Convergent evolution of worker policing by egg eating in the honeybee and common wasp

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Mutual policing, where group members suppress each others' reproduction, is hypothesized to be important in the origin and stabilization of biological complexity. Mutual policing among workers in social insects can reduce within-colony conflict. However, there are few examples. We tested for worker policing in the common wasp *Vespula vulgaris*. Workers rapidly removed worker-laid eggs but left most queen-laid eggs (four out of 120 worker eggs versus 106 out of 120 queen eggs remained after 1 h). Ovary dissection (1150 workers from six colonies) revealed that a small but significant number of workers have active ovaries (4%) equivalent to approximately five to 25 workers per colony. Consistent with effective policing of worker reproduction, microsatellite analysis of males (270 individuals from nine colonies) detected no workers' sons. Worker policing by egg eating has convergently evolved in the common wasp and the honeybee suggesting that worker policing may have broad significance in social evolution. Unlike the honeybee, relatedness patterns in *V. vulgaris* do not explain selection for policing. Genetic analysis (340 workers in 17 nests) revealed that workers are equally related to the queen's and other workers' sons (worker–worker relatedness was 0.51 ± 0.04, 95% confidence interval). Worker policing in *V. vulgaris* may be selected due to the colony-level benefit of conflict suppression.

Keywords: worker policing; levels of selection; reproductive conflict; social insects; Vespinae;
Vespula vulgaris

1. INTRODUCTION

Explaining the regulation of individual replication and reproduction is key to understanding the major transitions in evolution, including the origin and maintenance of sociality (Maynard Smith & Szathmáry 1995; Keller 1999; Michod 1999). The potential for individual selfishness exists in all non-clonal groups, which can lead to reproductive conflicts that are detrimental to group-level function. Mechanisms of conflict suppression are therefore central in explaining the rise and stabilization of biological complexity (Keller 1999). Theory suggests that mutual policing, where group members invest in the suppression of each others' reproduction, is an important mechanism of conflict suppression (Frank 1995; Keller 1999). Mutual policing may be particularly important in insect societies because their unique kin structure can favour the evolution of policing among workers in order to suppress worker male production (Hamilton 1964; Starr 1984; Ratnieks 1988; Ratnieks & Reeve 1992; Bourke & Franks 1995; Crozier & Pamilo 1996). In addition, the mobility of individuals in animal societies may facilitate the many-against-one interactions fundamental to policing, more than at other levels of organization such as the genome.

Workers in most eusocial Hymenoptera (bees, ants and wasps) cannot mate yet can lay unfertilized male eggs. This leads to potential reproductive conflict among the workers and between the workers and the queen over male production (Hamilton 1964; Woyciechowki & Łomnicki 1987; Ratnieks 1988; Ratnieks & Reeve 1992). However, in single-queen colonies, if the queen mates multiply and the workers are the daughters of more than two males, workers are more related to the sons of their mother queen (0.25) (brothers) than to their sister workers' sons (< 0.25) (nephews). In this situation, workers benefit from policing one another (worker policing), thereby reducing male production by workers (Starr 1984; Ratnieks 1988).

There are few data on worker policing. Worker policing by egg eating occurs in the honeybee *Apis mellifera* (Ratnieks & Visscher 1989), a derived eusocial species with multiply mated queens and low worker relatedness (Estoup *et al*. 1994). Workers rapidly kill worker-laid eggs but leave queen-laid eggs (Ratnieks & Visscher 1989) and, despite a significant amount of worker laying, very few workers' sons survive the egg stage (Visscher 1989, 1996; Ratnieks 1993). In queenless ants, experimentally introduced ovary-activated workers are selectively aggressed by non-reproductive workers, suggesting that worker policing regulates the number of reproductives (Gobin *et al*. 1999; Kikuta & Tsuji 1999; Liebig *et al*. 1999). Although policing occurs in both the honeybee and queenless ants, the mechanism is different. Queenless ant workers prevent worker laying by direct aggression that causes ovary regression, while honeybee workers remove the product of worker reproduction. However, it is possible that both mechanisms are important in the honeybee. Sakagami (1954) and Visscher & Dukas (1995) found that ovary-activated worker honeybees were aggressed by other workers, although to what extent this reduces their reproduction is unclear.

We investigated the possibility that worker policing by selective egg eating occurs in the common wasp *Vespula vulgaris*. We chose *Vespula* because it is a derived genus (Carpenter 1987; Foster *et al*. 1999) comparable to *Apis*, where multiple mating by queens and queen-only male production have previously been shown (*Vespula maculifrons* and *Vespula squamosa*; Ross 1986). Using a combination of genetic analysis of kinship, dissection of

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workers' ovaries and egg-eating bioassays, here we show that worker policing by egg eating occurs in *V. vulgaris* and that, despite some worker ovary activation, the queen is responsible for all or the vast majority of male production. From a non-social common ancestor, *Apis mellifera* and *V. vulgaris* have convergently evolved eusociality and remarkably similar worker policing systems .

2. METHODS

(a) *Study organism*

Nests of *V. vulgaris* were collected from pest control calls in the Sheffield area, UK, during late summer in 1996–1999. Policing assays were performed on free-flying colonies, which were relocated to polystyrene nest-boxes at the Laboratory of Apiculture and Social Insects, Sheffield, in September 1999.

(b) *Worker relatedness*

Twenty workers from each of 17 *V. vulgaris* nests were analysed at two microsatellite loci, Rufa 18 and 19 (Thorén 1998), using the protocol of Foster *et al.* (1999). Inspection of worker genotypes for each nest was used to estimate pedigree relatedness (r) and also to estimate sperm bias when multiple paternity occurred. The expected heterozygosities at the two loci were estimated from allele frequency estimates using the program Relatedness 4.2 (Goodnight & Queller 1994).

(c) *Effective paternity*

The population effective mating frequency (Me) was estimated after Starr (1984):

$$Me = \frac{n}{\sum_j \sum_i p_{ij}^2},\qquad(1)$$

where p_i is the proportional contribution of the ith male in the jth nest for n nests.

(d) *Do the queen or the workers produce the colony's males?*

In order to determine whether males were the queen's sons or workers' sons, 30 adult males from each of nine queenright colonies were analysed at one or both of the loci depending on whether both were informative. Loci are informative if the worker's paternal and maternal alleles differ, allowing a worker's son to be distinguished from the queen's sons when he receives the distinct paternal allele from his worker mother (Foster *et al.* 2000). The probability of detecting a worker's son, that is the probability that a worker's son possesses at least one distinct paternal allele (P_j), was then calculated for each nest from

$$\sum_1^n p_i(1 - 0.5^{l_i}),\qquad(2)$$

where n is the number of patrilines in the nest, p_i is the proportional representation of the ith patriline and l_i is the number of informative loci analysed at the ith patriline. This is a more general equation than the equation presented in Foster *et al.* (2000) since it can include more than one locus in which not all worker patrilines have informative genotypes. The number of assignable males (Na) for each species sample is then $\sum(P_j N_j)$ where P_j is the probability of detecting a worker-produced male and N_j is the number of males analysed for the jth nest. If workers produce a proportion x of the males, the probability of not sampling any worker-produced males is $(1 - x)^{Na}$.

(e) *Worker ovary activation*

One thousand, one hundred and fifty randomly selected workers from six mature queenright nests (150–200 per nest) were examined by dissection under a binocular microscope with a graticule eye-piece. The size of the largest egg was compared to the mean size of five eggs taken from cells and placed into one of three categories: < 50%, 50–90% and > 90% full size.

(f) *Policing assay*

Policing of worker-laid eggs was investigated by transferring worker-laid and queen-laid eggs into a test comb. This comb was then placed in a queenright test colony in the reproductive phase of the annual life cycle. A total of 120 queen-laid and 120 worker-laid eggs were introduced to two test colonies housed in polystyrene boxes in three trials of 80 eggs each. The number of eggs remaining was checked after 1 and 16 h.

The worker-laid eggs were obtained from four groups of *ca.* 30 queenless workers taken from the two test colonies. Each queenless group was isolated in a 15 cm × 15 cm × 40 cm wire mesh cage with a piece of comb and food (honey and honeybee pupae) *ad libitum*. The queenless workers activated their ovaries and began to lay unfertilized eggs after approximately ten days. The existence of queenless male-producing colonies in *V. vulgaris* shows that worker-laid eggs are viable (Edwards 1980). The queen-laid eggs came from the test colonies themselves. Because the wasp eggs were glued to the paper comb, we cut out small pieces of comb of *ca.* 3 mm × 3 mm each with an adhering egg and glued them individually into cells in the test comb with water-based polyvinyl acetate glue. The test combs were the lowest combs from each test colony and had large cells which are used to rear both males and queens.

(g) *Queen or worker policing?*

In order to exclude the possibility that the eggs were removed by the queen, the first hour of the last two trials was recorded through the clear plastic base of the nest-box using an infrared video camera.

3. RESULTS

(a) *Worker relatedness*

The mean effective paternity (Me) in the 17 colonies was 1.90, giving a worker–worker relatedness (r) of 0.51 ± 0.04 (95% confidence interval). One nest was found to contain two matrilines, which were treated separately for all paternity analyses. One queen was single-mated, 11 were double-mated, four were triple mated and one was quadruple-mated. One male contributed on average to 59% of the workers in multiple-paternity nests. The effects of non-detection and non-sampling error, which can cause relatedness to be overestimated (Boomsma & Ratnieks 1996; Foster *et al.* 1999), were low due to the high heterozygosities of the two loci (0.9 and 0.74), which gave a non-detection error of 0.03, and the sampling of 20 workers from each nest (Foster *et al.* 1999; Pedersen & Boomsma 1999). Non-detection error is the probability that two males share the same genotype by chance at all the loci studied. Because hymenopteran males are haploid, this is equal to the probability that a diploid individual is homozygous at all loci, i.e. $\prod(1 - H_i)$, where H_i is the expected heterozygosity at the ith loci of n.
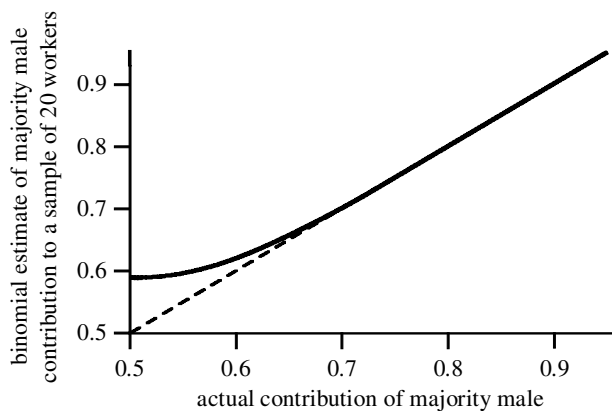
Figure 1. The effect of binomial sampling on the paternal contribution of the majority male to a sample of 20 workers as a function of his actual paternal contribution in the colony. As paternity bias increases, the binomial sampling effect rapidly becomes negligible.

(b) Binomial sampling error

Using a sample of 20 workers for estimating paternity in species where two patrilines are common can lead to error from binomial sampling effects. The binomial expansion allows this error to be estimated as

$$P_r \frac{n!}{r!(n-r)!} p^r (1-p)^{(n-r)}, \qquad (3)$$

where P_r is the probability of sampling r of patriline A and $n-r$ of patriline B in a sample of n workers, and p is the proportional representation of patriline A in the actual colony. The probability of each combination of paternities (20A, 19A:1B, 18A:2B, etc....) can then be calculated and averaged in order to generate the expected contribution of the majority male to the sample. For a sample of 20 workers from a colony with two equal patrilines, this predicts that the majority male will contribute to 0.58 of the workers. In our data, the paternity contribution of the majority male in the double-mated nests (mean = 0.64) was not significantly different from this binomial estimate (one-tailed t-test, $p = 0.08$). Therefore, the paternity bias found could be an artefact of sampling. Effective paternity estimates can also be affected by binomial sampling error. If actual paternity is skewed, however, the effect is negligible (figure 1). Furthermore, even in the worst-case scenario of sampling from two equal patrilines, which estimates paternal contributions as 0.58/0.42, effective paternity is estimated as 1.95 (equation (1)), which is very close to the actual value of 2. Therefore, the key conclusion, that effective paternity in *V. vulgaris* is close to 2, is unaffected.

(c) Do the queen or workers produce the colony's males?

No workers' sons were detected. The number of assignable males (Na) was estimated at 171. This means that there is a probability of less than 5% of missing a worker contribution to male production greater than 2% (Foster *et al.* 2000).

(d) Worker ovary activation

Five out of 1150 workers from three out of the six nests examined had fully activated ovaries. A further seven workers from four of the nests had eggs greater than half-size in their ovaries. This is a low but biologically significant proportion of ovary activation given that mature *V. vulgaris* nests contain 1000–5000 workers (Wilson 1971; Edwards 1980).

(e) Policing assay

Worker-laid eggs were rapidly removed but the majority of queen-laid eggs were spared (table 1). Summing all three trials, after 1 h only four out of 120 worker-laid eggs remained, whereas 106 out of 120 queen-laid eggs remained (χ^2-test, $p < 0.001$). Following the rapid egg removal in trials 1 and 2, trial 3 was checked after 15 min, by which time 75% of the worker-laid eggs had been removed. Trial 3 also showed that egg environment was not the basis for discrimination since all eggs came from a different colony to the test colony.

(f) Queen or worker policing?

The queen was never seen on the test comb during the first hour of trials 2 and 3 showing that workers were responsible for egg removal. Up to five workers at a time visited the 40 test cells. Workers made more and longer visits to cells containing worker-laid eggs (265 visits versus 172 visits, $p < 0.001$, χ^2-test, median visit duration 4.1 s versus 1.0 s, $p < 0.001$, Mann–Whitney U-test, analysis based on the first 15 min of the two videos).

(g) Is sex allocation biasing an alternative explanation for egg removal?

Workers in some social insect species have been shown to kill males selectively in order to favour their more related sisters (Sundström *et al.* 1996). Because all worker-laid eggs are male, it is therefore possible that such sex allocation biasing could produce results similar to worker policing. In order to test this possibility, 200 queen-laid eggs from the two colonies were sexed. The sex ratio was approximately even with 85 males and 83 females (the polymerase chain reaction failed for 32 eggs). The removal of all worker-laid eggs but only 33% of queen-laid eggs (table 1) cannot therefore be explained by workers selectively removing male eggs. Furthermore, genetic analysis of ten queen-laid eggs that remained after the trials showed that both male and female eggs had been left by workers, with six being male. Finally, the test combs had large cells that are used to rear both males and females and the test colonies were rearing both sexes at the time of study, giving no *a priori* reason why workers would discriminate eggs on the basis of sex.

4. DISCUSSION

Our data show that worker policing by egg eating occurs in the common wasp *V. vulgaris*. Workers rapidly remove worker-laid eggs but leave the majority of queen-laid eggs. Ovary dissections suggested that such egg removal occurs naturally because a low but significant (approximately five to 24) number of reproductive workers are present in reproductive-phase colonies (0.4% of 1000–5000 workers). The genetic analysis of adult males further suggested that policing is effective because we detected no workers' sons. Worker policing may be a general characteristic of *Vespula*. Like *V. vulgaris*, *V. squamosa*

Table 1. *Worker policing in* V. vulgaris

(Workers removed all worker-laid eggs and left the majority of queen-laid eggs in each trial. The number of queen-laid versus worker-laid eggs remaining after 16 h was significantly different for each trial (χ^2-test, $p < 0.001$).)

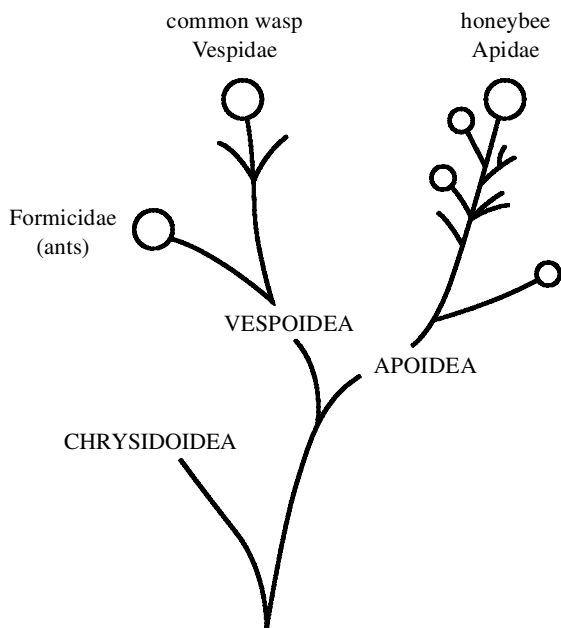| trial and egg source		colony		number of eggs in cells		
		test	egg source	start	1 h	16 h
1	queen	1	1	40	30	24
	workers	1	1	40	1	0
2	queen	2	2	40	39	34
	workers	2	2	40	2	0
3	queen	2	1	40	37	22
	workers	2	1	40	1	0
combined						
	queen	1 and 2	1 and 2	120	106	80
	workers	1 and 2	1 and 2	120	4	0

Figure 2. Convergent evolution of worker policing in the common wasp and honeybee illustrated by the phylogeny of the aculeate Hymenoptera (Brothers & Carpenter 1993). Formicidae represents ants, Vespidae includes vespine wasps and Apidae includes honeybees. Families containing eusocial species are shown by circles. Large circles denote families containing highly eusocial species (with large colonies and significant queen–worker dimorphism). Family branches within the Vespoidea and Apoidea crown groups are shown.

and *V. maculifrons* have multiple-mated queens and queen-only male production (Ross 1986) and low levels of reproductive workers occur in *V. maculifrons*, *Vespula flavopilosa*, *Vespula germanica* and *Vespula vidua* (0.6–2.6% of workers) (Ross 1985). In addition, workers in a colony of *Vespula atropilosa* killed an introduced ovary-activated worker but did not kill non-reproductive workers (Landolt *et al.* 1977), thereby raising the possibility that worker policing by aggression also occurs in *Vespula*. We therefore suggest that the following reproductive characteristics are found throughout *Vespula*: multiple mating, a small number of reproductive workers, males primarily the offspring of the queen and, we hypothesize, worker policing. Worker

policing appears to be widespread in vespines in general. Since this study, worker policing has also been found in *Vespa crabro* (Foster *et al.* 2001*b*) and *Dolichovespula saxonica* where policing is facultative in response to queen mating frequency (Foster & Ratnieks 2000).

The worker policing by mutual egg eating in *V. vulgaris* is strikingly similar to that found in the honeybee *A. mellifera* (Ratnieks & Visscher 1989). This is not due to common ancestry since the vespine wasps and honeybees belong to lineages that have evolved eusociality independently (Brothers & Carpenter 1993) (figure 2). From solitary origins, both lineages have evolved large complex societies, strong queen–worker dimorphism (Wilson 1971) and a worker policing system that appears to be highly efficient at reducing worker reproduction. The two species further share a low level of worker ovary activation (one in 10 000 worker honeybees have full-size eggs in their ovaries; Ratnieks 1993) suggesting that the majority of workers in both species exercise reproductive self-restraint. Self-restraint is probably due to effective policing. When policing is effective, workers receive little benefit from egg laying and may benefit more by working to increase total colony reproduction (Ratnieks 1988; Ratnieks & Reeve 1992).

Honeybee queens are highly polyandrous, causing very low worker–worker relatedness. This results in workers being more related to their mother queen's sons (0.25) than other workers' sons (*ca.* 0.15) (Estoup *et al.* 1994) and so selects for worker policing (Ratnieks 1988). Worker–worker relatedness is also low in *V. vulgaris* when compared to most other single-queen hymenopteran societies (Boomsma & Ratnieks 1996). However, the observed relatedness between workers of *ca.* 0.5 means that workers are equally related to the queen's sons and other workers' sons. Relatedness cannot therefore explain selection for worker policing in *V. vulgaris*. This makes the *V. vulgaris* system particularly interesting because it suggests that worker policing is selected for due to other factors, such as the colony-level benefits of reducing reproductive conflict (Ratnieks 1988; Frank 1995; Keller 1999). Such apparently costly conflict over male production occurs in *Dolichovespula*, the sister group of *Vespula*, where male production by workers and physical queen–worker conflict have been recorded (Ross & Matthews 1991; Foster *et al.* 2001*a*).

Mutual policing is thought to be important in the evolution of more complex biological organization because it suppresses conflict among lower-level units (Frank 1995; Maynard Smith & Szathmáry 1995; Keller 1999). The convergent evolution of worker policing in *Apis* and *Vespula* and the suggestion that, in *V. vulgaris*, worker policing is selected for because it enhances colony performance support this hypothesis. The honeybee and the common wasp are both well-studied, common and geographically widespread social insects. However, in neither was worker policing observed until suitable experiments were carried out. We suggest that worker policing by egg eating should be deliberately looked for in other eusocial Hymenoptera, particularly in the ants where large complex societies have evolved independently (figure 2). Studies of queenless ants have suggested that worker policing by aggression regulates the number of reproductives (Gobin *et al.* 1999; Kikuta & Tsuji 1999; Liebig *et al.* 1999), but data from the largest ant societies, such as the leafcutter, wood and weaver ants, are lacking. Mutual policing may also be important at other levels of organization. A recent model of the transition from single-celled to multicelled organisms predicted that mutual policing among cells should evolve once organisms reach a critical cell number (Michod 1997). At the intragenomic level, recombination (Haig & Grafen 1991) and meiosis (Hurst & Pomiankowski 1991) have been suggested as reflecting the policing of selfish genetic elements within a 'parliament of genes' (Leigh 1977, 1991). Although these mechanisms are unlikely to prove formally identical to worker policing (Hurst *et al.* 1996), they reflect a similar many-against-one suppression of reproductive conflict. Mutual policing may prove to be a widespread and important answer to what Leigh (1999) referred to as the 'fundamental problem in ethology'—how conflict is prevented in cooperative groups.

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