NOTES AND COMMENTS

Ovariole number and ovary activation of Russian honeybee workers (*Apis mellifera L.*)

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Reproductive altruism by workers is a defining characteristic for eusocial insects such as the honey bee (*Apis mellifera* L.). Nevertheless, honey bee workers have retained functional ovaries, although they are usually very small and inactive in the presence of a queen. Compared to queen ovaries with more than 120 parallel ovarioles that produce the eggs, worker ovaries contain usually only 2-12 ovarioles. The size of worker ovaries, measured as the number of ovarioles per ovary, has however been correlated to individual reproduction in the absence of a queen when workers compete for drone production (Makert *et al.*, 2006). In addition, the size of the worker ovary is related to various aspects of the non-reproductive division of labour among workers, such as the age of foraging initiation and foraging specialization (Page and Amdam, 2007). Considerable variation in worker ovary size exists within and among populations of *A. mellifera* (Ruttner and Hesse, 1981), and some selected crosses have revealed unexpected genetic variation for this trait, with workers containing over 140 ovarioles (Linksvayer *et al.*, 2009).

Russian honey bees were imported from the Primorski province (south eastern Russia) into the US from 1997 to 2002 for their superior resistance to *Varroa destructor*, and 18 separate lines have been maintained with systematic interbreeding (Bourgeois and Rinderer, 2009). These breeding stocks showed decreased mite reproduction but also might have a lower queen acceptance and a higher propensity to rear replacement queen cells than other *A. mellifera* populations (Cargel and Rinderer, 2004). Thus, Russian workers may be less responsive to queen signals, which may be influenced by ovary size or may influence ovary size by altering larval feeding (Makert *et al.*, 2006). For these reasons, we predicted relatively large worker ovaries in Russian honey bees and set out to study them.

Frames with brood combs of emerging worker brood from 18 colonies belonging to all 18 lines of the three blocks of the breeding programme (Bourgeois and Rinderer, 2009) were transferred to an incubator. Newly emerged workers were colour-marked to distinguish different colony origin and introduced into a queenless colony to stimulate ovary activation. All workers that remained at the age of two weeks were collected, frozen, and their ovaries were dissected. Ovary size was assessed by counting the number of ovarioles in each ovary, scoring the larger side as "maximum ovary size" and the smaller one as "minimum ovary size" for each worker. When one ovary was missing, we assumed to have lost the smaller ovary. "Total ovary size" was computed as the sum of the ovarioles in both ovaries and ovary asymmetry was calculated as the difference between the two ovarioles numbers divided by their sum. Ovarian activation was assessed on a 5-point scale, ranging from inactive ovaries with thin, translucent ovarioles (score 0) to ovarioles with fully developed oocytes (score 4). Non-parametric statistics (Spearman Rank correlation, Kruskal-Wallis ANOVA) were used when a Kolmogorov-Smirnov test indicated variables deviated significantly from normality.

Across all samples, ovariole number per ovary varied from 1 to 6, with a median of 2. "Minimum ovary size" and "maximum ovary size" were significantly correlated ($R_S = 0.78$, $n = 105$, $p < 0.001$) and both variables, as well as the "total ovary size" showed a positive correlation to the ovary activation score (min: $R_S = 0.26$, $n = 105$, $p = 0.008$; max: $R_S = 0.28$, $n = 126$, $p = 0.001$; mean: $R_S = 0.28$, $n = 105$, $p = 0.004$). Ovary asymmetry had a median of 0.2 (lower quartile: 0.0; upper quartile: 0.2) and ranged between 0.0 and 0.6.

While the differences in "mean ovary size" among the three blocks were not significant ($H = 5.7$, $df = 2$, $p = 0.058$), colonies ($H = 27.7$, $df = 17$, $p = 0.049$) differed significantly (Fig. 1). "Minimum ovary size" ($H = 28.3$, $df = 17$, $p = 0.042$) was also significantly different
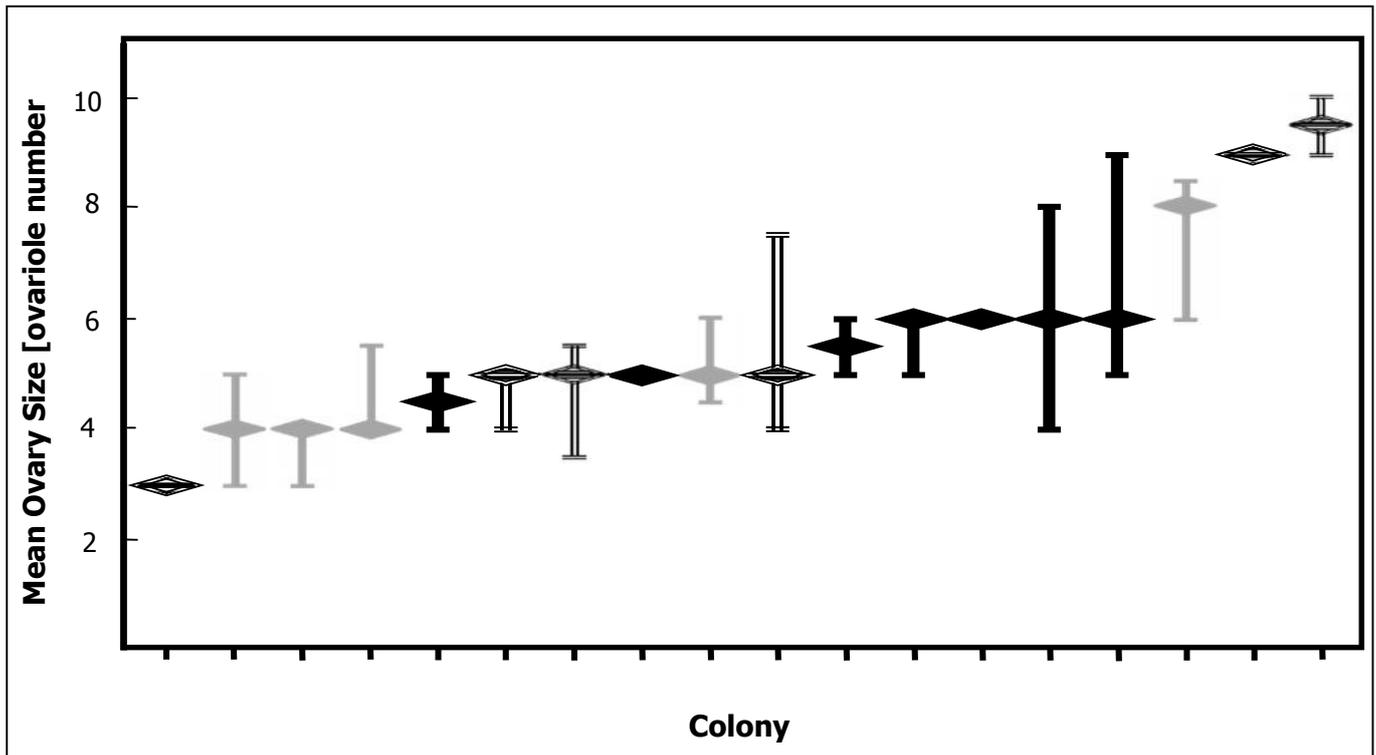
among colonies but "maximum ovary size" (H = 27.1, df = 17, p = 0.056) showed only a trend towards colony differences. "Ovary activation" was not different among the three blocks (H = 2.5, df = 2, p = 0.293), but colonies (H = 36.3, df = 17, p = 0.004) differed significantly. The rankings of the colonies according to average ovary size and ovary activation were significantly correlated for colonies (linear correlation, weighted by each colony's sample size: R = 0.42, n = 17, p = 0.048). Ovary asymmetry did not differ among colonies (H = 14.9, df = 17, p = 0.601) or blocks (H = 2.3, df = 2, p = 0.316).

In contrast to our prediction, worker ovaries of Russian honey bees contained few ovarioles compared to other *A. mellifera* populations. The reported ovary sizes are most similar to *A. mellifera mellifera* and *A. m.carnica* (Ruttner and Hesse, 1981). The result corroborates the general trend that *A. mellifera* workers in northern populations have fewer ovarioles than southern populations (Ruttner and Hesse, 1981), possibly suggesting an influence of climate. Climate could have multiple effects on the balance between individual- and colony-level selection that affects worker ovary size (Rueppell *et al.*, 2011).The result also suggests that the Russian's high propensity to build supersedure cells and lower acceptance of queens do not stem from a generally increased reproductive disposition of workers that might make them less responsive to reproductive suppression by other colony members (Makert *et al.*, 2006). Worker ovary size was positively correlated to ovary activation scores under our experimental, queenless conditions, as reported previously in a racial comparison (Ruttner and Hesse, 1981) and for Africanized honey bees (Makert *et al.*, 2006).

The 18 colonies were not drastically different (compare Linksvayer *et al.*, 2009), although some indirect or direct genetic influences on ovary size and activation were revealed as significant colony effects. These effects could also be explained by environmental differences such as the position in the apiary or management, but the impact of the external environment on developing larvae in the colonies is presumably low. The Russian breeding programme was designed to maintain genetic diversity, and the differentiation among the three blocks is low (Bourgeois and Rinderer, 2009). The genetic variation within blocks outweighs the effect of between-block differentiation (Bourgeois and Rinderer, 2009), which may explain the absence of significant block effects, particularly in the context of non-additive effects (Linksvayer *et al.*, 2009).

Acknowledgements

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