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Association between Larger Ovaries and Pollen Foraging in Queenless *Apis cerana* Workers Supports the Reproductive Ground-plan Hypothesis of Social Evolution

O. Rueppell · Evelyn Hunggims · Salim Tingek

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Introduction

Based on the ovarian ground-plan hypothesis (West-Eberhard 1987; West-Eberhard 1996) the reproductive ground-plan hypothesis of social evolution has recently been proposed as a theoretical framework to understand social evolution in honeybees (Amdam et al. 2004, 2006). The reproductive ground-plan hypothesis seeks to explain the evolution of honeybee social complexity from solitary ancestors by co-option of control elements of the female, gonadotropic reproductive cycle (Amdam et al. 2004). One of its central predictions, therefore, is that reproductive physiology and social behavior, in particular division of labor, be linked in particular ways (Amdam et al. 2006). Ovary size influences the level of reproductive hormones (Goodman and Granger 2005) and is therefore predicted to be correlated with differences in social behavior. Honey bee workers exhibit age-dependent division of labor between in-hive tasks and foraging (Beshers and Fewell 2001) but intrinsic worker differences affect the timing of behavioral transitions and further aspects of

O. Rueppell (✉)
Department of Biology, University of North Carolina, Greensboro, 312 Eberhart Building,
Greensboro, NC 27403, USA
e-mail: olav_rueppell@uncg.edu

E. Hunggims · S. Tingek
Agricultural Research Station, Tenom, Sabah, Malaysia

E. Hunggims
e-mail: arstnm@tm.net.my

S. Tingek
e-mail: arstnm@tm.net.my

the division of labor, such as the specialization during foraging on nectar or pollen collection (Page and Erber 2002).

Phenotypic associations of worker ovary size with foraging specialization and with the age of the transition from in-hive tasks to foraging in queenright hives of the Western honey bee, *Apis mellifera* (L.) have been proposed as important support for the reproductive ground-plan hypothesis (Amdam et al. 2006). However, the generality of these associations has been doubted (Oldroyd and Beekman 2008). We have studied these associations with two experiments in a different, but related species, the Eastern honey bee (*Apis cerana*). *Apis cerana* workers shows high levels of ovary activation and reproduction in the absence of a queen (Oldroyd et al. 2001) and therefore presents an ideal model to test whether the phenotypic associations between ovary size and foraging specialization and the age of foraging initiation persist when workers assume reproductive roles. This could substantiate the potential effect of reproductive hormonal differences among workers due to different ovary sizes (Makert et al. 2006) but it also changes the workers' social role, vitellogenin dynamics, and thus presumably the onset of foraging (Amdam et al. 2007).

Materials and Methods

The experiments were conducted at the Agricultural Research Station, Tenom (Sabah, Malaysia) from February to April 2007. *Apis cerana* colonies were housed in standard nest boxes and the five experimental colonies were spread over an area of 500 m × 250 m in an agricultural mixed-use area. Each hive consisted of a single box with 5,000–15,000 workers, brood, and food reserves. Three unrelated colonies containing a queen were used as sources for emerging workers, the two observational host colonies did not contain a queen.

In the first experiment, we collected emerging brood frames from three unrelated colonies and let them emerge overnight in a temperature-controlled incubator. After color-marking with enamel paint according to colony source, workers were introduced into a newly de-queened hive. After two days, it was established that 277 workers (source1: 73, source2: 124, source3: 80) survived the marking and were accepted into the unrelated host colony. Subsequently, we recorded all marked returning foragers for 25 minutes and captured returning foragers for another 30 minutes three times per week for a total of 35 days until no more marked workers were found. The captured foragers were dissected to determine ovary size (as number of ovarioles) and activation (Amdam et al. 2006). Dissections were performed under a stereomicroscope and Dupont-5 forceps (Fine Science Tools) and then transferred to a compound microscope for determination of ovariole number, developing eggs, and yellow bodies (Fig. 1). Left and right ovary were assessed and we computed the total number of ovaries. In addition, we recorded foraging age and whether the worker had foraged for pollen or not.

Since we did not record any marked pollen forager in the first experiment, we conducted a second experiment, specifically collecting pollen and non-pollen foragers to compare their ovary size and reproductive status. Over five days, equal numbers of unmarked foragers returning with or without pollen were collected from

Fig. 1 Dissected left and right ovary of an *A. cerana* forager from a queen-less colony. Note the highly asymmetric number of ovarioles and the large number of yellow bodies, indicating previous ovarian activation.

the entrance of another experimental queen-less hive and dissected as described above to quantify their ovary size and activation. To collect the samples, the hive was briefly moved to a different location and one to three returning foragers with and without pollen were collected at random. The results are presented using non-parametric statistics but the results of parametric tests did not differ significantly.

Results

No significant difference between the three colony sources was found in the age of first foraging (Mantel Cox Log-rank χ^2=0.99, n=48, p=0.611) or ovary size (Kruskal–Wallis test: χ^2=2.1, n=17, p=0.354). The age of first foraging and ovary size were not significantly correlated (Spearman's rho = 0.39, n=17, p=0.120). All dissected foragers except for two had activated ovaries and/or yellow bodies, but none was found to have collected pollen. Due to this lack of variation, the planned association tests between ovary activation status and foraging specialization, between ovary activation status and the age of first foraging, and between ovary size and foraging specialization could not be performed in the first experiment.

In the second experiment, the sample contained 17 pollen foragers and 16 foragers that returned without pollen. The pollen foragers had significantly larger ovaries (more ovarioles) than non-pollen foragers (Fig. 2, Mann–Whitney Z=2.6, n=33, p=0.012). This effect was even more pronounced when only considering the ovary with more ovarioles (Mann–Whitney Z=2.9, n=33, p=0.003). Ovary size and ovary activation (as measured by yellow bodies and developing oocytes) were not significantly correlated (Spearman's rho = 0.21, n=33, p=0.253) and reproductive status, scored as "active" (yellow bodies or developed ovaries) or "inactive", was not associated with foraging specialization (χ^2=0.08, df=1, p=0.776). However, a large number of returning foragers showed developing oocytes or yellow bodies as signs of previous reproductive activity (24/33).

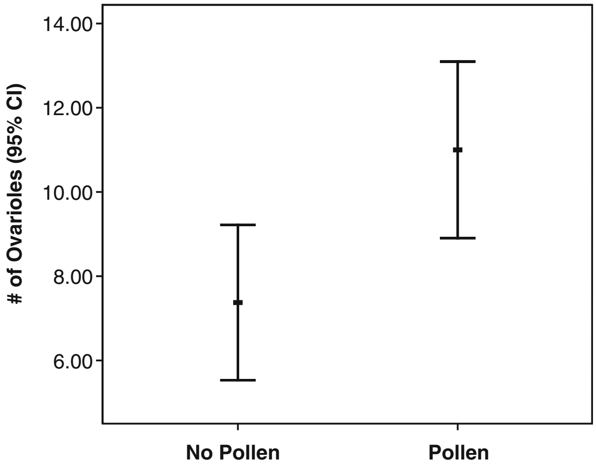

Fig. 2 The association between ovary size (total number of ovarioles in both ovaries) and foraging specialization in *Apis cerana* workers under queen-less conditions. Foragers returning with pollen had on average 3.6 ovarioles more than workers returning without pollen.

Discussion

This study significantly extends previous reports from *Apis mellifera* on the relationship between worker ovary size and social behavior (Amdam et al. 2006) to a novel species, *Apis cerana*, lending support to the generality of the reproductive ground-plan hypothesis. Specifically, the data support the prediction that reproductive traits are linked to foraging specialization in workers, which has recently been questioned (Oldroyd and Beekman 2008). Furthermore, the results show that the association between foraging specialization and ovary size also hold in the absence of a queen, which presents a drastically different social condition (Velthuis 1970; Oldroyd et al. 2001). Larger ovaries of queen-less *Apis cerana* workers were associated with pollen foraging, as in queen-right workers of *Apis mellifera* (Amdam et al. 2006). Ultimately, the association between pollen foraging and reproduction may be based on the high protein demands of egg production and larval feeding. Proximately, it is presumably mediated through the yolk protein vitellogenin (Page and Amdam 2007).

In *Apis mellifera*, workers ovary size was also positively correlated with two other hallmarks of the pollen hoarding syndrome that distinguishes nectar and pollen foragers and is believed to be caused by differences in reproductive physiology (Page and Erber 2002; Page and Amdam 2007). High ovariole number was correlated with high sucrose responsiveness and early onset of foraging (Amdam et al. 2006). We did not evaluate sucrose responsiveness in our study, but for the age of first foraging we found a non-significant trend in the opposite direction. This might be due to species-specific differences in the regulation of the division of labor between in-hive workers and foragers. However, in our view it is more likely that ovary size has an opposite effect on the temporal division of labor between in-hive tasks and foraging under queen-right and queen-less conditions. *Apis cerana* workers have a high propensity to develop their ovaries (Oldroyd et al. 2001) and we found in both experiments a high proportion of foragers with signs of current or past reproductive activity. Thus, workers may compete intensely for reproductive opportunity under queen-less conditions, leading to a breakdown of their regular temporal division of labor (Sakagami 1958). In accordance with this interpretation, our results show that only a small fraction of the marked cohort was actually recorded foraging in the first experiment. Workers with larger ovaries may delay foraging because they preferentially assume a reproductive role (Makert et al. 2006) and maintain high internal vitellogenin levels for longer, delaying the onset of foraging (Amdam et al. 2007).

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References

Amdam GV, Norberg K, Fondrk MK, Page RE Jr (2004) Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proc Nat Acad Sci U S A 101:11350–11355

Amdam GV, Csondes A, Fondrk MK, Page RE Jr (2006) Complex social behaviour derived from maternal reproductive traits. Nature 439:76–78

Amdam GV, Nilsen K-A, Norberg K, Fondrk MK, Hartfelder K (2007) Variation in endocrine signaling underlies variation in social life-history. Am Nat 170:37–46

Beshers SN, Fewell JH (2001) Models of division of labor in social insects. Annu Rev Entomol 46:413–440

Goodman WG, Granger NA (2005) The juvenile hormones. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science. vol. 6. Elsevier, Boston, pp 55–115

Makert GR, Paxton RJ, Hartfelder K (2006) Ovariole number—a predictor of differential reproductive success among worker subfamilies in queenless honeybee (*Apis mellifera* L.) colonies. Behav Ecol Sociobiol 60:815–825

Oldroyd BP, Beekman M (2008) Effects of selection for honey bee worker reproduction on foraging traits. PLoS Biology 6:e56

Oldroyd BP, Halling LA, Good G, Wattanachaiyingcharoen W, Barron AB, Nanork P, Wongsiri S, Ratnieks FLW (2001) Worker policing and worker reproduction in *Apis cerana*. Behav Ecol Sociobiol 50:371–377

Page RE Jr, Amdam GV (2007) The making of a social insect: developmental architectures of social design. BioEssays 29:334–343

Page RE Jr, Erber J (2002) Levels of behavioral organization and the evolution of division of labor. Naturwissenschaften 89:91–106

Sakagami SF (1958) The false-queen: fourth adjustive response in dequeened honeybee colonies. Behaviour 13:280–296

Velthuis HHW (1970) Ovarian development in *Apis mellifera* worker bees. Entomol Exp Appl 13:377–394

West-Eberhard MJ (1987) Flexible strategy and social evolution. In: Ito Y, Brown JL, Kikkawa J (eds) Animal societies, theories and facts. Japan Scientific Societies, Tokyo, pp 35–51

West-Eberhard MJ (1996) Wasp societies as microcosms for the study of development and evolution. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper wasps. Oxford University, New York, pp 290–317