Insect. Soc. 53 (2006) 399 – 402
0020-1812/06/040399-4
DOI 10.1007/s00040-005-0885-6

Insectes Sociaux

Research article

Worker policing in the common wasp *Vespula vulgaris* is not aimed at improving colony hygiene

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Received 30 January 2006; revised 2 May 2006; accepted 5 May 2006.

Abstract. In insect societies, eggs laid by workers are frequently killed by other workers – a behaviour known as ”worker policing”. The traditional explanation of worker policing is that it is a mechanism to resolve intra-colony conflict, and maintain the reproductive monopoly of the queen. Recently, Pirk et al. (2004) proposed that worker policing instead is aimed at removing unviable worker-laid eggs and is ultimately just another example of hygienic behaviour. Here we test this hypothesis for the common wasp *Vespula vulgaris*, a species with highly effective worker policing. We show that worker-laid eggs from queenless colonies have a lower hatch rate (68%) than queen-laid eggs (82%). Analysis of egg laying rates of queens and workers, however, shows that the difference is not big enough to explain the apparent absence of adult worker-derived males in this species.

Keywords: Worker reproduction, worker policing, egg viability, *Vespula vulgaris*, common wasp.

Introduction

Worker policing, whereby workers selectively kill eggs laid by other workers, is an important mechanism of conflict resolution in insect societies (Ratnieks and Wenseleers, 2005; Ratnieks et al., 2006). It prevents unfertilised worker-laid eggs from being reared into adult males, and thereby helps to maintain the reproductive primacy of the queen. Although the presence of worker policing is now known in many species of ants, bees and wasps (Ratnieks et al., 2006), its selective basis remains controversial (Gadagkar, 2004; Hammond and Keller, 2004; Wenseleers and Ratnieks, 2006b). At the ultimate level, several factors have been suggested which can select for worker policing, including the favouring of more closely related queen's sons, improving colony efficiency, or helping to cause a female-biased sex allocation (Ratnieks, 1988; Foster and Ratnieks, 2001b; Wenseleers and Ratnieks, 2006). In addition, a fourth explanation was recently proposed by Pirk et al. (2004). Based on the observation that worker-laid eggs had lower viability than queen-laid eggs in the honey bee *Apis mellifera*, Pirk et al. (2004) suggested that worker policing is simply aimed at removing dead worker-laid eggs and is ultimately just another example of hygienic behaviour (Gadagkar, 2004; Pirk et al., 2004). However, subsequent research showed that dead queen-laid eggs are not policed (Beekman and Oldroyd, 2005), unlike what would be expected if removing dead eggs was the reason for worker policing.

In this study we determine the viability of queen-laid and worker-laid eggs in the common wasp *Vespula vulgaris*, a species with highly effective worker policing (Foster and Ratnieks, 2001a). Furthermore, we analyse worker and queen egg-laying rates, to examine if the differences in viability can explain the rarity of adult worker-derived males. Our results clearly show that worker policing in the common wasp is aimed at resolving intra-colony conflict and not at removing dead eggs and improving colony hygiene.

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Methods

Study species

We studied the common wasp *Vespula vulgaris*, a species with highly effective worker policing, which rears zero or few workers' sons in colonies with a queen (Foster and Ratnieks, 2001a). However, workers' sons are routinely reared in orphan colonies (Potter, 1965; Ross, 1985). Colonies of the common wasp were collected near Sheffield, UK, and Leuven, Belgium in August 2005, transferred to wooden nest boxes and then placed on the outside wall of the laboratory where they could forage freely (cf. Wenseleers et al., 2005a, 2005b).

Comparison of hatch rates of queen-laid and worker-laid eggs

Queenless colony fragments or colonies which did not contain a mother queen when collected were used as sources of worker-laid eggs. Queenright colony fragments were used as sources of queen-laid eggs (we assumed that a negligible proportion of these eggs was worker-laid, since most worker-laid eggs are killed within 1 hour after having been transferred, Foster and Ratnieks, 2001a, and most of the eggs are older than 1h, given that we sampled eggs laid during a 24h period). When egg laying by workers commenced in the queenless colonies, both colony types were sampled for eggs to compare their hatching success. First, a comb of cells was removed from the colony and the eggs carefully removed using forceps. The comb was then put back into the colony and removed 24 hours later. Any newly-laid eggs were counted and the combs were put into a plastic box (4cm x 10 cm x 20 cm) containing a piece of moist tissue paper and incubated at 25°C, close to natural temperatures maintained in Vespinae wasp colonies (Edwards, 1980). The proportion of eggs which had hatched into first instar larvae was determined 5 days later (Edwards, 1980). Eggs that had not hatched after the five-day period had shrivelled and were clearly dead. Hatching success was determined blind as the person inspecting the comb did not know if it contained queen-laid or worker-laid eggs.

Estimating the expected percentage of workers' sons based on mortality effects

Based on our data for the hatching rates of queen-laid and worker-laid eggs, we also calculated what proportion of the adult males were expected to be workers' sons if workers only policed dead eggs, assuming no differences in viability of larvae. To estimate this, we first calculated the proportion of eggs that are worker-laid in queenright colonies as

$$\mathrm{A}=\mathrm{n_W.p.e_W}/(\mathrm{n_W.p.e_W}+\mathrm{e_Q}) \tag{1}$$

where n_W is the mean colony size (number of workers), p is the proportion of workers with active ovaries and e_W and e_Q are the mean numbers of eggs laid by a single reproductive worker and by the queen per day. The mean number of eggs laid per day by a single reproductive worker e_W was calculated from 5 small queenless units, each containing 10–50 workers, which were presented with an empty comb, allowed to lay eggs for 5 days and dissected at the end. Ovary active workers were defined as those having ovaries containing oocytes at least half the size of a freshly-laid egg (Foster and Ratnieks, 2001a). The proportion of worker with active ovaries in queenright colonies p is 1.04% (12/1,150, Foster and Ratnieks, 2001a). The mean colony size n_W of reproductive-stage queenright colonies in August / September is 2,042 workers (n=46 colonies, Spradbery, 1971). The number of eggs laid per day by the queen e_Q at the same time is 161, given the presence of an average of 805 eggs (n=46 colonies, Spradbery, 1971), and a development time of eggs of 5 days. From A, we calculated the proportion of male eggs that are worker-laid as

$$B=A/(A+(1\text{-}A).m) \tag{2}$$

where m is the proportion of the queen's eggs that are male, which has been estimated at 50.60% (85/168) (Foster and Ratnieks, 2001a). Given the observed hatching rates of queen-laid (h_Q) and worker-laid (h_W) eggs, the estimated proportion of adult males that would be workers' sons if workers only policed dead eggs is

$$C=B.h_W/(B.h_W+(1\text{-}B).h_Q) \tag{3}$$

95% confidence limits on A, B and C were calculated as the 2.5% and 97.5% percentiles of simulated distributions, whereby n_W and e_Q were taken as constants and p, m, h_W and h_Q as binomial parameters. e_W was calculated as one fifth of the total number of eggs laid during 5 days divided by the total number of reproductive workers present, and with the total number of eggs laid and the total number of reproductive workers present as Poisson distributed. In all cases was the distribution of the parameters consistent with the theoretical distributions used.

Our model assumes that egg laying rates of reproductive workers are similar in queenright and queenless colonies. This assumption is supported by the finding that the proportion of reproductive workers that had highly developed (>90%) ovaries was not significantly different between queenless and queenright colonies (34/53 and 5/12 respectively, Table 2 and Foster and Ratnieks, 2001a, Fisher's exact test p=0.20). Hence, the fertility of reproductive workers was likely the same in both. Furthermore, the model assumes similar viability of worker laid eggs in queenless and queenright colonies. In support of this assumption, several species of Vespine wasps successfully rear worker-laid eggs into adults in queenright colonies (Foster and Ratnieks, 2001c; Wenseleers et al., 2005a; Wenseleers and Ratnieks, 2006a).

Results and discussion

Hatching success was high in both colony types, and overall 75% of all eggs hatched (Table 1, n = 2,077 eggs from 10 queenright and 10 queenless colonies, mean number of eggs per colony = 103.9, s.d. = 76.1). The hatching success of worker-laid eggs was lower than that of queen-laid eggs (727/1,071 = 68% vs. 827/1,006 = 82%, Fisher's exact test, p = 10^{-14}, Mann-Whitney test for colony means, U = 81.5, p = 0.02, n = 20). The mean hatching success was not different between study localities (Fisher's exact test, p = 0.198, using colony means: Mann-Whitney test, U = 58.5, p = 0.52, n = 20). The same conclusions are reached if queenless 4 & 5 and queenright 1 from Sheffield) are left out. The greater mortality of worker-laid eggs was primarily due to fungus growth, which was more apparent in the combs collected from queenless colonies. In fact, a slight amount of fungus occurred in all but one (Sheffield 2) of the combs collected from queenless colonies, whereas fungus was present only in 3 of the 10 combs collected from queenright colonies (in colonies Leuven 1 and Sheffield 1 and 3). Presumably this was because workers in queenless colonies invest less time in colony maintenance activities (Potter, 1965; Ross, 1985). Thus, our measured hatching rates of worker-laid eggs are minimum estimates, and the true hatching success of worker-laid eggs under natural conditions in queenright colonies may well be higher, which would further support our conclusions. Alternatively, recessive deleterious alleles might also explain the slightly lower hatching success of worker-laid eggs, as all of those are haploid, whereas the queen's eggs would have comprised a mix and haploid and diploid eggs.

Table 1. Hatching rates of queen-laid and worker-laid eggs in colonies of the common wasp.

Colony	Collection locality	Eggs laid by	# of eggs	# hatched	% hatched
1	Sheffield	workers	25	13	52%
2	Sheffield	workers	100	82	82%
3	Sheffield	workers	87	53	61%
4	Sheffield	workers	5	2	40%
v5	Sheffield	workers	10	7	70%
1	Leuven	workers	149	89	60%
v2	Leuven	workers	182	128	70%
3	Leuven	workers	269	183	68%
4	Leuven	workers	145	107	74%
5	Leuven	workers	99	63	64%
Total / Avg.			1071	727	68%
1	Sheffield	queen	20	0	0%
2	Sheffield	queen	245	237	97%
3	Sheffield	queen	86	65	76%
4	Sheffield	queen	38	26	68%
5	Sheffield	queen	42	39	93%
1	Leuven	queen	58	45	78%
2	Leuven	queen	168	128	76%
3	Leuven	queen	79	71	90%
4	Leuven	queen	92	72	78%
5	Leuven	queen	178	144	81%
Total / Avg.			1006	827	82%

Table 2. Rates of ovary activation and reproductive rates of workers in queenless colony fragments of the common wasp.

Source colony	Locality	# eggs laid in 5 days	# workers with active ovaries[a]	total # of workers	# eggs laid per reproductive worker per day
1	Leuven	41	13	25	0.63
2	Leuven	103	15	35	1.37
3	Leuven	29	2	10	2.9
4	Leuven	45	5	28	1.8
5	Leuven	63	18	43	0.7
Total / Avg.		281	53b	141	1.63

[a] With oocytes in their ovaries at least 50% the size of a freshly laid egg. [b] 34 out of 53 (64%) had more than 90% developed ovaries.

The difference in hatching rates between queen-laid and worker-laid eggs is too small to account for the lack of workers' sons in queenright colonies. Our analysis of worker egg-laying rates shows that observed differences in hatching rate alone cannot explain the inferred absence or rarity of adult worker-derived males (Foster and Ratnieks, 2001a). In queenless colonies, a total of 53 reproductive workers laid 281 eggs over a period of 5 days (Table 2, n=5 colonies). Hence, the mean number of eggs laid per reproductive worker per day was 1.06. This leads to an estimated 12.3% (A) and 21.7% (B) of all eggs and male eggs, respectively, being worker-laid in queenright colonies (95% *C.L.* on A and B: 6.1%–20.1% and 11.3%–33.4%). Observation of a single queenright colony in Sheffield gave a figure that closely matches this estimate, with 4/29 (13.8%) of the eggs being laid by workers over a period of 16 hours. If workers only policed dead eggs, and allowed all viable eggs to develop, 18.6% of the adult males would hence be expected to be workers' sons (95% *C.L.* on C = 9.4%–29.5%). That Foster and Ratnieks (2001a) found none in a sample of 171 assignable males, despite a power of 95% to detect a worker contribution of at least 2%, provides clear evidence that policing in this species removes a large proportion of viable worker-laid eggs, and is not just carried out to remove dead eggs. Furthermore, in policing trials 88% of worker-laid eggs are removed within one hour of being introduced (Foster and Ratnieks, 2001a), and are thus presumably alive.

Our data also show that low egg viability does not ultimately explain the low proportion of egg-laying workers in queenright colonies of *V. vulgaris* (Foster and Ratnieks, 2001a). Inclusive fitness models predict such acquiescence only when the relative survival of worker-laid eggs is less than 10% of that of queen-laid eggs, for relatedness and colony size of *V. vulgaris* (Wenseleers et al., 2004a, b; Wenseleers et al., 2006b). Such a low survival of worker-laid eggs is consistent with effective policing, but not with the observed viability differences.

In conclusion, our results agree with those of Beekman and Oldroyd (2005) and show that in *V. vulgaris* the killing of worker-laid eggs is aimed at resolving intracolony conflict and not at killing dead eggs and improving colony hygiene. We also suggest that low viability of worker-laid eggs is unlikely to explain egg policing in other species. First, low viability of worker-laid eggs as such is unlikely to ever completely resolve conflict over male production, since even a small proportion of viable worker-laid eggs could lead to significant conflict. Second, viability of worker-laid eggs is clearly demonstrated by the high number of males reared in queenless colonies of wasps, bees and ants (e.g. Marchal, 1896; Ross, 1985; Bourke, 1988; Martin, 1990; Foster and Ratnieks, 2001c; Miller and Ratnieks, 2001; Villesen and Boomsma, 2003). Finally, if workers are able to lay eggs, natural selection should favour laying viable eggs, unless they have a trophic function.

Acknowledgements

We thank Kristel Vuerinckx for help with data collection and Johan Billen for support. This study was funded by grants from the Finnish Cultural Foundation to HH and the Fund for Scientific Research – Flanders to TW.

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