Males of the social wasp *Ropalidia marginata* can feed larvae, given an opportunity

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A striking feature of hymenopteran societies is the general absence of male workers. There are some reports of males feeding larvae in some social wasps, but not in the extensively studied *Ropalidia marginata*. By providing excess food and by removing the females, we investigated whether males are incapable of feeding larvae or lack opportunities to do so. Males fed larvae with probabilities and rates comparable to those of females, suggesting that lack of preadaptation to feed larvae does not explain the absence of male workers in social Hymenoptera. Although males were not as efficient as females at feeding larvae, they seemed capable of doing enough for natural selection to have promoted the evolution of male workers if there were not other factors preventing it. Genetic relatedness asymmetry may be one such factor. A third hypothesis for the absence of male workers in social Hymenoptera, concerning the increased susceptibility of haploid males to infection, is not relevant because males handle and masticate prey for their own consumption and otherwise interact with the larvae.

A striking feature of eusocial Hymenoptera is that the worker caste is always female. Males may stay on their natal nests or leave to lead a nomadic life but, with rare exceptions (see below), they do not participate in nest building, brood care or foraging. Workers, who are females, perform all these tasks. There are reports of occasional instances of males feeding larvae in some species of social wasps (West-Eberhard 1969; Yamane 1969; Jeanne 1972; Hunt & Noonan 1979; Makino 1983; Gadagkar & Joshi 1984; Cameron 1986; Kojima 1993; O'Donnell 1995, 1999) and during many years of study of the behaviour and social biology of the social wasp *Ropalidia marginata*, males have never been seen to feed larvae (Gadagkar 2001). To explain why male *R. marginata* do not feed larvae, we put forward three hypotheses. (1) Male *R. marginata* are incapable of feeding larvae. (2) Males never get access to enough food to satisfy themselves and have something leftover to offer to the larvae (males do not forage and have to depend on the females for food). (3) Females are so much more efficient at feeding the larvae that they leave no opportunities for the relatively

inefficient males to do so. To test these hypotheses, we observed unmanipulated nests, nests with males and females to which we offered excess food, and all-male nests (created by removing all the females) in which the males were hand fed to satiation.

METHODS

This study is based on 325 h of observations, using 14 postemergence nests of *R. marginata*, between August 2003 and February 2004. Nests were collected from their sites of natural initiation in Bangalore (13°00′N, 77°32′E), and transplanted to the vespiary, located in the Centre for Ecological Sciences, Indian Institute of Science, Bangalore (Gadagkar 2001). All adult wasps were individually marked with unique coloured spots of enamel paint. Behavioural observations were uniformly spread between 0800 and 1800 hours.

The 14 nests were arbitrarily assigned to one of four groups and subjected to different types of manipulations and observations. Type 1 nests were unmanipulated and had both males and females with no food supplement. Type 2 nests were also unmanipulated and had both male and female wasps, but they received a food supplement in

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addition to what they foraged on their own. The food supplement consisted of about 10 *Corcyra cephalonica* larvae, diluted honey and water, placed within 3 cm of the nest, every day. Type 3 nests had only male wasps because we removed all female wasps before the study (we maintained the removed females in a closed cage with ad libitum food, water and building material). These nests also received a food supplement; however, since the males do not forage for solid food, even from petri dishes placed nearby, we hand fed them with pieces of *C. cephalonica* larvae, which they readily accepted when they were hungry. Type 4 nests had only female wasps. To facilitate recording complete sequences of feeding behaviour by the females, we offered *C. cephalonica* larvae to the females in these nests.

We recorded behaviours in 5-min sessions followed by 1-min breaks. In each 5-min session we either scanned the behavioural state of each individual in the nest (scan) or recorded every performance, by every individual, of behaviours associated with dominant/subordinate interactions, bringing and sharing food, bringing and sharing building material, feeding larvae and other forms of brood care (these sessions are referred to as 'all occurrence sessions'). We analysed data on food mastication and feeding of larvae by the adult wasps. Equal numbers of scans and all occurrence sessions were randomly intermingled. This method of observation was used for nests of types 1, 2 and 3. To examine similarities and differences in the process of feeding larvae by males and females, we recorded entire feeding bouts using focal animal sampling in type 3 (for males) and type 4 (for females) nests. Because it was necessary to remove all the females to observe a significant frequency of feeding larvae by males, we had to study male and female feeding bouts in different nests. During the focal animal sampling sessions, we chose the first adult wasp, male or female, to acquire food as the focal animal and observed it until it exhausted the food by mastication and/or feeding larvae. Each such observation sequence beginning with acquiring food and ending with exhaustion of the food was referred to as a feeding 'bout'. Every time an adult wasp entered a larval cell and transferred solid food or regurgitated liquid to the larva, this act was counted as one instance of feeding a larva. Transfer of solid food was confirmed by the observation of solid food, first in the mandibles of the adult and later in the mandibles of the larva; the latter was seen to be masticating and later eating the solid food. Transfer of regurgitated liquid was inferred when the adult wasp inserted its head and thorax into a larval cell and made contact with the larva while pumping its gaster for at least 3–4 s. Male *R. marginata* are produced infrequently in relatively small numbers and they reside on their natal nest for only about a week, after which they leave to lead a nomadic life. Hence, we pooled data from a number of nests to accumulate a large enough sample. Larvae were classified into three size classes: L1 (small), L2 (medium) and L3 (large). Table 1 summarizes the nests used in this study.

Table 1. Different types of nests studied and their characteristics

Nest code (type)	Food supplement	Observation period	Duration of observation (h:min)	No. of males	No. of females*	No. of eggs	No. of larvae (L1:L2:L3)†	No. of pupae
V452 (type 1)	No	14–16 August 2003	10:00	1	16	16	13:07:06	8
V482 (type 1)	No	26 August–16 September 2003	42:30	22	33	10	13:09:14	21
V484 (type 1)	No	3–16 September 2003	32:30	8	14	50	2:02:04	10
V488 (type 1)	No	26 August–2 September 2003	16:20	3	12	1	4:11:13	12
V455 (type 2)	Yes, placed nearby	18–25 September 2003	40:00	37	11	17	13:12:38	61
V422 (type 2)	Yes, placed nearby	16–24 October 2003	20:00	7	07	24	19:08:08	9
V456 (type 3)	Yes, hand fed	22 October–5 November 2003	59:30	17	0 (22)	4	6:04:36	37
V499 (type 3)	Yes, hand fed	11–17 November 2003	25:00	8	0 (05)	9	19:06:05	2
V515 (type 3)	Yes, hand fed	28 December 2003–10 January 2004	67:30	35	0 (13)	31	27:13:36	64
V422 (type 4)	Yes, hand fed	3–4 December 2003	1:24	0	13	9	30:15:03	6
V506 (type 4)	Yes, hand fed	6 February 2004	1:23	0	17	4	24:12:12	0
V519 (type 4)	Yes, hand fed	27–29 January 2004	3:42	0	21	31	10:17:13	29
V522 (type 4)	Yes, hand fed	29 January–5 February 2004	3:01	0	18	26	8:13:22	10
V524 (type 4)	Yes, hand fed	4–6 February 2004	2:13	0	11	5	18:07:22	22

*Numbers in parentheses are the numbers of females that were removed from the nest.
†Larvae were classified as small (L1), medium (L2) and large (L3).

RESULTS

Males can Feed Larvae

Males were never seen to feed larvae in type 1 nests (without a food supplement). However, both males and females acquired and masticated food and the females fed larvae. In type 2 nests (with a food supplement), males were seen to feed larvae, although the proportion of males that participated in feeding larvae was not statistically different from zero (G test: $G_{adj} = 7.62$, $N = 36$, $P < 0.005$, $\alpha = 0.005$, after Bonferroni correction). Similarly, the frequency/h of feeding larvae by males was not significantly different from the corresponding rate in type 1 nests (Mann–Whitney U test: $U = 595$, $N_1 = 34$, $N_2 = 44$, $P = 0.12$, $\alpha = 0.005$, after Bonferroni correction). In type 3 nests (hand fed), males fed larvae much more often. The proportion of males that participated in feeding larvae and the frequency/h at which they did so in type 3 nests were indistinguishable or significantly greater than the corresponding values for females in type 1 and type 2 nests (proportion feeding larvae: type 1: $G_{adj} = 84.41$, $N_1 = 60$, $N_2 = 75$, $P < 0.001$; type 2: $G_{adj} = 13.42$, $N_1 = 60$, $N_2 = 18$, $P < 0.001$; frequency: type 1: $U = 663$, $N_1 = 60$, $N_2 = 75$, $P < 0.001$; type 2: $U = 320$, $N_1 = 60$, $N_2 = 18$, $P = 0.009$; $\alpha = 0.001$, after Bonferroni correction; Fig. 1a, b). Thus, hypothesis 1 (males cannot feed larvae) is not supported but hypotheses 2 (lack of access to sufficient food) and 3 (presence of females) are both supported.

We had several lines of evidence to suggest that male *R. marginata* were not simply getting rid of unnecessary food but that they actively sought out the most appropriate larvae to be fed. (1) Males did not simply drop unwanted food on to the floor but delivered it to the cells in the nest. (2) Males were never seen to deliver food to empty cells, egg cells or to cells with relatively small larvae. In 340 of 345 instances that males were observed to feed larvae, they fed the largest class of larvae. (3) The act of feeding larvae by males involved a fairly complex series of actions; males moved about the nest antennating various cells and their contents in search of cells bearing large larvae, with a piece of solid food in their mandibles, they inserted their head and thorax into the chosen larval cell, held on to the food with their mandibles at one end in a position that made it possible for the larvae to grab the food with their mandibles at the other end, and then let go of the food.

Males Masticate More and Feed Larvae Less

When males fed larvae, their feeding bouts were significantly longer than those of females (t test: $t_{340} = 2.38$, $P = 0.017$; Fig. 2). Mastication durations also differed significantly ($t_{340} = 9.63$, $P < 0.001$): for females, the mastication duration was about a third of the total feeding duration, whereas for males it was more than 90% (Fig. 2).

Males Feed Larvae Differently From Females

The results in this section come from our record of 266 feeding bouts with 345 instances of feeding larvae by 54 males and 60 feeding bouts with 1426 instances of feeding larvae by 39 females. In a typical feeding bout, a female wasp acquired solid food, masticated it for about 3–4 min, distributed the solid food to about two larvae, groomed herself, regurgitated liquid food to about six larvae and once again groomed herself. This second grooming was
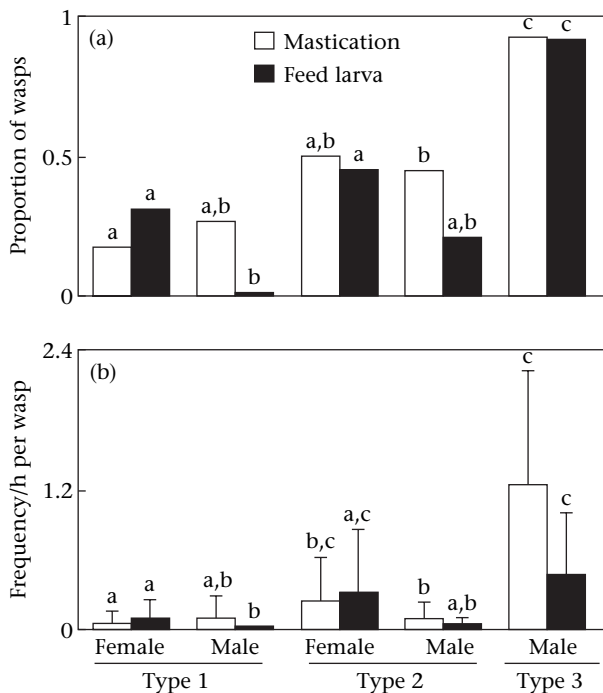

Figure 1. Comparison of males and females in different types of nests with respect to mastication of food and feeding larvae. (a) Proportion of wasps performing the behaviour. (b) Frequency of the behaviour/h per wasp. Statistical comparisons using G test (for proportion) and Mann–Whitney U test (for frequency) were done separately for mastication and feeding larvae. Bars carrying different letters are significantly different from each other at $P < 0.05$ (with Bonferroni correction). Type 1 nests had no food supplement, type 2 nests had a food supplement and type 3 nests were all-male nests with hand feeding of males (see Methods).
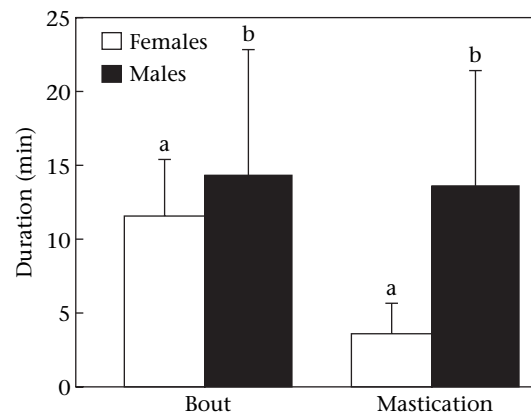

Figure 2. Durations of feeding bouts and mastication by males and females. Statistical comparisons are by t test, separately for bout duration and mastication. Bars carrying different letters are significantly different ($P < 0.05$).

not considered part of the feeding bout. Thus, feeding bout duration was measured from the time she acquired food until the beginning of the second grooming. For the females, the number of larvae fed, the proportion of feedings to large larvae (L3) and the proportion of feedings with solid food were calculated separately for the phase of the female bout before the first grooming (pregrooming phase) and the phase of the feeding bout after the first grooming (postgrooming phase; Fig. 3). In contrast, a typical feeding bout of a male consisted of acquiring solid food, feeding usually a single larva after over 10 min of mastication, followed by self-grooming but no regurgitation. Since males did not feed larvae after the first grooming, a single set of values is given for males, which corresponds to the pregrooming phase of the female's feeding bout (Fig. 3). Females fed significantly more larvae with regurgitated food than with solid food (Mann–Whitney U test: $U = 542$, $N_1 = N_2 = 60$, $P < 0.001$, $\alpha = 0.016$, after Bonferroni correction; Fig. 3a). Males fed fewer larvae than females in the pregrooming and postgrooming phases of their feeding bout (pregrooming: $U = 4168$, $N_1 = 60$, $N_2 = 266$, $P < 0.001$; postgrooming: $U = 1240$, $N_1 = 60$, $N_2 = 266$, $P < 0.001$; $\alpha = 0.012$, after Bonferroni correction; Fig. 3a). Males almost exclusively fed the largest larvae (L3), whereas females distributed food to all sizes of larvae, although females also fed a higher proportion of L3 than smaller larvae in the pregrooming phase of their feeding bout (G test: $G_{adj} = 96.95$, $N_1 = 147$, $N_2 = 1279$, $P < 0.01$, $\alpha = 0.016$, after Bonferroni correction; Fig. 3b). Females fed exclusively with solid food in the pregrooming phase of their feeding bout and exclusively with regurgitated food in the postgrooming phase. As mentioned above, males fed only with solid food (Fig. 3c). Perhaps for these reasons, in the nests from which the females had been removed, a substantial proportion of large larvae (which were fed) and almost all the small larvae (which were not fed) died. The cause of death was most likely to be starvation as almost no such deaths were recorded in nests with females.

Feeding Larvae by Females is More Complex

Compared to that of males, the process of feeding larvae by females appeared to be more complex because it was accompanied more often by other behaviours. These included fanning wings while entering the larval cell, antennal drumming of the larvae during feeding, and withdrawing from the larval cell with a rapid jerk of the body which was sometimes performed synchronously by many females. Females fanned their wings significantly more often while feeding larvae in the pregrooming phase than they did in the postgrooming phase (G test: $G_{adj} = 148.29$, $N_1 = 147$, $N_2 = 1279$, $P < 0.01$, $\alpha = 0.016$, after Bonferroni correction), but still significantly more than males did (pregrooming: $G_{adj} = 190.78$, $N_1 = 147$, $N_2 = 345$, $P < 0.01$; postgrooming: $G_{adj} = 33.56$, $N_1 = 1279$, $N_2 = 345$, $P < 0.01$; $\alpha = 0.016$, after Bonferroni correction; Fig. 4a). Females performed antennal drumming equally during the pregrooming and postgrooming phases ($G_{adj} = 1.05$, $N_1 = 147$, $N_2 = 1279$, NS, $\alpha = 0.016$, after Bonferroni correction), but males never performed
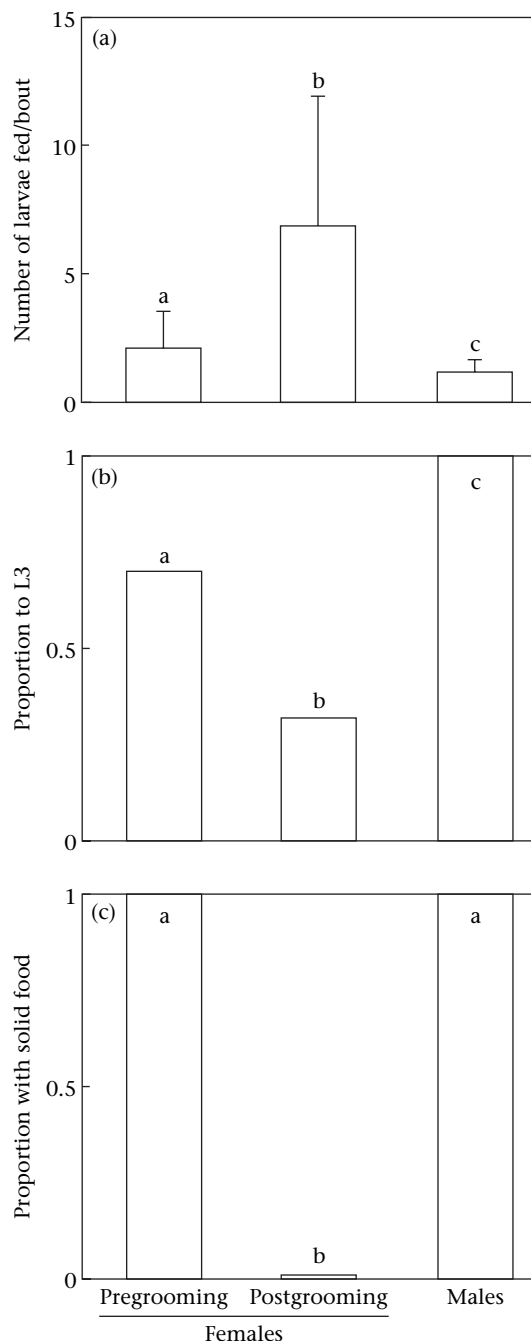

Figure 3. Differences between males and females in the behaviour of feeding larvae (see text for details). (a) Number of larvae fed/bout. (b) Proportion of feedings to the largest larvae (L3). (c) Proportion of feedings with solid food. Statistical comparisons using (a) Mann–Whitney U test and (b, c) G test were done separately for a–c. Bars carrying different letters are significantly different at $P < 0.05$ (with Bonferroni correction).

antennal drumming (Fig. 4b). Females withdrew from a larval cell with a body jerk primarily in the postgrooming phase, and significantly more often than in the pregrooming phase ($G_{adj} = 34.95$, $N_1 = 147$, $N_2 = 1279$, $P < 0.01$, $\alpha = 0.016$, after Bonferroni correction), which in turn was not statistically different from the value for males ($G_{adj} = 0.18$, $N_1 = 147$, $N_2 = 345$, NS, $\alpha = 0.016$, after
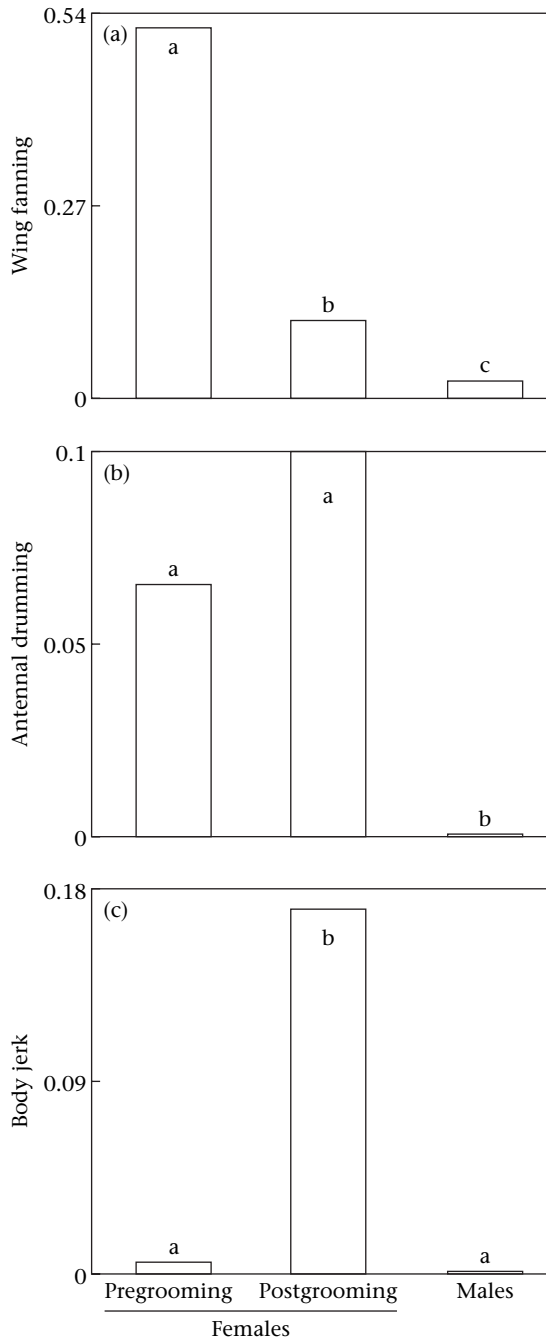
Figure 4. Differences between males and females in the proportions of feedings with (a) wing fanning, (b) antennal drumming and (c) body jerks (see text for details). Statistical comparisons using *G* test were performed separately for (a)–(c). Bars carrying different letters are significantly different at *P* < 0.05 (with Bonferroni correction).

Bonferroni correction; Fig. 4c). Several females feeding larvae with regurgitated liquid sometimes performed this body jerk synchronously but males never did.

DISCUSSION

We found clear evidence that when a food supplement is available, and especially when females are missing, male

R. marginata do feed larvae with a probability (proportion of individuals feeding larvae) and rate (frequency/h per wasp) matching those of females. Thus, we may conclude that male *R. marginata* are not incapable of feeding larvae and that they do not do so in natural colonies because they do not have access to enough food and/or because females leave no opportunity for them to do so.

Although male *R. marginata* fed larvae when given the opportunity and although we have argued that they are not merely dumping unwanted food (see Results), there is no doubt that from the point of view of the larvae, females are far more efficient at feeding than males. Unlike the females, males almost never fed younger larvae and did not feed regurgitated liquid. Furthermore, in each feeding bout males spent much more time masticating the food and much less time feeding larvae than the females did. There is evidence that adult wasps derive nourishment for themselves when they masticate food while feeding the larvae (Jeanne 1972; Hunt 1984). Therefore, we can assume that male *R. marginata* derive relatively more nutrition for themselves and provide less for the larvae than the females do. However, there is also the possibility that since males probably have weaker mandibles than females, they may need to masticate the food for longer than the females to make it useful for the larvae. At this time, we cannot decide whether by masticating the food for longer male *R. marginata* are being more selfish by imbibing more nutrition or whether they are being more altruistic by working hard to make the food useful for the larvae. Nevertheless it is clear that for the larvae, being fed by males is not adequate because many larvae under the care of males died (see Results). From our observation we suspect, however, that if a nest is devoid of females for a few days, males should be able to keep at least some of the large larvae sufficiently fed, at least until females eclose and take over brood care. There is of course the problem that males do not forage; in our nests, we hand fed them and it is this food that they fed to the larvae. On the other hand, we have observed males cannibalize some larvae in their nest. Therefore, they may be able to cannibalize some larvae and feed others at least for a short while. Thus, it seems reasonable to argue that there must be occasional situations for males to derive some inclusive fitness by feeding larvae and therefore occasional opportunities for natural selection to act on and promote this behaviour.

We found that the behaviour of feeding larvae by females was associated with a number of additional behaviours but feeding behaviour by males was not. Although we do not fully understand the significance of these associated behaviours, it does appear that they are somehow involved in adult–larval or adult–adult communication. Since males almost never feed larvae in natural nests and the entire task of feeding larvae is done by the females, it is not surprising that the behaviour of feeding larvae has evolved into a more complex and sophisticated process in females, whereas the same behaviour in males has remained simple.

Three classes of hypotheses have been proposed for the general absence of male workers in social Hymenoptera. Genetic asymmetries created by haplodiploidy make females more closely related to their full sisters than to

their offspring. On the other hand, males are more closely related to their daughters than to their siblings. According to such a relatedness hypothesis, females should prefer to care for their sisters rather than their offspring, whereas males should be selected to make efforts to mate and produce daughters rather than function as sterile workers that assist in rearing siblings (Hamiton 1964). There has been considerable discussion in the literature about whether or not these genetic asymmetries preclude the evolution of male workers (reviewed in Crozier & Pamilo 1996). Alternatively, the preadaptation hypothesis suggests that the evolution of male workers is difficult because nest building, brood care and all forms of parental care are restricted to the females in solitary Hymenoptera and because, unlike females, males are not equipped with strong mandibles useful for hunting and a sting useful for colony defence (reviewed in Bourke & Franks 1995; Crozier & Pamilo 1996). Our finding that male *R. marginata* can feed larvae, given an opportunity, does not lend support to the preadaptation hypothesis. Finally, the haploid susceptibility hypothesis argues that because the haploid males cannot be heterozygous at loci affecting resistance to disease, they should evolve adaptations to diminish the risk of infection (O'Donnell & Beshers 2004). However, it is not clear that feeding larvae increases the risk of infection because males routinely masticate solid food for their own consumption and also inspect and interact with larvae to drink larval saliva.

The ability to feed larvae (the raw material for natural selection) is certainly present in males. We therefore find it surprising that at least in species in which males stay on their natal nests for substantial periods of time, natural selection has not evolved feeding larvae by males into a more common and more efficient behaviour. A fuller understanding of why male workers have not evolved in Hymenoptera therefore awaits further theoretical and empirical investigation. Such future investigation should take into consideration our finding that males can feed larvae, given an opportunity.

Acknowledgments

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