature and day length) (Cherednikov, 1967; Huang and Robinson, 1995) and food availability (Huang and Robinson,

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1995; Fluri, 2012). It is also influenced by within-hive stimuli, such as pheromones and social contexts (Huang and Robinson, 1996; Leoncini et al., 2004; Amdam et al., 2009; Smedal et al., 2009; Amdam, 2011; Fluri, 2012). Previous research has shown that manipulating colony age demography could accelerate, delay, or even reverse the behavioral and corresponding physiological maturation of workers, thus influencing their ageing process and eventually their lifespan (Robinson et al., 1992; Huang and Robinson, 1996; Amdam et al., 2005; Rueppell et al., 2007). For instance, the production of pheromones by foragers hinders the behavioral maturation of in-hive nestmates (Leoncini et al., 2004), which thus benefit from a longer lifespan (Rueppell et al., 2007). The absence of larvae that require feeding with glandular secretions of workers has also been shown to increase worker lifespan (Dzierżon and Bruckisch, 1857; Amdam et al., 2009; Fluri, 2012). This effect has been attributed to the release from the metabolic costs of brood food production (Amdam et al., 2003, 2009), while other researchers have reported it could result from the lack of need for generating heat to ensure optimal brood development (Kleinhenz et al., 2003). Since brood removal also resulted in the absence of new generations of workers in all of these experiments, the effects of brood and young workers on worker lifespan could not be disentangled. Using an alternative experimental design in which larvae were present but pupae and hence emerging young workers were absent, previous studies reported prolonged physiological youth and longer lifespan of nestmates (Kratky, 1931; Haydak, 1963; Fluri, 2012). This prolongation suggests an effect of young adults on the lifespan of their nestmates. We tested this hypothesis by comparing the lifespan of workers in colonies deprived of larvae and pupae in the presence or in the absence of young workers. In broodless colonies, the long lifespan of workers (Maurizio, 1950; Fluri et al., 1982) is expected to be drastically reduced by the addition of young workers or brood and hence will be easily measurable. Since our aim was not to test the nature of interactions (additive or synergistic) between the effects of brood and young workers, but rather solely to investigate the possible effects of young workers on lifespan, we have omitted the group with the presence of both brood and workers. A third group of colonies with larvae, from which pupae were continuously removed and hence no young worker emerged, was used to determine whether the presence of young workers affects lifespan via the same physiological mechanisms as brood does. Expression of several genes related to ageing and to task performance of young individuals (nursing) was thus monitored in the three experimental groups as markers for the physiological status resulting from the different social contexts.

Juvenile hormone is a gonadotropin controlling important physiological and behavioral mechanisms in honey bees, such as the transition from in-hive to foraging tasks (Huang et al., 1991; Bomtorin et al., 2014). This transition was described as the major determinant of worker lifespan (Rueppell et al., 2007). Summer and winter workers engaged in in-hive tasks have low juvenile hormone titers, whereas foragers display high titers (Huang et al., 1991; Bomtorin et al., 2014). Since juvenile hormone titer is linked to transcription of juvenile hormone-biosynthetic genes, such as methyl farneosate epoxidase and methylase transferase (Bomtorin et al., 2014), we used the measure of their gene expression as a proxy for juvenile hormone quantification. Their low expression in long-lived individuals should result in low transcription levels in the absence of young workers. Vitellogenin is a glycolipoprotein synthesized in the fat bodies and is released into the haemolymph (Amdam et al., 2004; Corona et al., 2007). It governs multiple processes, including ageing and brood food production (Amdam et al., 2003, 2004; Seehuus et al., 2006; Münch et al., 2015). Vitellogenin titer is high in nurse and winter bees, but low in foragers. It is influenced by social interactions between nestmates (Amdam et al., 2004; Corona et al., 2007) and is thus likely to be influenced by the different social contexts experimentally generated here. Since vitellogenin titer correlates to vitellogenin gene expression and since lifespan in the absence of larvae and young adults is known to be long, we predict that vitellogenin gene expression should be high in workers exposed to this social context. In contrast, it should be lower in the presence of either brood or young workers. Major royal jelly proteins are a family of proteins with functions putatively extending beyond nutrition (Corby-Harris et al., 2016). They may be involved in ageing, behavior, development, and immunity mechanisms (Drapeau et al., 2006; Buttstedt et al., 2014; Chua et al., 2015). We thus measured transcript levels of *mrjp-1*. *Mrjp-1* levels are higher in nurses than in foragers (Kubo et al., 1996; Drapeau et al., 2006; Ji et al., 2014), which corresponds to the trend observed in the amount of major royal jelly protein measured in nurse- and forager-aged workers (Feng et al., 2009). We expect workers to express *mrjp-1* more in the absence of young nestmates and in the presence of brood, as these conditions are hypothesized to promote a phenotype characteristic for in-hive individuals.

Previous studies of the determinants of ageing used observation hives or colonies from which the queens were temporarily removed (Kratky, 1931; Haydak, 1963; Fluri, 2012). We chose to perform our experiment under more realistic conditions by using full-sized queenright colonies. We show that, in addition to brood and foragers, the presence of young workers is a factor affecting the lifespan of nestmates. We discuss our results in the context of the high plasticity of social organization and its link to the ageing mechanisms, which are central factors determining life history in the honey bee.

## 2. Methods

### 2.1. Experimental set up

The experiment was conducted from June to August 2013 in Bern, Switzerland using honey bee (*A. mellifera*) colonies of mixed European origin (N = 9, ~16,000 workers each) kept in Dadant hives. The queens heading these colonies were not related. To limit the effect of parasites and pathogens on worker lifespan, colonies without obvious clinical disease symptoms were used. Low infestation rates with ectoparasitic mites, *Varroa destructor*, were achieved by treating these colonies with the recommended formic and oxalic acid products the previous summer and winter, respectively (Imdorf et al., 1996; Charrière and Imdorf, 2002).

Prior to the experiment, all brood combs were removed from the experimental colonies to ensure that no young workers could emerge. Three to four combs partially filled with nectar and pollen were provided to each colony as food stores. Two to three empty combs previously drawn from foundation wax sheets were also placed in each hive to allow for additional food storing.

The colonies were randomly assigned to one of the following three groups (N = 3 colonies each) representing different social contexts (Fig. 1) obtained by experimental manipulation of colony demography:

- broodless without young workers
- broodless with young workers
- broodright without young workers.

The queens of the broodless groups were placed in cages preventing oviposition to ensure brood-free conditions (i.e., the absence of larvae and pupae) during the experimental period. The plastic queen cages [7.8 × 5 × 2.5 cm] were placed in a hole of the same size in a central comb. Openings enabled workers to enter and leave the cages, thereby ensuring that the sequestered queens received adequate feeding and grooming throughout the experiment.

The broodless group with young workers was created by adding newly emerged workers (N = 450 ± 213) once daily on 42 occasions during the first 50 days of the 60-day experiment. On eight of these 50 days, not enough workers emerged to be added to the colonies. The newly emerged workers originated from six donor colonies that were unrelated to those used in the experiment. We removed combs with ready-to-emerge workers from these donor colonies and placed

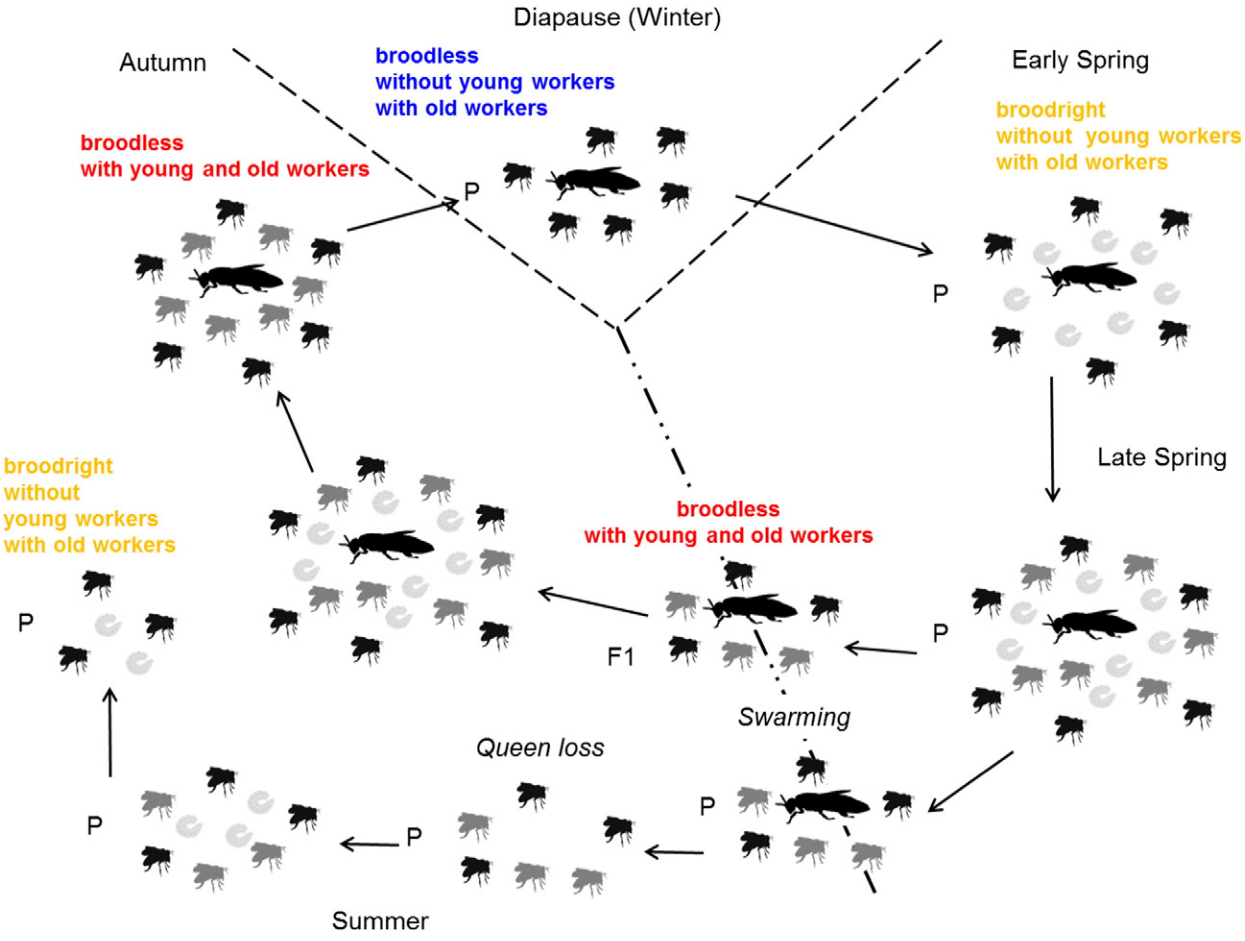


**Fig. 1.** Honey bee life history with different social contexts (in color fonts). In winter, only the queen and old workers are present. In early spring, the queen, old workers, and brood can be found. Later in spring, young workers are present. After reproductive swarming, parental (P) and first filial (F1) colonies only contain queens and old and young workers, but no brood. Then, the queen, brood, old and young workers are again present until autumn. At this time, brood disappears and only queens, old and young workers can be found. If, however, the queen is lost and colonies fail to rear a replacement, only old and young workers populate the colony. These workers can arrhenotokously produce male offspring until the colony dwindles and eventually dies. Large black individuals = queens; small black individuals = old workers; small medium grey individuals = young workers; small light grey individuals = brood. Solid arrows indicate life history transition events, and different colors represent social contexts reflecting those tested in our experiment. Note that queen loss may occur at any stage of the honey bee life history and that after swarming a parental nest may contain brood, but no young workers, depending on the timing of the daughter queen's mating flights.

them in an incubator at 35 °C and 65% relative humidity until adult emergence. Every 24 h, the newly emerged workers were weighed to determine their number (11 g = ~100 workers) and then equally distributed between the three colonies of the experimental group.

In the broodright group without young workers, the queens were placed on an empty comb kept in a frame cage [37.9 × 23.6 × 5.5 cm] that enabled workers, but not queens, to move freely to other combs. The queens were able to oviposit in the cells of the caged comb. After four days, the brood combs containing eggs and one-day old larvae were removed from the cages and placed into the same colonies for another nine days. On the removal day, new empty combs were placed into the frame cages with the queen. After 13 days, the previously caged combs, now populated with pupae in capped cells, were removed from the colonies to prevent the emergence of young adults. The repetition of this cycle ensured that no young workers emerged, but that open and capped brood was constantly present.

Three hours before manipulation of the social context in the experimental colonies, 200 workers that emerged from incubated combs (as described above) within 24 h were collected from all colonies of the three experimental groups. They were individually marked with Opalith-platelets on the thorax to enable the measures of their lifespan. Marking with Opalith-platelets was performed on three consecutive days, with the marking of one colony of each group each day. Marked workers (N = 1800) were reintroduced into their respective mother colonies. The identity of each marked worker was noted during screening of every comb and of the hive box after comb removal. Screenings were performed in all colonies at five-day intervals. Screening was performed during daytime when some of these workers could have been foraging. Even though worker longevity could have been underestimated due to consecutive absences of individuals during the last screenings, this would have occurred in all groups and therefore does not represent a bias. Marked workers that were not seen again after a particular date were considered dead at the date following their last recorded presence. When marked workers were found dead during hive inspections (e.g., on the bottom board), they were identified and counted. The same person conducted the screenings in all colonies with the help of a digital voice recorder (VN-7500; Olympus). The experiment was terminated when >90% of the marked workers in one of the groups had died, which occurred at day 60. In order to control for the potential impact of marking on lifespan, all workers that had died during the first 10 days were excluded from the analyses.

On the same days when workers were marked with Opalith-platelets, in each colony, an additional cohort of 200 newly emerged workers was marked on the thorax with a colony-specific color using commercial acrylic paints (N = 1800) and reintroduced into their maternal colonies. Five of these marked workers were recaptured from each

colony for gene expression analyses on days 0, 5, 14, 21, 28, and 35, encompassing the typical in-hive (days 0–14) and foraging (days 21–35) periods (Seeley, 1995; Rueppell et al., 2007; Amdam et al., 2009). After day 35, too few marked workers were available in one of the groups to collect complete samples of five individuals. Sampled workers were frozen at −20 °C immediately after collection for quantification of transcript levels of genes potentially associated with ageing.

### 2.2. Gene expression quantification by real-time qRT-PCR

Total RNA was extracted from whole workers using the NucleoSpin 96 RNA Kit (Macherey-Nagel) according to the manufacturer's protocol. Subsequently, cDNAs were synthesized using M-MLV Reverse Transcriptase (Invitrogen®) random hexamers (2.5 μM), oligo dT (0.1 μM), and dNTP (1.0 mM) in a final reaction volume of 20 μl. The cDNA was diluted, with molecular grade water, in a 1:10 ratio. Each sample was analyzed twice with a quantitative PCR assay (Kapa SYBR Green Fast) evaluating the transcript levels of genes, which have previously been associated with ageing in honey bees: vitellogenin (*Vg*), major royal jelly protein 1 (*mrjp-1*), methyl farneosate epoxidase (*mfe*), and methylase transferase (*mt*), using specific primers (Table 1). The thermal profile for all q-PCRs was programmed according to the manufacturer as follows: 95 °C (2 min), followed by 40 cycles of 95 °C (3 s) and 60 °C (20s). In parallel, the *β-actin* reference gene was analyzed in the same way to normalize each duplicate sample for its total RNA amount. Normalization, based on the delta Ct method, was performed for all targets by subtracting the target threshold cycle numbers from the cycle numbers of β-actin. Duplicate normalized Ct values were averaged for each sample for further analyses.

### 2.3. Statistical analyses

The parametric Weibull accelerated failure time model (Collett, 2003) was used to determine the influence of social context (presence of either workers or brood or their absence) on the lifespan of nestmate workers, which also provided the median survival probability of each group. The model fit was appropriate because the Cox-Snell residuals did not deviate systematically from the 45° line. These analyses were performed using SYSTAT 13. For the graphical illustration of the survival functions of the three groups differing in their social context, the R package "survival" was used (Therneau, 2013). In order to test whether the social context affected workers' lifespan, pairwise log-rank tests for interval-censored data were performed (Finkelstein, 1986; Chen et al., 2013) using the R package "glrt". We also used this test to perform pairwise comparisons between the three groups. Version 3.0.2 of R was used for these analyses and P-values below 0.017 were considered significant (applying Bonferroni correction). Prior to statistical comparisons of gene expression data (obtained from the qPCR-runs), all Ct values were centered using the bootstrap means (N = 10.000) of the treatment groups on day 0 as baseline values. The centered values of transcript abundance were tested for differences between experimental groups over the first 35 days. Since the data did not follow a normal distribution in some of the replicates (Anderson-Darling test $P < 0.05$), the estimates of transcript abundance between groups were compared with the non-parametric Kruskal-Wallis test. Pairwise comparisons were performed with the Conover-Iman test. Applying Bonferroni correction, P-values below 0.025 were considered significant. The same set of statistical tests was used for comparisons between groups for each sampling day. These analyses were performed using SYSTAT 13.

## 3. Results

### 3.1. Lifespan of workers under different social contexts

In one of the colonies of the broodless group with young workers, the queen died during the experiment and attempts at introducing a new one failed. This colony was therefore excluded from data analyses. In the broodless group without young workers, the Weibull model predicts a median survival probability of 57.6 days for workers [1st quartile: 38.7; 3rd: 79.0]. For the broodright colonies without young workers, the median survival probability was 39 days [1st quartile: 25.9; 3rd: 52.8]. The median survival probability obtained for the broodless colonies with young workers was the lowest with 34.3 days [1st quartile: 22.9; 3rd: 46.9]. Pairwise log-rank tests showed that both the presence of young workers and the presence of brood significantly negatively affected worker lifespan (young workers: $\chi^2 = 36.5$, $P < 0.001$; brood: $\chi^2 = 55.8$, $P < 0.001$). The survival functions of individuals in broodless colonies were significantly inferior in the presence of young workers compared to when they were absent ($\chi^2 = 104.05$, $P < 0.001$). The survival of workers was also significantly lower in broodright than broodless colonies without young workers ($\chi^2 = 127.06$, $P < 0.001$). However, there were no significant differences between the survival of workers in broodright colonies without young workers and in broodless colonies with young workers added ($\chi^2 = 0.58$, $P > 0.4$; Fig. 2).

### 3.2. Gene expression under different social contexts

The RT-qPCR analyses performed over 35 days revealed that transcript abundance of vitellogenin and *mrjp-1* was significantly different between workers of identical age that were exposed to different social contexts (vitellogenin: $K = 52.3$; $P < 0.001$; *mrjp-1*: $K = 46.6$; $P < 0.001$; Fig. 3). Post hoc pairwise comparisons revealed significant differences between all pairs of groups for both genes (Fig. 3). Vitellogenin expression increased in all groups until day 5 and decreased over time in the groups with either brood or young workers, but not in the broodless group without young workers. In the latter group, the initial decrease of vitellogenin expression to the level of the group with young workers was followed by an increase to a level that remained significantly higher than both of the other groups. Vitellogenin expression was overall the lowest in the broodright group without young workers (Fig. 3).

The analyses of the juvenile hormone precursor methylase transferase also revealed significant differences in the level of transcript abundance between the three groups of workers ($K = 28.1$; $P < 0.001$, Fig. 3). The Conover Iman pairwise comparisons demonstrated significant differences in the patterns of gene expression along time between the broodless groups with and without young workers ($P < 0.001$), between the broodright and broodless groups without young workers

**Table 1**
Forward and reverse primer sequences used for qPCR analyses of genes known to be associated with ageing in honey bee workers (vitellogenin = *Vg*, methyl farneosate epoxidase = *mfe*, methylase transferase = *mt*, major royal jelly protein 1 = *mrjp-1*). Respective references are shown in the last column on the right.

| Locus | Forward primer | Reverse primer | Reference |
|---|---|---|---|
| *β-actin* | CGT GCC GAT AGT ATT CTT G | CTT CGT CAC CAA CAT AGG | Lourenco et al., 2008 |
| *Vg* | AGT TCC GAC CGA CGA CG | TTC CCT CCC ACG GAG TCC | Corona et al., 2007 |
| *mfe* | CAT CTC AGA TCT TTC GGC CTA | AGA TTT TCA GCT TCT ACG GTT | Miguel Corona, unpublished |
| *mt* | CCT TCA CTG GTG CCA AAA CT | TGG CCT ATA TCG AGG ATT CG | Bomtorin et al., 2014 |
| *mrjp-1* | TGA CCA ATG GCA TGA TAA GAT TTT | GAC CAC CAT CAC CGA CCT | Miguel Corona, unpublished |

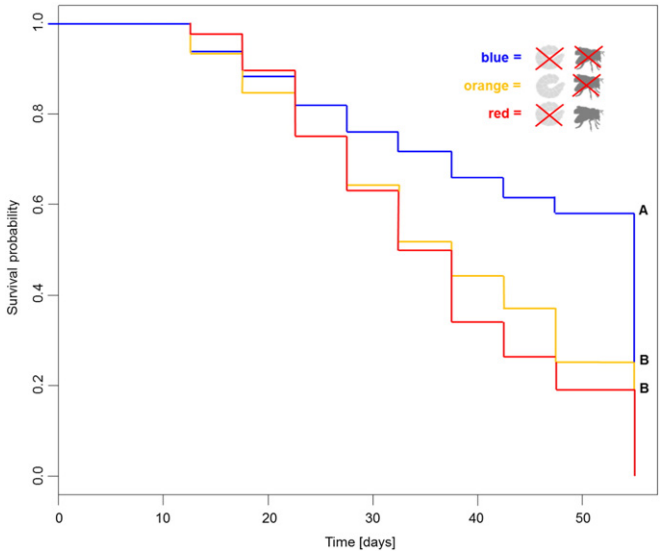


**Fig. 2.** Survival of workers exposed to different social contexts. The presence of young workers (=red line) and brood (=orange line) significantly reduced worker lifespan compared to the group where both brood and young workers were absent (=blue line). Significant differences using "glrt" pairwise log-rank tests ($P < 0.017$ after Bonferroni correction) are indicated with different uppercase letters.

($P < 0.001$), but not between the broodless group with young workers and the broodright group without young workers ($P = 0.17$; Fig. 3).

Significant differences in methylase transferase expression between two groups occurred at all time points except the last one (i.e., day 35), when there was no difference between the three groups (Fig. 3). A peak expression at day 5 in the broodless group with young workers was remarkable. The Kruskal-Wallis test revealed no significant differences in methyl farneosate epoxidase gene expression between the groups ($K = 1.3$, $P = 0.53$). The only significant differences between gene expressions occurred at days 5 and 28 when levels for the broodless group with young workers and the broodright group without young workers, respectively, were higher than in the other groups (Fig. 3). The general trend was similar to methylase transferase, including the peak expression at day 5 in the broodless group with young workers, but there were fewer days showing significant differences in gene expression between groups (Fig. 3).

## 4. Discussion

The data show that in honey bees, young workers can significantly reduce the lifespan of nestmates similar to the previously described effect of brood. However, the differential expression of all tested genes, with the exception of methyl farneosate epoxidase, suggests that young workers and brood influence ageing of adult nestmate workers via different physiological pathways.

The biology of the honey bee constrained our ability to control the size of our experimental units. Indeed, colonies only accept the addition of young workers deprived of colonial odor in colonies without fights that often result in deaths (Breed and Stiller, 1992; Breed et al., 2004). As a result, colony size in the group in which young workers were added grew during the experiment, whereas that of the other groups in which no older nestmates could be added did not. Given that lifespan decreases with increasing colony size (Rueppell et al., 2009), the reduction in worker lifespan could have been due to the change in colony size rather than to the addition of young workers. However, a doubling of population size resulted in a 4-day decrease in lifespan (Rueppell et al., 2009), whereas the continuous addition of young workers (resulting in less than doubling of colony size) resulted in a 5-fold

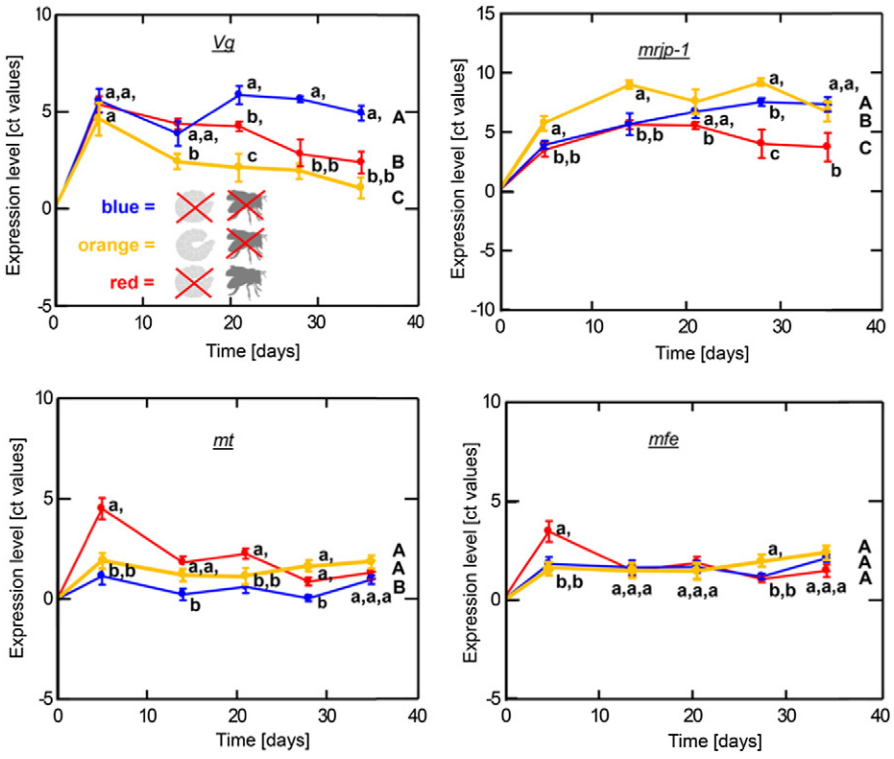


**Fig. 3.** Gene expression levels as centered Ct values of vitellogenin (*Vg*), major royal jelly protein 1 (*mrjp-1*), methylase transferase (*mt*), and methyl farneosate epoxidase (*mfe*) over time in workers of the three experimental groups (red line = broodless group with young workers; orange line = broodright group without young workers; blue line = broodless group without young workers). Significant differences using Conover Iman pairwise comparisons ($P < 0.025$, after Bonferroni correction) are indicated with different uppercase letters for the entire period and with lowercase letters for specific sampling days 0, 5, 14, 21, 28, and 35.

greater reduction of 23.3 days. The effect of population demography on worker lifespan thus appears stronger than that of colony size. Repeating our experiment with varied ratios of colony size to numbers of young workers added will allow for disentangling the effect of young workers and colony size.

Worker lifespan was significantly longer in the absence of young nestmate workers and brood than it was in the presence of either of them. This result is in accordance with previous studies showing that workers in broodless honey bee colonies, which in consequence are also deprived of young adults, can live longer than summer bees usually do (4–6 weeks; Dzierżon and Bruckisch, 1857; Maurizio, 1950; Omholt and Amdam, 2004; Fluri, 2012). Since the experiment was terminated after 60 days, the observed median survival probability (57.6 days) might be an underestimation of the maximum lifespan in the absence of brood, and it might even have reached the five to eight months typical for winter bees (Maurizio, 1950; Fluri et al., 1982). Since the long-living phenotype typical for winter was generated in summer, it can be concluded that environmental factors do not constrain the expression of prolonged worker lifespan (see also Fluri, 2012). Our results thus confirm that lifespan is significantly affected by population dynamics within the colony.

The long lifespan observed in the absence of brood was reflected in the physiological status of workers: from day 21 onwards, high expression levels of vitellogenin and low methyl transferase expression levels were typical for long-living bees usually found in winter (Maurizio, 1950; Fluri et al., 1977, 1982; Amdam et al., 2004). *Mrjp-1* expression increased to levels similar to that of the group with brood, suggesting that the workers retained the capacity to perform brood care once egg-laying by the queen resumes. The highest *mrjp-1* expression levels were found in the presence of brood, which is consistent with the hypothesis of a high demand for feeding immature nestmates. However, brood pheromones alone appear to be sufficient to reduce worker lifespan (Smedal et al., 2009), suggesting that the perception of a signal is enough to trigger an increased and earlier foraging to sustain brood rearing (Sagili et al., 2011), thus exposing workers to higher risks.

The presence of brood was associated with initially high vitellogenin expression levels decreasing over time. This corresponds to earlier observations that nurses show high levels of vitellogenin in their haemolymph, whereas foragers display low levels (Fluri et al., 1982; Hartfelder and Engels, 1998; Corona et al., 2007). We measured the highest *mrjp-1* expression levels in workers exposed to brood, matching with the nutritional role attributed to the major royal jelly protein-1 (Schmitzova et al., 1998; Buttstedt et al., 2014). However, expression patterns of vitellogenin and *mrjp-1* were unexpectedly asynchronous and peaked at different times, indicating roles beyond the mere nutritional contributions (Drapeau et al., 2006; Buttstedt et al., 2014; Chua et al., 2015).

Our results show for the first time that the presence of young workers alone can significantly reduce the longevity of nestmates. In contrast to the presence of brood, the presence of young workers is unlikely to shorten lifespan due to the metabolic cost of heat production. Moreover, it seems unlikely that the costs derived from jelly production are a significant factor here, since adult workers are fed much smaller amounts of proteins compared to larvae (Crailsheim, 1998). What else could explain this significantly reduced life expectancy in the presence of young workers? It has been proposed that age demography shapes division of labor (Robinson et al., 1992; Seeley, 1995; Huang and Robinson, 1996). In line with this hypothesis, the continuous emergence of young workers may accelerate the behavioral ontogeny of nestmates, i.e., workers may start foraging earlier in the presence of young adults, thereby decreasing their lifespan (Rueppell et al., 2007). Indeed, the foraging stage is ultimately linked to more risky tasks and thus to earlier death (Neukirch, 1982). An earlier foraging of nestmates in the presence of young workers but in the absence of brood is supported by reduced expression of both vitellogenin and *mrjp-1* genes from day 21 onwards. Moreover, methyl transferase and methyl farneosate epoxidase expression did peak in this group at day 5, which could correspond to the early onset of foraging (Elekonich et al., 2001; Bomtorin et al., 2014).

Methyl farneosate epoxidase expression was not affected by social context, while expression of methylase transferase and methyl farneosate epoxidase precursors of juvenile hormone varied differently according to social context. As a consequence, variations in their expression were not directly coupled with that of vitellogenin, suggesting that they modulate the production of juvenile hormone differentially. Although vitellogenin and juvenile hormone were shown to be interdependent (Barchuk et al., 2002; Amdam and Omholt, 2003; Guidugli et al., 2005; Münch and Amdam, 2010), their interaction does not seem to be mediated via methylase transferase and methyl farneosate epoxidase expression; furthermore, other factors might be involved in determining their hemolymph titers (e.g., Amdam and Omholt, 2003; Corona et al., 2007). More detailed studies are needed to disentangle the factors influencing the expression of these genes. Given that post-transcriptional regulation can affect the production of proteins and that various physicochemical factors can affect their function, such mechanisms should be considered to confirm the occurrence of the physiological processes deduced from gene expression.

In this study, we showed the lifespan-reducing effect of young workers on their nestmates in the absence of brood. Broodless stages in the presence of young workers regularly occur after the departure of the queen in a reproductive swarm and until her successor reproduces (Fig. 1). This is also the case in colonies unable to replace lost queens (for example, after her failure to return from a mating flight; Neumann et al., 1999). In these situations, the presence of young workers could function as a signal informing their elder nestmates that they can start or carry on foraging. The resulting maintenance of colonial organization could ensure the availability of the food supply necessary for rearing the brood that will be produced by the new queen or by the reproductive workers that develop following queen loss (Robinson et al., 1990). Such a regulatory mechanism would also be adaptive in broodright colonies, where both the effect of brood and young workers would co-occur. Although our experiment was not designed to compare the nature of the interaction between brood and young workers, our results provide some indications. The absence of a significant difference between the life expectancy of workers in the presence of young individuals but the absence of brood on the one hand and of workers in the presence of brood but the absence of young individuals on the other hand suggests that the effect of both factors is of similar magnitude. The 4–5-week lifespan measured in our groups is characteristic for workers in colonies rearing brood and indicates non-additive effects between brood and young workers.

Pheromones emitted by their elder nestmates were shown to be sufficient to delay maturation of young workers (Leoncini et al., 2004). It remains to be tested whether the observed reduced life expectancy in the presence of young workers may also result from pheromones they secrete. Alternatively, a high number of young workers could decrease the intensity of the signal emitted by foragers, thus allowing transitions of nestmates to the dangerous task and shortening their lifespan. Under both scenarios, our results support the idea that colony needs and social regulation (Huang and Robinson, 1996; Rueppell et al., 2008; Amdam, 2011) determine the lifespan of colony members.

The question arises regarding how nestmate behavioral ontogeny can be inhibited by the presence of foragers, while the presence of young workers favors this ontogeny. We propose that opposing activatory and inhibitory signals ensure optimal behavioral ontogeny. In an unbalanced social context (e.g., the excess of young workers or of foragers), one of the signals would predominate and re-equilibrate the balance between in-hive and foraging cohorts. Alternatively, these opposing signals could affect an intermediate cohort of workers that could idle before their work is needed (Winston, 1987; Hasegawa et al., 2016).

We have shown that the physiological mechanisms affecting longevity differ when brood or young workers are present, thereby adding to

our understanding of how social context affects the ageing of workers. The finding that young adults precipitate the demise of their elders provides evidence for a yet undescribed regulation mechanism of colony functionality. Adding to the complexity of organization within insect societies, our results suggest that nestmates signaling each other's presence might regulate age-related division of labor. Such regulation mechanisms might be common to all social insects showing this attribute and could provide the plasticity required for colonies to rapidly adapt to changing environments.

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