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Ovarian development in a primitively eusocial wasp: Social interactions affect behaviorally dominant and subordinate wasps in opposite directions relative to solitary females

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ABSTRACT

In many primitively eusocial wasp species new nests are founded either by a single female or by a small group of females. In the single foundress nests, the lone female develops her ovaries, lays eggs as well as tends her brood. In multiple foundress nests social interactions, especially dominance–subordinate interactions, result in only one 'dominant' female developing her ovaries and laying eggs. Ovaries of the remaining 'subordinate' cofoundresses remain suppressed and these individuals function as workers and tend the dominant's brood. Using the tropical, primitively eusocial polistine wasp *Ropalidia marginata* and by comparing wasps held in isolation and those kept as pairs in the laboratory, we demonstrate that social interactions affect ovarian development of dominant and subordinate wasps among the pairs in opposite directions, suppressing the ovaries of the subordinate member of the pair below that of solitary wasps and boosting the ovaries of dominant member of the pair above that of solitary females. In addition to being of physiological interest, such mirror image effects of aggression on the ovaries of the aggressors and their victims, suggest yet another mechanism by which subordinates can enhance their indirect fitness and facilitate the evolution of worker behavior by kin selection.

1. Introduction

Caste differentiation into a reproductive caste and a worker caste is the hallmark of eusociality. In highly eusocial species such as honey bees, vespine wasps and most ants, caste differentiation is pre-imaginal, i.e., the differentiation of individuals into fertile queens and sterile workers takes place in their early larval stage of development. In primitively eusocial species such as polistine wasps, bumble bees and stingless bees, caste differentiation takes place largely in the adult stage because queens and workers are morphologically similar and physiologically nearly totipotent. Here dominance–subordinate interactions among adults lead to the winners becoming fertile queens and losers becoming sterile workers (Pardi, 1948; Wilson, 1971; Keller and Nonacs, 1993). In many primitively eusocial species, new nests may be founded either by a solitary female or by small groups of females. Solitary nest foundresses develop their ovaries, lay eggs and also tend their brood to adulthood. In multiple foundress nests however, dominance subordinate interactions result in caste differentiation such that only one individual (the queen) develops her ovaries and the rest remain without ovarian development and function as workers (Ross and Matthews, 1991). It is reasonable to expect that the ovaries of the subordinate cofoundresses in these nests remain less developed not only compared to their dominant partners but even compared to that of solitary nest foundresses.

In the primitively eusocial wasp *Ropalidia marginata* however, there is reason to suspect that such social interactions may also enhance ovarian development in the dominant cofoundress, over and above that of a solitary foundress (Lamba et al., 2007). In this species one of the workers becomes very aggressive and takes over as the next queen soon after loss or experimental removal of the original queen (Chandrashekara and Gadagkar, 1991; Premnath et al., 1996; Sumana and Gadagkar, 2003). It turns out that such a successor takes more, not less time to lay her first egg if she has no other wasp to show dominance behavior. In contrast when a successor has to show dominance and suppress other wasps, she takes significantly less time to develop her ovaries and lay her first egg. Based on this result we have previously suggested that dominance behavior shown by the successor is helpful for her to

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rapidly develop her own ovaries (Lamba et al., 2007). We therefore hypothesize that during nest founding, dominance subordinate interactions should show opposite effects in the dominant and subordinate wasps such that dominants have better ovaries compared to solitary foundresses and subordinates have poorer ovaries compared to solitary foundresses. Here we perform a direct test of this hypothesis by following the early time course of ovarian development of solitary (without social interactions) as well as paired wasps (with social interactions) maintained in the laboratory.

2. Methods

2.1. Study animals

We collected twenty-five *R. marginata* colonies from in and around Bangalore, India (13°00′ N, 77°32′ E), removed all existing adult wasps, eggs and larvae from these nests (such that all eclosing females were already in the pupal stage), and monitored them daily in the laboratory to check for eclosing females. On the day of eclosion we collected the females from their nests and randomly assigned them into two categories: the paired category (two freshly eclosed females randomly paired and housed together in a plastic box) or solitary category (female wasps maintained in solitude in a plastic box). We further randomly assigned each category into one of the following four age classes: zero days, one week, two weeks or four weeks. We stored the females assigned to zero day category directly for dissection after being collected from their nests. We maintained females assigned to the one, two or four week categories (both paired and solitary) from the day of their eclosion until one, two, or four weeks as designated, in similar plastic boxes (22 cm × 11 cm × 11 cm; allowing ambient light and air to enter) with ad libitum food [*Corcyra cephalonica* (Lepidoptera: Pyralidae) larvae], honey, water and a piece of soft wood as building material. At the end of pre-designated age limits, we stored all females at −20 °C until they were dissected. The study used a total of 304 female wasps.

2.2. Experimental design

Among each pair of wasps housed together, one had higher ovarian development than the other. These wasps were labeled paired superior (PS) and paired inferior (PI) respectively. To study the effects of social interactions on female ovarian development we can compare females from the paired category (PS and PI) with age controlled solitary wasps, using a simple comparison of means. However, this will be inadequate because the PS and PI wasps may have higher or lower ovarian growth than solitary wasps merely by chance. Because there is considerable variation in ovarian development even among the solitary wasps, we need to compare PS wasps with those with better ones among solitary wasps and PI wasps with those with less developed ovaries among solitary wasps. To accomplish this, we created virtual random dyads of (non-interacting) solitary wasps to compare with the (socially interacting) paired wasps, at the end of the experiment. In each such dyad, we labeled the one with higher ovarian development as solitary superior (SS) and the one with smaller ovaries as solitary inferior (SI). We then compared PS wasps with SS wasps and PI wasps with SI wasps separately for each age class. Each female therefore belonged to only one of the four classes. Here, the adjectives 'superior' and 'inferior' therefore always refer only to higher and lower ovarian development.

It is necessary to rule out the possibility that our results could change if some other potential combinations of solitary females were used to create the solitary dyads. We therefore repeated the analysis 1000 times, drawing a new set of solitary dyads from the same data set (sampling without replacement), designating them as SS or SI depending on their relative ovarian indices, and compared them each time with the PS and PI females of the corresponding age classes, using the Mann–Whitney *U* test. We then counted the number of times out of 1000 trials that the results were similar to our original results. If these numbers were more than 950 (setting alpha = 0.05) we concluded that our original results could not have been obtained by chance alone.

2.3. Computation of ovarian index (OI) and body size index

We dissected all females immediately after they completed their pre-existing age limit (zero days; one, two, or four weeks) in Insect Ringer's solution and measured the following ovarian parameters for each female: number of mature (chorionic) oocytes, mean length of all six proximal oocytes, mean width of all six proximal oocytes, the total number of oocytes with yolk in all six ovarioles, and total number of oocytes in all ovarioles. Proximal oocytes included chorionic oocytes if present. We subjected these five parameters to Principal Components Analysis (PCA) and used Principal Component 1 as ovarian index for each female (Chandrashekara and Gadagkar, 1991; Gadagkar, 2001). Similarly, we subjected 27 body parameters per wasp (Gadagkar, 2001) to Principal Components Analysis and used Principal Component 1 from this analysis as an index of body size. PCA and Mann–Whitney tests were performed using StatistiXL version 1.8 (http://www.statistixl.com).

2.4. Behavioral observations

For a subset of the paired individuals (41 out of 73 pairs), we made quantitative behavioral observations to identify the behaviorally dominant individual of each pair. To facilitate such observations, we marked the paired females with unique paint spots to allow individual identification, placed them together in ventilated transparent glass beakers and observed for one hour. We performed behavioral observations on the paired wasps in either the first or the second weeks of their experimental lifespan. We only noted dominance behaviors *viz*. attack, peck, nibble, aggressive bite, crash, etc. (Gadagkar, 2001) during such observations, and calculated the total number of acts of dominance shown by each female at the end of the hour. A wasp was designated dominant if she showed at least two acts of dominance and at least twice the number of acts shown by her subordinate partner (if the subordinate showed any acts of dominance at all). Pairs failing to satisfy this criterion were considered non-interactive and omitted from the analysis. Out of 41 pairs on which behavioral data were collected, 13 pairs showed no interaction during the one hour of observations and 11 pairs did not qualify the above criterion. Of the 17 pairs that satisfied our criteria, ovarian data were available on 10 females, as at least one of the pair died before the completion of the experiment in the remaining pairs.

Only virgin females were used in the experiment. Since age is an important predictor of ovarian development in virgin *R. marginata* females (Shukla et al., 2013), all comparisons in this experiment were done between identically aged female wasps. Wasps were paired randomly, and therefore pairs comprised of nestmates as well as non-nestmates. We have shown previously that these wasps do not discriminate between nestmates and non-nestmates if removed from their nest at eclosion as we have done here (Venkataraman et al., 1988). We controlled for adult nutrition by providing all females with ad libitum food, and performed all experiments in the same season.