Behavioural and morphological dimorphism of the sexes: an account of two primitively eusocial wasps
Ruchira Sen*†, and Raghavendra Gadagkar

Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India; Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India

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In social Hymenoptera, foraging, nest building, brood care and all other colony maintenance functions are carried out by the females while males function solely as reproductives. This asymmetry in social roles of the two sexes has led social insect researchers to focus almost exclusively on the females whereas males have remained relatively neglected. We studied two sympatric, primitively eusocial wasps, *Ropalidia marginata* and *Ropalidia cyathiformis*, and compared the morphological and behavioural profiles of males and females. Males of both species are smaller in size and weigh less compared to females. Males of the two species live in the nest for different durations. Borrowing from the ecological literature we use novel methods to compute and compare behavioural diversity and behavioural richness and show that females of both species are behaviourally richer and more diverse than the males.

Keywords: social wasps; behavioural diversity; behavioural richness; sexual dimorphism

Introduction

Eusocial insects are generally defined as those that display overlap of generations, cooperative brood care and reproductive caste differentiation (Michener 1969; Wilson 1971). In hymenopteran societies, the female reproductive caste consists of one or more females, who reproduce with the assistance of a large number of temporarily or permanently sterile female workers. All functions related to colony maintenance, such as foraging, brood care and nest building, are largely performed by these female workers; males in such societies usually do not take part in colony maintenance and act solely as reproductives. The only known functions related to colony maintenance that are carried out by the social hymenopteran males are very rare instances of brood care in the form of sharing food with the larvae and pupal thermoregulation by fanning their wings (Hölldobler 1966; West-Eberhard 1969; Yamane 1969; Jeanne 1972; Makino 1983; Hunt 1984; Cameron 1986; O’Donnell 1995, 1999; Sinzato et al. 2003; Giannotti 2004; Sen and Gadagkar 2006). The striking phenomena of cooperation and altruism exhibited by the females have therefore led to a plethora of theoretical...
and empirical investigations focusing on them. The behaviour of males on the other hand has been described only sporadically and opportunistically.

Here we have conducted a comparative study of the morphological and behavioural attributes of males and females of two congeneric, sympatric, primitively eusocial wasps *Ropalidia marginata* and *R. cyathiformis*. *R. cyathiformis* is a typical primitively eusocial species; colonies are headed by a queen who is the most or one of the most dominant (physically aggressive) individual(s) of the colony (Kardile and Gadagkar 2002, 2003); workers reproduce occasionally in this species. *R. marginata*, on the other hand, is a primitively eusocial species but shows some attributes of highly eusocial societies (Gadagkar 2001). In a *R. marginata* colony, at any point of time there is only one queen (egg layer); the queen is docile and does not show physical aggression to the workers and appears to use a pheromone to regulate worker reproduction (Bhadra et al. 2010). The comparison of these two primitively eusocial species with different levels of eusociality promises to offer new insights on the evolution of eusociality. Earlier studies have compared the behaviour of the queens and the workers of these two species (Kardile and Gadagkar 2002, 2003; Deshpande et al. 2006) and males and females of *R. marginata* (Sen and Gadagkar 2010). Here, we compare the morphology and behaviour of males of these two species and compare males with their female counterparts. For morphological comparison, we measured various body size parameters and dry body weights of males and females. We quantitatively studied intranidal behaviour of the males and females of both species and report the behavioural repertoire of the males. By employing methods drawn from the ecological literature, such as Shannon–Wiener index of alpha diversity, we show that females are behaviourally more diverse compared to males. We estimate the behavioural distance between the two sexes within species and between the two species within sexes, by using the Morisita–Horn index for beta diversity and illustrate the behavioural proximity or distance of each group to the other.

**Materials and methods**

For comparison of body size, we collected 52 males and 97 females from seven nests of *R. marginata* and 42 males and 64 females from four nests of *R. cyathiformis*. For each wasp, we measured 27 body size parameters (interocellar distance, right ocello–ocular distance, left ocello–ocular distance, head width, head length, clypeus width, clypeus length, width of first segment of right antenna, length of first segment of right antenna, width of first segment of left antenna, length of first segment of left antenna, inter-antennal socket distance, width of mesoscutum, length of mesoscutum, alitrunk length, length of right wing, length of left wing, length of first marginal cell of right wing, length of first marginal cell of left wing, number of ham-muli on right wing, number of hammuli on left wing, width of first gastral segment, length of first gastral segment, height of first gastral segment, width of second gastral segment, length of second gastral segment and height of second gastral segment (Kardile and Gadagkar 2005). Univariate (t-test) and multivariate (principal components analysis) statistical tests were carried out to compare the body size of males and females.

For comparison of body weight, we collected 27 males and 77 females from three nests of *R. marginata* and 15 males and 45 females from three nests of *R. cyathiformis*. 
Table 1. Observational period and nest details.

| Nest code | Observation period         | Observation duration (hh:mm) | Number of males observed | Number of females observed |
|-----------|---------------------------|------------------------------|--------------------------|---------------------------|
| Ropalidia marginata |                           |                              |                          |                           |
| V452      | 14–16 August 2003         | 10:00                        | 1                        | 13                        |
| V482      | 26 August–16 September 2003 | 42:30                      | 17                       | 28                        |
| V484      | 3–16 September 2003       | 32:30                        | 6                        | 13                        |
| V488      | 26 August–2 September 2003 | 16:20                        | 2                        | 5                         |
| M001      | 28 September–1 October 2004 | 20:00                        | 5                        | 9                         |
| V587      | 3–6 October 2004          | 20:00                        | 4                        | 5                         |
| Total     |                           | 141:20                      | 35                       | 73                        |
| Ropalidia cyathiformis |                     |                              |                          |                           |
| C102      | 16–23 April 2004          | 20:00                        | 10                       | 23                        |
| C103      | 15–23 April 2004          | 20:00                        | 4                        | 30                        |
| C106      | 28 April–2 May 2004       | 20:00                        | 16                       | 38                        |
| C108      | 28 November–1 December 2004 | 20:00                    | 9                        | 20                        |
| Total     |                           | 80:00                        | 39                       | 111                       |

Each wasp was freeze killed, measured for fresh weight, oven dried at 72°C for more than 48 hours to constant dry weight and weighed. Univariate (t-test) statistical tests were carried out for body weight comparison of males and females.

For behavioural comparison, we have studied six *R. marginata* nests for 140 hours and four *R. cyathiformis* nests for 80 hours between August 2003 and December 2004 (Table 1). We collected *R. marginata* nests from their sites of natural initiation in Bangalore (13° 00′ N and 77° 32′ E) and transplanted them in the vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The vespiary is a room of dimension 9.3 m × 6 m × 4.8 m covered with a wire mesh screen of (mesh) dimension 0.75 cm × 0.75 cm, which permits *R. marginata* to fly in and out freely but prevents its main predator *Vespa tropica* from entering. Thus the wasps are free to forage on their own and also to leave and found or join other nests. One *R. marginata* nest however, was studied in its site of initiation (M001). We studied all *R. cyathiformis* nests at their natural site of initiation in Bangalore, because unlike *R. marginata*, this species cannot be successfully established in the vespiary.

All adult wasps were individually marked with small unique coloured spots of quick-drying non-toxic enamel paint. A census of all adult wasps in each nest and a record of nestmap were taken once in two or three days. Behavioural observations were conducted in 2 h 30 min to 5 h sessions in a day, uniformly spread between 0800 and 1800 hours using “instantaneous scan” and “all occurrences sessions” sampling methods. Observations were made in 5 min sessions followed by a 1 min break. During
each 5 min session, we recorded behaviours using one of the sampling methods. Equal numbers of instantaneous scans and all occurrences sessions were randomly intermingled during the observation session (Gadagkar 2001). During instantaneous scans, we recorded performance of every individual once (like a snap shot); during all occurrences we recorded every performance of behaviours associated with dominant/subordinate interactions, bringing and sharing food, and building material, feeding larvae and other forms of brood care. From the instantaneous scan data, proportions of time spent in different behaviours were calculated, while from the all occurrences session data, frequency (per hour) of the rare behaviours or events were computed.

We analysed five long duration behaviours (data from instantaneous scans), in which wasps spent more than 90% of their time cumulatively, and 22 rare behaviours (data from all occurrences sessions), which were shown by one or more groups at least once in 10 h (Table 2). Univariate analysis (Mann–Whitney U test) was carried out on all the 27 behaviours for comparisons within species, across sexes, and within sexes, and across species. We emphasize eight behaviours, where the differences were most drastic between males of the two species or males and females of the same species (Figure 1).

Here we propose some novel ways of comparing behavioural profiles of individual wasps that may also be useful and informative in other studies. In doing so we borrow from the ecological literature which has a vast array of powerful methods of quantifying and comparing ecosystem properties. We define behavioural richness as the total number of behaviours shown by each individual and behavioural diversity as the Shannon–Wiener index, $H'$ (Shannon and Weaver 1949), computed as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i,$$

where $p_i$ is the proportion of the $i$th behaviour in a collection of $S$ behaviours (Gadagkar and Chandrashekara 2005). The individual-based behavioural richness and diversity indices are then compared across the sexes and across species.

Continuing to borrow from the ecological literature, we quantify the behavioural similarity and dissimilarity between the sexes and the species using Morisita–Horn similarity index, $C_\lambda$ (Wolda 1981), computed as

$$C_\lambda = \frac{2 \sum (n_{1i} \cdot n_{2i})}{(\lambda_1 + \lambda_2) \cdot N_1 \cdot N_2},$$

where

$$\lambda_j = \frac{\sum n_{ji}^2}{N_j^2},$$

where $n_{ji}$ is the mean of behaviour $i$ in group $j$ and $N_j$ is the sum of all behaviours performed by group $j$. The index was calculated with data logarithmically transformed as $\ln(n_{ji} + 1)$.
Table 2. Performance of 27 behaviours by males and females of *R. marginata* and *R. cyathiformis*. Performance of a behaviour by one or more wasps of a group is represented by 1 and no performance is represented by 0. The first five behaviours are referred to as long duration behaviours and are measured by the “instantaneous scan” sampling method; the next 22 behaviours are referred to as rare behaviours and are measured by the “all occurrences sessions” sampling method.

| Behaviour                          | Individual/Interaction | *R. marginata* male | *R. marginata* female | *R. cyathiformis* male | *R. cyathiformis* female |
|-----------------------------------|------------------------|---------------------|----------------------|------------------------|--------------------------|
| 1 Sit and groom                   | Individual             | 1                   | 1                    | 1                      | 1                        |
| 2 Sit alert                       | Individual             | 1                   | 1                    | 1                      | 1                        |
| 3 Walk                            | Individual             | 1                   | 1                    | 1                      | 1                        |
| 4 Inspect cells                   | Individual             | 1                   | 1                    | 1                      | 1                        |
| 5 Away from nest                  | Individual             | 0                   | 1                    | 1                      | 1                        |
| 6 Dominance behaviour             | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 7 Subordinate behaviour           | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 8 Solicit                         | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 9 Being solicited                 | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 10 Snatch things                  | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 11 Lose things                    | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 12 Feed self                      | Individual             | 1                   | 1                    | 1                      | 1                        |
| 13 Feed larva                     | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 14 Antennate nest                 | Individual             | 1                   | 1                    | 1                      | 1                        |
| 15 Antennate another wasp         | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 16 Being antennated               | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 17 Bring things (foraging trip)   | Individual             | 0                   | 1                    | 1                      | 1                        |
| 18 Bring nothing (empty trip)     | Individual             | 0                   | 1                    | 1                      | 1                        |
| 19 Inspect pupa                   | Interaction            | 0                   | 1                    | 0                      | 1                        |
| 20 Mouth to mouth contact with larva | Interaction         | 1                   | 1                    | 1                      | 1                        |
| 21 Extend old cells               | Individual             | 0                   | 1                    | 0                      | 1                        |
| 22 Fanning wings                  | Individual             | 1                   | 1                    | 1                      | 1                        |
| 23 Mutual antennation             | Interaction            | 1                   | 1                    | 0                      | 1                        |
| 24 Approach                       | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 25 Being approached               | Interaction            | 1                   | 1                    | 1                      | 1                        |
| 26 Body jerk                      | Individual             | 1                   | 1                    | 0                      | 1                        |
| 27 Wing jerk                      | Individual             | 1                   | 1                    | 1                      | 1                        |

We divided the list of analysed behaviours in 10 individual and 17 paired behaviours. If the behaviour involved only one wasp, it was considered as an individual behaviour [sit and groom (SG), sit alert (SA), walk (WA), away from nest (FG), antennate nest (AN), bring nothing (BX), feed self (FE), fanning wing (FA), body jerk (BJ), wing jerk (WJ)], while if the wasp interacted with another wasp
or brood, the interaction was considered as a paired behaviour [inspect cells (IC), dominance behaviour (DB), subordinate behaviour (S-), solicit another wasp (SC), being solicited (SS), antennate another wasp (AE), being antennated by another wasp (BE), extend old cell (EO), feed larva (FL), snatch things (ST), lose things (LT), inspect pupa (IP), mouth-to-mouth interaction with larva (MC), bring things (BT), mutual antennation (M1), approach (AP) and being approached (PP)]. We calculated Morisita–Horn index of beta diversity separately with the individual behaviours and the paired behaviours. The Morisita–Horn indices of all pair of groups were then used to prepare two sets of distance matrices and dendrograms.

Results

The males and females of *R. marginata* and *R. cyathiformis* are distinguishable by their clypeus colouration, antennal segment number and structure. In *R. marginata*, the males have bright yellow clypeus while females have brown clypeus. *R. cyathiformis* males and females both have yellow clypeus, but only females have a crescent shaped brown spot on the clypeus. Males of both species have 13 antennal segments while females have 12. The male antennal segments have dented structures called tyloids and the apical segments look like a hook (Gadagkar 2001; Kardile and Gadagkar 2005)

Males are smaller than females

In *R. marginata* males are smaller in 14 parameters (out of 27) and larger in only one parameter (IOD, interocellar distance). In *R. cyathiformis*, males are smaller in 23 parameters and there is no significant difference in four parameters (all comparisons done by t-test, *a* =0.0018, after Bonferroni correction due to 27 comparisons). Males and females also make two distinct clusters when plotted in a two dimensional principal components space (Figure 1). The first two principal components together
explained more than 50% of the variation. Males of both species have significantly less body weight (dry) compared to the females (Figure 2).

**Males are Behaviourally Different than Females**

Females of both species performed all 27 behaviours analysed, while 22 and 23 behaviours were performed by the males of *R. marginata* and *R. cyathiformis*, respectively (Table 2). All the instances of nest building, brood care and foraging were performed by the females. Among the brood care behaviours, *R. marginata* males occasionally showed feed larva behaviour. Except feed larva behaviour, males never performed any colony maintenance activity. There is a prominent difference between the males of the two species. After 4–8 days of eclosion, males of *R. marginata* leave the nest permanently for a nomadic life in the wild but during the first few days they do not leave the nest. *R. cyathiformis* males on the other hand, live in their natal nest for a much longer time, perhaps their entire lives (19 ± 21 days, Gadagkar and Joshi 1984), with frequent trips although they return with nothing. We witnessed a single instance of a *R. cyathiformis* male bringing some liquid in its mouth.

On the nest, *R. marginata* males spent most of the time “sitting and grooming” (SG). The average proportion of time spent in SG by males of *R. marginata* was significantly more than both *R. marginata* females and *R. cyathiformis* males.
Since *R. marginata* males never left the nest during their first few days on the nest, *R. marginata* females and *R. cyathiformis* males both spent significantly more proportion of time “away from the nest” (FG) compared to the *R. marginata* males. Female *R. marginata* also spent more time in SG and less time in FG than *R. cyathiformis* females (Figure 3a, b).

The males of *R. marginata* masticated (or fed themselves, FE) food in equal frequency to that of females but more than *R. cyathiformis* males, but the males of both species rarely fed larvae (FL) (Figure 3c, d). *R. marginata* males solicited (SC) other wasps for food and liquid more than their counterparts in the other species. *R. marginata* males solicited other wasps more than females and they were “solicited” (SS) least by others (Figure 3e, f). Males of both species rarely showed any dominance behaviour, and such occasional dominance behaviours are better interpreted as attempts to snatch food or liquid from the females. *R. marginata* males showed subordinate behaviour comparable to females. *R. marginata* females performed significantly more dominance and subordinate behaviour compared to *R. cyathiformis* females (Figure 3g, h). Other than occasional food sharing male-to-male interactions were never witnessed.

Males of both species never attempted to mate with the females on the nest or in close proximity of the nest.

**Females are behaviourally more diverse and rich**

We calculated behavioural richness (number of behaviours) and behavioural diversity (Shannon–Wiener index) at individual level and compared them across sex within species and across species within sex. We found females of the two species with comparable behavioural richness and diversity, are more behaviourally rich and diverse than the males (Figure 4). *R. marginata* males are more rich and diverse than *R. cyathiformis* males.

**Males and females cluster differently according to behaviours**

The separate dendrograms for individual and paired behaviours revealed different patterns (Figure 5a, b). For individual behaviours, we found species-based clusters, i.e. males and females of each species grouped together while for paired behaviours we found sex-based clusters, i.e. males of the two species grouped away from the female group of the two species.

**Discussion**

**Morphological dimorphism**

Our study shows that in the two primitively eusocial species *R. marginata* and *R. cyathiformis*, sexual size dimorphism exists. In both species, males are smaller in size than the females. Hymenopteran insects are holometabolus, i.e. the adult insects eclose from the pupa and after the eclosion the adult insect does not further grow in size. The size difference of males and females are therefore preimaginal due to genetic and/or environmental (resource-based) factors. Body size does not influence mating probability or mate choice in either males or females of *R. marginata* (Sen et al. 2010;
Figure 3. Comparison of *R. marginata* and *R. cyathiformis* males and females for eight behaviours: (a) sit and groom (SG), (b) away from nest (FG), (c) feeding (FE), (d) feeding larvae (FL), (e) solicit, (f) being solicited (SS), (g) dominance behaviour (DB) and (h) subordinate behaviour. Letters represent comparison across sex within species; numbers represent comparison within sex across species. Bars carrying different letters/numbers are significantly different from each other (Mann–Whitney U test, *p* < 0.05; please see supplementary table S1 for detailed statistical results).

Shilpa et al. 2010), and it may be true for *R. cyathiformis* too. Therefore, if the males are smaller in size due to genetic factors, then this trait should be naturally selected because small males would need less food and/or will have faster growth, which would result from lesser resource investment. In hymenopteran eusocial insects, males do not take part in colony maintenance; their only known function is to mate, while females take part in either reproduction or colony maintenance or both. Hence a trait that does not influence mate choice but yet requires less resource investment in males and allows more investment in females should be favoured by natural selection. Females
Figure 4. Comparison of (a) behavioural richness (represented by number of behaviours performed) and (b) diversity (represented by Shannon–Wiener index of alpha diversity) in males and females of *R. marginata* and *R. cyathiformis*. Letters represent comparison across sex within species; numbers represent comparison within sex across species. Bars carrying different letters/numbers are significantly different from each other (Mann–Whitney U test, \( p < 0.05 \); please see supplementary table S2 for detailed statistical results).

...can also derive much more advantage from larger size. These species are primitively eusocial so most of the females under study are workers. Larger size can be helpful for colony maintenance functions such as hunting, carrying the foraged material and defending the colony from non-nestmates. In other words, whatever the optimum sex investment ratios for the species, the investment meant for males will be packaged into many small males and the investment meant for females needs to be packaged into few large females. But of course we cannot predict the numerical sex ratio because we do not know the optimum sex investment ratios for these species.

**Behavioural dimorphism**

When the behaviours are considered in an individual behaviour-based dendrogram, both males and females of the same species group more closely together than males and females of the other species (Figure 5a). This is probably due to the fact that males and females of *R. cyathiformis* spend significantly longer time away from the nest compared to their *R. marginata* counterparts. Conversely, in the dendrogram based on paired behaviours, males and females cluster together despite the species differences (Figure 5b). This is easy to understand because males of both species do not take part in behaviours that are concerned with colony maintenance and they interact less with other wasps and brood compared to the females. Furthermore, females from both species perform more behaviours, so they are behaviourally richer and more diverse. In our analysis, we have not included some of the behaviours solely done by...
Figure 5. Behavioural dendrograms of *R. marginata* and *R. cyathiformis* based on the Morisita–Horn index of beta diversity. The indices were calculated for each pair of groups based on frequency or proportion of time spent in (a) individual behaviours and (b) interaction-based behaviours.

It is not surprising that the behavioural profiles of the relatively inactive males and worker females of both *R. marginata* and *R. cyathiformis* are so different, because they represent the typical social hymenopteran behavioural profiles (Wilson 1971).
In social Hymenoptera, such a difference in the behaviour of males and females is predicted on the basis of theoretical arguments. The haplodiploidy sex determination mechanism in Hymenoptera makes females more closely related to their full sisters than they would be to their own offspring. This helps to promote the evolution of worker behaviour, which involves staying back in their mother’s nest and assisting in the rearing of siblings instead of leaving to produce their own offspring (Hamilton 1964). The asymmetry in relatedness between siblings on one hand and offspring on the other may disappear if siblings include brothers, but a female-biased sex ratio is expected to restore the asymmetry that favours the evolution of worker behaviour in females (Trivers and Hare 1976). Nevertheless, in an outbred population, males are expected to be related both to their sisters and brothers by ½ and to their daughters by 1, and of course males have no sons. Thus, males are expected to be selected to make efforts to mate and produce daughters rather than function as sterile workers that assist in rearing siblings (Hamilton 1964). Whether or not these genetic asymmetries preclude the evolution of male workers which would have produced a diverse behavioural profile of the males remain debatable (Charlesworth 1978; Charnov 1978; Bartz 1982; Craig 1982; Crozier and Pamilo 1996). Some authors have argued that the absence of preadaptations for parental care in males is more important in making it difficult for the evolution of male workers. The absence of preadaptation arises from the fact that nest building, brood care and all forms of parental care are restricted to the females in solitary Hymenoptera, suggesting that this is the ancestral condition. Besides, only the females but not the males are equipped with strong mandibles useful for hunting and a sting useful for defence (Wilson 1971; Lin and Michener 1972; Alexander 1974; Eickwort 1981; Craig 1982; Bourke and Franks 1995; Crozier and Pamilo 1996). But many authors have expressed scepticism for this theory of preadaptation (Hamilton 1972; West-Eberhard 1975; Bartz 1982; Kukuk et al. 1989; Kerr 1990; Sen and Gadagkar 2006). Absence of male workers has also been explained by a third hypothesis that considers the elevated susceptibility of males due to haploidy as a reason for female-only workers (O’Donnell and Beshers 2004).

Instead of their intranidal behavioural similarity, males of *R. marginata* and *R. cyathiformis* were very different in the proportions of time spent on and off the nest. The females of *R. marginata* often showed physical aggression to the males, once they were old enough to leave the nest and forced them to leave. Females of *R. cyathiformis* however, showed minimal physical aggression to the males and these males stay on in their natal nests, although we do not know which is the cause and which is the effect. During their stay on the nest, *R. marginata* males use the resource of the nest equally with the females, as the males perform the feed self (FE) behaviour in comparable frequency with the females (Figure 3). Females distribute their share of the food with the larvae with great efficiency (Sen and Gadagkar 2006), while males rarely feed larvae. Furthermore, males solicit for resources in a comparable rate to the females but they are not reciprocally solicited in similar rate to females, i.e. the other wasps do not turn to the males for resource. Since mating does not occur on or in the close proximity of the nest, the queens cannot accrue fitness through their sons unless the males leave the nest. The *R. cyathiformis* males, on the other hand, stay for a longer period on the nest with frequent trips to the wild and they use fewer nest resources compared to females, as they perform less feed self (FE) behaviour compared to females (Figure 3). Males of *R. marginata* do not die after mating (Sen et al. 2010) and can mate multiply.
(Shilpa et al. under revision); we expect a similar mating biology for *R. cyathiformis*. Therefore, extended shelter and access to food resources could assure a longer lifespan and more mating possibilities, which may assure a higher reproductive fitness of the colony.

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Note
Supplementary material can be viewed online.

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