OBSERVATIONS ON THE NATURAL HISTORY AND BEHAVIOUR OF THE PRIMITIVELY EUSOCIAL WASP *ROPALIDIA CYATHIFORMIS* (FAB.) (HYMENOPTERA: VESPIDAE)[1]

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This paper reports on natural history and behaviour of the primitively eusocial wasp *Ropalidia cyathiformis*, which builds small, open, paper carton nests and exhibits an aseasonal nesting cycle. The number of adult wasps on a nest ranges from one to about a hundred, and nests last from a few days to sixteen months or more. Most colonies have a single queen, who is morphologically similar to her workers but has better developed ovaries. Female wasps exhibit several dominance behaviours which are positively correlated with rates of snatching food and building material from incoming foragers, and also of feeding larvae and building the nest. This suggests that behaviourally dominant individuals specialize in performing intra-nidal tasks including brood care. Queens are the most behaviourally dominant individuals of their colonies and appear to inhibit worker reproduction and regulate non-reproductive activities of workers using dominance behaviours. Our observations suggest that *R. cyathiformis* is a typical example of a primitively eusocial species, in striking contrast to the congeneric *R. marginata* which exhibits some features reminiscent of more advanced eusociality. Queens of *R. marginata* are behaviourally docile and appear to use a non-behavioural (probably pheromonal) method of regulating worker reproduction. Non-reproductive activities of workers in *R. marginata* are regulated in a decentralised, self-organised manner without the involvement of the queen. A comparative study of *R. cyathiformis* and *R. marginata* will be valuable in understanding the evolutionary transition from primitive to advanced eusociality in general.

Key words: primitively eusocial wasps, social evolution, *Ropalidia cyathiformis*, *Ropalidia marginata*, nesting cycle, dominance behaviour

INTRODUCTION

Eusocial insects are characterised by overlap of generations, co-operative brood care and reproductive differentiation into fertile reproductive and sterile worker castes. Eusociality is seen in ants, bees, wasps, termites, aphids, thrips, ambrosia beetles, marine shrimps, a possible example among spiders and a lone vertebrate example – the Naked Mole Rat. Eusocial species may be classified as primitively or highly eusocial. Primitively eusocial species lack morphological caste differentiation and retain many behavioural features of their solitary ancestors. Highly eusocial species exhibit morphological differentiation between workers and reproductives, and have acquired many behavioural features not present in their solitary ancestors and inconsistent with solitary life (Michener 1969; Wilson 1971; Hölldobler and Wilson 1990; Bourke and Franks 1995; Crozier and Pamilo 1996; Gadagkar 2001). The emergence of a sterile, altruistic worker caste is one of the most challenging problems of the evolution of eusociality. Primitively eusocial species are appropriate model systems to investigate the early stages of evolution because reproductives and workers are usually not irreversibly committed to their respective roles and because individuals in many species have retained the ability to found nests and rear brood in the solitary mode.

Among social Hymenoptera, primitively eusocial species are found among bees and wasps. The old world tropical genus *Ropalidia* is thought to be of particular interest in understanding the evolution of eusociality because it includes both primitively as well as highly eusocial species. *Ropalidia marginata* and *Ropalidia cyathiformis* are the two most abundant primitively eusocial wasps in peninsular India. Of these, *R. marginata* has been studied extensively to yield a number of interesting insights into the evolution of eusociality (Gadagkar 2001). By comparison, *R. cyathiformis* remains poorly studied but promises to contribute to our understanding of the evolution of eusociality in new and interesting ways (Gadagkar 2001).

R. cyathiformis was first described by Fabricius (1804) as *Eumenes cyathiformis*. It was also described as *Icaria ceylonica* by Cameron (1898), as *Icaria cayaynensis* by Ashmead (1905a,b), as *Icaria bilineata* by Cameron (1905) and as *Icaria cyathiformis* by Schulz (1912). Vecht (1941, 1962) first used the combination *Ropalidia cyathiformis*. *R. cyathiformis* wasps are small in size, the females are 6.5 - 7.0 mm long and the males about 5.5 mm. Sexes are easily distinguishable. *R. cyathiformis* has been reported from Uttar Pradesh, Arunachal Pradesh, Bihar, Assam, Madhya Pradesh, Maharashtra and Karnataka in India and also from Nepal, Sri Lanka, Malaysia and Sulawesi and Sumba in Indonesia (Das and Gupta 1989).

We have initiated a long-term study of this species to develop an additional model system for investigating the evolution of altruism and eusociality. Here we describe some aspects of the natural history and behaviour of *R. cyathiformis* in Bangalore (13° 00' N and 77° 32' E), India.

METHODS

Nesting cycle

Selected buildings and other favourite nesting sites on the campus of Indian Institute of Science (IISc), Bangalore were surveyed once in about two weeks for the presence of nests of *R. cyathiformis*. When a nest was first encountered, the number of eggs, larvae, pupae, parasitized cells, empty cells, combs, pedicels, adult females and adult males present were recorded. This was done before 0700hrs or after 1900hrs when adult wasps are expected to be in the nest. On subsequent visits we noted only whether it was active or abandoned. A nest with brood and adults was considered active and one devoid of both was considered to have been abandoned.

Adults of old world primitively eusocial wasps remove the larval meconium (faecal matter) by chewing a small hole at the bottom of the cells. This is done immediately after the larva spins a silk cap on its cell, in preparation for pupation. After removing the meconium, the adult wasps seal the hole with salivary secretion. Thus, transparent windows can be seen at the bottom of those cells in which larvae have pupated at least once. Since the cells are reused, empty cells as well as egg and larva bearing cells may have transparent windows. Hence the presence of any one or more transparent windows at the bottom of empty cells, egg cells or larval cells indicates a post-emergence nest. Based on this criterion, every nest was classified either as a pre-emergence or post-emergence. When a pre-emergence nest had only eggs and young larvae, it was designated as having been initiated in that month. The numbers of nests seen to have been initiated or abandoned in different months of the year were compiled from such data. The survey was done for most months during four consecutive years (Table 1). For those months in which survey was done in more than one year, the numbers of nests initiated and abandoned in that month was averaged over all the years during which survey was done. During this study we located and recorded data on 33 pre-emergence nests and 53 post-emergence nests.

Behaviour

We observed 10 post-emergence nests (Table 2) from April 2002 to January 2004. All adults on each nest were uniquely marked with spots of quick drying, non-toxic enamel paints. Five minute observation sessions were made, during which every performance by each individual was recorded for the following behaviours: dominance behaviour, bring food, snatch food, lose food, feed larva, bring building material, snatch building material, lose building material and build. Observation sessions were evenly distributed from 0630 hrs to 1830 hrs. Five sessions of 5 minute observations were randomly performed each hour during four to six hours per day. Each nest was thus studied for 16-20 hours over a period of four to six days.

Table 1: Monthly census records. √ denotes nest census was taken in that month for that year while — denotes no census was taken in that month for that year

Month	1999	2000	2001	2002	No. of events of census
January	√	√	√	√	4
February	√	√	√	—	3
March	√	√	√	—	3
April	√	√	√	√	4
May	√	√	—	√	3
June	√	√	√	√	4
July	√	—	√	—	3
August	—	—	√	√	2
September	—	√	—	√	2
October	—	√	√	√	3
November	√	√	√	√	4
December	√	—	√	√	3

Table 2: Number of females, males, eggs, larvae, pupae, empty cells and parasitized cells present in the 10 nests used for the behavioural observations

Nest	Females	Males	Eggs	Larvae	Pupae	Empty cells	Parasitized Cells
C76	14	0	17	20	5	0	0
C79	33	0	37	35	16	1	0
C80	26	0	14	26	6	0	0
C81	14	0	22	16	7	1	2
C85	15	0	19	17	12	1	0
C90	21	0	14	29	9	0	1
C93	17	0	14	25	4	0	0
C96	21	1	25	19	13	0	1
C97	26	12	18	23	9	0	2
C98	18	2	11	31	8	0	0

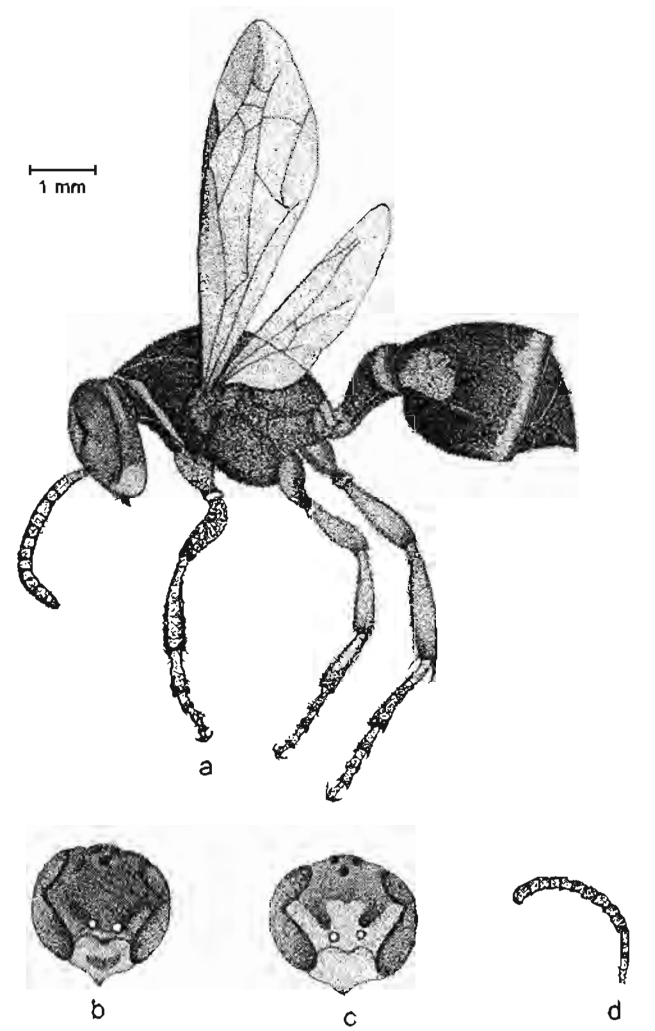

Fig. 1: Camera lucida drawings of (a) female *R. cyathiformis* in profile, frontal view of (b) head of female, and (c) head of male and (d) side view of male antenna showing curved apical segment and tyloids. (By Thresiamma Varghese)

Body size

All adult wasps were collected at the end of the study, and stored at -20° C for measurement and later dissection. For each wasp, the following were measured: 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 distance, width of mesoscutum, length of mesoscutum, alitrunk length, length of right wing, length of left wing, length of 1st marginal cell of right wing, length of 1st marginal cell of left wing, number of hammuli on right wing, number of hammuli on left wing, width of 1st gastral segment, length of 1st gastral segment, height of 1st gastral segment, width of 2nd gastral segment, length of 2nd gastral segment and height of 2nd gastral segment. These 27 body measurements were subjected to principle components analysis using a correlation matrix, separately for each colony as well as for data pooled from all the 10 colonies. The results were used either as an index of body size (defined as the magnitude of the first principle component) or to plot the relative positions of different wasps in a two dimensional principle components space.

Ovarian development

Female wasps were dissected to evaluate the state of ovarian development. The following measurements were made: width of the largest oocyte, length of the largest oocyte, average width calculated over all proximal oocytes, average length calculated over all proximal oocytes, total number of oocytes, number of oocytes with yolk and number of mature oocytes. The ovarian measurements were subjected to principal components analysis and the results were utilized as for body size.

Dry weight and fat content

After taking all measurements the wasps were oven-dried at 72 °C for 36 hours and weighed. Fat content was then estimated using the method of Folch *et al.* (1957).

RESULTS

Females have a crescent shaped brown mark on the clypeus, which males' lack. The apical segment of the antenna is more curved in males. Only males have tyloids on the third and the subsequent segments of the antennae. The first of these differences is easily seen, without disturbing wasps sitting on the nest, making field identification easy (Fig. 1).

Nesting habits

Like most primitively eusocial polistines, *R. cyathiformis* builds simple, stelocyatarus (suspended by a pedicel) and gymnodomous (un-enveloped) nests. Each nest has a single pedicel only, and generally a single comb. Of the 86 nests observed 82 had a single comb, three had two combs and one had three combs. Each comb was suspended by a single pedicel, situated either approximately at the centre (60 combs) or at the periphery (31 combs). Stone pillars and walls (40 nests), cement walls (11), croton bushes (10), wooden door and window frames (9), iron beams (7), asbestos sheets (3), underside of leaves (3), glass panes (2) and brick wall (1) were used as nesting sites in decreasing order of preference. Nests were invariably built in relatively open spaces and were never seen in crevices or in closed places with only a narrow entrance. Active nests were seen throughout the year (Fig. 2a). Nests were also abandoned at all times of the year, but

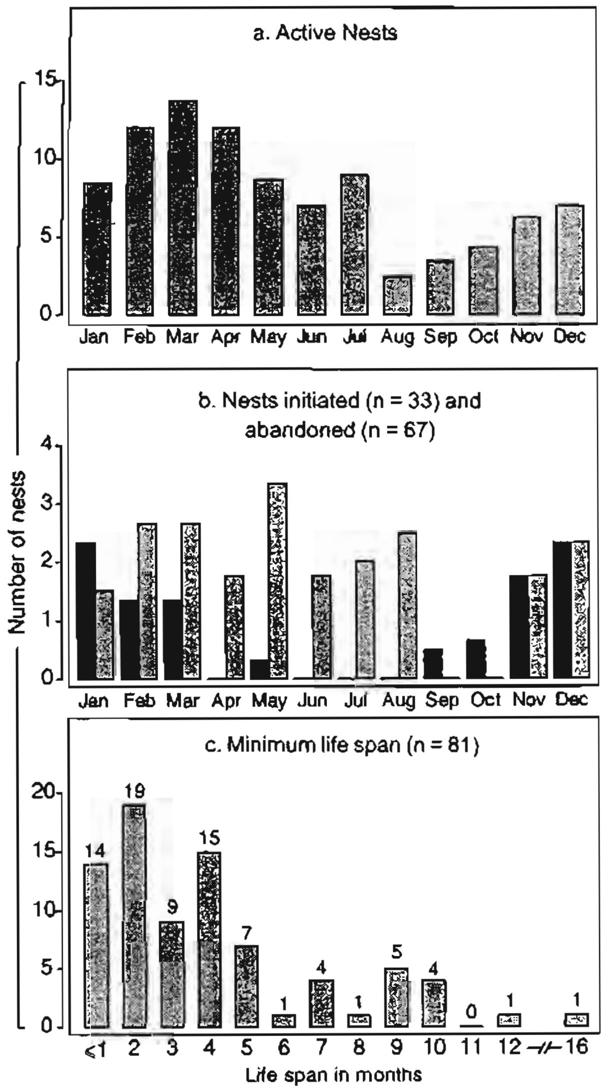

Fig. 2: Frequency distribution of (a) active nests, (b) nests initiated (black bars) and abandoned (grey bars), (c) minimum life span of nests. Sample sizes of nests studied are shown in parentheses in (b) and (c) and also above the bars in (c)

nest initiation occurred usually between October and March (Fig. 2b). For most nests we either recorded the initiation or the abandoning, but not both. This is because either the nests were already initiated at the beginning of our study or our study was terminated before the nest was abandoned. Hence we can only assess the minimum life span of the nest which ranged from one month or less to 16 months, with six out of 81 nests lasting for 10 months or more. The median of the minimum life span was three months and mode was two months.

The total number of adult wasps on a nest ranged from 1 to 93 (Fig. 3a) and the number of females ranged from 1 to 72 (Fig. 3b). 22 out of 33 newly initiated nests had a single founder while the remaining 11 had two founders each. Males were never seen on pre-emergence nests. Out of 53 post-emergence nests, 11 had from 1 to 21 males (Fig. 3c). Information on nest size in terms of cells and brood is summarised for 75 nests in Fig. 3d-g. Although ants and an unidentified ichneumonid wasp occasionally prey upon/ parasitize *R. cyathiformis* nests, the hornet *Vespa tropica* is undoubtedly its major predator (not counting humans), keeping its population in check.

Body size

Intracolony variation in the seven most variable parts of the body and in the index of body size is depicted for a representative colony in Fig. 4a. Intracolony variation in body size is rather small and continuous. By no measure of body size is the queen the largest individual. Multivariate statistical analysis confirms that intracolony variation in body size is relatively continuous and that the queen is intermediate; there are individuals with lower as well as higher values than the queen along principal component 1 as well as principal component 2 (Fig. 5a). The intermediate position of the queen relative to workers is even more clear when data from all the 10 colonies are pooled. The 10 queens are scattered among the 136 workers along the two principal component axes (Fig. 5b). In colony C97 there were 12 males in addition to 18 females. In two others (C96 and C98) there were males but only one and two respectively. Intracolony variation in body size for males, workers and queen for the colony C97 is depicted in Fig. 6 as the relative positions of different wasps in principal components space. Males and females form two distinct clusters with all females (with one exception) having higher values of principal component 1 than males. Males and females have similar variation in values of principal component 2. Since wing length has the maximum weightage in principal component 1, this means that males are smaller than females when measured by wing length although they may be comparable to females in some other measures of body size.

Ovarian condition

In sharp contrast to body size, ovarian condition varies discontinuously within colonies. Intracolony variation in ovarian condition for seven measurements of the ovaries, as well as by a composite index of ovarian condition for the same representative colony is depicted in Fig. 4b. When measured by length and width of the largest oocyte, average length and width of proximal oocytes or total number of oocytes, three kinds of individuals can be recognized - the queen with a very high value, about half the workers with low and the remainder with zero values. Mature oocytes and oocytes with yolk were generally seen only in the queen. When the data are subjected to principle components analysis

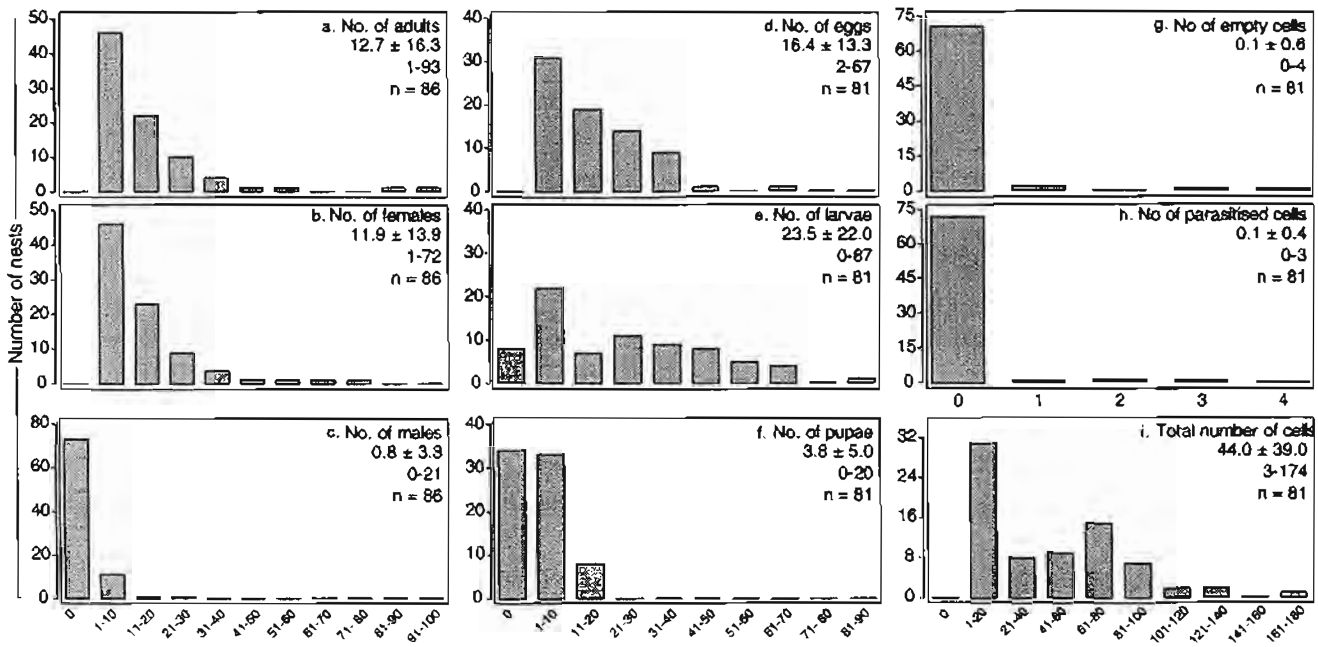

Fig. 3: Size distribution of nests. The measure of nest size, mean ± S.D., range and sample size are indicated in each panel

and the relative positions of individual wasps are plotted in the first and second principal components space, the discontinuous variation is even better emphasized. The workers form one cluster and the queen alone lies far away (Fig. 7a). Even when data are pooled across all colonies the queens (with one exception) form a distinct cluster at one end while workers (with two exceptions) form a tight cluster at the other end (Fig. 7b). The one exceptional queen that lies in the cluster of workers was labelled as the queen because she was the only individual observed to lay eggs in her colony. However, upon dissection she was found to have no mature oocytes. The workers with higher values of ovarian index had proximal oocytes similar to or smaller than that of the queen, but they had higher numbers of oocytes. We suspect that the queen in this colony had approached the end of her tenure.

Dominance behaviour

As in other primitively eusocial wasps, adults of *R. cyathiformis* exhibit several kinds of aggressive or agonistic behaviours towards each other on the basis of which one member of the interacting pair can be unambiguously designated as 'dominant' and the other as 'subordinate'. Six distinct dominance behaviours were observed; peck, nibble, chase, attack, hold in mouth and sit on another wasp. The sum of the frequencies of these behaviours was designated as the frequency of dominance behaviour. The relative abundance of the six behaviours is shown in Table 3. When the frequencies of dominance-subordinate behaviours were used to compute a dominance index and construct a dominance hierarchy, the queen was always at the top of the hierarchy except in colony C97 in which we suspected the queen to be approaching the end of her tenure (see section on ovarian condition). These data are not given here because similar results have been published before (Kardile and Gadagkar 2002, 2003). The frequency of dominance behaviour shown by the wasps had a significantly positive correlation with the frequency with which they snatched food, fed larvae, snatched building material and built the nest, as well as with their state of ovarian development and fat content. The frequency of dominance behaviour was not significantly correlated with body size and dry weight (Table 4).

DISCUSSION

The main motivation for studies on *R. cyathiformis* from our laboratory comes from the desire to identify a species of

Table 3: Types of dominance behaviour and their relative abundance, measured as percentage of total dominance behaviour

Types of dominance behaviour	Relative abundance (mean ± S. D.)
Peck	42.9 ± 22.7
Nibble	30.8 ± 11.7
Chase	2.3 ± 2.3
Attack	10.4 ± 8.5
Hold in mouth	10.6 ± 9.7
Sit on another wasp	3.0 ± 3.4

Data pooled from the 10 colonies used to study dominance behaviour

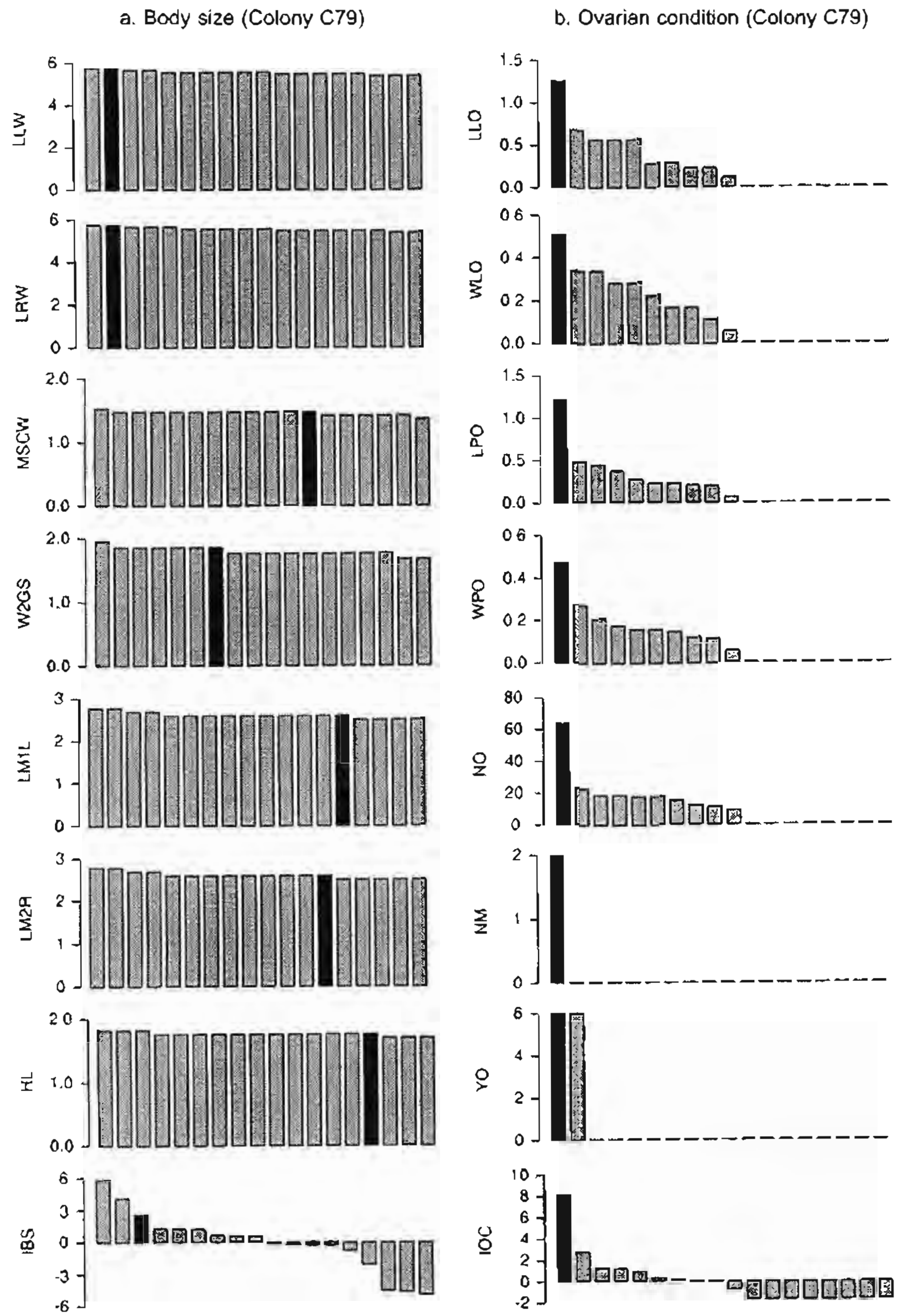

Fig. 4: (a) Body size and (b) Ovarian condition of wasps in a representative colony C79. The seven most variable measures of body size are on the left panel and all the seven measurements of ovarian development are on the right panel. Grey bars represent workers while black bars represent queens. Left panel: LLW, length of left wing; LRW, length of right wing; MSCW, width of mescoscutum; W2GS, width of second gastral segment; LM1L, length of 1st marginal cell of left wing; LH1R, length of 1st marginal cell of right wing; HL, head length; IBS, composite index of body size. Right panel: 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.

primitively eusocial polistine wasp that would be suitable for comparison with *R. marginata*. The latter is also classified as a primitively eusocial wasp because of the absence of morphological differentiation between queens and workers. *R. marginata* is one of the most extensively studied social wasps, whose natural history, ethology, nesting biology and social biology have been documented in considerable detail during the past 25 years. This species has served as an

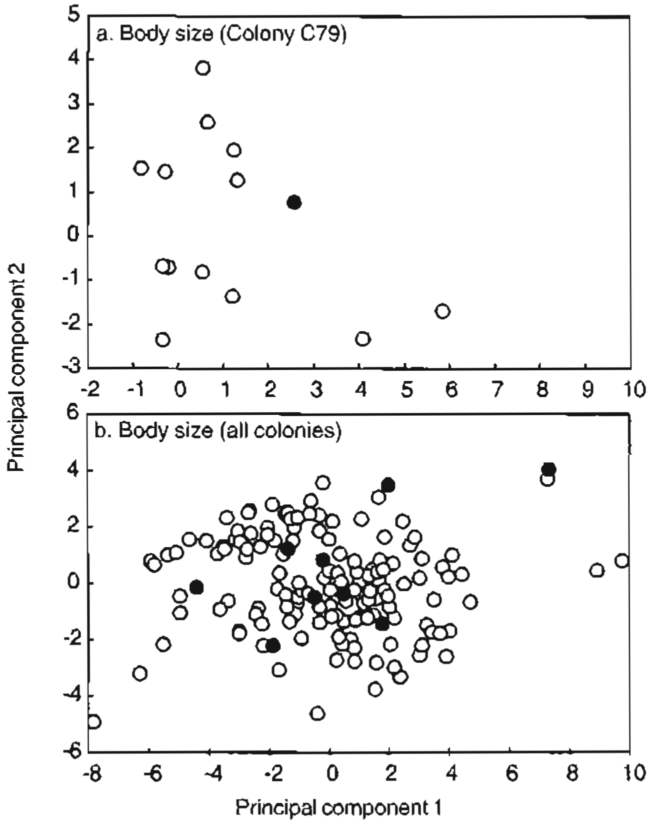

Fig. 5: Intra- and inter-colony variation in body size (a) colony C79 and (b) all colonies. Closed circles = queens, open circles = workers

excellent model system for understanding the evolution of eusociality and the apparent paradox of altruism (Gadagkar 2001). These investigations have, however, yielded a major surprise. In primitively eusocial species queens are known to be the most behaviourally dominant, active and interactive individuals who are always at the top of the dominance hierarchies of their colonies and use dominance behaviour to suppress worker reproduction as well to coerce workers to undertake non-reproductive activities such as foraging for food and building material. Because of these roles, queens in primitively eusocial species have sometimes been labelled as central pacemakers of their colonies (West-Eberhard 1969, 1977; Wilson 1971; Breed and Gamboa 1977; Brothers and Michener 1974; Buckle 1982; Dew 1983; Reeve and Gamboa 1983, 1987; Fletcher and Ross 1985; Gamboa *et al.* 1990; Gadagkar 1991; Reeve 1991; Röseler 1991).

Table 4: Kendall's coefficient of rank correlation (tau) between dominance behaviour and other variables

Variable	Tau	N
Bring food	-0.12	205
Snatch food	0.25*	205
Lose food	0.01	205
Feed larvae	0.17*	205
Bring building material	0.03	205
Snatch building material	0.15*	205
Lose building material	0.02	205
Extend walls of cells + build new cells	0.19*	205
Body size index	0.04	146
Ovarian index	0.20*	146
Dry weight (mg)	0.15	136
Fat (mg)	0.23*	136

To test significance of tau, α was set at 0.05. After Bonferroni correction (12 tests), $p < 0.004$ was considered significant and is indicated with an asterisk. Because data on all variables were not available for all wasps, sample sizes (N) varied between different correlations

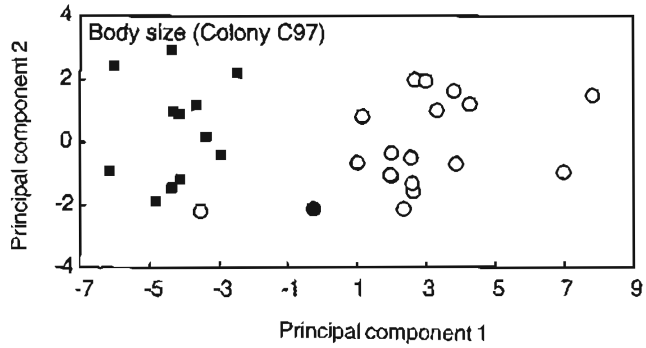

Fig. 6: Intra-colony and inter-sex variation in body size. Closed circle = queen, open circles = workers, closed squares = males

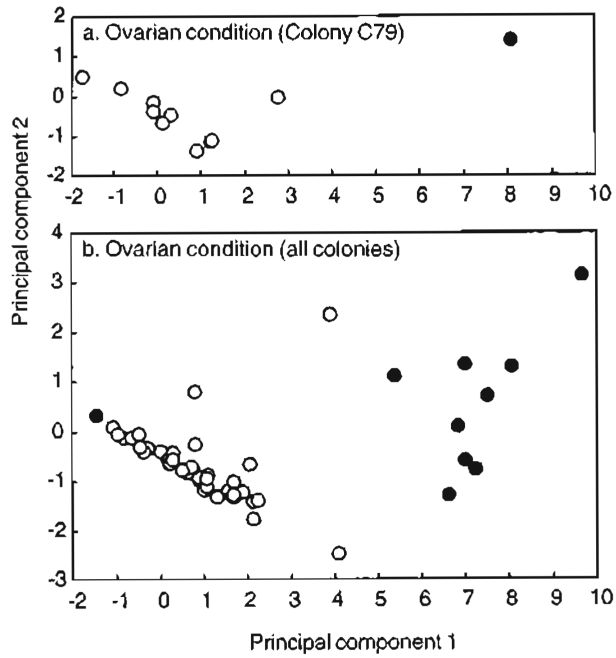

Fig. 7: Intra- and inter-colony variation in ovarian condition (a) colony C79 and (b) all colonies. Closed circles = queens, open circles = workers

By contrast, queens of *R. marginata* are classified as meek sitters who are never at the top of dominance hierarchies

(Gadagkar 2001). Nevertheless they are completely successful in maintaining reproductive monopoly and there is suggestive evidence that they may do so by using pheromones to regulate worker reproduction (Sumana *et al.* in preparation). Queens of *R. marginata*, by virtue of their physical inactivity and lack of behavioural dominance are also not involved in regulating non-reproductive activities of their workers. This appears to be achieved by the workers in a decentralised, self-organised manner (Premnath *et al.* 1995). *R. marginata* also exhibits a well-developed, remarkably honeybee-like age polyethism that is not usually expected in primitively eusocial species (Naug and Gadagkar 1998). *R. marginata* may perhaps be described as a relatively more socially advanced species among the primitively eusocial species (Gadagkar 2001).

Such unusual features of *R. marginata* merit comparative investigations with another closely related species which is more typically primitively eusocial. There is already some evidence that *R. cyathiformis* may be the appropriate species for enhancing our understanding of the unusual properties and evolutionary position of *R. marginata* (Gadagkar 2001; Kardile and Gadagkar 2002, 2003). We have, therefore, commenced detailed investigations on the biology of *R. cyathiformis* similar to previous studies with *R. marginata*. Here we report our observations on the natural history and behaviour of *R. cyathiformis.*

There are several features of *R. cyathiformis* that are very similar to *R. marginata*. Both exhibit an aseasonal nesting cycle and lack morphological caste differentiation. Most nests are monogynous (have a single egg-layer). As in *R. marginata* most of the dominant individuals stay on the nest, snatch food and building material from incoming foragers and specialise in performing intranidal activities, including brood care. An important difference is that the adult wasps, as well as the nests, are much smaller in *R. cyathiformis*. Another difference is that *R. cyathiformis* colonies can be polygynous (although all ten studied here were monogynous), which is never so in *R. marginata* (Gadagkar and Joshi 1982; Gadagkar 2001). We already know that queens of *R. cyathiformis* are indeed the most dominant, active and interactive individuals and behave as if they regulate worker reproduction and activities by using dominance behaviour (Kardile and Gadagkar 2002). Unlike in *R. marginata*, regulation of foraging appears to be a centralised process with a major role for the queen (Kardile and Gadagkar 2003).

The observations reported here combined with the previous studies make *R. cyathiformis* an ideal model system to compare with *R. marginata*. A comparative study of *R. cyathiformis* and *R. marginata* is expected to help understand the evolutionary transition from physical to chemical control of reproduction, centralised to decentralised regulation of worker activity and from the primitive to the highly eusocial state in general.

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