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Research article

Worker policing in the European hornet *Vespa crabro*

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Summary. Although generally capable of producing males, workers in most hymenopteran societies (bees, ants and wasps) perform little or no reproduction in the presence of the queen. We investigated why workers do not reproduce in the European hornet *Vespa crabro* (L.). Previous genetic and behavioural work on this species had shown that, although queen mating frequency is low (effective paternity, 1.1) causing workers to be more related to workers' sons than to the queen's sons, workers do not lay eggs and the males reared are all the queen's sons. This suggested that workers are under queen pheromonal control. Here we show that this is not the case. Using egg introduction experiments, we show that worker policing behaviour occurs. We introduced queen-laid and worker-laid eggs into four discriminator colonies in five trials. In colonies with a queen, workers removed significantly more worker-laid than queen-laid eggs (1/79 worker-laid eggs but 46/72 queen-laid eggs remained after 16 h, $p < 0.001$). In colonies without a queen, workers removed significantly more queen-laid than worker laid eggs (30/44 worker-laid eggs but 13/41 queen-laid eggs remained after 16 h, $p \leq 0.001$). The presence of worker policing in queen-right hornet colonies provides a proximate explanation for the absence of worker reproduction. Workers are not under queen control but instead are collectively enforcing their own sterility. Worker policing at low paternity may have been selected for because it enhances colony productivity by eliminating costly conflicts over reproduction.

Key words: Worker policing, worker reproduction, *Vespa crabro*, hornets, reproductive conflict.

Introduction

Workers in many hymenopteran societies perform little or no reproduction (Bourke, 1988; Bourke and Franks, 1995). However, in almost all species workers are capable of reproduction. In species with morphologically distinct queens and workers, workers are generally unable to mate but possess ovaries and will lay haploid, male eggs in the absence of the queen (Wilson, 1971; Bourke, 1988). Furthermore, workers are generally more related to each other's sons than to the queen's sons (Hamilton, 1964; Starr, 1984). The absence of worker reproduction in the presence of the queen, therefore, requires explanation (Bourke and Franks, 1995).

Queen control is likely to be important in some species (Wilson, 1971; Ratnieks, 1988). Physical queen control seems to occur in small colony species (e. g., *Polistes*, Arevalo et al., 1998). However, in larger colonies physical control seems unlikely (Ratnieks, 1988). It has been suggested that in large colonies physical queen control gives way to pheromonal control (Wilson, 1971; Hölldobler and Bartz, 1985). However, this idea was criticised by Seeley (1985) and Keller and Nonacs (1993) who argued that workers should simply ignore such a queen control pheromone if it was against their fitness interests. In support of this, workers have been shown to adjust the sex ratio against the queen's interest by selectively killing brothers (e. g., Sundström et al., 1996).

The absence of worker reproduction may alternatively be explained by worker policing (Ratnieks, 1988) or reproductive self-restraint (Bourke and Franks, 1995). Worker policing is especially likely to be selected in single-queen societies when the mother queen is inseminated by multiple males (effective paternity > 2), because workers will be more related to the queen's sons ($r = 0.25$) than other workers' sons ($r < 0.25$) (Starr, 1984; Ratnieks, 1988). However, worker policing, and also reproductive self-restraint, may be selected for at any paternity if worker reproduction significantly

reduces colony productivity (Ratnieks, 1988; Bourke and Franks, 1995). Selection for worker policing at paternity < 2 may also result from an interaction between the conflicts over male production and sex allocation (Foster and Ratnieks, 2002). In ants, it is known that workers perform sex allocation biasing by killing male *larvae* (e.g., Sundström et al., 1996). This favours worker policing of eggs because policing reduces the cost of male larvae killing by removing some of the males at very low cost at the egg stage (Foster and Ratnieks, 2002).

Worker policing at paternity < 2 has been found in in two vespine wasp species, *Vespula vulgaris* (L.) and *Dolichovespula saxonica* (F.). In egg introduction experiments into two queenright discriminator colonies of *V. vulgaris* (effective paternity = 1.9), all worker-laid eggs were removed by workers whilst the majority of queen-laid eggs remained (0/120 worker-laid versus 80/120 queen-laid eggs remained after 16 h) (Foster and Ratnieks, 2001). Furthermore, genetic analysis of 270 males from 9 colonies revealed only queens' sons despite the presence of a significant number of reproductive workers (5–25 per colony). In *D. saxonica*, evidence for facultative policing was found with workers policing more in multiple paternity colonies than single paternity colonies (Foster and Ratnieks, 2000). However, paternity was biased in the multiple paternity colonies such that policing was often also occurring at paternity < 2.

The aim of this study was to investigate the absence of worker reproduction in another vespine wasp, the European hornet, *Vespa crabro*. Most colonies are headed by a singly mated queen and population-wide effective paternity is low (1.11, Foster et al., 1999) leading to the prediction of worker reproduction in queenright colonies. However, a subsequent study suggested that workers do not lay eggs, rarely have activated ovaries and that 282 males from 15 colonies were all queens' sons (Foster et al., 2000). This raised the possibility that hornet workers were under queen pheromonal control (Foster et al., 2000). Here we test the alternative hypothesis that workers do not lay because their eggs will only be removed by other workers, as suggested by the discovery of worker policing in the other vespine genera (above). Using the same egg transfer techniques used by Foster and Ratnieks (2001) on *Vespula vulgaris*, we again find that workers in queenright colonies remove worker-laid eggs but leave the majority of queen-laid eggs. This suggests that workers are not under queen control but instead are collectively enforcing their own sterility.

Methods

Relocation of hornet colonies

We collected ten colonies of the European hornet, *Vespa crabro*, in the New Forest, Hampshire, UK from pest control calls during July to September 2000. All were removed at the request of residents as they were in close proximity to human habitation. We relocated them (as described in Foster et al., 2000) to wooden nest boxes (30 × 30 × 40 cm) attached to trees in private woodland near Ashurst, New Forest. The boxes had a 4cm diameter entrance at the front and were hinged at the top allowing experimental access. We studied two additional colonies. One we relocated by moving the rabbit hutch it was in to Ashurst and the other we studied *in situ* in a barn near Holmsley, New Forest.

Study colonies

Two of the ten nests relocated to boxes became well established and developed to reproductive status, but none remained queenright. We used these two successful nests as queenless discriminator colonies (see below) and as a source of worker-laid eggs. The rabbit hutch and barn colonies both remained queenright and developed to reproductive status. These were used as queenright discriminator colonies and as sources of queen-laid eggs. Previous work had shown that workers never or very rarely lay eggs in queenright colonies (Foster et al., 2000), so we can be confident that eggs from queenright colonies were queen-laid. An additional two colonies which failed to establish were used as sources of worker-laid (Colony 1, queenless) and queen-laid eggs (Colony 2, queenright), immediately after their collection (Table 1).

Policing assay

We investigated worker policing using the egg transfer techniques applied by Foster and Ratnieks (2001) on *Vespula vulgaris*. Foster and Ratnieks (2001) showed that in *V. vulgaris* workers' eggs were preferentially removed over queens' eggs for two conditions. (1) Eggs from own colony: queen-laid eggs were from the test colony itself and worker-laid eggs were from isolated groups of workers from the test colony. (2) Eggs from different colonies: queen-laid and worker-laid eggs came from two additional colonies to the colony that was tested for worker policing. In this study we used only the latter test where all eggs are from foreign colonies. This is more conservative test because in (1) discrimination against worker eggs could be due to their foreign origin (the isolated worker groups) compared to queen-laid eggs which were obtained from the test colony itself (Foster and Ratnieks, 2001).

We introduced queen and worker-laid eggs from foreign colonies into discriminator colonies in five trials in late August/early September (Table 1). Each trial involved three colonies, a discriminator colony, a queen-laid eggs source colony and a worker-laid eggs source colony. A policing trial was started by removing the bottom comb from each of the three colonies. We removed and discarded all the eggs and brood in the discriminator test comb. Next, eggs were removed from the source combs using a pair of forceps. Eggs in vespine wasps are glued to the

Figure 1. Photographs of the discriminator test comb. After the foreign queen-laid and worker-laid eggs had been glued into the comb, the comb itself was glued to a piece of dowel, which was inserted into soil in a flower pot (left). The comb could then be reintroduced into the colony in its original position (right)

paper comb and so were removed with a small piece of paper attached, ca. 3 × 3 mm. We then glued the eggs by their paper attachments, using water-based PVA glue, into the test comb in alternating rows of queen-laid and worker-laid eggs. Finally, we replaced the test comb now containing the foreign queen-laid and worker-laid eggs into the discriminator colony (Fig. 1). The numbers of queen-laid and worker-laid eggs remaining were checked after 45 min and 16 h. The glued eggs' paper attachments made it possible to locate the experimental rows, even when the eggs had been removed. To exclude the possibility of queen policing in the queenright colonies, we checked the test comb for the queen's presence every 10 min prior to the first check.

Results

Queenright colonies removed significantly more worker-laid eggs and queenless colonies removed significantly more queen-laid eggs. This was highly significant in every trial at both the first and the second check, except for Trial 5 which was only significant at the second check (Table 1). The dissociation between queenright and queenless colonies is most strongly illustrated by trials 2 and 5 where the discriminator colonies were given eggs from the same two source colonies. The Oak Tree colony was slower to remove eggs than the other colonies (Trial 5). This can be explained by a its small size – approximately 20 workers at the time of testing. The other colonies all had in excess of 100 workers during the tests.

In the queenright colonies, 10–15 workers were seen on the test comb at each 10-minute check, while the queen was only seen on a test comb once and this was at the time of the first check (trial 4). This strongly suggests that workers were responsible for the egg removal that had occurred at the first check. The queen, however, may have contributed to the removal of eggs between the 45-minute and 16-hour checks. Newly laid eggs could be identified as they were not on the small paper attachments that we glued in. These were found after 16 h, in trials 3 and 4 and were not included in the data.

Discussion

Our data suggest that worker policing by egg eating occurs in queenright colonies of the European hornet, *Vespa crabro*. Egg removal in the two queenright colonies is highly comparable to that found in two colonies of *Vespula vulgaris* (0/120 worker-laid eggs versus 80/120 queen-laid eggs remaining after 16 h, Foster and Ratnieks, 2001) and is consistent with genetic data showing the absence of worker sons in 282 males from 15 colonies of *V. crabro* (Foster et al., 2000). These data provide a proximate explanation for the absence of attempted worker reproduction in hornet societies (Foster et al., 2000). Effective policing will favour workers investing in the colony rather than in reproduction because attempted reproduction is likely to fail and so provide little benefit to the laying worker. Indeed the low level of worker ovary activation in the species suggests that policing proves an effective deterrent to reproduction (Foster et al., 2000; Foster and Ratnieks, 2001). However, with single mating by queens the norm in *V. crabro* (effective paternity 1.11, Foster et al., 1999) worker policing is not predicted on relatedness grounds. Workers are considerably more related to the nephews that they kill (r = 0.35) than to the brothers that they spare (r = 0.25). This suggests that other factors, such as costs to colony productivity associated with worker reproduction (Ratnieks, 1988) or sex allocation biasing by workers (Foster and Ratnieks, 2002) favour worker policing (see introduction).

Hornet workers in the two queenless nests performed the opposite discrimination to workers in the two queenright nests, removing significantly more queen-laid eggs than worker-laid eggs. This strengthens the conclusion that worker policing in queenright colonies is a real phenomenon by suggesting that the policing response is facultative. At this stage it is not clear whether the discrimination by queenless workers is adaptive. However, it may have evolved to combat

Table 1. Removal of introduced eggs in colonies of *Vespa crabro*. The number of introduced eggs at the start of each trial, after 45 min and after 16 h is shown

Trial	Discriminator colony	Queen present	Egg type	Egg source colony	Start	1st check	2nd check	Q v W at 2nd check
1	Rabbit Hutch	Yes	W	Colony 1	26	5	0	
			Q	Colony 2	17	14	14	$p < 0.001$
2	Rabbit Hutch	Yes	W	Orchard	25	0	0	
			Q	Martin's Barn	25	12	12	$p < 0.001$
4	Martin's Barn	Yes	W	Oak tree	28	11	1	
			Q	Rabbit Hutch	30	26	20	$p < 0.001$
3	Orchard	No	W	Colony 1	20	12	7	
			Q	Rabbit Hutch	20	2	0	$p = 0.004$*
5	Oak Tree	No	W	Orchard	24	24	23	
			Q	Martin's Barn	21	20	13	$p = 0.006$*

Workers selectively discriminate against worker-laid eggs in *queenright* colonies but against queen-laid eggs in *queenless* colonies. The trials are numbered in the order that they were performed. * Indicates that a 1-tailed Fisher's exact test was used as expected values in some cells were less than five. For all others trials, a Chi-square test was used. All tests used a contingency table of the form Queen laid eggs/Worker Laid eggs × Eaten/Not Eaten. This comparison is also highly significant at the 1st check in all trials ($p < 0.01$), except for trial 5

intraspecific parasitism. Genetic and behavioural evidence suggest that usurpation of queenright nests by foreign queens is common in *V. crabro* (see Foster et al., 2000). Queens, therefore, may also usurp already queenless nests. If this is true, workers will be unrelated to the new queen and will benefit from removing her eggs to favour their own offspring. This contrasts with perennial societies such as the honeybee *Apis mellifera* (L.) in which workers raise a new sister queen when the old queen dies, and queenless workers must tolerate queen-laid eggs in order to allow the new queen's offspring to be reared.

Our evidence for worker policing in *Vespa crabro,* which has mean paternity close to one, suggests that worker policing can evolve in societies of any kin structure. This contributes to a growing body of evidence suggesting that worker policing is of widespread importance in the eusocial Hymenoptera (Foster and Ratnieks, 2001). Worker policing has been demonstrated in the honeybee, *Apis mellifera* (Ratnieks and Visscher, 1989) and two vespine wasp genera in addition to *Vespa* (Foster and Ratnieks, 2000; Foster and Ratnieks, 2001). Furthermore, worker policing by mutual aggression occurs in queenless ants (Liebig et al., 1999; Gobin et al., 1999), where it also seems to occur both in line with and against relatedness predictions (Kikuta and Tsuji, 1999; Monnin and Ratnieks, 2001). Just how common worker policing is remains to be seen. However, it seems likely to prove a major piece in the puzzle of why workers do not reproduce.

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