Worker interests and male production in *Polistes gallicus*, a Mediterranean social wasp

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Abstract

The resolution of social conflict in colonies may accord with the interests of the most numerous party. In social insect colonies with single once-mated queens, workers are more closely related to the workers' sons than they are to the queens' sons. Therefore, they should prefer workers to produce males, against the queen's interests. Workers are capable of producing males as they arise from unfertilized eggs. We found *Polistes gallicus* to have colonies of single, once-mated queens, as determined by microsatellite genotyping of the workers, so worker interests predict worker male production. In colonies lacking queens, workers produced the males, but not in colonies with original queens. Thus worker interests were expressed only when the queen was gone. The high fraction of missing queens and early end to the colony cycle relative to climate so early in the season is surprising and may indicate a forceful elimination of the queen.

Introduction

One of the important questions of biology is how advanced sociality, represented by functionally sterile helping individuals, is maintained in nonclonal groups with conflicting genetic interests. Hamilton (1964a, b) derived the inclusive fitness framework for addressing this question and was also the first to appreciate the consequences of within-group conflicts, a field subsequently greatly expanded by others (Hamilton, 1972; Trivers & Hare, 1976; Ratnieks, 1988; Seger, 1991; Ratnieks & Reeve, 1992; Bourke & Franks, 1995; Crozier & Pamilo, 1996; Queller & Strassmann, 1998). One of the key arenas for exploring within-colony conflicts is over male production in hymenopteran colonies (Woyciechowski & Lomnicki, 1987; Ratnieks, 1988; Reeve & Ratnieks, 1993; Bourke, 1994). All females, in all colony structures, prefer to produce males themselves because relatedness to sons (1/2) is higher than relatedness to any other male. If there is a single, once-mated queen, worker relatedness to brothers (1/4) is lower than relatedness to nephews, the progeny of other workers (3/8), so workers should favour male production by workers, in conflict with the queen. If the queen mates more than twice, workers are more closely related to brothers than to the progeny of other workers that are a mix of full and half sisters. This leads to the famous policing prediction, that workers should prevent each other from laying eggs in favour of the queen when queens are multiply mated (Woyciechowski & Lomnicki, 1987; Ratnieks, 1988). However, most social insects have queens that mate only once, a situation that can cause worker/queen conflict over male production (Strassmann, 2001). The resolution of conflict in colonies with single, once-mated queens can be assessed by evaluating who actually produces the males.

The outcome of the hypothetical genetic conflict between workers and the queen depends on several factors. First, is the genetic conflict a real conflict? The inclusive fitness interests of workers and queens depend

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not only on relatedness, but also on costs and benefits of alternatives (Hamilton, 1964a, b). If the cost of worker production is too high, then queens will continue to produce males (Foster & Ratnieks, 2001). Second, it could be that workers have a clear interest favouring worker male production, but they are not able to exercise that interest because of the power advantage of the queen. Perhaps the only way they can effectively exercise their interests is by killing the queen (Bourke, 1994; Foster & Ratnieks, 2001).

Polistes is a cosmopolitan genus of wasps that is particularly interesting for studies of conflict of interests within colonies because of their small size and flexible social organization (Reeve, 1991; Strassmann, 1996). In *Polistes bellicosus* and *P. dorsalis* Arévalo *et al*. (1998) found that males were produced by the queen, not by the workers. Thus, the interests of the queen, and not the workers, were met. We studied *P. gallicus,* a common circum-Mediterranean species characterized by colonies started by a single queen in open locations (Cervo & Turillazzi, 1989; Dani & Cervo, 1992). *P. gallicus* is particularly interesting because workers have been documented to have significantly more developed ovaries on nests where an usurpation (take-over by a foreign queen) occurred in the period just before worker emergence (Cervo & Turillazzi, 1989). Workers in usurped colonies are also more aggressive towards an usurping queen than they are towards their own mother if she is still queen (Dani *et al*., 1994). The capability of workers to develop their ovaries and behave aggressively in response to a social event which reduced relatedness of the workers to the brood suggests that they might also do so in other social circumstances. Another factor which makes this species particularly interesting for worker reproduction is its high rate of nest predation which might also increase queen mortality (Dani & Cervo, 1992). As there are no subordinate foundresses in this singly founded species, worker reproduction could fill this void.

Materials and methods

We identified a wild population of *P. gallicus* for study in a 1-ha plot of *Inula viscosa* south-east of Florence. We censused all colonies for presence of the queen on 6 July 1996, and collected the colonies and their associated wasps on 23 July 1996, 16 days later. Queens were identified by their larger size and frayed wingtips (Dani, 1994). In all, we collected 19 colonies. Missing numbers in the series represent colonies predated between the census and collection date (Table 1).

We counted the number of mature and, separately, nearly mature eggs in the collected females' ovaries, and measured the length of the longest oocyte (whether mature or immature). Females had mated if we observed sperm in their spermathecae. We also noted if they had copious fat bodies common in prehibernating females destined to be the next year's foundresses.

We attempted to genotype eight workers, eight male pupae and eight eggs from every colony. Morphological traits were used to identify male pupae (males have 13 antennal segments; females have 12). These identities

Table 1 Male production and colony characteristics. See text for an explanation of likelihood-based estimates of male production.

Nest ID	Queen present 23 July	Queen present 6 July	Proportion queen prod. pupae	Proportion queen prod. eggs	No. workers	No. adult males	Proportion future gynes	No. pupae	No. larvae	No. eggs	No. empty cells	No. genotyped workers	No. genotpyed male pupae	No. genotyped male eggs	No. unique worker alleles
1	no		0.08	1.0	28	9	0.000	64	32	17	36	16	8	1	2
2	no	yes	0	0	9	0	0.222	7	26	17	8	9	1	2	0
3	no		1.0	0	8	0	0.000	8	13	12	45	8	5	6	0
4	yes	yes	1.0	0.44	18	0	0.000	37	38	23	42	9	3	5	0
5	no		0	0	38	1	0.432	52	36	32	29	22	8	2	2
6	yes	yes	1.0		38	2	0.000	44	26	11	70	12	8	0	2
7	no	yes	1.0	0	8	0	0.000	14	18	20	22	8	1	1	1
8	no		0.42	0.52	19	0	0.000	49	67	35	51	11	8	5	3
9	yes		1.0	1.0	28	0	0.000	26	45	9	80	9	5	4	1
10	no	yes	1.0		21	2	0.095	49	38	43	40	17	8	0	2
12	yes	yes	1.0	0.72	28	0	0.448	81	17	14	101	15	7	4	1
13	no	yes	0	0	13	1	0.231	3	23	12	15	12	3	3	0
14	yes	yes	1.0	1.0	34	3	0.273	47	51	24	76	11	8	5	0
16	no	yes	0.01		22	5	0.048	28	26	21	32	15	8	0	2
17	no	no	1.0	0	53	1	0.020	75	60	64	86	27	8	5	3
18	no	yes		0.20	36	1	0.114	37	44	20	106	14	0	2	1
21	no	no	0.17	0.30	8	7	0.375	19	26	14	37	8	7	5	2
22	no	yes	1.0	1.0	20	0	0.250	27	33	14	16	9	2	3	0
23	no		1.0	0	47	3	0.043	52	38	15	123	10	8	1	0

were later confirmed by their haploid genotypes which was how we identified male eggs (see below). In addition to the eight workers, we genotyped all females with ovarian development. Some colonies had fewer than eight male pupae. Samples with fewer than three loci were dropped from the analysis. In all we successfully genotyped 242 female adults, 106 male pupae, 51 male eggs and 22 female eggs, a total of 426 individuals.

We used four polymorphic DNA microsatellite loci, Pbe430AAT, Pbe411AAT, Pbe269AAG and Pbe205AAG (Strassmann *et al.*, 1997). These loci, originally developed for *P. bellicosus*, had 12, 4, 9 and 7 alleles, respectively, in our population of *P. gallicus*. In polymerase chain reactions we used the annealing temperatures close to those originally optimized for these loci in *P. bellicosus* and standard techniques (Strassmann *et al.*, 1996, 1997).

We used these loci to infer the queen and her mate's genotypes when she was not collected, to determine which eggs were male, who produced the males, and in whose genetic interests this allocation of reproduction was. We were able to infer the queen and her mate's genotype even when she was not collected by inspecting the genotypes of the collected workers. In all, 238 of 242 workers could be assigned to a single once-mated queen.

We were able to identify male eggs by their single alleles at all loci. The chance that a female would be homozygous at all four loci, and thus appear to be a male, was only 0.0175, so we are unlikely to have misassigned our 73 eggs. This was supported by our observation that none of the adult females we genotyped were homozygous across all loci.

We used a maximum likelihood method to determine who produced the males, because it is more powerful than exclusion methods (Tóth *et al.*, 2002). With this method we calculated the most likely value of the parameter, *Q*, which is the fraction of males produced by queens as opposed to workers (Arévalo *et al.*, 1998), using a version of the program modified for colonies with a single once-mated queen (Tóth *et al.*, 2002). For each of the colonies we calculated the relative likelihood of values of *Q* as: $L = K \prod_{males}\left(Q \prod_{loci} f_{qi} + (1-Q) \prod_{loci} f_{wi}\right)$. *K* is a multinomial constant that never has to be calculated because it multiplies all *L*'s by a constant. For each male allele considered in turn, f_{qi} and f_{wi} are the expected frequencies of that allele in the queen and in the workers. We calculate *L* for all values of *Q* between 0 and 1 at increments of 0.01 (Excel spreadsheet for this available from DCQ). For each colony, all *L*'s were divided by the maximum *L* in order to represent them on a single scale from zero to one.

Information on genetic relatedness is necessary to assess whose genetic interests are being met by the observed male production. We estimated genetic relatedness within colonies from our DNA microsatellite data using the computer program Relatedness 5.07 for Macintosh (Goodnight & Queller, 1999; available at http://Gsoft.smu.edu/Gsoft.html). Standard errors were based on jackknifing over loci for within-colony estimates and over colonies for population estimates (Queller & Goodnight, 1989). We weighted colonies equally in all analyses. We estimated 95% confidence intervals assuming the jackknifed pseudovalues followed a *t*-distribution with degrees of freedom equal to one minus colony number.

Results

Colony condition and queen presence

At the census on 6 July 1996, we determined that 11 colonies still had queens, two did not and six were ambiguous. We collected all females and their nests before dawn on 23 July 1996. At this time only five colonies still had queens. There were no demographic differences between colonies with queens and those lacking queens using either queen presence on 6 July or 23 July. The two types of colonies did not differ in numbers of workers, males, proportion future queens, cells, eggs, larvae, pupae, empty cells or proportion empty cells (Mann–Whitney *U*-tests, *P*-values all above 0.08).

There was no genetic evidence of usurpation on any of the colonies. Only two colonies had any evidence of females that were not full sisters, daughters of a single once-mated queen. In one of these colonies (no. 22) there was a single worker whose genotype did not match those of the other females, so she probably drifted from a nearby nest. The other colony (no. 13) had three misfit females that could not have been explained by multiple mating by the queen. In all, 238 females were full sisters with their nestmates (98.3%).

A number of indicators placed these colonies close to the end of their cycle (Table 1). All nests had empty cells, making up 14–58% of total cells. All nests were producing males. Eleven had adult males. All but one colony (no. 18) had male pupae and all had male eggs. Thirteen colonies had young mated females with enlarged fat bodies indicating preparation for hibernation.

What are worker interests in male production?

Worker relatedness was higher to worker-produced males than it was to queen-produced males (Fig. 1). This is true overall, considering all colonies, and also separately for colonies with males produced by workers and colonies with males produced by queens. Thus, when queens are present, worker genetic interests favour worker male production over queen male production.

Who produced the males?

Who produced the males depended on whether or not the queen was present. All five colonies with a collected

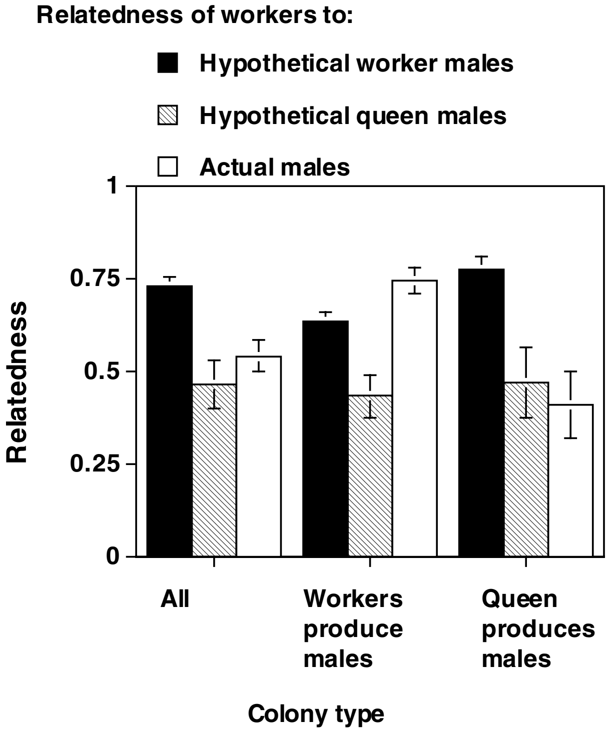

Fig. 1 Histogram showing the comparison of overall relatedness of workers to hypothetical worker-produced males (the same as worker–worker relatedness, black bars), to hypothetical queen-produced males (the same as worker–queen relatedness, striped bars), and to actual males (clear bars). Shown separately are these values for the whole population, for colonies with queens and for colonies lacking queens. Standard errors on the relatedness values are also indicated.

queen, who was always the mother of the workers, had male pupae and eggs that were most likely queen-produced (Table 1; colonies 4, 6, 9, 12 and 14). The queen had also produced the male pupae on an additional six colonies (Table 1; colonies 3, 7, 10, 17, 22 and 23). Three of these colonies had a queen at the 6 July census and one did not; queen presence was not determined on the other two. On four of these six colonies lacking queens, but having queen-produced pupae, the eggs were produced by the workers, indicating that queens died between the time of pupa and egg production on these colonies (Table 1; colonies 3, 7, 17 and 23). One of the other two colonies had queen-produced eggs (colony 22), and the other lacked genotyped male eggs (colony 10).

In the remaining eight colonies, all lacking a collected queen, workers produced most of the males. The only apparent exception was colony 8 where queenship was not determined on 6 July and this colony had a mix of queen- and worker-produced pupae and eggs. Four of these colonies clearly had worker-produced eggs.

Evidently, most male production in *P. gallicus* follows quite a simple rule: queens usually produce males when they are present. Otherwise workers produce the males. We might expect that there are more workers with developed ovaries in colonies lacking queens than in colonies with queens. But this was not the case. All colonies had some workers with ovarian development. Colonies with queens averaged 34% of workers with ovarian development, not significantly less than the 44% of workers with ovarian development in colonies lacking queens (Mann–Whitney *U*, $P > 0.31$). Furthermore, the degree of development was not greater in colonies lacking queens, measured by average length of the longest oocyte ($P > 0.64$) or by average number of mature and nearly mature eggs in ovaries ($P > 0.20$).

Discussion

Workers in our sample of colonies of *P. gallicus* were overwhelmingly full sisters, consistent with a single once-mated queen. This result is in accord with the observation that mated *P. gallicus* queens begin nests alone. We did not expect this simple relatedness structure, because of the frequency of usurpation reported for this species. Dani & Cervo (1992) observed 11 cases of usurpation in a population of about 40 colonies that survived to the time just before worker emergence. The usurping queens generally came from colonies whose nests had been taken by predators, and predation rates were also high in our population. If our study had contained usurped colonies we should have detected it through genetic analyses.

The relatedness structure that we found in *P. gallicus* means that it would be in the worker's interests to produce the males instead of letting the queen produce them. However, *P. gallicus* workers do not produce males in the presence of the queen, so their relatedness interests are not met. This was also the case for the North American *P. bellicosus* and *P. dorsalis* (Arévalo *et al.*, 1998). These results can be explained in several ways. The queen herself may prevent workers from producing males, either by eating worker-laid eggs, or by preventing workers from laying eggs. Workers may actually refrain from male production because it has a cost in overall productivity of the colony.

Although the workers did not produce males in the presence of the queen in *P. gallicus*, queens were present in only 26% of collected colonies. Early loss of the queen has also been reported in earlier studies of *P. gallicus* (Turillazzi & Conte, 1981; Dani, 1994). Comparing colonies with and without queens in our sample does not clarify how queens were lost. If queens are attacked by workers, we would expect it in colonies where worker interests favour worker male production but, our colonies did not differ in relatedness structure. We also expect queen eviction where workers might have more power, but colonies lacking queens did not have more workers than those with queens. We do know that workers actually have the ability to kill usurping queens by stinging them, something observed in two of 32 usurped nests from another study of *P. gallicus* (Cervo & Dani, unpublished data). What we do not know is whether they use this ability to evict their natural queens.

Several indirect lines of evidence support the hypothesis that the workers kill or evict the queen so that workers can produce males. Queen death is very high in this species, compared with co-occurring congeners, *P. dominulus* and *P. nimphus*. In *P. dominulus,* 20% (five of 24) in 1995, and 15% (three of 20) in 2000, of single foundress colonies had lost their queens by mid to late July (unpublished data). In *P. nimphus* 46% (six of 13) in 1995 and 0% (zero of nine) in 2000 of single-foundress colonies had lost their queens by mid to late July (unpublished data). Greater queen mortality in *P. gallicus*, compared with *P. dominulus* and *P. nimphus* could be explained by the more open nest sites of *P. gallicus,* if open nests increase queen mortality. Open nest sites clearly increase predation on the nests and the brood contained in them, but our nests were not predated during July when they lost so many queens.

One of the possible consequences of matricidal worker/queen conflict over male production is for queens to produce males earlier and earlier, and for workers to respond by killing queens earlier and earlier. The colony cycle ends very early in *P. gallicus*, with male production in July, when it is still hot, and prey are still abundant. This is earlier than male production in *P. dominulus* and *P. nimphus.*

Matricide in social insects can occur in a variety of contexts, one of which appears to be to allow worker production of males (Bourke, 1994). The model developed by Bourke (1994) predicted matricide for worker male production to be most common in annual species where males are produced in a specific period near the end of the colony cycle, so there is no cost of lost female production. Other factors which favour matricide are declining queen productivity, a queen that is producing only males, and single mating by the queen. *Polistes gallicus* fits two of these conditions, annual nesting cycle and single mating, but these conditions also fit congeners that show no signs of matricide.

Matricide for male production has been reported in bumblebees and vespine wasps (Bourke, 1994; Foster & Ratnieks, 2001). In vespine wasps, species where queens mate once were more likely to have a missing queen late in the season than were colonies where queens had mated with two or more mates, in accord with kin-conflict predictions (Foster & Ratnieks, 2001). It is hard to say whether matricide occurs because of declining queen productivity or for worker male-production interests if it occurs when queens are producing only males.

Polistes gallicus has a simple rule for male production. Queens produce males when they are present; when queens are gone workers do. Although there are some intriguing circumstantial indicators that workers cause the disappearance of queens in *P. gallicus,* future behavioural studies will more clearly reveal if this is the case.

Acknowledgments

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