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Research Paper

Evolution of Reproductive Dominance in Animal Societies – Lessons From a Social Wasp

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Most insect societies can be classified as either primitively or highly eusocial. Primitively eusocial insect societies are usually led by queens who are morphologically indistinguishable from the workers and use aggression to control the workers, thereby typically holding top positions in the colony's dominance hierarchy. Highly eusocial species have morphologically large queens who regulate worker reproduction through pheromones and achieve larger colony sizes than their primitively eusocial counterparts. However, it is not clear whether this switch from aggression to pheromone took place in a single step in which a population as a whole evolved chemical regulation, or in two steps in which a queen used physical regulation when the colony size was small and switched to chemical regulation when the colony became larger. *Ropalidia marginata* is a primitively eusocial wasp, which also has some characteristics that are typically seen in highly eusocial species. The queens in this species do not usually lead the colony's dominance hierarchy and use pheromones to signal their presence to workers. Since new colonies are founded by one or a few individuals and grow through time, young colonies are small enough to permit suppression of worker reproduction through aggression. Queens in small colonies indeed sometimes occupy the top position in the colony's dominance hierarchy, thus providing a unique opportunity to test the above-mentioned hypotheses. We analysed data from 100 colonies of *R. marginata* to test these two competing hypotheses and found support for the former. Our findings are consistent with the hypothesis that the evolution of highly eusocial societies from primitively eusocial ones involved a one-step transition from physical control to chemical regulation of worker reproduction.

Keywords: Social Insects; Dominance Behaviour; Pheromones; Colony Size; Worker Control; Statistical Artefacts

Introduction

The formation of groups or communities often helps to achieve efficiency in performing complex tasks, and the performance of such groups largely depends on the efficient administration of individual tasks by group members. Group living is ubiquitous in the animal kingdom across taxa as diverse as insects, fishes, birds and mammals (Wilson 1975); and diverse control mechanisms can be recognized in social organizations

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of such group-living animals. Group activities like the schooling of fish, termite mound building or flight pattern regulation of bird flocks occur through self-organization, without the intervention of any external directing influence (Camazine *et al.*, 2003; Bhattacharya and Vicsek 2010). Activities like cooperative breeding, on the other hand, are in general actively regulated by one or a few individuals, usually through physical aggression. For example, in species like the dwarf mongoose (Keane *et al.*, 1994), common marmoset (Abbott *et al.*, 1998), meerkat (Clutton-Brock *et al.*, 1998), African wild dog (Creel *et al.*, 1997), spotted hyena (Frank 1986), woodpecker (Koenig *et al.*, 1998), grackle (Poston 1997) etc., dominant individuals enjoy the bulk share of reproduction by denying the others. An extreme example of such regulation is seen in insect societies such as honeybees, where the queen bee has evolved to use chemical signals that help her to achieve complete reproductive monopoly by regulating reproduction in the workers (Wilson 1971; Keller and Nonacs 1993). Studying mechanisms by which complex social organization is maintained can lead to a better understanding of the evolutionary processes by which social behaviours might have been selected. Eusocial insects like ants, bees, termites and wasps offer an excellent array of social organization, from solitary individuals to highly organized complex societies, with myriad intermediate levels of complexity.

Eusocial insects are characterized by colonies with reproductive caste differentiation, cooperative brood care and overlap of generations (Batra 1966; Michener 1969; Wilson 1971), and are broadly categorized into the primitively and highly eusocial species. Primitively eusocial species have small colonies headed by one or a few queens who bear little or no morphological differences with the workers. Though the workers in these species remain functionally sterile in the presence of the queen, they are potentially capable of mating and reproduction and can replace the queen to assume the role of the queen (West-Eberhard 1969; West-Eberhard 1977; Fletcher and Ross 1985; Reeve 1991; Gadagkar 1991). The queens in these species are usually very active and are known to use physical aggression and intimidation to suppress reproduction in the workers (Pardi 1948; Reeve and Gamboa 1983, 1987; Theraulaz *et al.*, 1989; Keller and Nonacs 1993; Monnin and Peeters 1999; Dietemann *et al.*, 2005). Physical aggression takes the form of dominance interactions, based on which a dominance hierarchy can be recognized in the colony, and in some species, the relative positions of the workers in the hierarchy reflect their chances of becoming future queens (Pardi 1948; West-Eberhard 1969; Jeanne 1972).

Highly eusocial species on the other hand, usually have complex societies, with large colonies and striking morphological differences between the workers and the queens. The workers in these species are physiologically incapable of mating and therefore are not able to produce female offspring. The queens typically do not participate in any nest maintenance activities, and they maintain their reproductive monopoly by signalling their presence to the workers by means of pheromones which serve as honest signals of their fertility (Wilson 1971; Ross and Matthews 1991; Keller and Nonacs 1993). Thus, transition from a primitively eusocial to a highly eusocial colony organization, which occurred repeatedly within independent social insect lineages (Noirot and Pasteels 1988; Hölldobler and Wilson 1990), involves a set of behavioural changes with respect to the control of worker reproduction, along with a set of physiological and morphological changes, and an increment in colony size. Though the differences between primitively and highly eusocial societies are well documented, the process of this transition from the simpler to more complex social organization is not well understood and is thus an interesting premise for scientific enquiry.

Ropalidia marginata is classified as a primitively eusocial wasp on account of the complete absence of morphological differences between the reproductive and non-reproductive castes (Gadagkar 2001).However, it has been shown that the queens in this species are remarkably meek and docile and use pheromones to signal their presence to the workers (Premnath *et al.*, 1995; Gadagkar 2001; Kardile and Gadagkar 2002; Sumana and Gadagkar 2003; Bhadra *et al.*, 2010). There is nevertheless a certain amount of dominance behaviour in the colony on the basis of which a dominance hierarchy can be constructed (Premnath *et al.*, 1990). However, the queen does not usually occupy the topmost rank in the hierarchy, and the position of workers in the hierarchy are also not correlated with their chances of future reproduction

(Gadagkar 2001). Experimental and theoretical studies have revealed that the dominance interactions among the workers are used for the decentralized self-organization of the non-reproductive activities of the workers (Premnath *et al*., 1995). Thus we have argued that *R. marginata*, along with other such primitively eusocial species with pheromonal control of worker reproduction (Sledge *et al*., 2001; Dapporto *et al*., 2007; Sumana *et al*., 2008; Bhadra *et al*., 2010) represents an intermediate stage in the evolutionary transition from the primitively eusocial to the highly eusocial stage and thereby qualifies as a perfect model system to investigate the evolutionary processes associated with such transition.

Though the queens in *R. marginata* rarely indulge in dominance interactions with their workers, a closer look at the data collated from several previous studies revealed that occasionally, the queens might occupy the alpha rank in the dominance hierarchies of their colonies. Moreover, we also observed that such unusual colonies seem to be typically very small in size. This observation permits two different hypotheses related to the evolutionary changes in the worker control. 1) *R. marginata* queens may function like queens of typical primitively eusocial species, using physical aggression to suppress worker reproduction in the founding stage of the colony, switching over to chemical regulation when the colonies become large. There may thus be a transition from a primitively eusocial queen-like behaviour to a highly eusocial queen-like behaviour within the life cycle of a colony. 2) *R. marginata* queens may always use pheromones to regulate worker reproduction irrespective of the size of the colony, and the occasional topmost ranks of queens in *R. marginata* may be a statistical artefact of small colony size. To discriminate between the two hypotheses, we analyse data on dominance-subordinate interactions and the resulting dominance hierarchies in 100 colonies of *R. marginata*, of different colony sizes, studied over many years for different purposes. We compare the observed results with simulated patterns obtained assuming queen's dominance rank determined solely by chance. Our analysis favours the later hypothesis which is consistent with the idea that the evolutionary transition from physical control to chemical regulation of worker reproduction is a one-step process, not requiring an intermediate step within. We also believe that our analysis can shed light on the proximate mechanisms involved in the process of evolution of complex social organizations.

Methods

The Observational Data

In the present study, we used data obtained from observations on 100 colonies of *R. marginata*, collected from in and around Bangalore over many years, by several members of our research group. In each colony, all wasps were uniquely marked with coloured spots of quick-drying enamel paint. Behavioural observations were made using instantaneous scans and all occurrences sessions (Gadagkar 2001). Total observation duration per colony ranged from 5 to 20 hours, in which instantaneous scans and all occurrences sessions were interspersed randomly (Gadagkar, 2001).

Although almost 100 behaviours were recorded in these colonies, we used dominance and subordinate interactions in the present analysis. Dominance behaviour was calculated as the frequency per hour of the sum of nine different behaviours i.e., attack, chase, nibble, peck, crash land on another individual, sit on another individual, being offered regurgitated liquid, aggressively bite, and hold another individual in mouth. Subordinate behaviour was defined as an event of receiving the above mentioned nine behaviours and was calculated as the frequency per hour of their sum (Chandrashekara and Gadagkar 1991; Gadagkar 2001). Based on these frequencies, a dominance index was computed for each individual using the method of frequency based dominance index (FDI) (Premnath *et al*., 1990), which has been shown to give more unique ranks than most other such dominance indices (Bang *et al*., 2010). Arranging these indices in descending order, a dominance hierarchy was arrived at for each colony. Thus, all wasps in a colony including the queen obtained a rank ranging from 1 to n, where n is the colony size. In cases where the queen was tied with some other worker(s), her rank was decided by taking an average of her own rank with that of the tied individual(s).

We analysed the data set in two ways. First, we divided the colonies into two groups: a set of colonies where the queen held rank one (QR1) and the complementary set of colonies in which the queen was not ranked one ($QR1^c$). These two groups were then compared with each other in terms of their sizes. Secondly, Queen rank r was plotted against the colony size n, and the regression slope was estimated. To detect a possible behavioural transition at low colony sizes, we defined a critical colony size n_c to split the data set into two categories. If the size of a colony was less than or equal to n_c, we categorized it as 'small' colony, otherwise it was a 'large' colony. Values for n_c were taken as 5 to 14 and for each case the regression slopes were calculated for both the categories and compared with each other ($n_c<5$ resulted in a sample size of 5, and $n_c=14$ was the median colony size). If the queen switches to chemical regulation for controlling the workers in larger colonies, then the slope found in the small colony category is expected to be smaller than that in the large colony category, for at least one n_c. For each n_c we used t-test to compare the 'small' and 'large' colonies.

The Simulation

To test whether the pattern we observed from the experimental colonies could be obtained by chance (hypothesis 2), we ran a simulation. We used the same distribution of colony sizes as in the 100 experimental nests. For each run of the simulation, assuming equal probability for the queen to have any rank from 1 to n in the dominance hierarchy, we assigned her a random integer rank using a uniform random number generator. Once this was done for all the 100 nests, the colonies were grouped into those where the queen was ranked one (QR1) and those where she was not ranked one ($QR1^c$). Then these two groups were compared with each other. Simulated QR1s and $QR1^c$s were also compared with observed QR1 and $QR1^c$ respectively. This process was repeated 1000 times. In order to uphold hypothesis 2, we needed to obtain patterns similar to the observed ones. Also, for each run of the simulation, a regression slope between the colony sizes and the corresponding simulated ranks were estimated. This estimated slope, averaged over 1000 realizations, was considered as the 'hypothesized slope' and compared with the slope from the observational data. We categorized the simulated data set into small and large colonies following the same steps used for the observational data, did the regression analysis for each category, and compared the obtained slopes with their observational counterparts. If hypothesis 2 is true, no differences between simulated and observed slopes for any n_c were expected. To achieve this, we calculated the 95% confidence interval for the overall data as well as for the 'small' and 'large' colonies at each $n_{c.}$ and investigated whether the respective point estimate form natural colonies lie within this interval.

Results

Data Analysis

The data set of N=100 colonies used in this analysis consisted of colonies ranging from 3 to 77 adult wasps with mean = 20.43, median = 14 and mode = 9 & 14. The ranks obtained by the queens in the dominance hierarchies of their colonies ranged from 1 to 44 (mean = 8.725, median = 4.250 and mode = 1). The sizes of the group of colonies where the queen occupied the top rank [N_{QR1} = 16, mean ± SD = 14.875 ± 17.877] and where queens did not occupy the top rank [N_{QR1^C} = 84, mean ± SD = 21.488 ± 14.729] are shown in a box and whiskers plot (Fig. 1). We found that the colony sizes in the category of QR1 were significantly smaller than those belonging to $QR1^c$ [Wilcoxon rank sum test, W=355.5, p=0.001]. This finding favours the impression that queens in *R. marginata* hold the top ranks in the dominance hierarchies of the smaller colonies more frequently than the larger ones.

In the regression analysis, a significant positive correlation was observed between queen rank and colony size [B = 0.474, $r^2 = 0.498$, F = 97.175, $p < 0.0001$] (Fig. 2A). From the diagram, the data appears to be *heteroscedastic*, i.e., the standard deviation in r is increasing with n. A regression technique is only valid for data with homogeneous variance, so we repeated the regression analysis after performing a logarithmic transformation on our data (Fig. 2B). This time we tried to fit the equation of the form $\log(r) = C+D.\log(n)$ and again found a significant regression coefficient D = 0.948 ($r^2 = 0.410$, F = 68.092, $p < 0.0001$).

The regression slopes of our categorized colony data, both for the small colonies ($n \leq n_c$) and the large colonies ($n > n_c$), are furnished in Table 1. We compared the slopes of small and large colonies for each critical colony size n_c by means of t-test (column d_1 vs. d_2 in Table 1), and for the whole range of the

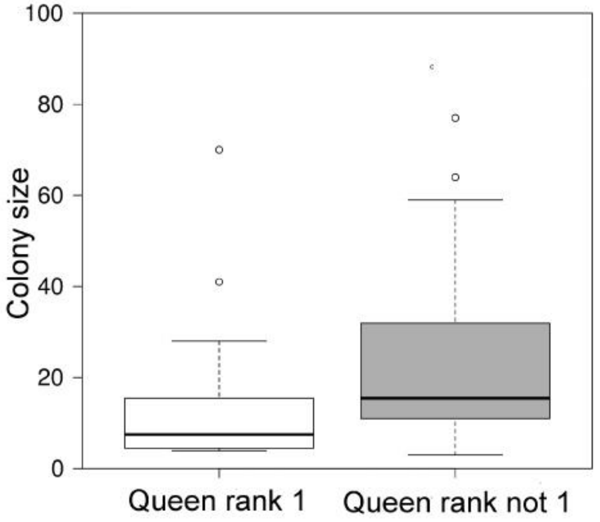

Fig. 1: Comparisons of observed colony sizes where queen occupies rank one (QR1) and where queen occupies ranks other than one ($QR1^c$) in the dominance hierarchy in the primitively eusocial wasp *R. marginata*. The box represents the inter-quartile range and the whiskers represent the non-outlier range (1.5 times the inter-quartile range). The bold lines within the boxes depict the median values. Outliers are shown by small circles

n_c, we found no significant differences between the categories. Therefore, we failed to detect the existence of any critical colony size below which the queen uses physical aggression to control her workers (and thereby holds top ranks) and above which she does not need to use the same (holds arbitrary ranks). The queen, therefore, does not change her strategy from physical aggression to chemical control within the life cycle of a colony; thereby, hypothesis 1 is not upheld.

The Simulation Results

For the simulated colonies, the regression slopes between the assigned queen ranks r and the colony sizes n were found to follow a Gaussian distribution with a mean of 0.507 ± 0.096 [Shapiro-Wilk's test, W = 0.999, p = 0.830]. A linear relationship with a slope of 0.5 can also be estimated analytically (please refer to the appendix). It is also predicted that the variance would increase with colony size n, therefore a log-transformed data would be preferred in this case. For each run of the simulation, both the nest size n and the assigned rank r were log-transformed and a regression equation of the form of log(r)=C'+D'.log(n) was fitted. Averaging over 1000 such runs, the `hypothesized' slope D' was estimated as 0.882. The 95% CI of the values were determined as D'±1.96SD. Such hypothesized slopes for small and large colonies, along with their confidence intervals, categorized by

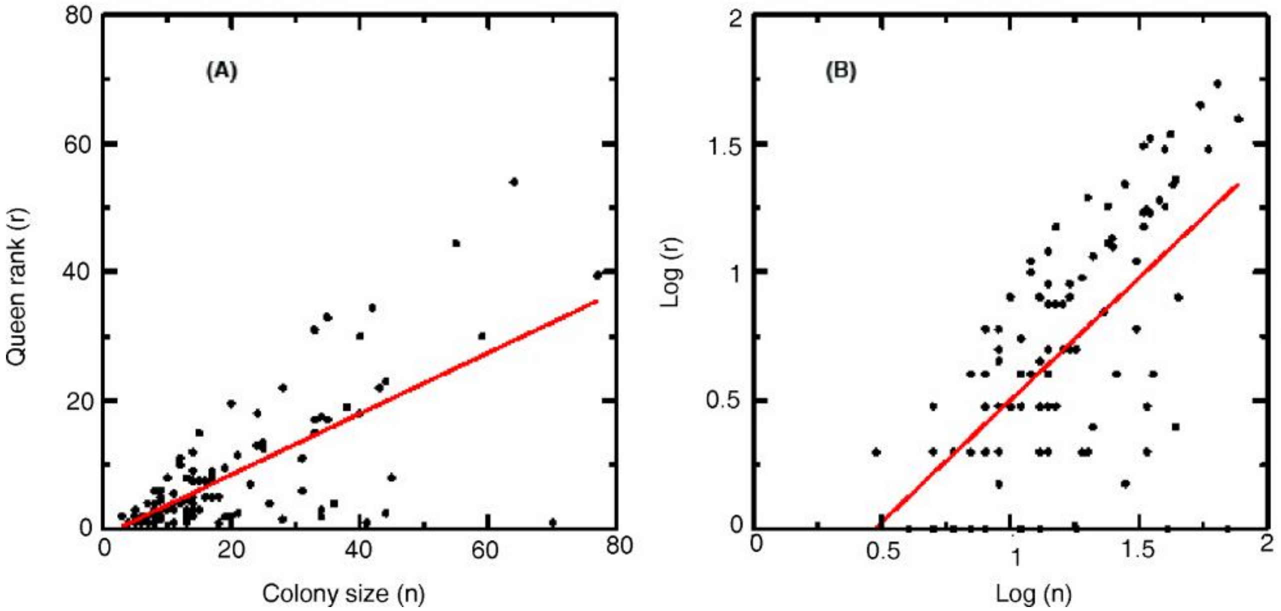

Fig. 2: a) Queen ranks r for all the 100 observed *R. marginata* nests plotted against respective colony sizes n. The solid line corresponds to the fitted regression equation of the form of r=A+B.n with A=-0.963 and B=0.474. b) The same plot with log-transformed data. The corresponding regression equation of the form of log(r)=C+D.log(n) with C=-0.445 and D=0.948 is also shown

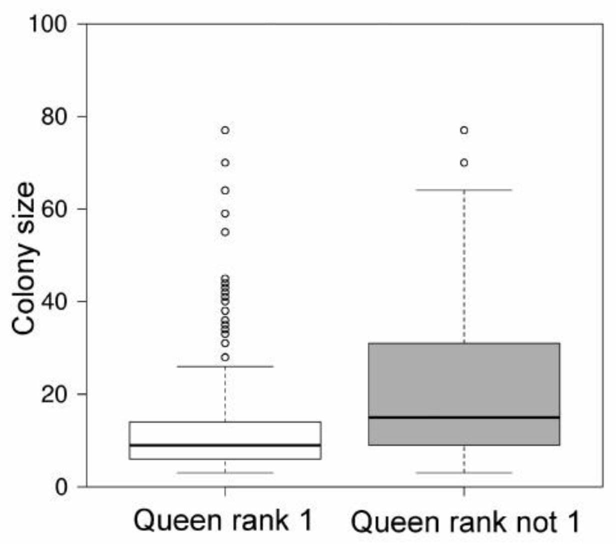

Fig. 3: Comparison of simulated colony sizes where queen occupies rank one (QR1) and where queen occupies ranks other than one (QR1c) in the dominance hierarchy in the primitively eusocial wasp R. marginata, for 1000 realizations. The box represents the inter-quartile range and the whiskers represent the non-outlier range (1.5 times the inter-quartile range). The bold lines within the boxes depict the median values. Outliers are shown by small circles

n_c, are furnished in Table 1. QR1 and QR1c for all 1000 realizations put together are shown with a box and whiskers plot in Fig. 3, and QR1 [mean ± SD = 12.371 ± 10.079] was found be significantly smaller than QR1c [mean ± SD = 21.150 ± 15.472, Wilcoxon rank sum test, W = 2X108, $p < 0.0001$].

The mean colony sizes for these two queen rank categories were also estimated analytically, just like the mean slope (refer to the appendix). For each run, we also calculated the mean sizes for both the categories and the set of means corresponding to the simulated QR1s [mean ± SD=12.381 ± 3.818] were also found to be significantly smaller than that of QR1d' [mean ± SD = 21.145 ± 0.386, Wilcoxon Signed Rank test, W = 4488, $p < 0.0001$]. These analyses show that the observed patterns, which gave an impression that the queens occupy top ranks in smaller colonies, can also be obtained from a situation where queen ranks are purely determined by chance.

The Comparison

The regression slope for the log-transformed data of 100 nests was found to be 0.948 with standard error 0.115 (Fig. 2B). This was compared to the 95% CI of the slopes obtained through simulation [d',Upper CI, Lower CI = 0.882, 1.086, 0.678]. The experimental slope was found to lie well within this interval. Hypothesis 2 was upheld as we compared observed slopes of small colonies with their simulated counterparts for each nc (column d1 vs. $d^{1'}$ in Table 1) and found no evidence for a difference. Differences were also not detected in the large colonies (column d^2 vs. $d^{2'}$ in Table 1). For the

Table 1: Comparisons of regression slopes in categorized colonies. The observational data are split into small and large colony categories according to different critical colony sizes, and their estimated regression slopes are compared. Slopes for the colonies in each category are also compared with corresponding hypothesized value obtained from simulations. All comparisons were found to be not significant at α=0.05

Critical colony sizenc	No. of nest $n \leq n_c$	Estimated slope (from data) d'±SE	Hypothesized slope (from simulation) d'_1 [Lower CI, Upper CI]	No. of nest $n > n_c$	Estimated slope (fromdata) d'±SE	Hypothesized slope (from simulation) d'_2 [Lower CI, Upper CI]	p-value for t-test (d^1 vs.d^2)
5	8	0.383±1.031	0.683 [-1.485, 2.850]	92	0.944±0.141	0.893 [0.640, 1.146]	0.760
6	11	0.040±0.624	0.680 [-0.814, 2.174]	89	0.899±0.150	0.889 [0.607, 1.171]	0.477
7	14	0.462±0.517	0.748 [-0.432, 1.928]	86	0.880±0.158	0.900 [0.622, 1.178]	0.643
8	19	0.872±0.423	0.745 [-0.102, 1.592]	81	0.874±0.172	0.908 [0.600, 1.216]	0.998
9	28	1.080±0.298	0.788 [0.143, 1.433]	72	0.882±0.208	0.922 [0.561, 1.283]	0.707
10	30	1.176±0.287	0.765 [0.138, 1.392]	70	0.914±0.217	0.919 [0.541, 1.297]	0.604
11	34	1.045±0.269	0.770 [0.207, 1.333]	66	0.872±0.234	0.910 [0.512, 1.308]	0.710
12	37	1.242±0.257	0.795 [0.272, 1.318]	63	0.961±0.246	0.927 [0.502, 1.352]	0.523
13	42	1.032±0.244	0.800 [0.330, 1.270]	58	0.883±0.270	0.917 [0.468, 1.366]	0.718
14	51	0.990±0.211	0.799 [0.391, 1.207]	49	0.817±0.340	0.924 [0.369, 1.479]	0.668

comparisons of grouped colony sizes, we used Wilcoxon rank sum tests in each run of the simulation and found that in 99.7% cases simulated QR1s are not different from observed QR1s with an average p-value of 0.607 ± 0.258. Simulated $QR1_c$s were also found to be comparable with the observed counterparts in 100% cases with 0.656 ± 0.150 as the average p-value. This non-significance also added evidence in favour of hypothesis 2. Therefore, the observed pattern, in which the queens in small colonies of *R. marginata* hold top ranks in the dominance hierarchy, can occur due to chance alone and there is no evidence that the queen switches her strategy from physical aggression to chemical control as the colony grows in size.

Discussion

The sheer constraint of controlling nestmates through physical aggression limits the sizes of primitively eusocial insect colonies (Wilson 1971, Gadagkar 2001). In contrast, more efficient control through pheromones has allowed colonies of highly eusocial species such as hornets, honeybees and ants to reach gigantic sizes. A relevant example in this context would be the primitively eusocial species from the same genus as discussed here – *Ropalidia cyathiformis*. The queen in this species maintains her reproductive monopoly though aggression, i.e., the queen is always the alpha individual in the dominance hierarchy. Moreover, when the queen is replaced, the beta individual becomes the next queen. Due to this physical regulation of worker reproduction, *R. cyathiformis* colonies are much smaller than *R. marginata* colonies (Kardile and Gadagkar 2003). Although the consequences of such enlargement of colonies have been much discussed in the context of the formation of complex animal societies (Alexander *et al*., 1991; Bourke 1999; Monnin *et al*., 2003), the associated behavioural changes required to control the increased number of colony members have not been fully explored.

In insect societies, an evolutionary transition from physical control to chemical regulation is linked with the transition from primitively eusocial species to highly eusocial species; and *R. marginata*, owing to its intermediate social organization, is an excellent system to study the behavioural changes associated with this transition. While *R. marginata* lacks a morphologically distinct queen caste, much like the typical primitively eusocial species, there are many evidence that the queen uses pheromones to control her workers (Bhadra *et al*., 2010; Mitra and Gadagkar 2011; Mitra and Gadagkar 2012a), which is more reminiscent of the behaviour of highly eusocial queens. Since queens in small colonies of *R. marginata* are sometimes found to occupy top ranks in the dominance hierarchies of their colonies, it was not hitherto clear whether queens in small colonies use aggression and only those in large colonies use pheromones (hypothesis 1) or whether all queens use pheromones, and the occasional top ranks of queens in small colonies is only a statistical artefact (hypothesis 2). The behavioural transition of the queen from aggressive control to pheromonal regulation of worker reproduction is a key step in the evolution towards the highly eusocial organization in social insects. Hence we endeavoured to discriminate between these two competing hypothesis in order to understand the evolutionary process that led to the intermediate level of social organization observed in *R. marginata*. In order to achieve this, we employed a combination of approaches. We collated data from 100 colonies and performed regression analysis both for the entire dataset as well as for 'small' and 'large' colonies at different critical colony sizes (n_c). We did not find significant difference in the slopes of 'small' and 'large' colonies for any of then_cs. We also ran a simulation assigning random ranks to the queen, taking the same colony sizes as in the natural colonies, and compared the simulated distribution of regression slopes with the respective point estimate from the natural colonies. The experimental estimates were not different from the null distributions.

Therefore, all the approaches we employed failed to provide any support in favour of hypothesis 1 or against hypothesis 2. Although we observed that colonies with queen rank 1 were significantly smaller than those with queen rank more than 1, we failed to find a critical colony size (n_c) beyond which there was a significant decrease in the slope of queen ranks over colony sizes, as would be expected if there was a transition from physical to chemical control with an increase in colony size. On the other hand, using both simulations and analytical techniques, we found that the regression slope (0.47) for observed data was well within the confidence interval (0.32-0.70) of the expected value assuming that the queen was being decided randomly with respect to her aggression. We further showed using simulations that for each critical colony size for both subsets of colonies the observed slope always lies within the simulated confidence interval. Moreover, we showed that even if the queen was being decided randomly with respect to her dominance rank, one could still observe colonies with queen rank 1 to be smaller than colonies with queen rank more than 1.

Therefore, we conclude that the occasional top ranks of queens could indeed be a statistical artefact of the small colony sizes in which they occur. This suggests that *R. marginata* queens use pheromones to regulate their workers in both small as well as large colonies. Consistent with this conclusion we now have evidence that even solitary foundresses have a queen-like pheromone profile and that workers who become replacement queens go through a transition from a worker-like pheromone profile to a queen-like pheromone profile within about a week (Mitra and Gadagkar 2012b). Interestingly, when individual wasps are randomly paired in a closed environment, creating a premise for contests of dominance, aggression appears to be a strong predictor of egg-laying (Brahma *et al*., 2018, Bang 2010). It has been demonstrated that aggression helps the potential queen to develop her ovaries, and an individual left alone on the nest without the queen and other workers takes much longer to develop her ovaries (Lamba *et al*., 2007). Though in similarly created triplets, one of the non-egg-layers shows more dominance behaviour than the egg-layer, who in turn is indistinguishable in dominance behaviour from the other non-egg-layer (Brahma *et al*., 2018). Hence aggression is likely to play a complex role in the social dynamics of *R. marginata*, definitely for the establishment of reproductive hierarchy and perhaps also its maintenance in some cases.

Based on the results of the current study, we therefore speculate that the evolutionary transition from physical to chemical control of reproduction is a one-step transition applicable to all colony sizes, rather than requiring an intermediate step with physical control in small colonies and chemical control only in large colonies, though the role of dominance in the social dynamics of the species is yet to be clearly understood. This suggestion is best treated as a hypothesis requiring further tests in this and other lineages of social insects.

The present accepted paradigm in our current understanding of the evolution of social behaviour comes from the field of evolutionary developmental biology. Several studies using this approach have shown that complex social behaviour such as division of labour evolved through changes in genetic regulatory mechanisms, rather than changes in genes (Toth and Robinson 2007; Page and Amdam 2007). *R. marginata* provides the perfect system to carry out investigations on the molecular mechanism of transition from primitively to highly eusocial animal societies. Our analyses started with an impression that the queens of the primitively eusocial wasp R. marginata hold top ranks in the dominance hierarchies only in small colonies. Our comparison of observed colony sizes, where the queen holds top rank and where the queen does not hold the top rank, actually confirmed the impression. But with a more detailed and rigorous analyses, we have been able to establish that the observation was a statistical artefact of small colony size. This kind of statistical artefact is not uncommon in the literature of ecology and evolution (Nachman and Heller 1999; Huston et al., 2000; Lytle 2001). Therefore, biologists should be aware of such artefact and any pattern observed should be verified by constructing appropriate null models.

Appendix

Let us consider a nest with n individuals with unique hierarchical ranks for each of them. If the rank of the queen is determined randomly, then the occurrence of each of the ranks 1,2,3,...,n will be equally likely. Then the average rank would be r_{av}=(1+2+3+....+n)/n = ($\Sigma_i\, i$)/n = (n(n+1)/2)/n = (n+1)/2. Therefore the theoretical curve describing the relationship between the average rank of the queen (Y) and the corresponding nest size (X) would follow a straight line Y=(X+1)/2 with the slope of m=0.5. The variance of the ranks can be calculated by using $\Sigma_i\,(i - r_{av})^2/n$ and with the help of the arithmetic series formula, the variance would come as $(n^2 - 1)/12$.

We have observational data from N=100 nests of varying sizes. We consider the size of each nest as ni where i runs from 1 to N and n_i varies from 3 to 77. The mean nest size where the queen holds the top rank would be $\mu=(\Sigma_i n_i P(n_i))/(\Sigma_i P(n_i))$ where $P(n_i)$ is the probability that the queen will hold the top rank in a nest of size n_i. If the ranks are distributed randomly, the probability $P(n_i)$ could be estimated as $1/n_i$. Then the required average is $N/\Sigma_i 1/n_i$ =100/8.180=12.225, where $\Sigma_i 1/n_i$=8.18 is being calculated from the data. The standard deviation can be calculated by using the formula $\sigma^2=\Sigma_i/n_i^2P(n_i)/\Sigma_i/P(n_i) - ((\Sigma_i/n_iP(n_i))/\Sigma_i/P(n_i))^2$ and since the data yields $\Sigma_i n_i$ =2043, σ becomes 10.015. The mean and standard deviation of the nest sizes in which the queen is not rank 1 can also be calculated by using the same formula with $P(n_i)=1-1/n_i$. From the data $\Sigma_i n_i^2$ =65127 is calculated, and the mean and s.d. turn out to be 21.161 and 15.468 respectively.

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References

Abbott D H, Saltzman W, Schultz-Darken N J and Tannenbaum P L (1998) Adaptations to subordinate status in female marmoset monkeys *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* **119** 261-274

Alexander R, Noonan K and Crespi B (1991) The evolution of eusociality. Pp. 3-44 *in* P W Sherman, J U M Jarvis and R D Alexander eds. *The Biology of the Naked Mole Rat* Princeton University Press, Princeton, New Jersey

Bang A, Deshpande S, Sumana A and Gadagkar R (2010) Choosing an appropriate index to construct dominance hierarchies in animal societies: A comparison of three indices *Anim Behav* **79** 631-636

Bang A (2010) Thesis: Determinants of behavioural and reproductive dominance in the primitively eusocial wasp *Ropalidia marginata*. Indian Institute of Science

Batra S W T (1966) Nests and social behavior of halictine bees of India (Hymenoptera: Halictidae) *Indian J Entomol* **28** 375-393

Bhadra A, Mitra A, Deshpande S A, Chandrasekhar K, Naik D G, Hefetz A and Gadagkar R (2010) Regulation of reproduction in the primitively eusocial wasp *Ropalidia marginata*: On the trail of the queen pheromone *J Chem Ecol* **36** 424-431

Bhattacharya K and Vicsek T (2010) Collective decision making in cohesive flocks *New J Phys* **12** 093019

Bloch G, Hefetz A and Hartfelder K (2000) Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*) *J Insect Physiol* **46** 1033-1040

Brahma A, Mandal S and Gadagkar R (2018) Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata Proc Natl Acad Sci* USA **115** 756-761

Bourke A (1988) Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis Behav Ecol Sociobiol* **23** 323-333

Bourke A (1999) Colony size, social complexity and reproductive conflict in social insects *J Evol Biol* **12** 245-257

Camazine S, Deneubourg J-L, Franks N R, Sneyd J, Theraulaz G and Bonabeau E (2003) *Self-Organization in Biological Systems* Princeton University Press, Princeton, New Jersey

Chandrashekara K and Gadagkar R (1991) Behavioural Castes, Dominance and Division of Labour in a Primitively Eusocial Wasp *Ethology* **87** 269-283

Clarke F and Faulkes C (1997) Dominance and queen succession in captive colonies of the eusocial naked mole–rat, *Heterocephalus glaber Proc R Soc Lond B Biol Sci* **264** 993-1000

Clutton-Brock T, Brotherton P, Smith R, McIlrath G, Kansky R, Gaynor D, M O'riain and Skinner J (1998) Infanticide and expulsion of females in a cooperative mammal *Proc R Soc*

Lond B Biol Sci **265** 2291-2295

Creel S, Creel N M, Mills M G and Monfort S L (1997) Rank and reproduction in cooperatively breeding African wild dogs: Behavioral and endocrine correlates *Behav Ecol* **8** 298-306

Dapporto L, Santini A, Dani F R and Turillazzi S (2007) Workers of a Polistes paper wasp detect the presence of their queen by chemical cues *Chemical senses* **32** 795-802

Dietemann V, Peeters C and Holldobler B (2005) Role of the queen in regulating reproduction in the bulldog ant *Myrmeciagulosa*: control or signalling? *Anim Behav* **69** 777-784

Faulkes C G and Bennett N C (2001) Family values: Group dynamics and social control of reproduction in African mole-rats *Trends in Ecol Evol* **16** 184-190

Fletcher D J and Ross K G (1985) Regulation of reproduction in eusocial Hymenoptera *Ann Rev Entomol* **30** 319-343

Frank L G (1986) Social organization of the spotted hyaena *Crocuta crocuta*. II. Dominance and reproduction *Anim Behav* **34** 1510-1527

Gabathuler U, Bennett N and Jarvis J (1996) The social structure and dominance hierarchy of the Mashona mole rat, *Cryptomys darlingi* (Rodentia: Bathyergidae) from Zimbabwe *Journal of Zoology* **240** 221-231

Gadagkar R (1991) *Belonogaster*, *Mischocyttarus*, *Parapolybia* and Independent-founding *Ropalidia*. Pp. 149-190 *in* K G Ross and R W Matthews eds. The Social Biology of wasps. Cornell University Press, Ithaca, New York

Gadagkar R (2001) The social biology of *Ropalidia marginata* : Toward understanding the evolution of eusociality. Harvard University Press, Cambridge, MA

Heinze J, Holldobler B and Peeters C (1994) Conflict and cooperation in ant societies *Naturwissenschaften* **81** 489-497

Hölldobler B and Wilson E O (1990) The Ants. Springer, Berlin

Huston M, Aarssen L, Austin M, Cade B, Fridley J, Garnier E, Grime J, Hodgson J, Lauenroth W and Thompson K (2000) No consistent effect of plant diversity on productivity *Science* **289** 1255a-1255

Jacobs D, Bennett N, Jarvis J and Crowe T (1991) The colony structure and dominance hierarchy of the Damaraland mole rat, *Cryptomys damarensis* (Rodentia: Bathyergidae), from Namibia *J Zool* **224** 553-576

Jeanne R L (1972) Social biology of the neotropical wasp *Mischocyttarus drewseni Bull Mus Comp Zool* **144** 63-150

Kardile S and Gadagkar R (2002) Docile sitters and active fighters in paper wasps: a tale of two queens *Die Naturwissenschaften* **89** 176-179

Kardile S and Gadagkar R (2003) Regulation of worker activity in the primitively eusocial wasp Ropalidia cyathiformis *Behaviour* **140** 1219-1234

Keane B, Waser P, Creel S, Creel N, Elliott L and Minchella D (1994) Subordinate reproduction in dwarf mongooses *Anim Behav* **47** 65-75

Keller L and Nonacs P (1993) The role of queen pheromones in social insects: Queen control or queen signal? *Anim Behav* **45** 787-794

Koenig W D, Haydock J and Stanback M T (1998) Reproductive roles in the cooperatively breeding acorn woodpecker: Incest avoidance versus reproductive competition *Am Nat* **151** 243-255

Kolmer K and Heinze J (2000) Rank orders and division of labour among unrelated cofounding ant queens *Proc R Soc Lond B Biol Sci* **267** 1729-1734

Lytle D A (2001) Convergent growth regulation in arthropods: biological fact or statistical artifact? *Oecologia* **128** 56-61

Michener C D (1969) Comparative social behavior of bees *Ann Rev Entomol* **14** 299-342

Mitra A and Gadagkar R (2011) Can Dufour's gland compounds honestly signal fertility in the primitively eusocial wasp *Ropalidia marginata*? Naturwissenschaften **98** 157-161

Mitra A and Gadagkar R (2012a) Queen signal should be honest to be involved in maintenance of eusociality: chemical correlates of fertility in *Ropalidia marginata Insectes Soc* **59** 251-255

Mitra A and Gadagkar R (2012b) Road to Royalty - Transition of Potential Queen to Queen in the Primitively Eusocial Wasp *Ropalidia marginata Ethology* **118** 694-702

Monnin T and Peeters C (1999) Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant *Behav Ecol* **10** 323-332

Monnin T, Ratnieks F L and Brandão C R (2003) Reproductive conflict in animal societies: hierarchy length increases with colony size in queenless ponerine ants *Behav Ecol and Sociobiology* **54** 71-79

Nachman G and Heller K E (1999) Fluctuating asymmetry as an index of fitness: Causality or statistical artifact? *Oikos* 357-365

Noirot C and Pasteels J (1988) The worker caste is polyphyletic in termites *Sociobiology* **14** 15-20

Page R E Jr and Amdam G V (2007) The making of a social insect: Developmental architectures of social design *Bio Essays* **29** 334-343

Pardi L (1948) Dominance order in *Polistes* wasps *Physiol Zool*

21 1-13

Poston J P (1997) Dominance, access to colonies, and queues for mating opportunities by male boat-tailed grackles *Behav Ecol Sociobiol* **41** 89-98

Premnath S, Chandrashekara K, Chandran S and Gadagkar R (1990) Constructing Dominance hierarchies in a primitively eusocial wasp. In: Social Insects and the Environment. Pp. 80 *in* G K Veeresh, B Mallik and C A Viraktamath, eds. Proceedings of 11th International Congress of IUSSI, Bangalore, India, August, 1990. Oxford & IBH Publishing Co. Ltd., New Delhi

Premnath S, Sinha A and Gadagkar R (1995) Regulation of worker activity in a primitively eusocial wasp, Ropalidia marginata *Behav Ecol* **6** 117-123

R Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Reeve H K (1991) *Polistes*. Pp. 99-148. *in* K G Ross and R W Matthews eds. The social biology of wasps. Cornell University Press, Ithaca

Reeve H K and Gamboa G J (1983) Colony activity integration in primitively eusocial wasps: the role of the queen (Polistes fuscatus, Hymenoptera: Vespidae) *Behav Ecol Sociobiol* **13** 63-74

Reeve H K and Gamboa G J (1987) Queen regulation of worker foraging in paper wasps: A social feedback control system (Polistes fuscatus, Hymenoptera: Vespidae) *Behaviour* **102** 147-167

Röseler P (1991) Reproductive competition during colony establishment. Pp. 309-335 *in* K G Ross and R W Matthews eds. The Social Biology of Wasps. Cornell University Press, Ithaca

Rosenthal C, Bennett N and Jarvis J (1992) The changes in the dominance hierarchy over time of a complete field captured colony of *Cryptomys hottentotus hottentotus J Zool* **228** 205-226

Ross K G and Matthews R W (1991) The Social biology of wasps. Cornell University Press, Ithaca

Schwarz M P and Woods R E (1994) Order of adult eclosion is a major determinant of reproductive dominance in the allodapine bee *Exoneura bicolor Anim Behav* **47** 373-378

Sledge M F, Boscaro F and Turillazzi S (2001) Cuticular hydrocarbons and reproductive status in the social wasp Polistes dominulus *Behav Ecol Sociobiol* **49** 401-409

Stacey P B and Koenig W D (1990) *Cooperative breeding in birds: Long term studies of ecology and behaviour* Cambridge University Press

Sumana A, Deshpande S A, Bhadra A and Gadagkar R (2008) Workers of the primitively eusocial wasp Ropalidia marginata do not perceive their queen across a wire mesh partition *J Ethol* **26** 207-212

Sumana A and Gadagkar R (2003) *Ropalidia marginata* – a primitively eusocial wasp society headed by docile queens *Curr Sci* **84** 1464-1468

Theraulaz G, Pratte M and Gervet J (1989) Effects of removal of α-individuals from a Polistes dominulus Christ wasp society: Changes in behavioural patterns resulting from hierarchical changes *Actes des Colloques Insectes Soc* **5** 169-179

Toth A L and Robinson G E (2007) Evo-devo and the evolution of social behavior *Trends Genet* **23** 334-341

Turillazzi S (1985) Colonial cycle of *Parischnogaster nigricans serrei* (Du Buysson) in west Java (Hymenoptera Stenogastrinae) *Insectes Soc* **32** 43-60

Van Doorn A and Heringa J (1986) The ontogeny of a dominance hierarchy in colonies of the Bumblebee *Bombus terrestris* (Hymenoptera, Apidae) *Insectes Soc* **33** 3-25

Wallace E and Bennett N (1998) The colony structure and social organization of the giant Zambian mole-rat, *Cryptomys mechowi J Zool* **244** 51-61

West-Eberhard M J (1969) The social biology of polistine wasps. Museum of Zoology, University of Michigan, Ann Arbor

West-Eberhard M J (1977) The establishment of reproductive dominance in social wasp colonies. Pp. 223-227. 8th International Congress International Union Study of Social Insects., Wageningen,The Netherlands

Wilson E O (1971) The Insect Societies. The Belknap Press of Harvard University Press, Cambridge, MA

Wilson E O (1975) Sociobiology: The New Synthesis. Pp. 697. Belknap Press of Harvard University Press, Cambirdge, MA.