

RESEARCH ARTICLE



Genetic relatedness does not predict the queen's successors in the primitively eusocial wasp, *Ropalidia marginata*

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Abstract. *Ropalidia marginata* is a social wasp in which colonies consist of a single fertile queen and several sterile workers. If the queen is removed, one of the workers, potential queen (PQ), becomes hyperaggressive and becomes the next queen. The identity of the PQ cannot be predicted in the presence of the queen. The probability of a worker succeeding the queen is uncorrelated with her body size, dominance rank, ovarian or mating status, but imperfectly correlated with her age. Here, we investigate whether genetic relatedness help to predict the queen's successors. We constructed models based on successors being (i) most closely related to the queen, (ii) most closely related to the immediate predecessor queen/PQ, or (iii) having the highest relatedness to the majority of the workers; and (iv) having the highest average relatedness to all the workers. We predicted five successors from each of these models using pair-wise genetic relatedness estimated from polymorphic microsatellite loci. We independently performed serial queen/PQ removal experiments and compared the observed sequence of successors with the predictions from the models. The predictions of none of the models matched the experimental results; on an average 5–6 individuals predicted by the models were bypassed in the experiment. Thus, genetic relatedness is inadequate to predict the queen's successors in this species. We discuss why relatedness sometimes predicts the patterns of altruistic behaviour and sometimes not, and argue that the cost and benefit terms in Hamilton's rule, i.e. ecology, should be vigorously investigated when relatedness does not have adequate explanatory power.

Keywords. genetic relatedness; microsatellites; social hymenoptera; queen succession; queue jumping; *Ropalidia marginata*.

Introduction

Social Hymenoptera, such as ants, bees and wasps organize themselves into colonies consisting of one or a small number of reproductives and a large number of non-reproductive workers. Males generally do not participate

in social life. In the so called advanced insect societies, such as those of honey bees, most ants and some wasps, the queen is a morphologically differentiated individual compared to the workers (Michener 1969; Wilson 1971; Johnson and Linksvayer 2010). Queen-destined larvae experience a different nutritional and hormonal environment leading to their differentiation during development. If a colony loses its queen, a new queen has to be generated from the larval stage. In so called primitively eusocial societies, on the other hand, queens and workers are morphologically undifferentiated and physiologically nearly so. Reproductive caste differentiation is therefore, a largely

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postimaginal phenomenon, i.e. caste differentiation takes place in the adult stage (De Wilde and Beetsma 1982; Fletcher and Ross 1985; Strambi 1985; Wheeler 1986; Gadagkar *et al.* 1988; O'Donnell 1998). This makes it possible for queen and worker roles to be reversible. If a primitively eusocial colony loses its queen for any reason, one of the workers can become the next queen of the colony. Indeed, most queens arise by replacing old queens in this manner.

In tropical species with perennial colony cycles, most queens may in fact be replacement queens (West Eberhard 1969; Chandrashekara and Gadagkar 1992; Shakarad and Gadagkar 1995; Gadagkar 2001). Because only queens reproduce and workers forego all or nearly all reproduction, and because most workers may be potentially capable of becoming queens, we might expect more competition among the workers to become queens. It is therefore of great interest to understand the factors that make some workers more likely to become queens and others less so. Although several investigators have studied the phenomenon of queen succession (Jeanne 1972; Litte 1979, 1981; Pardi and Marino Piccioli 1981; Hughes *et al.* 1987; Mead *et al.* 1995; O'Donnell 1998; Strassmann *et al.* 2004), perhaps it has not received the attention it deserves.

Ropalidia marginata is a primitively eusocial wasp widely distributed in peninsular India. Colonies of this wasp consist of a single fertile queen and varying numbers (1 to about 100) of sterile workers. The queen lays all eggs, both haploid male eggs as well as diploid female eggs. The workers perform all tasks required for building and maintaining the nests, and care for the brood. This requires them to leave the nest to forage for food, building material and water. Male wasps stay on their natal nest only for about a week and then leave to lead a nomadic life (Gadagkar 2001). Although the queen maintains reproductive monopoly during her tenure, she is replaced from time to time by one of the workers who functions as the next queen. We have studied the phenomenon of queen succession both in the laboratory and in the field (Chandrashekara and Gadagkar 1992; Premnath *et al.* 1996; Sumana and Gadagkar 2003; Deshpande *et al.* 2006; Bang and Gadagkar 2012). Upon experimental removal or natural loss of the queen, one and only one worker becomes hyperaggressive and become the next queen. This individual can be identified in about 30 min after the queen is removed (Bang and Gadagkar 2012). We refer to the queen's successor as potential queen (PQ) during her hyperaggressive phase before she lays her first egg. It is quite remarkable that the PQ is almost never challenged by other workers. Her hyperaggression is unidirectional, from her to all or most of the workers, but not from the workers to her (Chandrashekara and Gadagkar 1992; Gadagkar *et al.* 1993; Premnath *et al.* 1995, 1996). If the PQ is removed as soon as her identity becomes apparent, yet another worker (but only one) from the remaining set become hyperaggressive and goes on to become the next

queen. In this manner, we have demonstrated that there are at least five PQs (PQ1–PQ5) waiting their turn to become future queens (Bang and Gadagkar 2012).

We have made many attempts to predict the identity of the PQ during the presence of the previous queen, but so far we have met with little success. PQs appear to be undistinguished—they are not unique in any way detected so far, not in their body size, behavioural profile, pheromonal profile, ovarian development, mating status or dominance rank (Chandrashekara and Gadagkar 1992; Sumana and Gadagkar 2003; Deshpande *et al.* 2006; Mitra and Gadagkar 2012). Age is a statistically significant predictor of PQs although not a perfect predictor; it is not that the oldest individual becomes the PQ. On an average, at least one older individual is bypassed during every queen succession (Bang and Gadagkar 2012). Our inability to predict the identity of the PQ (in the presence of the previous queen) is all the more striking because we have evidence that the workers themselves seem to know the identity of the PQ (Bhadra and Gadagkar 2008). However, we have so far not tested the possibility that the PQ is chosen based on her genetic relatedness to the queen or to other workers in her colony. To test this possibility, we have now constructed and tested the predictions of four different models described below.

Materials and methods

Collection and maintenance of R. marginata nests

Four natural *R. marginata* colonies with 12–17 individuals were collected from Bengaluru, India. Once located in the wild, each of the nests were collected along with the stock and placed inside plastic boxes with the combs facing above. Individuals that were not sitting on the nest during this process were collected separately in glass vials and released into the boxes. The nests were affixed on a wooden board with its natural stock using super glue. These nests were then placed inside a 45 × 45 × 45 cm wooden cage with wire-meshed doors and all the individuals were released in it. The cages were kept closed for a few days for the wasps to settle before beginning experimental manipulation. During this period the wasps were fed with hand. Once settled, each individual was uniquely marked with quick drying enamel paints. Subsequently, serial queen/PQ removal experiments were performed on these nests.

Serial PQ removal experiment

Serial PQ removal experiment was performed on four such colonies. Before removing any wasp, the colony was observed for 10 h spread across 2–3 days, during which the egg layer in the colony was identified as the queen. On the morning of the third day, the queen was removed from the colony and stored at –20°C and

simultaneously, the nest was continued to be observed and all the dominance behaviours were recorded. Dominance/subordinate behaviours are computed as the sum of the frequencies per hour at which the following nine behaviours namely, peck, nibble, attack, chase, sit on top of another individual, being offered food, crash, hold another individual by mouth and aggressive biting are observed (Chandrashekar and Gadagkar 1991; Gadagkar 2001). The PQ (PQ1) was identified as the worker that has shown at least five acts of dominance and at least twice the number of dominance than any other worker (Bang and Gadagkar 2012). These criteria were evaluated after observing the nest for a minimum of 30 min or as long as was necessary for the criteria to be fulfilled, following the removal of the queen. Once the PQ (PQ1) was identified, she was removed and stored at -20°C and the colony was observed to identify the next PQ (PQ2). Once the PQ2 was identified, she was also removed and stored at -20°C . This process was continued until the PQ5 could be identified, following which, the PQ5 and rest of the workers in the colony were collected and stored for further analysis.

DNA extraction and PCR

DNA was extracted from the head, thorax or legs of individual wasps using the 'hot sodium hydroxide and Tris' method or 'HotSHOT' method (Truett *et al.* 2000). Briefly, a piece of the tissue was taken in a PCR tube and 75 μL of lysis reagent (25 mM NaOH and 0.2 mM EDTA) was added to it. This was then heated at 95°C for 30 min in a thermal cycler and cooled to room temperature. Following this, the supernatant from the tube was pipetted out into a new tube and 75 μL of neutralization buffer (40 mM Tris HCl) was added to the tube. This was then stored at -20°C and used for PCR reactions. Microsatellite markers for *R. marginata* were developed and previously described (Johny *et al.* 2009), seven of which were used in this study, i.e. Rmsat11, Rmsat13, Rmsat19, Rmsat65, Rmsat67, Rmsat74 and Rmsat83. We attempted to amplify all the individuals for these seven loci. Each PCR reaction consisted of 2 μL DNA, 1 μL PCR buffer, 1 μL 1 mM dNTP, 0.8 μL MgCl_2 , 0.2 or 0.3 μL of the forward and reverse primers and 0.1 μL of the thermostable Taq polymerase enzyme. The forward primer was fluorescently labelled (see Johny *et al.* 2009 for details). The final volume was made up to 10 μL with water. The PCR reactions were performed in an Applied Biosystems GeneAmp PCR System 9700. The same temperature profile was used for all the loci, which consisted of an initial denaturation at 94°C for 3 min, followed by amplification cycle of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min 30 s, repeated 35 times, and a final extension of 72°C for 10 min. The reaction was then cooled to 4°C and used for genotyping the amplicons (Johny *et al.* 2009).

Genotyping

The amplified products were genotyped using capillary electrophoresis in ABI PRISM 3730 DNA Analyzer. To each well of a 96 well genotyping plate, 12 μL HiDi formamide and 0.3 or 0.5 μL of either GeneScan 500 ROX or GeneScan 500 LIZ were added. Subsequently, 0.5 μL of the amplified product was added to each of the wells. Here, HiDi formamide stabilizes the single stranded DNA following denaturation due to the heating of the plate performed in the next step, and ROX/LIZ acts as size standard for each sample. Following the addition of the PCR product, the plate was covered with a septum and heated to 96°C for 5 min and snap-frozen in ice. This was then processed in ABI PRISM 3730 DNA Analyzer. The machine uses POP-7 as the sieving matrix for the electrophoretic reaction. The results of each run were viewed and scored in ABI PRISM GeneMapper ver. 3.0. The allele sizes for each sample were entered in a Excel sheet which was later formatted to a Convert (Glaubitz 2004) readable file. Tandem was used to bin the allele sizes (Matschiner and Salzburger 2009).

Description of the genetic data and estimation of relatedness

Descriptive statistics about the genetic data were estimated using Genetic Data Analysis (Lewis and Zaykin 2001). The number of individuals (n), number of alleles per locus (A), the expected heterozygosity (H_e) and observed heterozygosity (H_o) and inbreeding coefficient (f) following Weir and Cockerham (1984) are provided in table 1 in sections A and B in electronic supplementary material at <http://www.ias.ac.in/jgenet/>. ' A ' varied from 2 (Rmsat67) to 7 (Rmsat19). Observed heterozygosity was maximum for Rmsat18 (0.90) and minimum for Rmsat67 (0.15). Exact tests for Hardy–Weinberg equilibrium (HWE) were performed in ML-Relate (Kalinowski *et al.* 2006) with the alternative hypothesis of heterozygote deficiency (H1, heterozygote deficient; 10,000 randomization steps) (Guo and Thompson 1992; Rousset and Raymond 1995). All the seven loci were observed to be in HWE. Pair-wise genetic relatedness values were calculated using ML-Relate (Kalinowski *et al.* 2006). Statistical analyses were performed using Excel, Statistica 7 (StatSoft 2006) and R (R Core Team 2015; R Studio Team 2015).

Models tested

Here, we tried to determine whether the queen's successor can be predicted from nestmate relatedness. To achieve this we constructed the following four models. (i) Model 1: the worker with the highest relatedness with the queen becomes PQ1, the next highest becomes PQ2 and so on. (ii) Model 2: the worker with the highest relatedness with the queen becomes PQ1, the one with the highest relatedness with PQ1 becomes PQ2 and so on. (iii) Model 3:

in this model, each individual ‘votes’ for the nestmate she is maximally related to. The worker who gets the maximum number of votes becomes PQ1, the next highest becomes PQ2 and so on. (iv) Model 4: for this model, we define average individual pair-wise genetic relatedness, $R_i = \sum_{j=1}^N r_{ij}/N$, where r_{ij} is the relatedness between individuals i and j , and N is the total number of individuals in the colony. The worker with the highest R_i becomes PQ1, the next highest becomes PQ2 and so on. Details of these models are given in sections A and B in electronic supplementary material and are summarized in table 2 in electronic supplementary material.

Queue jumping analysis

A predicted hierarchy was constructed based on each of the four models described above, and it was compared with the sequence of successors observed in the serial PQ removal experiments. For each model, the number of wasps in the predicted hierarchy that were bypassed in the observed hierarchy was counted for each successor of each colony, and a grand mean was calculated.

Results

We know from previous work that if the queen of an *R. marginata* colony is experimentally removed, one and only one worker becomes hyperaggressive and will become the next queen, if the original queen is not returned. This hyperaggressive individual has therefore been labelled as PQ. We also know that if a PQ is removed soon after she is identified by her hyperaggression, one and only one individual from the remaining set of workers will become hyperaggressive and will become the next queen, and so on. We have thus, previously identified five successive potential queens (PQ1–PQ5) in several colonies and demonstrated that there is a reproductive queue which is implemented upon the loss of the queen (Premnath *et al.* 1996; Sumana and Gadagkar 2003; Bhadra and Gadagkar 2008; Bang and Gadagkar 2012). Here, we have used this experimental paradigm to identify five successive potential queens in each of four experimental colonies of *R. marginata* (figure 1).

After completing the serial queen/PQ removal experiments, we genotyped all individuals for seven polymorphic microsatellite loci in the above four colonies and computed genetic relatedness between all pairs of individuals in a colony. From the pairwise-relatedness values, we predicted five successive potential queens for each colony based on four theoretical models. The models were based on the following four different ways in which genetic relatedness might potentially influence queen succession: successors (i) being most closely related to the queen (model 1), (ii) being most related to the immediate predecessor queen/PQ (model 2), (iii) having the highest relatedness to the majority of the workers (model 3), and (iv) having the

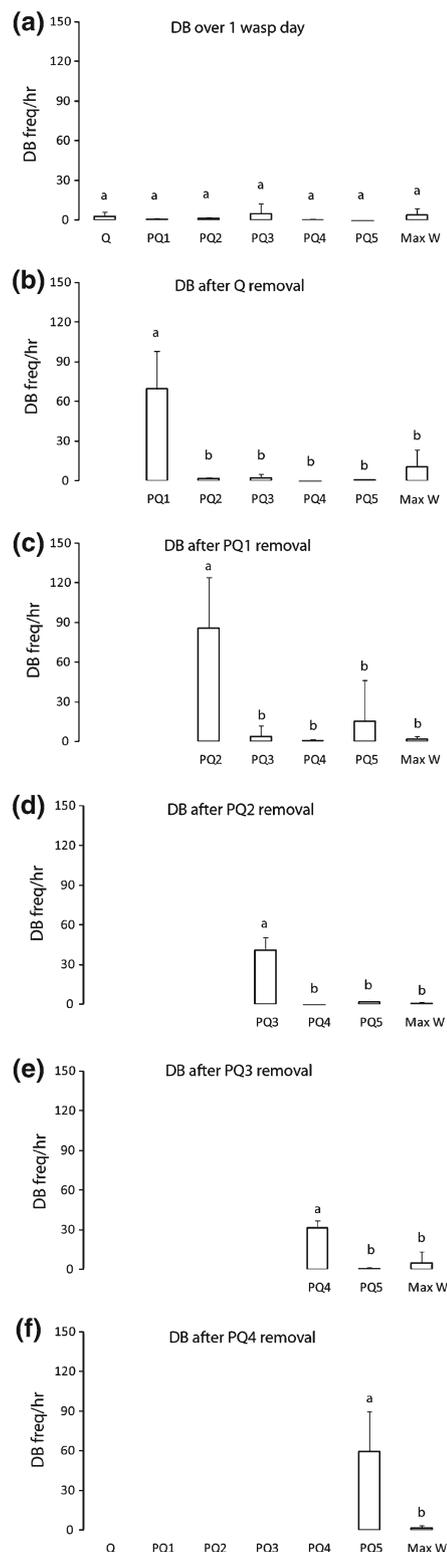


Figure 1. Frequency of dominance behaviour (Mean \pm SD of DB freq./hr) displayed by the queen, the potential queens (PQ1–PQ5) and max worker (Max W), i.e. the worker that showed maximum DB other than the queen and the PQs. Panels (a) represents a queen right colony, and the scenarios at the sequential removal of the successors are shown in (b–f).

highest average relatedness to all the workers (model 4). Finally, we compared the five successive potential queens identified in each of the four colonies in the serial queen/PQ removal experiment with the predictions of each of the models. We found many mismatches between the PQs identified in the queen/PQ removal experiment and the PQs predicted by each of the models. We quantified these mismatches through a queue jumping analysis in which we counted, for each PQ in the queen/PQ removal experiment, the number of individuals predicted to be the successor by the model which she bypassed. The distribution of relatedness for each of the colonies is provided in section D of the in electronic supplementary material. Average (± 1 SD) colony relatedness studied in the four colonies ranged from 0.49 ± 0.26 to 0.55 ± 0.19 .

The result of the queue jumping analysis is summarized in section C of the electronic supplementary material. In case of model 1, on an average, 2.00 ± 2.37 individuals were bypassed by the PQ1, although overall 5.08 ± 3.44 individuals were bypassed across the five PQs. In case of model 2, the result for the queen was expectedly identical as the same hierarchy was tested in both the cases. However, for PQs 2–5, on the averages of 9.25 ± 5.91 , 5.25 ± 2.87 , 4.88 ± 3.01 and 6.63 ± 3.04 , respectively, were bypassed. In this case, the overall mean was 5.60 ± 4.10 . In case of model 3, the average queue jumping ranged from 3.38 ± 1.65 (PQ5) to 8.00 ± 2.35 (PQ3) with the overall mean being 5.85 ± 3.59 . For model 4, the overall mean was 5.73 ± 3.92 with PQs 1–5 on average the bypassing respectively, 7.13 ± 5.17 , 6.75 ± 5.56 , 6.63 ± 1.60 , 3.75 ± 1.63 and 4.38 ± 3.45 , individuals. A summary of this queue jumping analysis is shown in table 1 and the complete results are in section C of the electronic supplementary material.

Discussion

The seminal papers of Hamilton (1964a,b) focussed attention on genetic relatedness as an important modulator of social interactions, especially those involving self-sacrificing altruistic behaviour. Although Hamilton's rule ($b \cdot r > c$) (also referred to as kin selection or inclusive fitness theory) predicts the spread of an altruistic allele depending on the relative values of the cost of altruism to the actor (c), the benefit of altruism to the recipient (b) and the genetic relatedness between actor and recipient (r), the major conceptually novel idea in Hamilton's theory of inclusive fitness was that self-sacrificing, altruistic behaviours could be favoured by natural selection, if they are directed to sufficiently close genetic relatives, i.e. if $b/c > 1/r$. Not surprisingly, many studies have since examined the effect of genetic relatedness on social behaviour, often ignoring the benefit and cost terms. The conceptual justification for focussing exclusively on genetic relatedness is based on the idea that relatedness can tilt the balance between selfish and altruistic behaviours, if the benefit and cost are roughly

equal. It follows therefore that if relatedness alone does not predict the observed patterns of altruistic behaviour, then the costs and benefits are not equal. As might be expected from this argument, many studies have found that relatedness predicts patterns of altruistic behaviour, while many others have found that relatedness fails to do so.

Cooperative breeding in birds and mammals offers an attractive paradigm for testing the role of relatedness in modulating altruistic behaviour. In cooperatively breeding species, one or more individuals potentially capable of reproducing by themselves, forgo the opportunity to do so and instead devote their time and energy to assist a different individual/s (usually their parents or siblings) to reproduce. Such helping behaviour was found to be broadly consistent with the prediction that altruism should be directed to close relatives rather than distant relatives in the pied kingfisher (Reyer 1984), bell miners (Clarke 1984, 1989), white-fronted bee eaters (Emlen and Wrege 1988), Galapagos mockingbirds (Curry 1988), pinyon jays (Marzluff and Balda 1990), Seychelles warblers (Komdeur 1994), long-tailed tits (Russell and Hatchwell 2001), African wild dogs (Girman *et al.* 1997) and brown hyenas (Owens and Owens 1984), to cite just a few examples. Similar consistency with altruism being preferentially directed to close relatives was seen in many communally breeding species, where the altruists may not forgo reproduction altogether, but may nevertheless expend some time and energy to help other individuals who are not their offspring. Examples include giving alarm calls in ground squirrels (Sherman 1977), allonursing in mouse lemurs (Eberle and Kappeler 2006) and lions (Bertram 1976; Pusey and Packer 1994), and feeding in Japanese macaques (Yamada 1963). Conversely, agonistic behaviour, such as aggression in yellow-billed marmots (Armitage and Johns 1982) seems to be preferentially directed to nonrelatives. Evidence for the role of relatedness was also found in the very fact that groups and coalitions in lions (Bertram 1976; Pusey and Packer 1994) and bats (Wilkinson 1987) are usually formed with close relatives rather than distant relatives or nonrelatives. Indeed it has sometimes been claimed that kinship itself can be predicted from behavioural interactions (Walters 1981).

On the other hand, in cooperatively or communally breeding cichlid fishes (Awata *et al.* 2005; Stiver *et al.* 2008), Venezuelan striped-back wren (Piper 1994), dunlocks (Davies 1992), carrion crows (Canestrari *et al.* 2005), fairy-wrens (Dunn *et al.* 1995), Arabian babblers (Wright *et al.* 1999), white-browed scrubwrens (Magrath and Whittingham 1997; Whittingham *et al.* 1997), greater ani (Riehl 2011), Galapagos hawks (DeLay *et al.* 1996), subdesert mesites (Seddon *et al.* 2005), meerkats (Clutton-Brock *et al.* 2000, 2001), moustached tamarins (Huck *et al.* 2005) and common marmoset (Faulkes *et al.* 2003), helpers are either unrelated to the breeders, helping propensities are uncorrelated with relatedness, or groups do not necessarily consist of close kin.

Social Hymenoptera are expected to offer even better paradigms for testing the role of relatedness. Because of their haplodiploid genetics, relatedness asymmetries such as those between sisters versus daughters, theoretically create more opportunities for relatedness to modulate altruistic behaviour even when costs and benefits are not exactly equal. There are at least four contexts in which the role of relatedness in promoting altruistic behaviour was studied. Firstly, colonies of social Hymenoptera (within which altruism is typically shown) are almost always family groups with relatively high genetic relatedness (Strassmann *et al.* 1989; Tsuchida 1994; Crozier and Pamilo 1996; Goropashnaya *et al.* 2001; Feldhaar *et al.* 2005; Sundström *et al.* 2005; Bolton *et al.* 2006; Seppä *et al.* 2011; Uddin and Tsuchida 2012). Secondly, in Epiponine wasps, although there are a number of queens per colony, cyclical variation in queen number and their close relatedness to each other leads to a relatively high genetic relatedness among the workers (Queller *et al.* 1993a). Thirdly, when relatedness to males and females is unequal, differential allocation of resources to favour the more highly related sex is predicted (Trivers and Hare 1976). There is evidence in favour of this prediction in several species of ants (Ward 1983; Herbers 1984; Chan and Bourke 1994; Sundstrom 1994; Deslippe and Savolainen 1995; Sundström *et al.* 1996), bees (Mueller 1991; Packer and Owen 1994) and wasps (Queller *et al.* 1993b). Fourthly, in yet another effect of relatedness, it was predicted that worker policing should be observed in species with multiply-mated queens and not in species with singly-mated queens (Starr 1984; Woyciechowski and Łomnicki 1987; Ratnieks 1988). There is considerable evidence in support of these predictions (Wenseleers and Ratnieks 2006), although the predictions themselves may not be so robust when the effect of worker policing on colony efficiency is simultaneously considered (Olejarczyk *et al.* 2016).

On the other hand, colony relatedness is quite low in many species of ants (Chapuisat *et al.* 1997; Beye *et al.* 1998; Cole and Wiernasz 1999; Brown and Keller 2000; Wiernasz *et al.* 2004; Kellner *et al.* 2007), wasps (Strassmann *et al.* 1997; Landi *et al.* 2003) and bees (Estoup *et al.* 1994; Danforth *et al.* 2003). Moreover, in several species of ants (Hagen *et al.* 1988; Carew *et al.* 1997; Gadau *et al.* 1998; Bernasconi and Strassmann 1999; Schlüns *et al.* 2009; Cahan and Helms 2012) and wasps (Queller *et al.* 2000; Liebert and Starks 2006; Gadagkar 2011; Leadbeater *et al.* 2011; Seppä *et al.* 2011), cofoundresses can be unrelated. Similarly, results contrary to the expectations of sex allocation theory were observed in a number of ants (Trivers and Hare 1976; Nonacs 1986; Bourke 1989; Helms 1999; Brown and Keller 2000; Hammond *et al.* 2003). Worker policing theory has also had its failures, e.g. in honeybees (Pirk *et al.* 2003, 2004; Loope *et al.* 2013), the vespine wasp *Dolichovespula saxonica* (Bonckaert *et al.* 2011) and the ant, *Leptothorax acervorum* (Hammond *et al.* 2003). In the tropical, primitively eusocial wasp

R. marginata, the subject of this study, colonies consist of a single queen at any given time and this queen can mate with up to three males lowering relatedness among daughters from the theoretically expected 0.75 to about 0.5 (Muralidharan *et al.* 1986). Queens are periodically replaced by workers who become new queens in the same colony so that there is a system of serial polygyny (Gadagkar *et al.* 1990, 1993). New queens may be daughters, sisters, nieces or cousins of their immediate predecessor queen resulting in highly variable worker–brood relationships ranging from full-siblings all the way up to cousins and cousins' offspring. All of this can significantly lower intracolony relatedness among workers and brood with values ranging about 0.2–0.4 (Gadagkar *et al.* 1991). Young unrelated conspecifics are readily accepted on to young colonies at least in the laboratory. Such alien intruders may also go on to become future queens of their foster colonies (Venkataraman and Gadagkar 1995; Arathi and Gadagkar 1996; Arathi *et al.* 1997). There is no evidence of intracolony kin discrimination or nepotism (Venkataraman *et al.* 1988). Therefore, it was argued that relatedness by itself has little explanatory power in understanding the patterns of social behaviour in this species. It was also shown that ecological, physiological and demographic factors play an important role in tilting the balance between selfishness and altruism, therefore, suggesting that the *b* and *c* terms in Hamilton's rule may be more important than the *r* term (Gadagkar 1990, 1991, 2001, 2016).

In this paper, we have examined whether queen succession in *R. marginata* can be predicted based on genetic relatedness alone. To do this, we have estimated pair-wise genetic relatedness for all wasps in four colonies after conducting serial queen/PQ removal experiments and experimentally identified five successive potential queens in each colony. Comparing the observed succession with the order of succession predicted from four models based on genetic relatedness, we find considerable mismatch between the order of succession observed and that predicted by relatedness. Unlike in *Polistes dominulus* (Cant *et al.* 2006), we did not find support for the prediction that the queen's or the PQ's successor is most closely related to her (models 1 and 2 in this study). We also did not find support for the prediction that the successor is chosen, so as to maximize the inclusive fitness of the highest number of nestmates (model 3 in our study). Neither did we find support for the prediction that the successor is chosen so as to maximize the average inclusive fitness of the colony (model 4 in our study). This is reminiscent of the observation in *P. dominulus*, where subordinate females were observed to accept successors unrelated to themselves (Monnin *et al.* 2009). Clearly, relatedness alone is inadequate to predict the order of queen succession in *R. marginata*.

We therefore see that, whether among birds and mammals or among social Hymenoptera, relatedness by itself can explain altruism in some cases, but not in others. Why should this be so? In *R. marginata*, for example, although

Table 1. A summary of the queue jumping analysis.

	PQ1	PQ2	PQ3	PQ4	PQ5	Mean
Model 1	2.00 ± 2.37	7.13 ± 3.47	6.88 ± 2.25	6.50 ± 2.62	2.88 ± 1.67	5.08 ± 3.44
Model 2	2.00 ± 2.37	9.25 ± 5.91	5.25 ± 2.87	4.88 ± 3.01	6.63 ± 3.04	5.6 ± 4.10
Model 3	6.63 ± 3.71	7.13 ± 5.36	8.00 ± 2.35	4.13 ± 3.09	3.38 ± 1.65	5.85 ± 3.59
Model 4	7.13 ± 5.17	6.75 ± 5.56	6.63 ± 1.60	3.75 ± 3.50	4.38 ± 3.45	5.73 ± 3.92

Mean (\pm SD) of number of individuals predicted to be successors in each of the models, bypassed by each of the PQs in the queen/PQ removal experiment, across all the four colonies are given. Mean in the last column refers to the average queue jumping across all the five PQs and across all the four colonies for that particular model.

relatedness by itself has little explanatory power, when combined with an analysis of the costs and benefits, Hamilton's rule is indeed a powerful explanatory framework for understanding the evolution of social behaviour. In other words, the *b* and *c* terms in Hamilton's rule are rather important in some situations perhaps because the *b* and *c* terms are very different from each other. Such inequality between costs and benefits are expected under rather harsh ecological conditions. In a mild environment, for example, individuals may be easily capable of leaving their natal nests and founding their own new nests. In such a situation, whether they stay back in their natal nests as nonreproductive helpers or leave to reproduce on their own would depend largely on their relatedness to their nestmates as compared to the relatedness to their own offspring in the new nests. On the other hand, in an ecologically harsh environment, the probability of successfully founding a new nest would be very low and individuals would therefore be selected to stay back in their natal nests even if relatedness to their nestmates is rather low. Because their success outside is likely to be low, the cost they pay by foregoing direct reproduction is relatively low compared to the (inclusive fitness) benefits they gain by staying back. It follows therefore, that ecological and other factors that might make costs and benefits unequal should be investigated, especially vigorously in species, where relatedness by itself is inadequate to understand the forces that mould the evolution of social behaviour.

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