

# Colony kin structure and male production in *Dolichovespula* wasps

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## Abstract

In annual hymenopteran societies headed by a single outbred queen, paternity (determined by queen mating frequency and sperm use) is the sole variable affecting colony kin structure and is therefore a key predictor of colony reproductive characteristics. Here we investigate paternity and male production in five species of *Dolichovespula* wasps. Twenty workers from each of 10 colonies of each of five species, 1000 workers in total, were analysed at three DNA microsatellite loci to estimate paternity. To examine the relationship between kin structure and reproductive behaviour, worker ovary activation was assessed by dissection and the maternal origin of adult males was assessed by DNA microsatellites. Effective paternity was low in all species (*D. media* 1.08, *D. maculata* 1.0, *D. sylvestris* 1.15, *D. norvegica* 1.08 and *D. saxonica* 1.35), leading to the prediction of queen–worker conflict over male production. In support of this, workers with full-size eggs in their ovaries (four out of five species) and adult males that were workers' sons (all five species) were found in queenright colonies. However, workers were only responsible for a minority of male production (*D. media* 7.4%, *D. maculata* 20.9%, *D. sylvestris* 9.8%, *D. norvegica* 2.6% and *D. saxonica* 34.6%) suggesting that the queen maintains considerable reproductive power over the workers. Kin structure and reproductive conflict in *Dolichovespula* contrast with their sister group *Vespula*. *Dolichovespula* is characterized by low paternity, worker reproduction, and queen–worker conflict and *Vespula* by high paternity, effective worker policing and absence of worker reproduction. The trend revealed by this comparison is as predicted by kin selection theory suggesting that colony kin structure has been pivotal in the evolution of the yellowjacket wasps.

**Keywords:** *Dolichovespula*, kin selection, paternity, relatedness, social insects, Vespinae

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## Introduction

The study of reproductive conflict is central to our understanding of social evolution (Keller 1999). In nonclonal groups, the divergent genetic interests of group members can cause within-group conflicts that disrupt social organization (Hamilton 1964; Ratnieks & Reeve 1992; Keller 1999). By identifying parties of common and differing interests (Ratnieks & Reeve 1992), kin structure is central to

understanding the structure of these conflicts, and their resolution. In single-queen hymenopteran societies (ants, bees and wasps) paternity, which is determined by queen mating frequency and sperm use, is a key determinant of colony relatedness patterns. Therefore, the study of paternity is central to the study of hymenopteran social evolution (Bourke & Franks 1995; Boomsma & Ratnieks 1996; Crozier & Pamilo 1996).

There is great potential for conflict over male production in hymenopteran societies (Hamilton 1964; Starr 1984; Woyciechowski & Łomnicki 1987; Ratnieks 1988). Although workers are generally unable to mate, because of haplodiploidy, their unfertilized eggs become males. With both the queen and the workers individually most related

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to their own sons, extensive conflict over male production is predicted. Paternity is of importance in understanding whether this conflict will actually be expressed, because multiple mating by queens is predicted to result in the suppression of worker reproduction by worker policing (Starr 1984; Ratnieks 1988). When the workers in a colony are offspring of a single queen but several fathers, a worker is more related to the queen's sons (0.25) than to other workers' sons (< 0.25). This shifts the workers' collective interest into line with the queen's interest in that workers are expected to police each other's reproduction resulting in enforced co-operation (Ratnieks 1988).

The wasp subfamily Vespinae (*Vespa*, *Provespa*, *Dolichovespula*, *Vespula*) has proved highly informative in the study of reproductive conflicts (Ratnieks 1988; Foster *et al.* 1999, 2000). The main reason for this is that the four genera are generally very similar in their basic biology (annual life cycle, single queen, physically distinct queen caste; Ross & Matthews 1991), yet paternity varies allowing contrasting predictions to be made concerning male production and reproductive conflict (Ross 1986; Foster *et al.* 1999, 2000; Foster and Ratnieks 2000). This has allowed fruitful comparison between taxa (Foster *et al.* 2000). Because a well-supported phylogeny of the Vespinae is available (Carpenter 1987), inferences on the evolution of reproductive traits in the group can also be made (Foster *et al.* 1999).

*Dolichovespula* is the sister group of *Vespula*. Together they form the yellowjackets, the most derived clade of the Vespinae (Carpenter 1987). *Dolichovespula* wasps make characteristic ball-shaped grey paper nests, consisting of a queen and typically about 100 workers (Greene 1991). Despite extensive study of their biology (reviewed by Greene 1991), little is known of paternity or worker reproduction. Using DNA microsatellites, we studied paternity and male production in five *Dolichovespula* species. Paternity was low in all species, leading to the prediction of queen-worker conflict over male production. Worker ovary dissection and genetic analysis of males showed that workers do indeed attempt to reproduce, producing a small but significant proportion of the adult males in queenright colonies.

## Materials and methods

### Study organisms

*Dolichovespula sylvestris* (13 nests), *D. norvegica* (18 nests) and *D. media* (13 nests) were collected from the Sheffield area, UK in 1996 to 1998. *D. sylvestris* (nine nests) and *D. media* (22 nests), along with *D. saxonica* (15 nests), were also collected in the New Forest, Hampshire, UK in 1999. *D. maculata* (19 nests) were collected in and around Ithaca, New York in 1991. All nests were collected from pest control calls and would otherwise have been destroyed.

### Genetic methods

Twenty workers and the queen, if collected, from each of 10 nests of each species (1000 workers in total) were analysed at three DNA microsatellite loci (Rufa 5, 13, 15 for *D. sylvestris*, *D. norvegica* and *D. maculata*, Rufa 5, 13, 18 for *D. saxonica*, *D. media*, see Appendix) using methods as in Foster *et al.* (1999). The majority of nests of *D. sylvestris* (8/10) and *D. media* (9/10) analysed were from the New Forest sample. Eighteen to 40 males from seven to 10 nests of each species (1125 males in total) were analysed at all informative loci (Foster *et al.* 2000 and below) to determine whether they were the queen's or workers' sons. All males analysed came from nests with female pupae. The presence of female pupae confirmed that the nests were queenright when the eggs that gave rise to the males were laid. In *D. sylvestris* and *D. saxonica*, male pupae were also available for a few colonies that were queenright on collection. These were analysed instead of adult males because they provide additional information on rearing location, showing in which combs workers were laying. The number of males analysed for each species was designed to give approximately 150 assignable males per species (assignable numbers ranged from 148 to 153, see *Male non-detection error* below).

### Worker-worker relatedness

The program RELATEDNESS 4.2 (Goodnight & Queller 1994) was used to calculate regression relatedness among workers ( $b$ ), inbreeding ( $F$ ) and allele frequencies. Assuming outbreeding, pedigree worker-worker relatedness ( $r$ ) was estimated by inspection of progeny genotypes. Inspection also allows the number of fathers and their relative paternities to be determined when multiple paternity occurs.

### Effective paternity

The population effective mating frequency ( $M_e$ ) was estimated after Starr (1984),

$$M_e = \frac{n}{\sum_j \sum_i p_{ij}^2} \quad (1)$$

where  $p_{ij}$  is the proportional contribution of the  $i$ th male in the  $j$ th nest for  $n$  nests.

### Male nondetection error

The mean probability of detecting a worker-produced male present in a sample was calculated for each nest from:

$$P = \sum_1^n p_i (1 - 0.5^{l_i}) \quad (2)$$

**Table 1** Genetic variation and the probability of detecting a second father for the microsatellite markers studied (nondetection error,  $d_n$ )

Species	Expected heterozygosity at <i>Vespula rufa</i> locus				$d_n$
	5	13	15	18	
<i>D. media</i>	0.738	0.812	—	0.733	0.010
<i>D. maculata</i>	0.900	0.865	0.826	—	0.002
<i>D. sylvestris</i>	0.860	0.792	0.841	—	0.005
<i>D. norvegica</i>	0.817	0.804	0.687	—	0.010
<i>D. saxonica</i>	0.874	0.887	—	0.821	0.003

where  $n$  is the number of patrines in the nest,  $p_i$  is the proportional representation of the  $i$ th patriline and  $l_i$  is the number of informative loci analysed at the  $i$ th patriline. An informative locus is one where the queen and her mate have different alleles so that the workers carry an allele which the queen does not (Foster *et al.* 2000). This equation is more general than that in Foster *et al.* (2000), which although correct for the analysis performed, is not applicable when more than one locus in a colony has both informative and uninformative patrilines. The number of assignable males ( $N_a$ ) for each species is then  $\Sigma(P_j N_j)$  where  $P_j$  is the probability of detecting a worker-produced male and  $N_j$  is the number of males analysed for the  $j$ th nest.

#### Worker ovary activation

Twenty workers from all genetically analysed queenright nests of each species were dissected and their ovaries were inspected under a binocular microscope using a reticule eyepiece. The mean size of five eggs taken from cells was taken for comparison and the largest egg(s) in worker ovaries was classed as < 50% full size (inactive), 50–90% full size (half), > 90% full size (full) (see Table 3).

## Results

### Queen loss in reproductive nests

It has been suggested that workers in reproductive nests may kill their queen to allow them to reproduce (Bourke 1994). No queen was found on collection of 5/14 *Dolichovespula sylvestris*, 12/14 *D. norvegica*, 2/10 *D. saxonica*, 6/19 *D. media* and 14/19 *D. maculata* nests at the reproductive stage of their lifecycle. The mother queen was recognized as different from new queens by her heavily worn wings (Edwards 1980).

### Allelic diversity

Allelic diversity and heterozygosity were high in all loci studied, with 5–15 alleles at each locus and a mean heterozygosity of 0.82 (Table 1). This is particularly interesting in the case of *D. media* and *D. saxonica* as both have recently invaded Britain from central Europe (1980 for *D. media*; 1987 for *D. saxonica*, Else 1994). The high heterozygosity suggests either a rapid recovery of genetic diversity at the loci studied through mutation or an absence of founder effects during this colonization, probably because Britain was colonized by many queens.

### Worker-worker relatedness

Regression and pedigree relatedness were high and effective paternity was low in all five species (Table 2). Across species, the paternity contribution of the majority male in double patriline nests (0.73) was significantly greater than that expected from sampling 20 workers from a nest with two equal patrilines (0.58), calculated using the binomial expansion ( $H_0 = 0.58$ ,  $t$ -test,  $P < 0.01$ , Foster & Ratnieks 2001):

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

**Table 2** Colony kin structure in five species of *Dolichovespula*

Species	Paternity				$P_{major}$	No. of mothers detected	Worker relatedness	
	$M_e$	No. of fathers detected					pedigree $r$	regression $b \pm SE$
		1	2	3				
<i>D. media</i>	1.08	9	2	0	0.73	1–2	0.71	0.72 ± 0.04
<i>D. maculata</i>	1.0	10	0	0	—	1	0.75	0.74 ± 0.02
<i>D. sylvestris</i>	1.15	7	4	0	0.68	1–2	0.68	0.62 ± 0.06
<i>D. norvegica</i>	1.08	8	2	1	0.83	1–2	0.71	0.65 ± 0.06
<i>D. saxonica</i>	1.35	4	5	1	0.68	1	0.62	0.57 ± 0.04

$M_e$  is the effective paternity,  $P_{major}$  is the mean proportional contribution of the majority male in multiple paternity nests.

**Table 3** Worker ovary activation and number of adult workers in colonies of five species of *Dolichovespula*. The three categories of ovary activation are defined in the text

Species	No. of workers analysed	No. of colonies analysed	Worker ovary activation (% workers)			Mean no. workers in each colony	Estimated no. reproductive workers per colony
			inactive	half	full		
<i>D. media</i>	140	7	94	1	5	74	4
<i>D. maculata</i>	40	2	100	0	0	181	—
<i>D. sylvestris</i>	140	7	91	2	7	76	5
<i>D. norvegica</i>	20	1	90	0	10	44	4
<i>D. saxonica</i>	160	8	88	1	11	69	8

	Worker male production			
	No. colonies analysed	% total males	$N_a$	% colonies
<i>D. media</i>	8	7.4	148.0	12.5
<i>D. maculata</i>	7	20.9	153.3	57.1
<i>D. sylvestris</i>	10	9.8	153.5	40.0
<i>D. norvegica</i>	8	2.6	151.8	12.5
<i>D. saxonica</i>	9	34.6	150.1	77.8

**Table 4** Adult male production by workers in five species of *Dolichovespula*

Percentage of total males produced by workers is estimated as  $(\text{no. detected}/N_a) \times 100$ , see *Male nondetection error* in Methods section.  $N_a$  is the number of assignable males; % colonies is the percentage of colonies in which at least one workers' son was detected.

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 to generate the expected contribution of the majority male to the sample. The probability that any two randomly chosen males are identical at all loci studied (nondetection error,  $d_n$ ) was calculated from expected heterozygosity. This estimate of  $d_n$  gives an approximate but reliable indication of the degree of error in estimating paternity (Boomsma & Ratnieks 1996; Foster *et al.* 1999). For all species  $d_n$  is extremely low, so that very few, or probably no, cases of multiple mating will be missed (Table 1). The effects nonsampling error was kept to low levels by the analysis of 20 workers per nest (Boomsma & Ratnieks 1996; Foster *et al.* 1999). Two matriline were detected in a single nest each for *D. sylvestris*, *D. norvegica* and *D. media*, with the majority matriline representing 0.65, 0.7 and 0.85 of workers sampled for each species, respectively.

#### Worker ovary activation

Workers with full-size eggs in their ovaries were found in all species except *D. maculata* (Table 3). However, only two

colonies of this species were analysed as ovary dissections were only performed on queenright colonies. This is because worker ovary activation is likely to be affected by the queen's presence (Edwards 1980; Bourke 1988).

#### Male production

Adult or pupal male production by workers was detected in some nests of each species (Table 4). Analysis of male pupae in the two *D. sylvestris* colonies studied revealed that all worker-produced males came from only one comb. However, in the single *D. saxonica* colony worker-produced males came from two of three combs (Table 5). In the colonies of *D. sylvestris* and *D. norvegica* that had two matriline, all queen-produced males analysed came from the majority queen. No males were analysed in the two matriline *D. media* colony.

#### Effect of colony size and collection date on worker male production

The percentage of males that were workers' sons did not correlate with date of nest collection or colony size. Spearman's rank correlation tests were performed for '% workers' sons vs. date of collection' and '% workers' sons

**Table 5** Male production by workers on different combs in two nests of *Dolichovespula sylvestris* and one of *D. saxonica*

Nest	Comb #		
	Top	Middle	Bottom
<i>D. sylvestris</i>	6/10	0/10	—
<i>D. sylvestris</i>	0/7	0/7	5/6
<i>D. saxonica</i>	6/9	2/4	0/1

In both *D. sylvestris* nests the difference in worker male production between the combs where male production was detected and those where it was not was highly significant (Fisher's exact test,  $P < 0.01$ ). The data shown are the number of worker-produced males detected over the total number of males analysed for each comb.

vs. number of workers'. These two tests were performed for each species individually and upon the entire data set by combining the within-species ranks ( $n = 42$ ). For all tests  $P > 0.10$ .

## Discussion

Our data show that the majority of *Dolichovespula* queens mate just once and that even when they mate multiply, most sperm comes from a single male. Across the four species in which multiple mating was detected paternity was biased, with the majority male fathering an average of 73% of female offspring. As a result there was high relatedness among workers in all five species. Paternity is significantly more biased in these *Dolichovespula* than in a comparable data set of the common wasp, *Vespula vulgaris* (majority male contribution in double paternity colonies = 0.64, Foster & Ratnieks 2001) ( $t$ -test comparing the proportional majority male contribution in 11 double paternity *Dolichovespula* colonies with 10 double paternity *V. vulgaris* colonies,  $P = 0.037$ ). This suggests that the high worker relatedness in *Dolichovespula* is due not only to the lower frequency of multiple mating, but also to more biased paternity when multiple mating occurs. In *D. maculata* all queens were singly mated, resulting in the highest possible worker relatedness, 0.75, without inbreeding. In the other species paternity and worker relatedness varied somewhat between colonies but only in one colony of the 50 studied (*D. saxonica*) did worker relatedness fall below 0.5.

Two matriline were found in one colony each of *D. sylvestris*, *D. norvegica* and *D. media*. Because only one adult queen was found in each of these colonies, this suggests a successful nest take-over by a second queen. Although observations of nest usurpation are numerous in *Vespula* (Greene 1991), this is the first evidence suggesting that take

over successfully occurs in *Dolichovespula*. Molecular evidence for queen take-overs has also been found in the hornet *Vespa crabro* (two of 33 nests, Foster *et al.* 2000) and *V. vulgaris* (one of 17 nests, Foster & Ratnieks 2001). It appears therefore to be a widespread strategy of vespine wasp queens.

Low paternity in all five *Dolichovespula* species supports the conclusion made by Foster *et al.* (1999) that high paternity is derived in the vespine wasps. Foster *et al.* (1999) also suggested that there were two origins of multiple paternity in the vespine wasps, once at the base of the genus *Vespula* and once in *D. saxonica*. However, the more extensive analysis presented here reveals that although moderate multiple mating does occur in *D. saxonica*, extreme multiple mating (effective paternity  $> 2$ ) is restricted to *Vespula* (Ross 1986). The conclusion that multiple mating is a derived trait in the Vespinae is therefore still strongly supported.

With their high relatedness, *Dolichovespula* workers are more related to each other's sons than to the queen's sons (with the exception of one *D. saxonica* colony). Therefore, they are not expected to police each other's male production and conflict with the queen is expected (Starr 1984; Ratnieks 1988). In line with this prediction, reproductive workers were found in queenright colonies in four out of five species. Observation nest studies of *D. media* (23 queen-laid and 12 worker-laid eggs observed across three colonies; Foster personal observation), *D. saxonica* (101 queen-laid and 164 worker-laid eggs observed across four colonies, Foster & Ratnieks 2000) and *D. maculata* (Balduf 1954; Greene 1979) have also shown that worker laying is common in queenright *Dolichovespula* colonies. Importantly, the genetic data show that workers in all five species successfully produce adult males in queenright colonies. Actual queen-worker conflict over male production therefore occurs in *Dolichovespula* as predicted by kin structure. Interestingly, in the two *D. sylvestris* colonies where pupae were available workers, only produced males on one comb suggesting that the nest was divided into areas of queen and worker control.

In spite of their numerical advantage *Dolichovespula* workers only produce a minority of males. How can the queen's power over male production be explained? Traditionally, pheromonal control by the queen has been invoked to explain the reproductive primacy of queens in insect societies. This has been criticised because such control is evolutionarily unstable because workers will be selected to ignore the pheromone (Seeley 1985; Keller & Nonacs 1993). Recent work on the hornet *Vespa crabro*, which also has low paternity (1.11), showed that workers do not activate their ovaries, lay eggs, or aggress the queen, indicating a possible role for pheromonal control (Foster *et al.* 2000). However, additional work has shown that workers preferentially remove worker-laid over queen-laid

eggs (Foster *et al.* submitted for publication). This suggests that worker policing and not queen pheromonal control causes worker sterility.

An alternative explanation for the queen's near monopolization of male production in *Dolichovespula* is that worker reproduction is costly and reduces colony reproduction. This could cause workers to police each other at low paternity (Ratnieks 1988; Franks 1995) as occurs in *Vespa crabro*, although if policing is occurring it is less effective than in *Vespa*. Alternatively, such a cost to worker reproduction may be causing workers to exercise reproductive self-restraint. The cost may arise simply because reproducing workers work less (Ratnieks 1988; Bourke & Franks 1996). Additionally, worker reproduction may interfere with colony female reproduction. If unable to tell the gender of eggs, reproducing workers risk killing their more valuable sisters as well as their brothers (Nonac & Carlin 1990). This seems likely in vespine wasps where males and females are often reared in the same cells.

The cost of worker reproduction in queenright colonies is likely to be further exacerbated by the occurrence of queenless colonies. In queenless colonies, workers develop their ovaries and produce many males (Bourke 1988). The large proportion of queenless colonies in *Dolichovespula* means that this extra male production is likely to be significant. This will reduce the mating success of males, further reducing the benefits of worker reproduction, particularly in queenright colonies if it is detrimental to female production. With so many colonies without a queen, queen killing by workers (matricide) is a possibility in *Dolichovespula* wasps. Matricide is predicted in low-paternity annual societies after sufficient workers have been raised and queen-destined eggs have been laid, as it allows workers to monopolize male production (Ratnieks 1988; Bourke 1994). Without observation of workers killing the queen, however, other causes of mortality cannot be excluded.

The ultimate cause of the low level of worker reproduction in *Dolichovespula* societies is not yet clear, but what proximate mechanisms are involved? It appears that both self-restraint by workers and egg removal are important. Our ovary dissections show that the majority of workers have inactive ovaries, suggesting that only a few are actually reproducing (Table 3). This may be established by a dominance hierarchy among the workers (Greene 1991). Removal of workers eggs by queens (queen policing) has been observed in *D. maculata* (Balduf 1954), *D. saxonica* and *D. media* (Foster, personal observation) and, with worker policing found in *D. saxonica* (see below) worker removal of other workers eggs is also potentially important.

*D. saxonica* is exceptional and is the subject of a separate paper (Foster and Ratnieks 2000). It has the greatest range of worker relatedness and significant male production by workers in several colonies. Most importantly, worker relatedness is positively correlated with worker male pro-

duction. In line with theory, this suggests that workers are policing each other's reproduction in the multiple-paternity colonies but not in the single-paternity colonies (Foster and Ratnieks 2000). In the other species, less worker reproduction occurs despite worker relatedness comparable to the *D. saxonica* colonies with high worker-relatedness where workers dominate male production. This suggests that the conflict over male production has been resolved in a unique way in *D. saxonica*. Whether worker policing also reduces worker reproduction in the other four species is unknown. Testing for the existence of worker policing in other *Dolichovespula* is a key future step in investigating the nature of sociality in the group.

Despite the difference between *D. saxonica* and the other species, as a genus *Dolichovespula* is a sharp contrast with its sister genus *Vespula*. *Dolichovespula* is characterized by low paternity, significant worker reproduction and queen-worker conflict over male production while *Vespula* displays high paternity, absence of worker reproduction (Ross 1986) and worker policing (Foster & Ratnieks 2001). This comparison reveals a trend exactly as predicted by kin selection suggesting that kin structure, specifically paternity frequency, has been pivotal in the social evolution of the yellowjacket wasps.

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## References

- Balduf WV (1954) Observations on the white-faced wasp *Dolichovespula maculata* (Linn.) (Vespidae, Hymenoptera). *Annals of the Entomological Society of America*, **47**, 455–458.
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society of London, Series B*, **351**, 941–975.
- Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. *Quarterly Review of Biology*, **63**, 291–311.
- Bourke AFG (1994) Worker matricide in social bees and wasps. *Journal of Theoretical Biology*, **167**, 283–292.
- Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.
- Carpenter JM (1987) Phylogenetic relationships and classification of the Vespinae (Hymenoptera: Vespidae). *Systematic Entomology*, **12**, 413–431.
- Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies*. Oxford University Press, Oxford.
- Edwards R (1980) *Social Wasps. Their Biology and Control*. Rentokil Ltd, East Grinstead, West Sussex, UK.

- Else GR (1994) Identification: Social Wasps. *British Wildlife*, **5**, 304–311.
- Foster KR, Ratnieks FLW (2000) Facultative worker policing in a social wasp. *Nature*, **407**, 692.
- Foster KR, Ratnieks FLW (2001) Convergent evolution of worker policing by egg eating in the honey bee and common wasp. *Proceedings of the Royal Society of London B*, **268**, 169–174.
- Foster KR, Ratnieks FLW, Raybould AF (2000) Do hornets have zombie workers? *Molecular Ecology*, **9**, 735–742.
- Foster KR, Seppä P, Ratnieks FLW, Thorén PA (1999) Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioral Ecology and Sociobiology*, **46**, 252–257.
- Franks SA (1995) Mutual policing and the repression of competition in the evolution of cooperative groups. *Nature*, **377**, 520–522.
- Goodnight KF, Queller DC (1994) *Relatedness 4.2*. Goodnight Software, Houston, TX.
- Greene A (1979) Behavioural characters as indicators of yellowjacket phylogeny (Hymenoptera: Vespidae). *Annals of the Entomological Society of America*, **72**, 614–619.
- Greene A (1991) *Dolichovespula* and *Vespula*. In: *The Social Biology of Wasps* (eds Ross KG, Matthews RW), pp. 263–304. Cornell University Press, Ithaca, NY.
- Hamilton WD (1964) The genetical evolution of social behaviour. *Journal of Theoretical Biology*, **7**, 1–52.
- Keller L, Nonacs P (1993) The role of queen pheromones in social insects: queen control or queen signal? *Animal Behaviour*, **45**, 787–794.
- Keller L (1999) *Levels of Selection in Evolution*. Princeton University Press, Princeton, NJ.
- Nonac P, Carlin NF (1990) When can ants discriminate the sex of brood? A new aspect of queen-worker conflict. *Proceedings of the National Academy of Sciences of the USA*, **87**, 9670–9673.
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist*, **132**, 217–236.
- Ratnieks FLW, Reeve HK (1992) Conflict in single queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *Journal of Theoretical Biology*, **158**, 33–65.
- Ross KG (1986) Kin selection and the problem of sperm utilisation in social insects. *Nature*, **323**, 798–800.
- Ross KG, Matthews RW (1991) *The Social Biology of Wasps*. Cornell University Press, Ithaca, NY.
- Seeley TD (1985) *Honeybee Ecology. A Study of Adaptation in Social Life*. Princeton University Press, Princeton, NJ.
- Starr CK (1984) Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith RL), pp. 427–464. Academic Press, Orlando, FL.
- Thorén PA, Paxton RJ, Estoup A (1995) Unusually high frequency of (CT)<sub>n</sub> and (GT)<sub>n</sub> microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). *Insect Molecular Biology*, **4**, 141–148.
- Woyciechowski M, Lomnicki A (1987) Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *Journal of Theoretical Biology*, **128**, 317–327.

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This study forms part of Kevin's recently completed PhD on 'Kin selection and reproductive conflict in vespine wasp societies'. The Laboratory of Apiculture and Social Insects at Sheffield is headed by Francis Ratnieks and focuses on social evolution, behavioural ecology, work organization in insect societies and apiculture. Niclas studies the population genetics of Formicine ants with Pekka Pamilo. Peter Thorén is a Senior Research Scientist in the Department of Virology of the National Veterinary Institute, Uppsala.

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## Appendix – Primer sequences

The primers in this study were designed using *Vespula rufa* as described in Thoren *et al.* (1995). However, the sequences have not yet been published and so are included below. Amplification success in one species from each vespine genus is shown. Ten workers, each from a different nest, were analysed for each species except for *Provespa anomola* for which all 10 workers came from a single nest (N = no or nonspecific amplification). *Vespa* is the most basal genus in the vespine wasps and hence, phylogenetically, the furthest from *Vespula rufa*, followed by *Provespa*, *Dolichovespula* and then *Vespula vulgaris* (Carpenter 1987)

Locus	Direction	Sequence 5'–3'	No. of alleles detected in 10 workers			
			<i>Vespa crabro</i>	<i>Provespa anomola</i>	<i>Dolicho sylvestris</i>	<i>Vespula vulgaris</i>
1	F	TTCTGAACTGCAATAATCAITTC	N	N	N	N
	R	GTTCTGAATAATCTTTTCATA				
2	F	GATCGACTTAAGCAGGAATG	2	N	N	N
	R	TCGTGTAATTCGTTGATTC				
3	F	GTCTGCAGATTAGGGAACG	N	N	N	1
	R	CTCCATGACCCGAGAATAAAG				
4	F	TTCAATGCTATTACATTATAATATTC	N	N	N	2
	R	GTCCAAGGAAGTTTCGAC				
5	F	GAGCAAATTTTACGACGTAGG	4	2	6	1
	R	CTGCCAATCGCATGTCG				
6	F	GGACACGTTTACGTAGAAGGATG	3	N	N	N
	R	CGCAGTGACGAGTTTCCAC				
7	F	CGTTGCTCGTGAAACTGTTAAC	1	2	1	1
	R	CCATCTTCGTTTCATCTTCGTTTC				
8	F	CCTGTCATCGTGCCACG	N	N	N	N
	R	TCCGATCGTTTGAATCCTG				
9	F	GACGAGATGTATGTAGTAGTGACG	N	N	N	N
	R	GTGATGCTATGCATTCCGG				
10	F	GATCGAACCTTAATTACACGATTC	N	N	N	N
	R	CAGGAAACAGCTATGACCATG				
11	F	GCATCGACGGATGAATTG	N	N	N	2
	R	GATGGAACAAGGAAGCATG				
12	F	CTTTCTAGGAGATACTTCGTATAAAG	N	N	N	1
	R	AAGATGGAACAAGGAAGC				
13	F	GATCTGTGTACGTAATTCTCTCC	3	2	2	1
	R	GAAAATCGGGAACGATG				
14	F	TGATGCTCGTTAGTTGCAC	N	N	N	N
	R	GATCAGATAATCAAAGAGACAGAC				
15	F	GATCAGAAATCTGATTAAGTCGAG	5	N	5	4
	R	GGTCGATTCGTTTGAAAATAG				
16	F	GTACAGTATACGTAAAACGCATAAGG	N	N	N	1
	R	GAGTGTCCGTCGCTCITTATG				
17	F	GAGCAGGAGGGATGTGAG	N	N	1	1
	R	GAGATATCGATAAATGTGATTCCG				
18	F	CGAATTTTCAACAGCAATATTG	4	2	N	6
	R	GATCGAAGTGGCTGATACTTTG				
19	F	CTCCATTCGGAACCTCTCG	N	N	N	3
	R	CTGAGGGATTTTATGGTGG				