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Honeybee workers with higher reproductive potential live longer lives

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A R T I C L E I N F O

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A B S T R A C T

Social insects, especially honeybees, have received much attention in comparative gerontology because of their peculiar and flexible ageing patterns that differ across genetically similar individuals. The longevity of honeybee individuals varies and depends on patterns of gene expression during development; females developing into reproductive individuals (queens) live longer than facultatively sterile workers. Here, we show that rebel workers, which develop under queenless conditions after swarming and have high reproductive potential, live approximately 4 days longer in hives and approximately 3 days longer in cages than individuals that develop in queenright colonies and have lower reproductive potential; this difference in longevity occurs in both free-flying and caged workers. Moreover, we show that both rebel and normal workers live longer when their ovaries contain more ovarioles. Longer-living rebel workers can benefit the colony because they can fill the generation gap that emerges between workers after queen exchange during swarming. Our findings provide novel evidence that the fecundity of workers in a social insect colony impacts their intrinsic longevity.

1. Introduction

Understanding the ageing processes is important from a medical perspective, so the question of why some individuals of the same species senesce at different rates is of special interest (Kirkwood and Austad, 2000). Social insects are excellent models for such studies because they form colonies of genetically similar individuals that differ in their physiology, behaviour and longevity (Bourke, 2007). In these insects, the reproductive females usually remain safely inside the nest and exhibit extraordinary longevity (queens can live > 20 years in some species), while the non-reproductive helpers, which perform all tasks inside and outside the nest, including foraging for food, are usually characterized by short lifespans (Keller and Genoud, 1997). As stated by the theory of ageing, the evolutionary explanation for this phenomenon is that high extrinsic mortality rates in age-structured populations promote rapid organismal ageing, thus increasing intrinsic mortality (Williams and Day, 2003).

The honeybee (*Apis mellifera* L.) is one of the social insect models that has drawn the attention of gerontologists due to the large differences in lifespan between castes (Münch et al., 2008; Page and Peng, 2001). Its colonies consist of a single queen and many workers, and the expression of the queen or worker phenotype depends on larval nutrition (Winston, 1987). Larvae fed with relatively small amounts of royal jelly by nurse bees develop into workers, which are small in size and facultatively sterile, whereas larvae fed large amounts of royal jelly develop into queens that are large with an active reproductive system (Winston, 1987). The queen and workers differ not only in their reproductive potential but also in terms of their lifespan; queens are extraordinarily long-lived and can survive up to 8 years (100 times longer than a worker) (Winston, 1987). However, the longevity of workers differs between individuals and depends on the geographic location and season. The summer generation of workers (summer bees) consists of short-lived individuals (longevity of 15–38 days) (Free and Spencer-Booth, 1959), while the winter generation is longer-lived (winter, *diutinus* bees; longevity of 6–8 months) (Mattila et al., 2001).

Multiple biotic and abiotic factors are related to the longevity of honeybee workers. Workers characterized by high nurse activity have shorter lifespans than workers with low nurse activity (Amdam et al., 2013). Similarly, the time of the onset of foraging significantly impacts worker longevity; individuals that begin foraging earlier usually have shorter lifespans than those that start later in life due to higher mortality outside the nest and greater physiological exploitation during foraging (Rueppell et al., 2007). Engagement in nurse activity and the onset of foraging are often determined by seasonally variable factors, e.g., the amount of nectar and pollen in the field, weather and/or colony energetic status (Winston, 1987).

One of the most spectacular events in the life of a honeybee colony is swarming. This is the only natural way for colonies to multiply, and it is associated with several changes in the nest. During swarming, the old mother queen leaves the nest with some workers to search for a new nest, while other workers are left behind in the old nest to care for the eggs, larvae and pupae of younger worker cohorts and the new,

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developing sister queens. New virgin queens begin to emerge approximately a week after the prime swarm is issued, and the first few virgin queens very often leave the nest when the afterswarm is issued. In the few days following the last afterswarming, the new queen takes over; kills other newly hatched and unhatched sister queens; performs nuptial flights, during which she mates with unrelated drones; and then begins laying eggs nursed by her sister and half-sister workers in the old nest (Winston, 1987). The time between the issuing of the prime swarm and the establishment of the new queen is approximately 3–4 weeks (Winston, 1987), during which new eggs are laid in the colony and no adults emerge. This can cause problems, as the typical longevity of workers during the swarming season is approximately 15–38 days (Free and Spencer-Booth, 1959), meaning that swarming causes a generation gap.

This generation gap can potentially be filled by workers that, as larvae, developed immediately after swarming under queenless conditions. These individuals, called rebel workers (Woyciechowski and Kuszewska, 2012), are characterized by their life strategy; compared to normal workers, they invest more into their own reproduction as they have more ovarioles in their ovaries as well as more developed mandibular glands and underdeveloped hypopharyngeal glands (Kuszewska and Woyciechowski, 2015; Woyciechowski and Kuszewska, 2012). Moreover, their ovaries are activated regardless of whether they live in queenless or queenright colonies (Woyciechowski and Kuszewska, 2012). We posited that rebels live longer than normal workers and thus fill the generation gap after swarming.

2. Methods

We performed our experiments in May and June 2015 in an experimental apiary at the Institute of Environmental Sciences of Jagiellonian University. Five queenright honeybee (*A. m. carnica*) colonies were studied, each consisting of 20,000–40,000 workers. All colonies were treated in the same way, and the experimental design was previously described by Woyciechowski and Kuszewska (2012). The experiment began when the queen was confined/restricted to two experimental frames to produce eggs of a similar age (day 0), and three days later, the colony was divided into queenright and queenless subunits, each contained in one experimental frame (day 3). When the worker cells in the experimental frames were sealed (day 12), the subunits were reunited so that the experimental broods experienced the same conditions during their prepupal and pupal stages. Twenty-one days after the experiment began, the frames containing newly emerged workers reared as larvae under queenright and queenless conditions were placed in an incubator in the laboratory (34 °C, 90% RH). Newly emerged workers from the two experimental frames were collected (day 19), and 30 from each frame (two groups) were killed by freezing (− 16 °C) and dissected to assess whether they developed into normal or rebel workers. A second set of workers were marked on the thorax with a spot of paint (Marabu–Brilliant Painter, 50 from each group and colony) and placed in wood-frame cages (13 × 9 cm and 5 cm high with glass and steel mesh sides) and provided with a small piece of honeycomb to assess their longevity under artificial conditions. Each cage, which contained 50 workers from each of the two groups (reared with and without a queen), corresponded to one experimental colony. The cages were incubated at 36 °C and 50–60% RH, and the bees were provided with a 50% sucrose solution and water ad libitum. The cages were checked every day, and dead bees were counted and removed. A third set of workers (100 from each group and colony) were marked individually on the thorax and returned to their native colonies to assess their longevity under free-flying conditions. In this case, we checked only four colonies because one of the colonies swarmed, and most of the marked bees were lost.

2.1. Examination of anatomical parameters

We dissected the hypopharyngeal glands (HPGs) and ovarioles of the frozen workers and examined them under a stereomicroscope. The size of the HPG was calculated from the average size of 10 acini, which are saclike dilations that compose the compound HPG (square root of the longest × shortest diameters of 5 right-gland and 5 left-gland acini). We also counted the ovarioles in both ovaries (sum) and evaluated ovary development (Kuszewska and Woyciechowski, 2015; Woyciechowski and Kuszewska, 2012).

2.2. Statistics

Mixed model two-way ANOVA was used to compare parameters (ovariole number, hypopharyngeal gland size) with the conditions during the larval stage (reared with or without the queen) as a fixed effect and colony as a random effect. If the effect of an experimental treatment was statistically significant, the ANOVA was followed by multiple comparisons using the post hoc Tukey HSD test with $P = 0.05$ considered significant. Differences in survival between normal and rebel workers in cages and hives were analysed using the generalized linear/nonlinear model (GLZ) module with a Poisson distribution and the log link function, which is a semiparametric statistical test. The colony was a random effect, and the worker type was a fixed effect. If a factor was statistically significant, the GLZ was followed by multiple comparisons using the post hoc Tukey HSD test with $P = 0.05$ indicating significance. Associations between the reproductive potential and longevity of bees were compared using separate simple regressions for rebel and normal workers and for all colonies (10 analyses in total). For this calculation, only caged bees were used because it was only possible to collect dead bees in this case. The assumption was that the reproductive potential of workers can linearly influence the longevity of individuals regardless of whether they come from groups of rebel or normal workers. All calculations were conducted in STATISTICA 9.0.

3. Results and discussion

Similar to previous studies (Kuszewska and Woyciechowski, 2015; Woyciechowski and Kuszewska, 2012; Woyciechowski et al., 2017), we found that the workers that developed under queenless conditions had more ovarioles (Fig. 1A; Table S1A) and smaller hypopharyngeal glands (Fig. 1B; Table S1B) than workers that developed under queenright conditions, confirming that we successfully obtained both normal and rebel workers. We found that workers reared without a queen lived significantly longer than workers reared in the presence of a queen under both free-flying (Fig. 2A; Table S2A) and caged conditions (Fig. 2B; Table S2B). The mean longevity ranged from 18.8 to 25.0 days for normal workers and from 22.6 to 30.2 days for rebel workers, depending on the hive, while the mean longevity ranged from 20.6 to 24.8 and from 24.2 to 28.5 days for these two groups of workers in the laboratory, respectively.

Rebel workers live only 4.5 days longer in the hive and 2.7 days longer in the cage than normal workers, but the generation gap is approximately 21–28 days long. Thus, our results do not fully answer the question of how this generation gap is filled. A possible explanation is that workers remaining in the orphaned colony after the first swarm is issued have no brood for which to care, and since nurse activity and the occurrence of brood pheromone shorten worker longevity (Amdam et al., 2013; Smedal et al., 2009), these workers may partially fill the generation gap. However, the absence of a brood increases the longevity of workers by only 1.5 days (Amdam et al., 2013), which is even shorter than the differences we report here, so another explanation is that the temporary lack of young workers after swarming significantly increases the lifespan of the older workers left behind after the colony was orphaned. Indeed, the presence of young workers has been shown to reduce the lifespan of nestmates by approximately 20 days (Eyer