THE EVOLUTION OF EUSOCIALITY: INSIGHTS FROM COMPARING TWO INDIAN PAPER WASP SPECIES

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ABSTRACT

Eusocial species live in colonies with a reproductive division of labour into fertile reproductive castes and sterile non-reproductive castes, an overlap of generations and cooperative brood care. A further distinction can be usefully made between primitively eusocial species which do not have morphological caste differentiation and highly eusocial species which do. *Ropalidia marginata* is a tropical primitively eusocial wasp that has been extensively studied, especially compared to other tropical social wasps. *R. marginata* has several distinct traits, such as a docile queen, well-developed age polyethism, and decentralized work regulation, which makes it different from other primitively eusocial wasps and reminiscent of highly eusocial species. Since tropical wasps, especially those belonging to the genus *Ropalidia* have been poorly studied, we cannot be sure whether *R. marginata* is unique or its traits are more common among tropical *Ropalidia* species. To begin to overcome this problem, we have extended our research to the congeneric and sympatric *Ropalidia cyathiformis*. Here, we compare and contrast what we now know about these two species, especially concerning their reproductive and non-reproductive division of labour. We find that *R. cyathiformis*, unlike *R. marginata*, has a behaviourally dominant queen, weak and rigid age polyethism, likely uses behavioural dominance to regulate worker reproduction and individual workers self-regulate their own non-reproductive activities. We, therefore, conclude that *R. marginata* is indeed unique and argue that *R. marginata* is intermediate between primitively and highly eusocial wasps.

Key words: *Ropalidia marginata*, *Ropalidia cyathiformis*, primitively eusocial, highly eusocial, dominance behaviour, social wasps

Many animals, especially insects, live in social groups and depend on each other for survival and reproduction. The extent of social interaction and integration vary widely. To focus on the most social species, the term eusocial, originally coined by Suzanne Batra (Batra, 1966), is used for species that display reproductive caste differentiation into fertile reproductives (queens and kings) and sterile non-reproductives (workers), an overlap of generations and cooperative brood care (Michener, 1969; Wilson, 1971). In addition to the division of reproductive labour between queens and workers, there is often a further division of non-reproductive labour among subsets of workers, although the extent of the latter may vary greatly.

Based on the reproductive division of labour, eusocial insects are classified as primitively eusocial, with no morphological differentiation between reproductive and non-reproductive and highly eusocial insects, where the reproductives, and non-reproductive are morphologically distinct. In the highly eusocial species reproductive caste differentiation occurs in the pre-imaginal (larval) stage as a process of developmental regulation so that adults are quite fixed in their roles at eclosion. In the primitively eusocial species, by contrast, reproductive caste differentiation largely occurs in the post-imaginal (adult) stage by a process of social regulation, leading to considerable flexibility in the roles that an individual can adopt.

All termites and all ants (except perhaps the queenless ants in the sub-family ponerinae), honey bees and some wasps are considered highly eusocial while many bees and wasps are primitively eusocial. Among the wasps, examples of highly eusocial species include most epiponine and vespine wasps (Wilson, 1971; Ross and Matthews, 1991) while examples of primitively eusocial species include all polistine wasps and bumble bees (Fletcher and Ross, 1985; Wheeler, 1986; Gadagkar et al., 1988; O'Donnell, 1998).

Flexibility in the social roles of adults in the primitively eusocial species results in the potential for conflict among individuals for the position of a reproductive in the colony. Studies on the mechanism

of queen succession and determinants of becoming a queen in primitively eusocial wasps have revealed age and dominance behaviour as the two most important factors in predicting whether an individual becomes a queen or not (Pardi, 1948; Yoshikawa, 1963; West-Eberhard, 1969, 1978; Jeanne, 1972; Litte, 1979; Dew and Michener, 1981; Strassmann and Meyer, 1983; Miyano, S., 1986; Hughes and Strassmann, 1988; Giannotti and Machado, 1997; Suzuki, 2003). In addition, the queens in such primitively eusocial societies appear to use dominance behaviour to maintain their reproductive monopoly in the colony. A clear exception to this rule is the primitively eusocial wasp *Ropalidia marginata,* where the queen is docile (Gadagkar and Joshi, 1983; Chandrashekara and Gadagkar, 1992; Gadagkar, 2001; Sumana and Gadagkar, 2003) and uses pheromones to regulate reproduction amongst the workers of her colony. The usage of chemicals to regulate reproduction is a trait that is more commonly observed in highly eusocial species (Winston and Slessor, 1992; Keller and Nonacs, 1993; Katzav-Gozansky et al., 2002; D'Ettorre et al., 2004; Monnin, 2006)1993; Katzav-Gozansky et al., 2002; D Ettorre et al., 2004; Monnin, 2006). However, recent studies have shown that perhaps the usage of pheromones for maintaining reproductive control might not be restricted to just highly eusocial species and used by some primitively eusocial species as well (Dani and Turillazzi, 2018; da Silva et al., 2020; Tsuchida et al., 2020; Oi et al., 2021)

Non-reproductive division of labour could be based on morphology, with individuals of different sizes performing different tasks, as in the case of termites and several species of ants (Oster and Wilson, 1978; Noirot, 1989; Hölldobler and Wilson, 1990) or based on age as in the case of honey bees, stingless bees and several species of ants (Oster and Wilson, 1978; Seeley, 1982; Sommeijer, 1984). In the age-based division of labour or age polyethism, individuals perform different tasks as they age, with younger individuals performing tasks within the colony such as nursing and maintenance of the nest and older individuals doing tasks outside the colony such as foraging. In the case of primitively eusocial insects, since there is no morphological differentiation among individuals, an age-based division of labour is expected. Studies on primitively eusocial insects reveal a rudimentary and not very well-developed age polyethism, although it has been explored relatively less (Jeanne, 1991). Again, an exception to the rule is the primitively eusocial wasp

Ropalidia marginata, which has a strongly developed and flexible age polyethism similar to what is observed in highly eusocial insect species such as honey bees and stingless bees (Naug and Gadagkar, 1998a, 1998b).

Ropalidia marginata therefore, emerges as a species of interest, considering its similarities to highly eusocial insects despite being primitively eusocial (Fig. 1). However, one cannot yet draw rigorous conclusions regarding the levels of sociality of *R. marginata* for two main reasons - 1) the study methodology between *R. marginata* and other primitively eusocial wasps differ greatly, making the comparisons difficult and 2) the majority of studies in other primitively eusocial wasps are mainly on temperate wasps, whereas *R. marginata* is a tropical species. Temperate and tropical wasps have important differences in their nesting cycles, with the former having an annual colony cycle and the latter a perennial cycle (Reeve, 1991). These different life cycles lead to several specific adaptations. In the temperate species for example, reproductives are produced only at certain times of the year and are different in size with more fat deposits from other workers, reducing flexibility in their social roles (Haggard and Gamboa, 1980; Strassmann, 1981; Reeve, 1991). Mated female wasps overwinter in hibernacula and start new colonies the following spring. In tropical wasps on the other hand, nests can be initiated throughout the year, and individuals do not differ in morphology. Hence, any worker is potentially capable of becoming a queen, resulting in higher flexibility in social roles.

The best andonly way to work around these difficulties and understand whether *R. marginata* is perhaps at a level of sociality between primitive and advanced societies, would be a comparable

Fig. 1. Picture of *Ropalidia marginata*

study, with similar methodology, focussed on another tropical primitively eusocial species. We have therefore begun to focus our attention on the tropical primitively eusocial wasp *Ropalidia cyathiformis*. *R. cyathiformis* (Fig. 2) is a congeneric species to *R. marginata* and shares the same habitat range (Gadagkar and Joshi, 1982; Kardile and Gadagkar, 2005). *R. cyathiformis* possess an aggressive and dominant queen (Gadagkar and Joshi, 1982, 1984; Deshpande et al., 2006), already indicating that it might be similar to its more studied temperate counterparts. These features make *R. cyathiformis* a suitable model system to compare and contrast with *R. marginata* and the other temperate paper wasps. In this review, we compare and contrast the results and inferences of three separate studies on *R. cyathiformis* on the reproductive (Unnikrishnan and Gadagkar, 2017) and non-reproductive division of labour (Unnikrishnan and Gadagkar, 2020) as well as on the regulation of division of labour (Unnikrishnan and Gadagkar, 2021) with similar studies in *R. marginata*. We also discuss how the two species compare with other primitively eusocial wasps.

2. Behaviour of the queens

2.1. *R. cyathiformis*

Although there is no morphological differentiation between queens and workers in primitively eusocial species, the behaviour of the queens is nevertheless of particular interest because they are usually the sole egg layers of the colony. (Gadagkar and Joshi, (1984) constructed time-activity budgets for all individually identified wasps in colonies of *R. cyathiformis*. Subjecting these data to principal components analysis and cluster analysis, they demonstrated the presence of three rather distinct clusters of wasps. Based on their behavioural profiles, they called these clusters 'behavioural castes' and labelled the 3 clusters as sitters, fighters and foragers (Fig. 3). Sitters spend
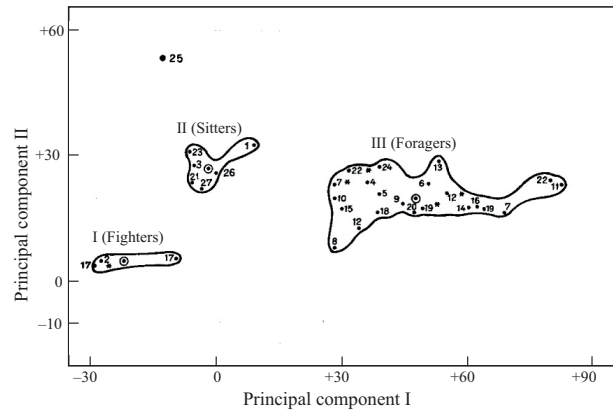

Fig. 2. Picture of *Ropalidia cyathiformis*

Fig. 3. Behavioural clusters of *R. cyathiformis*. Time-activity budget of 32 wasps is shown as points in the coordinate space of the first two principal components. The points fall into the 3 clusters by the criterion of the nearest centroid. Circled dot = centroid (Reproduced with permission from Gadagkar and Joshi, 1984)

most of their time sitting on the nest, apparently doing nothing. Fighters most frequently indulge in dominance-subordinate interactions with colony members.

In contrast, foragers spend more time away from the nest than the other behavioural castes, bringing back food and building material. Since the queens were not treated any differently during this analysis, the researchers could post-facto identify the behavioural caste of the queens. The *R. cyathiformis* queens were found to belong to the fighter caste. This was not surprising, since queens of primitively eusocial wasps are known to be aggressive individuals and use dominance behaviour to regulate the activities of the workers (Pardi, 1948; West-Eberhard, 1969; Jeanne, 1972; Litte, 1979; Strassmann, 1981; Strassmann and Meyer, 1983; Reeve and Gamboa, 1983; Miyano, S., 1986; Yamane, 1986; Hughes and Strassmann, 1988; Keeping, 1990; Giannotti and Machado, 1997; Tindo et al., 1997; O'Donnell, 1998; Tindo and Dejean, 1998; Giannotti, 1999; Noda et al., 2001; Suzuki, 2003; Prezoto et al., 2004; Oliveira et al., 2014). Consistent with this, (Kardile and Gadagkar, (2002) showed that *R. cyathiformis* queens are consistently at the top of the dominance hierarchies of their colonies. All subsequent studies have repeatedly re-confirmed that *R. cyathiformis* queens are active and interactive, aggressive fighters, usually at the top of the dominance hierarchies of their colonies (Deshpande et al., 2006; Gadagkar, 2001; Unnikrishnan and Gadagkar, 2017, 2021).

2.2. *R. marginata*

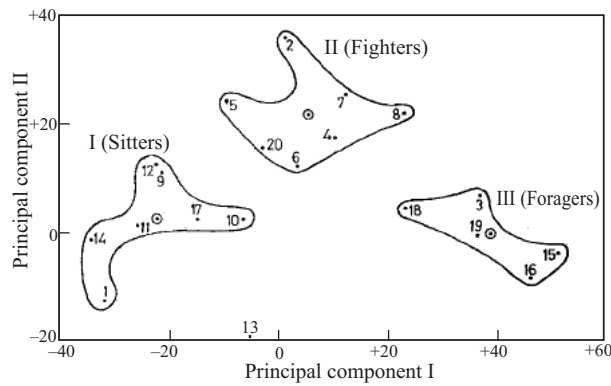Gadagkar and Joshi (1983) constructed time-

Fig. 4. Behavioural clusters of *R. marginata*. Time-activity budget of 20 wasps is shown as points in the coordinate space of the first two principal components. The points fall into the 3 clusters (castes) by the criterion of the nearest centroid. Circled dot = centroid (Reproduced with permission from Gadagkar and Joshi 1983)

activity budgets for all individually identified wasps in two colonies of *R. marginata*. Subjecting these data to principal components analysis and cluster analysis, they demonstrated the presence of three rather distinct clusters of wasps, sitters, fighters and foragers, based on their behavioural profiles (Fig. 4). It might have been expected that the queens also belonged to the fighter caste in this species, as in the case of *R. cyathiformis*. Instead, it turned out that the queens of both colonies of *R. marginata* belonged to the sitter caste. (Chandrashekara and Gadagkar, (1991a) re-confirmed this surprising result using 12 additional colonies. Chandrashekara and Gadagkar (1991) also constructed dominance hierarchies for each colony and showed that queens in *R. marginata* are seldom at the top of the dominance hierarchy. *R. marginata* queens have sometimes been found to be at the top of the dominance hierarchies of their colonies in small colonies, but this has been shown to be due to chance alone where the probability of any individual being on the top of the hierarchy is high (Chakraborty et al., 2018). All subsequent studies have repeatedly re-confirmed that *R. marginata* queens are meek and docile sitters, seldom at the top of the dominance hierarchies of their colonies, rarely interacting with the workers (Chandrashekara and Gadagkar, 1992; Gadagkar, 2001; Sumana and Gadagkar, 2003).

3. Reproductive division of labour

3.1. *R. cyathiformis*

Most colonies of *R. cyathiformis* contain a single egg layer, although workers have been seen to lay 1 or 2 eggs on rare occasions, despite the queen's presence. However, it is not clear whether it should be
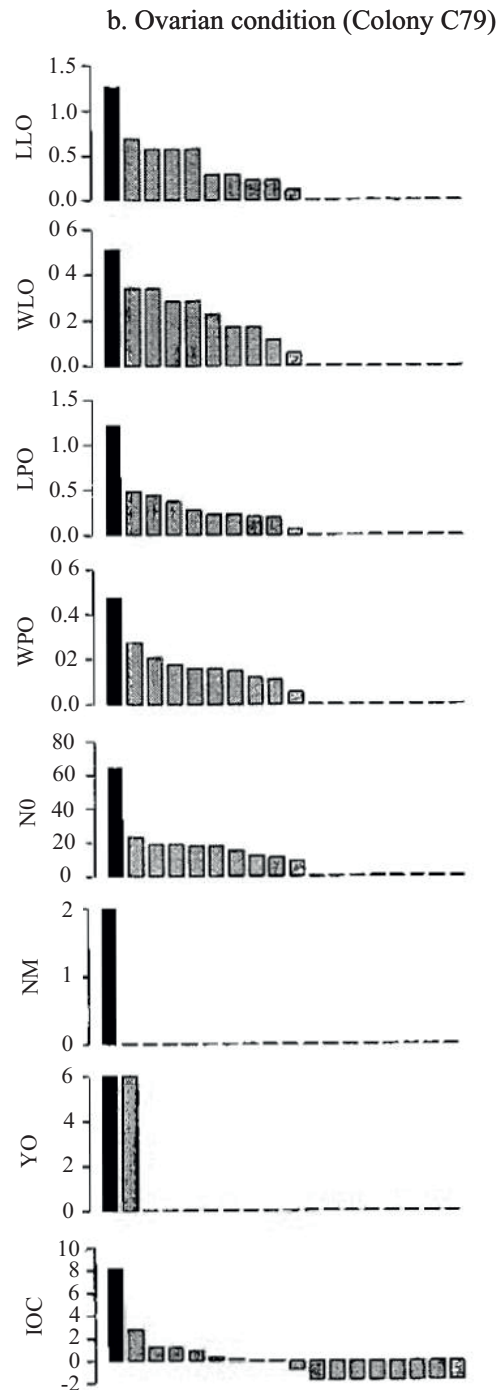
b. Ovarian condition (Colony C79)

Fig. 5. Ovarian condition of wasps in a representative colony. All the seven measurements of ovarian development are given. Grey bars represent workers while black bars represent queens. LLO, length of the largest oocyte; WLO, width of the largest oocyte; LPO, average length of proximal oocytes; WPO, average width of proximal oocytes;.NO, total number of oocytes; NM, number of mature oocytes; YO, number of oocytes with yolk; IOC, composite index of ovarian development
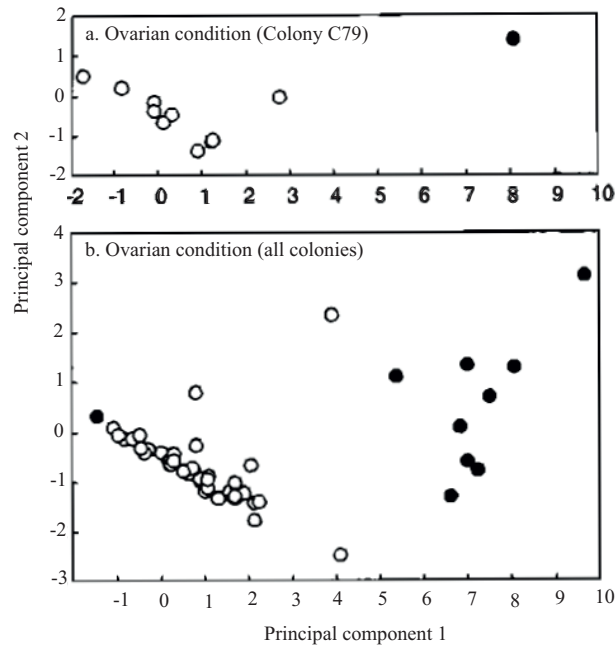
Fig. 6. Intra- and inter-colony variation in ovarian condition (a) representative colony and (b) all colonies. Closed circles = queens, open circles = workers "Reproduced with the kind permission of the Bombay Natural History Society from the "Journal of the Bombay Natural History Society – Vol 102: 266–273

treated as rare evidence of egg-laying workers or the presence of multiple queens in this species (Gadagkar and Joshi, 1982). In the absence of morphological caste differentiation between queens and workers, the difference between the labels 'egg-laying workers' and 'multiple queens' may only be semantic. The near reproductive monopoly of the queen is confirmed by data on ovarian development showing that queens have the best-developed ovaries, by any measure of ovarian development, and that workers seldom reach the queen's level of ovarian development even when compared across colonies (Kardile and Gadagkar, 2005) (Figs. 5, 6).

It is interesting to note, however, that workers often have some low level of ovarian development, suggesting that they are not permanently sterile but that their ovarian development is suppressed in the presence of the queen. If this is true, workers should be able to develop their ovaries and lay eggs if the suppressing effect of the queen is removed. This prediction has been borne out by the observation that, upon experimental removal of the queen, one of the workers develops her ovaries and takes over the role of the queen as the sole, or near-sole egg-layer of the colony (Deshpande et al., 2006; Unnikrishnan and Gadagkar, 2017). There are several remarkable features of such queen succession. Upon removal of the queen, one and only one worker becomes hyper-aggressive within a few hours, displaying high

levels of dominance behaviour towards almost all the remaining workers, and she is almost never the recipient of any dominance behaviour from her nestmates (Deshpande et al., 2006; Unnikrishnan and Gadagkar, 2017). This hyper-aggressive 'potential queen' develops her ovaries and begins to lay eggs within 1–9 days (mean± SD = 5.2± 3.5) (Unnikrishnan and Gadagkar, 2017). The potential queen (PQ) reduces her aggression at about the time she lays her first egg, and she subsequently remains about as aggressive as is typical for an *R. cyathiformis* queen.

The fact that the PQ is never challenged by the rest of the workers in the colony indicates that perhaps she has been pre-designated in some way to be the next queen of the colony. Indeed, studies show that there is a reproductive queue of potential queens who replace their predecessor queens in a remarkably conflict-free process of queen succession (Unnikrishnan and Gadagkar, 2017) (Figs. 7, 8). The reproductive queue, however, is not very long and ends with the 2nd (1 colony) or 3rd potential queen (10 colonies); no subsequent hyper-aggressive PQs were immediately identifiable, and observations have therefore been usually terminated at this point (Unnikrishnan and Gadagkar, 2017). The dominance behaviour shown by an individual in the presence of the queen is a statistically significant predictor of whether an individual will become a queen and also of her position in the reproductive queue. No other parameter, such as age, ovarian status or body size, has any role in determining whether an individual would become a queen or not, nor in determining the position of an individual in the queue. However, even dominance behaviour is not a perfect predictor because the next most dominant individual does not always become the next queen (Unnikrishnan and Gadagkar, 2017). Instead, potential queens are drawn from among the most dominant workers in the colony, but the order in which this subset of individuals succeed the dying or removed queens of the colony, cannot be predicted.

Nevertheless, dominance behaviour is important in queen succession as it is the only significant predictor of the identity of the potential queen. And this is not surprising as in all primitively eusocial species studied so far, dominance behaviour is an important predictor of whether or not an individual becomes a queen. On the other hand, it is surprising that age is not a significant predictor of the identity of the potential queen. In most primitively eusocial wasps studied, age is important in queen succession. In the so-called 'temperate pattern' of queen succession, the oldest wasps in the colony become future queens,
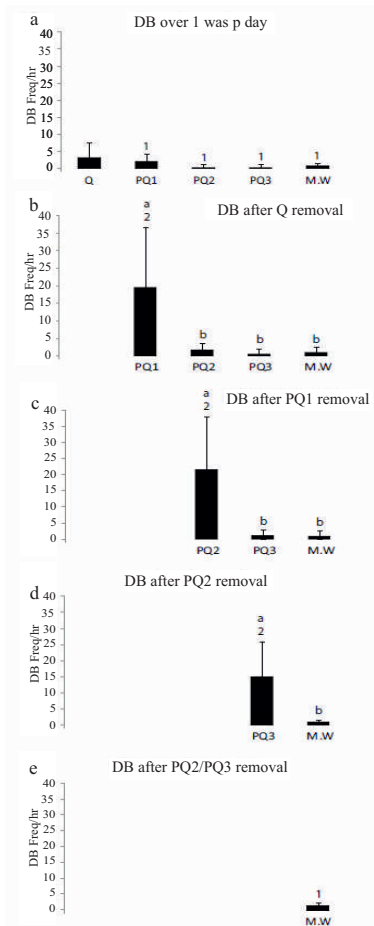
Fig. 7. Reproductive queue in *R. cyathiformis*. The mean and standard deviations of the frequencies per hour of dominance behaviour of the queen, 3 PQs and M.W (max worker, which is the individual who showed the highest frequency of dominance behaviour other than the Q and all three PQs), in normal queen-right colonies (a) and in the absence of the queen and the preceding PQs (b–d) (n = 12 for a–c and 10 for d and 8 for b). Bars carrying different alphabets are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test; to achieve P>0.05,   was set to 0.008, 0.01 and 0.05 on account of Bonferroni correction for b, c and d, respectively). Bars carrying different numbers show the significant difference in the frequency per hour of dominance behaviour shown by the focal PQ in the queen-right session with itself after the removal of the queen or previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve P>0.05,   was set to 0.01 on account of Bonferroni correction). All the PQs showed significantly higher dominance behaviour after the removal of the queen and the previous PQs compared to itself in the presence of the queen. Also, after the removal of PQ3 (and in two nests after PQ2) there was no identifiable PQ4. The dominance shown by the M.W was not significantly different from the M.W in the queen-right session. Also there was no significant difference in aggression shown between the M.W and the next most dominant individual in the colony (data not shown, Wilcoxon matched-pairs, signed-ranks test; P*0.05). DB dominance behaviour, Q queen, PQ1–PQ3 potential queens 1–3, M.W max worker (Teproduced with permission from Unnikrishnan and Gadagkar 2017)
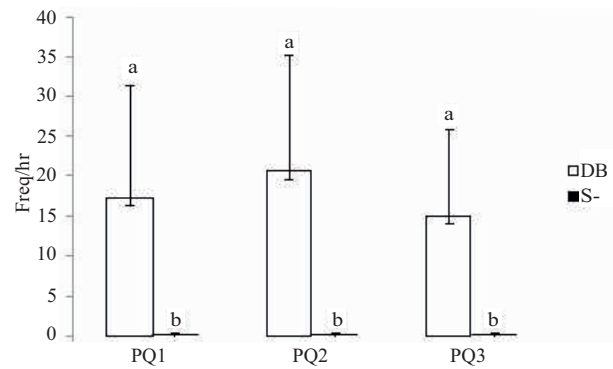

Fig. 8. Mean and standard deviations of dominance behaviour shown and received by PQs, after the removal of queen/previous PQs. Bars carrying different alphabets are significantly different from each other for each PQ (Wilcoxon matched-pairs, signed-rank test; P\0.05; n = 19, 15 and 10 colonies each for PQ1, PQ2 and PQ3). DB dominance behaviour, S- dominance received, PQ1–PQ3 potentials queens 1–3. [(to be reproduced with permission from Unnikrishnan and Gadagkar 2017)

as in the case of *Polistes dominulus* (Hymenoptera: Vespidae) (Pardi, 1948; Taylor et al., 2020), *P. jadwigae* (Hymenoptera: Vespidae) (Yoshikawa, 1963), *P. fuscatus* (Hymenoptera: Vespidae) (West-Eberhard, 1969), *Mischocyttarus. flavitarsis* (Hymenoptera: Vespidae) (Litte, 1979), *P. metricus* (Hymenoptera: Vespidae) (Dew and Michener, 1981), *P. exclamans* (Hymenoptera: Vespidae) (Strassmann and Meyer, 1983), *P. chinensis antennalis* (Hymenoptera: Vespidae) (Miyano, 1986), *P. annularis* (Hymenoptera: Vespidae) (Hughes et al., 1987), *P. instabilis* (Hymenoptera: Vespidae) (Hughes and Strassmann, 1988), *P. lanio* (Hymenoptera: Vespidae) (Giannotti and Machado, 1997), *Belonogaster petiolata* (Hymenoptera: Vespidae) (Keeping, 1997), *Liostenogaster flavolineata* (Hymenoptera: Vespidae) (Bridge and Field, 2007) and *Mischocyttarus cassununga* (Hymenoptera: Vespidae) (Murakami and Shima, 2009). In the so-called 'tropical pattern' of queen succession, relatively younger wasps supersede the queen, as in the case of *Polistes canadensis* (Hymenoptera: Vespidae) (West-Eberhard, 1969), *Mischocyttarus drewseni* (Hymenoptera: Vespidae) (Jeanne, 1972), *Metapolybia aztecoides* (Hymenoptera: Vespidae) (West-Eberhard, 1978) and *Parapolybia indica* (Hymenoptera: Vespidae) (Suzuki, 2003).

3.2. *R. marginata*

In thousands of hours of observation by dozens of observers, workers in *R. marginata* have not been seen to lay eggs in the presence of a healthy queen. Hence, to the best of our knowledge, the queen is always the sole egg layer of the colony (Chandrashekara and Gadagkar,

1991b; Gadagkar, 2001; Gadagkar et al., 1982). This is consistent with all available data on the ovarian development of queens and workers. Only queens have fully developed ovaries by any measure of ovarian development, while workers have significantly under-developed ovaries. Even when queens and workers from different colonies are considered simultaneously, the ovarian development of queens is significantly better than that of workers (Figs. 9, 10) (Chandrashekara and Gadagkar, 1991b; Gadagkar, 2001).

As in *R. cyathiformis*, upon experimental removal of the queen, one of the workers develops her ovaries and takes over the role of the queen as the sole egg-layer of the colony, in *R. marginata* also (Chandrashekara and Gadagkar, 1992; Kardile and Gadagkar, 2002; Premnath et al., 1996; Sumana and Gadagkar, 2003). The process of such queen succession has been studied in more detail in *R. marginata*. There are several remarkable features of queen succession in this species. Immediately upon removal of the queen, one and only worker becomes hyper-aggressive, displaying high levels of dominance behaviour towards almost all the remaining workers, but she herself is almost never the recipient of any dominance behaviour from her nestmates (Chandrashekara and Gadagkar, 1992; Sumana and Gadagkar, 2003; Bang and Gadagkar, 2012). This hyper-aggressive 'potential queen' develops her ovaries and begins to lay eggs within 3-11 (5.62± 0.29) days (Lamba et al., 2007). The potential queen loses almost all her hyper-aggression and behaves like a docile sitter queen, characteristic of the species, at about the time she lays her first egg (Fig. 11) (Premnath et al., 1996; Sumana and Gadagkar, 2003). Although the identity of the potential queen becomes evident within minutes of removing the queen (sooner than in *R. cyathiformis,* where it takes several hours to identify the PQ), all attempts to predict her identity before removing the queen have so far failed. And yet, there is clear evidence that the potential queen is a 'cryptic heir designate' known to all the workers (Bhadra and Gadagkar, 2008). As in *R. cyathiformis*, there is a reproductive queue of potential queens who replace their predecessor queens in a remarkably conflict-free process of queen succession; indeed, the reproductive queue in *R. marginata* is even longer, extending at least up to 5 PQs, perhaps it is even longer (Figs. 12, 13) (Bang and Gadagkar, 2012). Potential queen/s cannot be identified in the presence of the queen because they are not unique by any criterion, not in their dominance rank in the colony, not in their behaviour, nor their residual levels of ovarian development (Chandrashekara
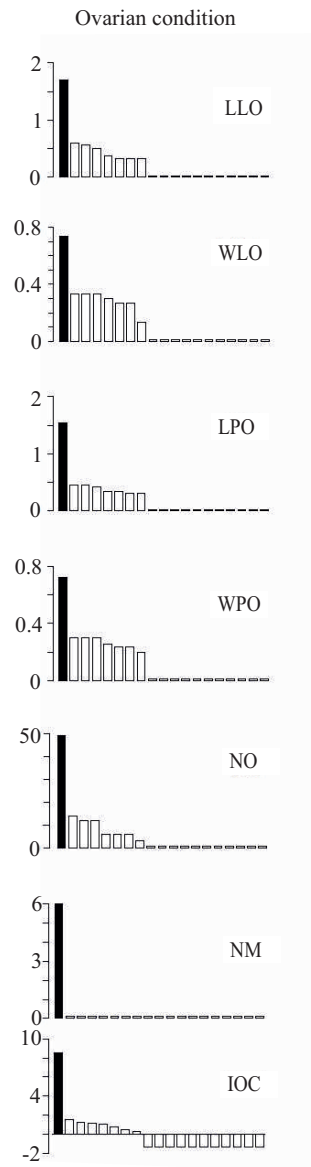

Fig. 9. Ovarian condition in *R. marginata*. The individual values used to assess ovarian condition are shown for all the workers (open bars) and the queen (black bars) in a typical colony. LLO, length of the largest oocytes; WLO, width of the largest oocyte; LPO, mean length of the proximal oocytes; WPO, mean width of the proximal oocytes; NO, total number of oocytes; NM, number of mature eggs (proximal oocytes that, on microscopic examination, appeared to have a fully formed chorion, which gave them a characteristic pearly white appearance); and IOC, index of ovarian condition. For each panel, the values for different individuals are arranged in descending order. Each bar represents one individual. Note that the queen always has the highest value for all measures of ovarian condition; several individuals have intermediate values; and a large number of individuals (all those other than the queen for the number of mature eggs) have a value of zero. All measurements except total number of oocytes and number of mature eggs are in mm (Data from Chandrashekara and Gadagkar, 1991a.) (To be reproduced with permission from Gadagkar 2001)
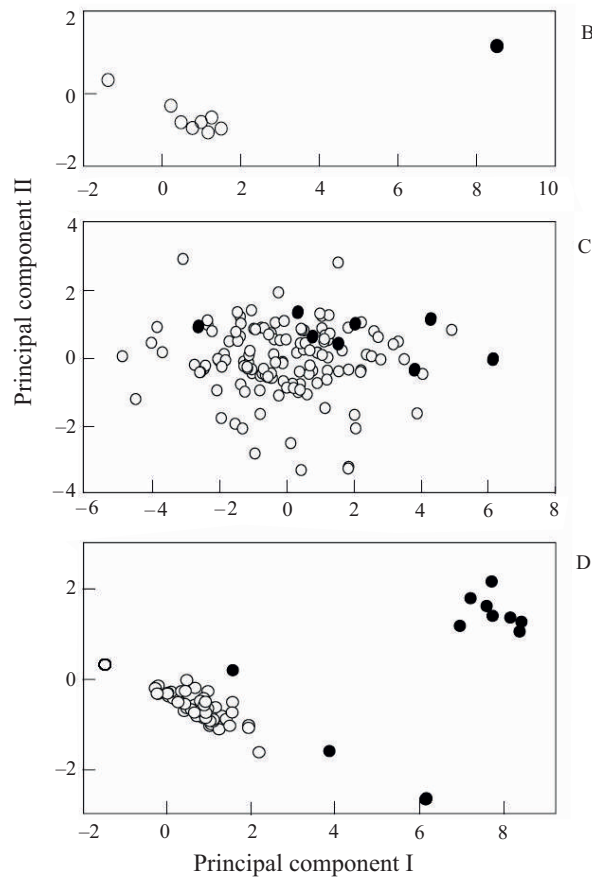
Fig. 10. B: Relative positions of queen and workers, with reference to their ovarian condition in a typical colony. Data from 5 are subjected to principal components analysis and the relative positions of the queens and workers are plotted in the space of the first two principal components. C and D: The relative positions of queens and workers from all the 11 colonies derived from a similar analysis. Note that queens and workers are well separated from each other. (Data from Chandrashekara and Gadagkar, 1991a)
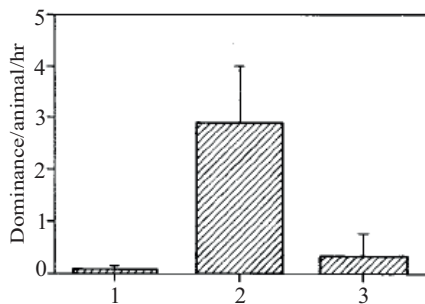

Fig. 11. Frequency of dominance behaviour of the founding queen, the individual next in the hierarchy on the day groups were formed and of the founding queen on the natal nest. Dominance acts per animal per h of a founding queen on the day groups were formed (2) is significantly greater than that of the wasp next in the hierarchy on this day (3) and than that exhibited by the founding queen earlier on the natal nest prior to nest-removal (1) (Wilcoxon matched-pairs signed-ranks test, P < 0.001 for 1 and 2; 2 and 3) (Reproduced with permission from Premnath et al. 1996)
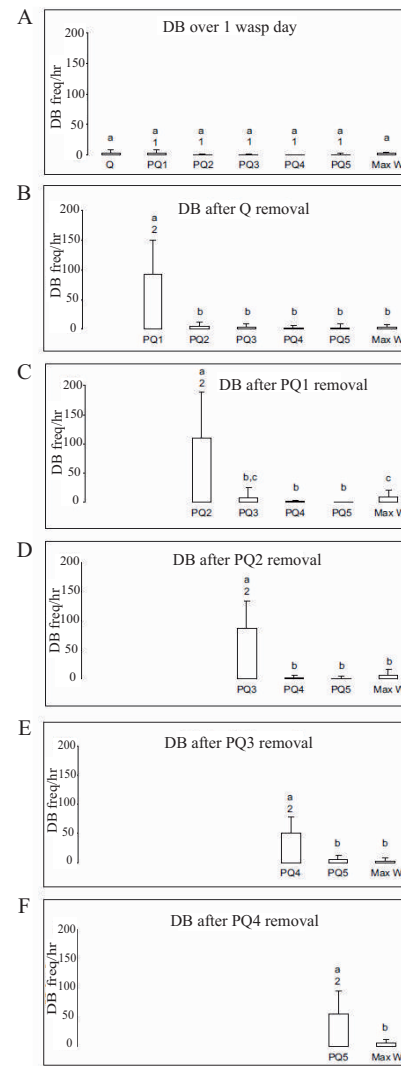

Fig. 12. Evidence for a reproductive queue in *R. marginata*. Means and SDs of frequencies per hour of behavioural dominance of the queen, five PQs, and the max worker (the individual that showed maximum behavioural dominance in the colony apart from the queen and the five PQs) in normal queenright colonies (A) and the PQs and the max workers in the absence of the queen and the preceding PQs (B–F) (n = 19 colonies). Bars that carry different letters are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test; to achieve P < 0.05, α was set to 0.002, 0.003, 0.005, 0.008, 0.017, and .05 on account of Bonferroni correction, for A, B, C, D, E, and F, respectively). Bars that carry different numbers represent significant differences in freq/h of behavioural dominance of the focal PQ in normal queenright colony with her own value after removal of the queen or previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve P < 0.05, α was set to 0.01 on account of Bonferroni correction). Note that each PQ showed significantly higher aggression after the queen and the previous PQs were removed than what she showed in the queenright colony and also compared with any other individual in the queenless colony. DB, behavioural dominance; Q, queen; PQ1 to PQ5, PQs 1–5; Max W, max worker (Reproduced with permission from Bang and Gadagkar 2012).
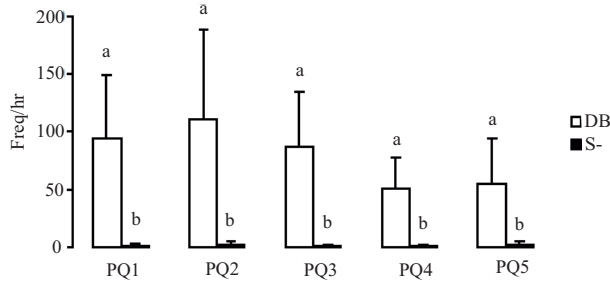
Fig. 13. Behavioural dominance shown and received (mean± SD) by different PQs in the absence of the queen and previous PQs (Wilcoxon matched-pairs, signed-ranks test; to achieve P < 0.05, α was set to 0.01 on account of Bonferroni correction; n = 19 colonies). Note that each PQ showed significantly higher behavioural dominance than what she received. On an average, dominance shown by each PQ was ~40–180-fold higher than the dominance she received. DB, behavioural dominance shown; S-, behavioural dominance received; PQ1 to PQ5, PQs 1–5 (Reproduced with permission from Bang and Gadagkar 2012)

and Gadagkar, 1992; Deshpande et al., 2006). The lack of a role for dominance rank in determining the next queen makes *R. marginata* quite different from other primitively eusocial wasps, including *R. cyathiformis*. Here the age of workers is a statistically significant predictor of their position in the reproductive queue. But age is not a perfect predictor because it is not as if the next oldest individual always becomes the next queen; potential queens often jump the age-based queue (Bang and Gadagkar, 2012). Instead, potential queens are drawn from among the oldest workers in the colony and the order in which they succeed the dying or removed queens of the colony cannot be predicted. Hence, it appears that queen succession in *R. marginata* follows a temperate pattern despite being a tropical species. A reversal of the pattern of queen succession, though in the opposite direction, has also been observed in the temperate species, *Parapolybia indica*, where queen succession followed a tropical pattern despite being a temperate species (Suzuki, 2003).

4. Non-reproductive division of labour - age polyethism

4.1. *R. cyathiformis*

In highly eusocial insects such as honey bees, workers perform various non-reproductive tasks in an orderly manner, shifting from one task to another in a predictable sequence as they grow older. This is referred to as age polyethism (Lindauer and Watkin, 1953; Seeley, 1982; Winston, 1987). *R. cyathiformis* displays a rudimentary age polyethism compared to honey bees (Unnikrishnan and Gadagkar, 2020). Changes in the performance of the two important intranidal tasks,
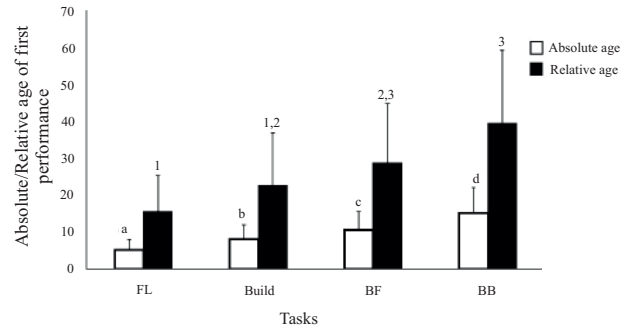

Fig. 14. Kruskal Wallis for both Absolute and Relative ages, absolute age – χ2 = 35.4, df = 3 P < 0.001; relative age – χ2 = 15.5, df = 3, P = 0.001. Mann Whitney U test showed significant difference between mean ages for successive tasks in absolute age (FL vs BU - U = 576, P = 0.001; BU vs BF – U = 332, P < 0.03; BF vs BB – U = 219, P = 0.03). Relative age showed no difference between mean ages for successive tasks (Mann Whitney U test, FL vs BU – U = 435, P = 0.14; BU vs BF – U = 298, P = 0.19; BF vs BB – U = 202, P = 0.12), but showed significant difference between, FL and BF and FL and BB and Build and BB (Mann Whitney U test, FL vs BF – U = 520, P = 0.003; BU vs BB – U = 228, P = 0.016). N = 100 (FL), 91 (Build), 44 (BF) and 26 (BB)

namely, building the nest and feeding the larvae, and two important extranidal tasks, namely, foraging for food and foraging for building materials, have been studied as a function of the age of the workers. These four tasks are performed by most individuals in the sequence - feed larva, build, bring food and finally bring building materials - as they age from about five days to about 60 days (Fig. 14). This trend of initiating intranidal tasks before extranidal tasks is common in other primitively eusocial wasps as well

(Yoshikawa, 1963; Dew and Michener, 1981; Jeanne, 1991; Tsuchida, 1991; Torres et al., 2012, 2013; Murakami et al., 2013; Cecílio et al., 2016). Task performance can be measured in two different ways. The frequency of task performance (FTP) is defined as the absolute frequency with which a task is performed. The probability of task performance (PTP) is defined as the probability of performing a task relative to other tasks. The age of an individual in the colony can be measured both as its absolute age in the number of days since eclosion and as its relative age with respect to the age distribution of the rest of the colony.

Both FTP as well as PTP vary with absolute and relative age, although not always statistically significantly. Age explains the changes in FTP better than it explains the changes in PTP. The sequence of the task performance and their dependence on age mentioned above are valid both for absolute and relative

age. However, absolute age is a better predictor of both FTP and PTP (Fig. 15). The best statistical fit is obtained when FTP is regressed against absolute age (Unnikrishnan and Gadagkar, 2020). In other words, the clearest evidence for age polyethism in *R. cyathiformis* comes from the observation that individuals modulate the absolute frequency of performing different tasks based on their absolute age. The frequency of performing intranidal tasks has a bell-shaped curve, with the maximum frequency obtained around middle age and then decreasing with age. The same pattern is observed in the case of extranidal tasks as well, indicating no clear-cut trade-off between performing intranidal tasks and extranidal tasks. Thus, individuals continue to perform both intranidal and extranidal tasks simultaneously (Unnikrishnan and Gadagkar, 2020). Taken together, we find that - (a) the division of labour is based on absolute age and not relative age, thereby reducing the flexibility of individuals to respond to changes in the colony demography and (b) the lack of trade-off between intra- and extranidal tasks suggests that individuals perform both simultaneously, indicating that the age polyethism in *R. cyathiformis* is not so well developed, as has also been observed in other primitively eusocial wasps (Yoshikawa, 1963; Dew and Michener, 1981; Jeanne, 1991; Tsuchida, 1991; Torres et al., 2009, 2012, 2013; Murakami et al., 2013).

In the case of *Belonogaster petiolata* (Keeping, 1992) and *Mischocyttarus latior* (Cecílio et al., 2016), age polyethism appears to be completely absent.

4.2. *R. marginata*

As in the case of *R. cyathiformis*, there is clear evidence of age polyethism in *R. marginata*; indeed, age polyethism appears to be much better developed in this species (Naug and Gadagkar, 1998). In a slight departure from *R. cyathiformis*, most *R. marginata* workers perform tasks in the sequence - feed larva, build, bring building materials and finally bring food - as they age from about five days to about 60 days or more (Fig. 16). As in the case of *R. cyathiformis*, both FTP and PTP vary significantly with both the absolute and relative ages of the individuals. However, in contrast to *R. cyathiformis*, age explains changes in PTP better than it explains changes in FTP, and relative age is a better predictor of both FTP and PTP in this species (Fig. 17). The best statistical fit in *R. marginata* is obtained when PTP is regressed against relative age (Naug and Gadagkar, 1998). In other words, the clearest evidence for age polyethism in *R marginata* comes from the observation that individuals modulate the probability of performing different tasks, relative to other tasks,
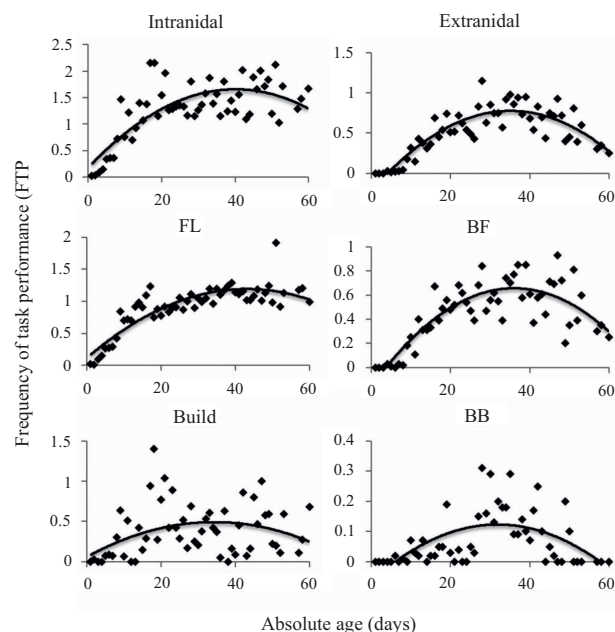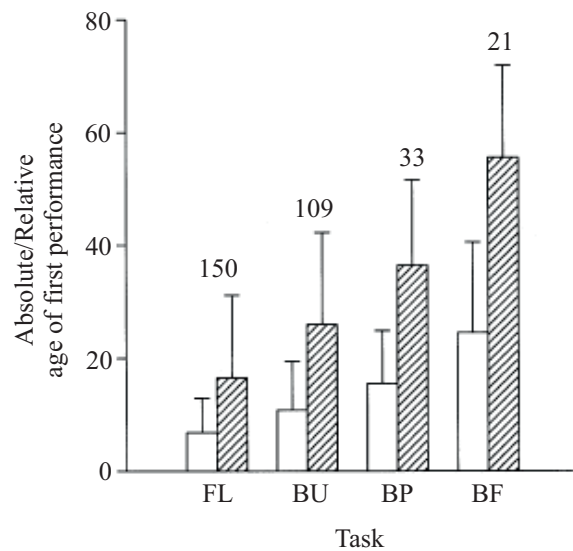

Fig. 15. Frequency per hour of task performance (FTP) as a function of absolute age fitted with second order polynomial regression lines. Data points represent the mean value (in frequency/ hr) for all individuals in that age class in 4 colonies

Fig. 16. Mean (+/ - SD) age of first performance for each task in terms of absolute age (open bars) and relative age (shaded bars). Sample size for each task is given above the respective bars. Multiple comparisons of mean ages of first performance using Tukey-Kramer method indicated significant differences across tasks (p< 0:05). The first performance of a task was significantly influenced by absolute age (one-way ANOVA, F 33:47, P < 0:0001) and relative age (F 49:12, P < 0:0001). Mean ages for successive tasks were also significantly different (p< 0:05) by a Mann-Whitney U-test (Reproduced with permission from Naug and Gadagkar 1998a)
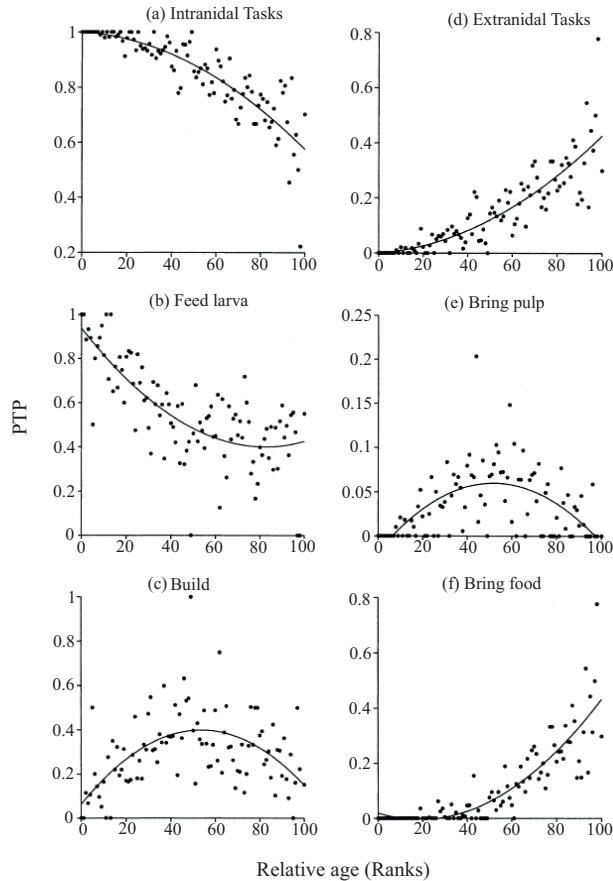
Fig. 17. Probability of task performance (PTP) as a function of absolute age (top) and relative age (bottom) fitted with second-order polynomial regression lines. Data points represent the mean value for all individuals in that age class in five colonies (Reproduced with permission from Naug and Gadagkar, 1998a)

based on their age relative to the age of other members of the colony, thereby providing flexibility to respond to changes in the colony demography. In *R. marginata,* the probability of performing intranidal tasks decreases with age.

In contrast, the probability of performing extranidal tasks increases with age such that there is a clear trade-off between performing intranidal tasks versus extranidal tasks. In other words, individuals begin to perform extranidal tasks by gradually giving up intranidal tasks as they grow older (Naug and Gadagkar, 1998a). The well-developed age polyethism in *R. marginata* is in contrast to other primitively eusocial wasps where age polyethism, if it exists, is poorly developed.

5. Non-reproductive division of labour - foraging and feeding larva

5.1. *R. cyathiformis*

Queens and workers show a clear-cut division of labour in foraging; queens perform no foraging, and workers are entirely responsible for this task. There is, however, no such clear-cut division of labour in the case of feeding the larvae; this task is shared between the queens and workers, such that queens are similar to an average worker in the rates at which she feeds larvae (Kardile and Gadagkar, 2003). Since there is no clear-cut partitioning of intranidal and extranidal tasks between different workers, as seen above, both foraging and feeding larvae are performed by most of the workers. This is in contrast to the possibility that some workers forage and bring food, hand it over to intranidal workers who then feed the larvae, a situation seen in the congeneric *R. marginata* (see below) much more strikingly than in *R. cyathiformis*. In the case of the queen, however, she unloads food from incoming foragers, some of which she feeds to the larvae. It is interesting to note that the removal of the queen does not affect the rates at which workers bring food and feed the larvae (Kardile and Gadagkar, 2003; Unnikrishnan and Gadagkar, 2021) (Fig. 18).

5.2. *R. marginata*

As in *R. cyathiformis*, there is a clear-cut division of labour between queens and workers such that queens never forage and workers perform all the foraging tasks. Unlike in *R. cyathiformis,* however, there is also a division of labour in the task of feeding the larvae such that queens rarely feed larvae, and workers are
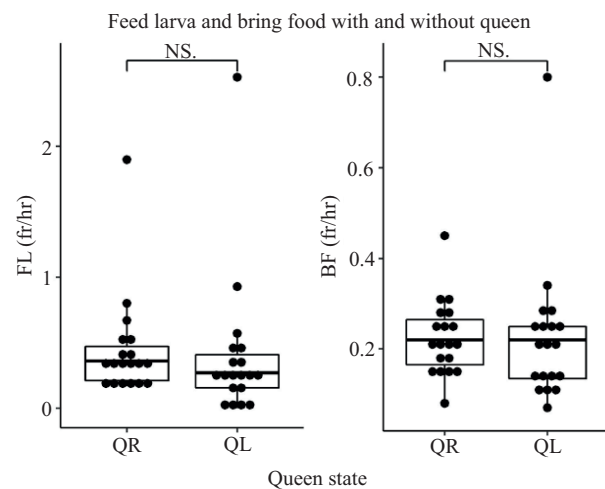

Fig. 18. Frequency per hour of feed larva (FL) (left panel) and bring food (BF) (right panel) behaviours in queen-right (QR) and queen-less (QL) sessions. There was no difference in the frequency of FL between QR and QL sessions (LMM − Estimate = 0.05, CI − 0.07 to 0.17, P value = 0.39) and also in frequency of BF between QR and QL sessions (LMM − Estimate = − 0.002, CI − 0.06 to 0.05, P value = 0.94). N = 19 colonies (Reproduced with permission from Unnikrishnan and Gadagkar, 2021)
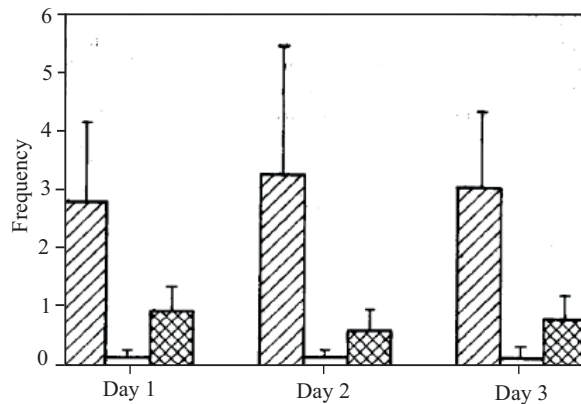
Fig. 19. Frequencies per hour of total food brought (hatched bars), food brought per animal per hour (open bars) and feed larvae per animal per hour (cross-hatched bars) are all not significantly different among days 1 (with queen), 2 (without queen), and 3 (after re-introduction of queen). Comparisons are by two tailed Wilcoxon matched-pairs signed-rank test (Reproduced with permissionfrom Premnath et al. 1995)

responsible for performing most of this task. Among the workers, there is again a more clear-cut partitioning of intranidal and extranidal workers. Foragers or extranidal workers bring food to the colony, which is then unloaded by the intranidal workers (Premnath et al., 1995; Lamba et al., 2008). This food is then distributed amongst the other intranidal workers, who then feed the larvae. Similar to *R. cyathiformis*, removal of the queens results in no difference in the rate of workers foraging and feeding the larvae in *R. marginata* also (Premnath et al., 1995) (Fig. 19).

6. Regulation of reproductive division of labour

6.1. *R. cyathiformis*

Since the queen in *R. cyathiformis* is a behaviourally dominant individual, she might be expected to suppress reproduction by the workers by means of her dominance behaviour. A detailed examination of the colony's initiators and recipients of dominance behaviour should help verify this conjecture. It turns out that the queen preferentially directs her dominance behaviour to the potential queen, and the potential queen preferentially directs her dominance behaviour to the workers; neither the potential queen nor the workers show any dominance behaviour to the queen, nor do the workers show any dominance behaviour towards the PQ. It, therefore, appears that the queen may be regulating worker reproduction using her dominance behaviour, but she seems to do so via the PQ. Such a strategy of using the PQ to help maintain her reproductive monopoly in the colony may save her much time and energy, which can be channelled into her own reproduction (Unnikrishnan

and Gadagkar, 2021). Although the use of dominance behaviour to regulate reproductive activities of other workers in the colony by the queen is common in other primitively eusocial wasps (Pardi, 1948; West-Eberhard, 1969; Jeanne, 1972; Litte, 1979; Strassmann and Meyer, 1983; Hughes et al., 1987; Hughes and Strassmann, 1988; Keeping, 1992, 1997; Giannotti and Machado, 1997), the strategy of utilizing the PQ for this task is something that has not been reported before. Whether this is because it is not present or because it has not been studied yet is unclear. In addition, recent studies have shown some evidence for non-physical modes of reproductive regulation such as a possible connection between juvenile hormone and CHCs, alpha females having certain distinguishing features in their CHC profiles etc. This has led to the belief that perhaps queens do use cuticular hydrocarbons as a means to regulate reproduction in workers in some species of *Polistine* wasps (Kelstrup et al., 2015; Smith and Liebig, 2017; Dani and Turillazzi, 2018; da Silva et al., 2020; Tsuchida et al., 2020; Oi et al., 2021). Whether this is true also in the case of *R. cyathiformis* is not known and would have to be studied.

Linear hierarchy formation requires repeated interaction by aggressive individuals, and this was prevented on a nest with a despotic queen that suppressed the aggressive behavior of nestmates. The existence of pheromonally maintained hierarchies, perceived by wasps, but invisible to the human observer, remains an intriguing possibility (West-Eberhard 1986).

6.2. *R. marginata*

Unlike *R. cyathiformis*, the queens of *R. marginata* are meek and docile individuals who rarely participate in the dominance-subordinate interactions of the colony. Hence, she cannot possibly be regulating the reproduction of her workers by means of dominance behaviour. Nevertheless, since *R. marginata* queens maintain a complete reproductive monopoly in their colonies, worker reproduction is somehow suppressed. One hypothesis is that, as in the case of highly eusocial species, the queen may suppress worker reproduction or at least signal her presence and fertility using pheromones.

Experiments on *R. marginata* show that the queen may be using a non-volatile pheromone to suppress worker reproduction or at least to signal her presence. Workers do not perceive the presence of the queen when separated from her by a wire mesh screen (Sumana et al., 2008). To address the question of how the workers

might perceive the non-volatile pheromone of the queen, Bhadra et al. (2007) examined the possibilities of direct or indirect physical interactions between queens and workers. They found that the time required for the workers to perceive the absence of the queen if she used direct or indirect interaction would be much more than the observed 30 minutes that the workers take to detect the queen's absence. This led them to conclude that the queen might be depositing her pheromone by rubbing her abdomen on the nest. Additional experiments showed that the Dufour's gland in the queen contains the pheromone that the queen uses to communicate to the workers in the colony. The Dufour's glands of the queens and workers contain at least 30 detectable hydrocarbons. Although no compound seems to be unique to the queens, the relative proportions of the different compounds vary significantly between queens and workers. Discriminant function analysis separates the queen and workers into two distinct groups based on the composition of their Dufour's gland (Bhadra et al., 2010; Mitra et al., 2011) (Fig. 20), although there is no difference in the morphology of the gland (Mitra and Gadagkar, 2012). A bioassay showed that the PQ reduced her aggression when a macerate of the queen's Dufour's gland was applied to the nest surface in the absence of the queen. The idea that workers perceive the queen pheromone that she applies to the nest surface is reinforced by experiments in which queens and workers are observed in the absence of a nest. In such a situation, the queen rubs her abdomen on the walls of the cage. But the workers nevertheless attack the queen presumably because the pheromone concentration is now reduced (Saha et al., 2012). The use of pheromones
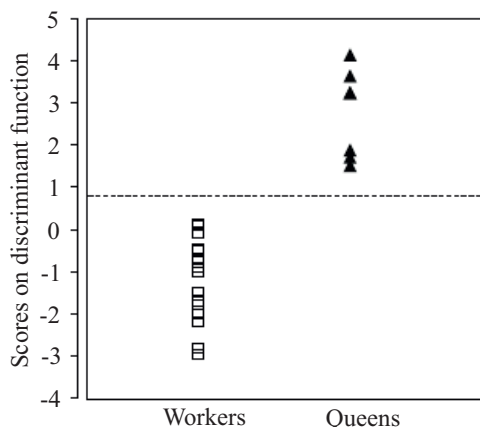

Fig. 20. Scores on discriminant function analysis for gas chromatographic analyses of Dufour's glands of *Ropalidia marginata* queens and workers (n. queens=7, n. workers=16, Wilk's λ=0.214, P<0.001, classification analysis: 100% correct classification) (Reproduced with permission from Bhadra et al., 2010)

by *R. marginata* queens is reminiscent of highly eusocial species where the queens use chemical means to regulate worker reproduction (Winston and Slessor, 1992; Keller and Nonacs, 1993; Katzav-Gozansky et al., 2002; D'Ettorre et al., 2004; Monnin, 2006; Chapuisat, 2014; Van Oystaeyen et al., 2014) and unlike primitively eusocial wasps who predominantly use dominance behaviour. However, recent studies in primitively eusocial wasps indicate that perhaps they also use chemical means to regulate reproduction, suggesting that non-physical means of reproductive regulation are not limited to highly eusocial species.

7. Regulation of non-reproductive division of labour - age polyethism

7.1. *R. cyathiformis*

As we have seen above, there is a rudimentary age polyethism in *R. cyathiformis,* but the tasks that individuals perform are a function of their absolute age and not relative to the ages of the other members of the colony (Unnikrishnan and Gadagkar, 2020). Such dependence on absolute age is bound to make the system much less flexible than a relative age-based division of labour. Age polyethism based on relative age allows the colony to respond to any changes in its age demography. In the event of the loss of workers of a particular age group due to predation or harsh environmental conditions, the colony can compensate for this by employing the remaining individuals based on their relative age. Such flexibility is not possible when an individual performs the task depending on its absolute age and not relative age in relation to the other individuals present in the colony. However, since there is no clear-cut partitioning of intranidal and extranidal tasks, indicating that individuals perform both simultaneously (Unnikrishnan and Gadagkar, 2020). Therefore, in the case of *R. cyathiformis*, although age polyethism is much less flexible, the colony is resistant to any demographic changes because individuals simultaneously perform all tasks.

7.2. *R. marginata*

Unlike in *R. cyathiformis*, individuals of *R. marginata* modulate the relative frequency of task performance with respect to their relative age instead of their absolute age (section 4.2). A relative age-based polyethism, unlike an absolute age-based polyethism, allows individuals to compensate for any changes in the colony age demography making the age polyethism a highly flexible one. This flexibility in age polyethism was explicitly shown in experiments that changed

the colony demography and observed changes in age polyethism. When colonies consisting of only very young individuals were studied, precocious foragers were observed as individuals started foraging at a much younger age than they would have in a colony with older individuals present (Naug and Gadagkar, 1998b). Interestingly, when this experiment's converse was performed, with colonies where young individuals were removed, it did not result in over-aged nurses nursing at very old ages; instead, it resulted in hard-worked nurses nursing at abnormally high rates (Agrahari and Gadagkar, 2004). To better explain the flexibility in age polyethism in *R. marginata,* an activator-inhibitor model was developed (Naug and Gadagkar, 1999). The model clearly shows how increasing task demands can be met by a decrease in the ages of first performance and an increase in the proportions of individuals engaged in various tasks. When the role of an important regulator of age polyethism from studies on honey bees, the juvenile hormone (JH), was studied, it revealed that JH increased ovarian development in *R. marginata* workers but did not play a role in regulating age polyethism (Agrahari and Gadagkar, 2003). In *R. marginata,* as there is a strict separation of intranidal and extranidal tasks, a flexible age polyethism will allow the colony to adjust and compensate for any changes in the colony demography.

8. Regulation of non-reproductive division of labour - foraging and feeding larva

8.1. *R. cyathiformis*

Since the queen in *R. cyathiformis* is a behaviourally dominant individual (see section 2.1), and she seems to suppress worker reproduction using dominance behaviour (see section 6.1), she might also be expected to regulate the non-reproductive activities of the workers, such as foraging and feeding of the larvae. However, we saw in section 5.1 that even when the queen was removed, foraging and feeding of the larvae continued unabated. In contrast, the absence of the queen results in lower activity levels of the workers in some other species such as *Polistes instabilis*, *P. dominulus* (Jha et al., 2006) and *P. versicolor* (Souza and Prezoto, 2012) but not in the case of *P. fuscatus* (Reeve and Gamboa, 1983, 1987). How are their activities regulated if the queen is unnecessary for workers to forage and feed the larvae in *R. cyathiformis*? In other words, how do the workers know how much to forage and feed the larvae? For the workers to regulate their foraging and feed the larvae, they need information about the colony's hunger level. How do they get this information? It is conceivable that information about

the colony's hunger level can be obtained while feeding the larvae. We find a significant positive correlation between the rates at which workers forage and feed larvae (Unnikrishnan and Gadagkar, 2021), implying that the same individuals who forage for food feed the larvae well (Fig. 21). Thus, foragers can directly obtain information about the colony's hunger levels while feeding larvae and regulating their own foraging rates. Based on the aggressive behaviour of the queen Kardile and Gadagkar (2003) had previously speculated that the queen may act as the central pacemaker in *R. cyathiformis*. And based on the finding that the PQ increased her levels of feeding unloading the foragers and feeding the larvae, they speculated that role of the pacemaker passes on from the queen to the PQ after queen removal. This interpretation would be reasonable if the queen was indeed a pacemaker in queen-right colonies. However, we now find that the levels of dominance received by the workers do not predict their foraging levels. On the other hand, we find a weak, but significant positive correlation between the rates at which individual wasps bring food and their own rates of feeding the larvae. We therefore speculate that foragers in this species may regulate their own foraging levels by obtaining direct information about larval hunger levels through their significant contribution to larval feeding.

8.2. *R. marginata*

Similar to *R. cyathiformis*, the absence of the queen results in no difference in the rates of foraging and feeding the larvae by the rest of the workers in *R. marginata*, indicating that the queen does not regulate the non-reproductive tasks of the colony. But in contrast to *R. cyathifrmis*, in *R. marginata*, there is a positive
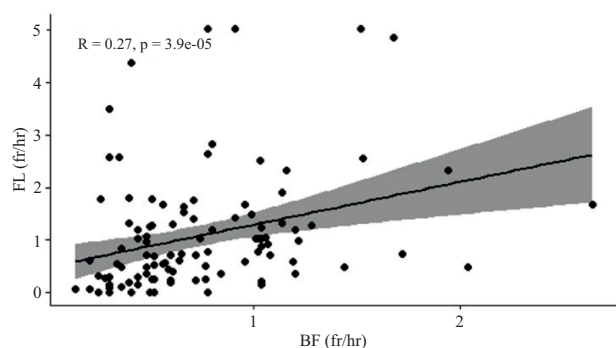

Fig. 21. Visualisation of correlation between Bring Food (BF) behaviour and Feed Larva (FL) behaviour. Kendall's correlation coefficient, τ = 0.27, P < 0.0001. Data points represent fr/ hr of FL for each forager for that particular freq/hr of BF for 23 colonies (Reproduced with permission from Unnikrishnan and Gadagkar, 2021)

correlation between dominance behaviour received and bring food behaviour with several acts of dominance behaviour directed at foragers (Premnath et al., 1995). To better understand how dominance behaviour is used to regulate foraging, colonies kept inside wooden boxes in the lab were observed after excess feeding them. On the first day, unmanipulated colonies were observed, and all behaviours, including all dominance behaviour shown and received, were recorded. On the second day, the colony was fed in excess, with individuals being hand-fed with food in addition to their normal foraging. Interestingly when the demand for food was less in the colony, there were fewer acts of dominance-subordinate behaviour (Bruyndonckx et al., 2006) (Fig. 22). Hence this experiment suggests that the dominant-subordinate interactions are used for the regulation of foraging. To further understand this, the converse of this experiment was performed, where instead of excess-feeding the

colony, the colony was starved on the second day. It was observed that when the colony was starved, the individuals that received the most aggression from other workers were the foragers (who were identified on day one as having brought food). And on the third day, once the colony was again fed, this aggression towards foragers came down (Lamba et al., 2008) queens are strikingly docile and non-aggressive individuals, never at the top of the behavioural dominance hierarchy of their colonies. Nevertheless, these queens are completely successful at suppressing worker reproduction, suggesting that they do not use aggression but employ some other mechanism (e.g. pheromones. This aggression was directed towards the foragers (Fig. 23). Hence this experiment further confirms the role of dominance behaviour in the regulation of foraging by indicating that intranidal workers use dominance behaviour to communicate the colony hunger status to the extranidal workers or foragers. In *R. marginata*, workers regulate foraging among themselves in a decentralized manner of work regulation with intranidal workers communicating colony hunger levels using dominance behaviour to the extranidal workers or foragers.

9. Insights into the evolution of eusociality

It is clear from the above comparisons that *R. cyathiformis* behaves as expected of a typical primitively
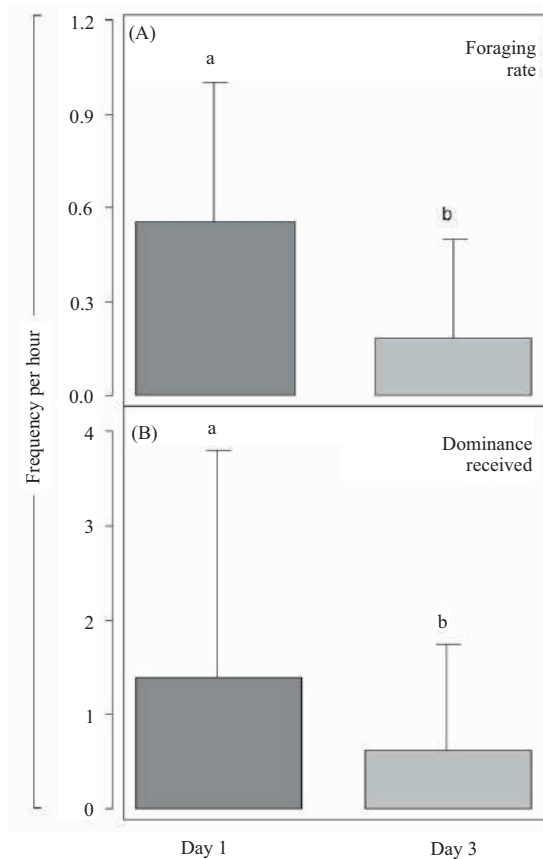

Fig. 22. Comparison of: (A) foraging rate; (B) dominance received by the 49 active foragers on day 1 (un-manipulated nests) and day 3 (one day after excess feeding). Bars shown are the means and S.D.'s for the 49 foragers. Both variables on day 1 are significantly greater than the corresponding variables on day 3 (two tailed Wilcoxon matched-pairs, signed-ranked tests; p < 0.05) (Reproduced with permission from Bruyndockk et al., 2006)
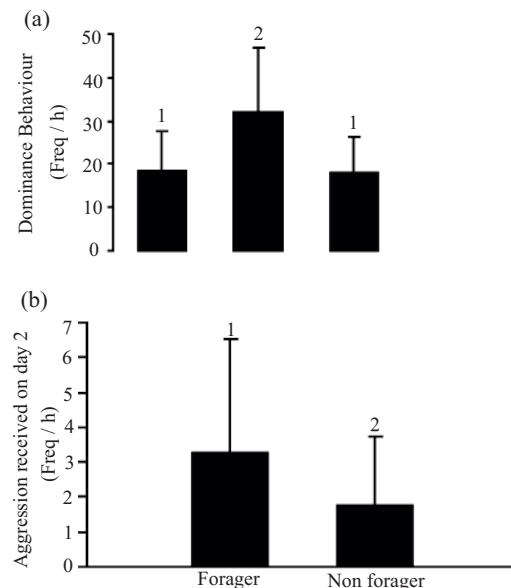

Fig. 23. (A) Mean and SD of a frequency per hour of dominance behaviour, on days 1, 2 and 3. (B) Mean and SD of frequencies per hour of aggression received on day 2 by foragers and non-foragers of day 1. Different numbers indicate a significant difference between the bars (Reproduced with permissionfrom Lamba et al., 2008)

eusocial wasp, with a behaviourally dominant queen, dominance-based queen succession, weak and rigid age polyethism, dominance-based reproductive regulation and lack of clear-cut separation of intranidal and extranidal task performance schedules. *R. marginata,* on the other hand, does not always behave as expected of a typical primitively eusocial wasp and displays some unusual traits. Queens are meek and docile, queen succession is not based on dominance behaviour, there is a well-developed and flexible age polyethism, clear-cut separation of intranidal and extranidal task performance schedules and the use of pheromones for reproductive regulation. Most of these traits displayed by *R. marginata* are reminiscent of highly eusocial species. However, *R. marginata* is also quite different from a typical highly eusocial species because it does not display queen-worker dimorphism, colony sizes are relatively small (rarely exceeding 100 individuals), and cast differentiation is largely post-imaginal (although there is some pre-imaginal caste bias (Gadagkar et al., 1988, 1990). *R. marginata*, being somewhat different both from primitively eusocial as well as highly eusocial species, may be thought of as being intermediate (Kardile and Gadagkar 2003; Gadagkar 2001; Deshpande et al. 2006; Saha et al. 2012; Chakraborty et al. 2018). We do not claim that eusociality necessarily involves a linear trajectory starting from a typical primitively eusocial species such as *R. cyathiformis,* passing through an intermediate stage such as *R. marginata* and finally evolving into a typical highly eusocial species such as the honey bees. That eusociality could have evolved by different trajectories and that we should not commit ourselves to a single, "ladder-like" trajectory has recently been emphasized by Linksvayer and Johnson (2019). Rather than suggest a trajectory going from *R. cyathiformis* through *R. marginata* to honey bees, we make the following very different point. Our comparison of *R. cyathiformis* and *R. marginata* concerning what is known about typical primitively and highly eusocial species suggests that traits such as pheromonal regulation of reproduction and age polyethism can evolve even without queen-worker dimorphism and increase in colony size. In other words, pheromonal regulation of reproduction and age polyethism could, at least sometimes, be the cause and queen-worker dimorphism and large colony size be the effect, rather than the other way around.

We are conscious that our conclusions are placed on comparing only two species, *R. cyathiformis and R. marginata,* and that one may arrive at different conclusions by studying other primitively eusocial species. We believe that detailed studies of even a small number of species could be very valuable (Goymann and Schwabl, 2021) and we, therefore, call for more detailed studies of many different primitively eusocial species, both in the tropics and in the temperate regions. We emphasize that we need careful documentation of phenomena such as queen-worker dimorphism, queen succession, the role of dominance behaviour, regulation of reproduction, age polyethism and task partitioning in a large number of species. Comparative analyses of the kind we are advocating are served better by behavioural data on many species rather than uncovering detailed physiological and molecular mechanisms of behaviour. In any case, the latter can only be attempted in a small number of species by relatively well-endowed research groups. Behavioural data of the kind we are seeking can be relatively easily obtained by large numbers of researchers, in a large number of habitats, without the need for expensive laboratory and technology. In addition to calling for such behavioural studies, preferably by researchers sympatric with their species of study, we also plead that such behavioural studies should not be considered less fashionable and 'sexy' than studies that attempt to understand the molecular basis of behaviour (Gadagkar, 2019, 2021).

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