A TEST OF WORKER POLICING THEORY IN AN ADVANCED EUSOCIAL WASP, *VESPULA RUFA*

T. Wenseleers,[1,2,3] N. S. Badcock,[4] K. Erven,[4] A. Tofilski,[4] F. S. Nascimento,[5] A. G. Hart,[4] T. A. Burke,[4] M. E. Archer,[6] and F. L. W. Ratnieks[3,4]

[1]*Zoological Institute, University of Leuven, Naamsestraat 59, 3000 Leuven, Belgium*
[2]*E-mail: tom.wenseleers@bio.kuleuven.ac.be*
[4]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom*
[5]*Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 Ribeirão Preto, Brazil*
[6]*York St John College, Lord Mayors Walk, YO31 7EX York, United Kingdom*

Abstract.—Mutual policing is an important mechanism for maintaining social harmony in group-living organisms. In some ants, bees, and wasps, workers police male eggs laid by other workers in order to maintain the reproductive primacy of the queen. Kin selection theory predicts that multiple mating by the queen is one factor that can selectively favor worker policing. This is because when the queen is mated to multiple males, workers are more closely related to queen's sons than to the sons of other workers. Here we provide an additional test of worker policing theory in Vespinae wasps. We show that the yellowjacket *Vespula rufa* is characterized by low mating frequency, and that a significant percentage of the males are workers' sons. This supports theoretical predictions for paternities below 2, and contrasts with other *Vespula* species, in which paternities are higher and few or no adult males are worker produced, probably due to worker policing, which has been shown in one species, *Vespula vulgaris*. Behavioral observations support the hypothesis that *V. rufa* has much reduced worker policing compared to other *Vespula*. In addition, a significant proportion of worker-laid eggs were policed by the queen.

Key words.—Queen policing, reproductive conflict, Vespinae wasps, worker policing, worker reproduction.

Individuals in social groups frequently have dissimilar interests, leading to conflict (Hamilton 1995; Frank 1998; Keller 1999). Unchecked selfishness can be detrimental to group living (Hardin 1968; Frank 1995; Heckathorn 1996). To maintain social harmony, mechanisms have evolved that reduce individual selfishness and promote group efficiency. One such mechanism is social policing, whereby mutual enforcement limits the success of selfish individuals (Frank 1995, 1996, 2003; Ratnieks 1988; Wenseleers et al. 2003, 2004a,b; Ratnieks and Wenseleers 2005).

In insect societies, policing plays an important role in resolving conflict, including conflict over caste fate (Zimmerman 1983; Wenseleers et al. 2003, 2004a; Wenseleers and Ratnieks 2004; Ratnieks and Wenseleers 2005), breeder replacement (Monnin and Ratnieks 2001; Monnin et al. 2002), and male production (Ratnieks 1988; Wenseleers et al. 2004a,b). In eusocial Hymenoptera (ants, some bees and wasps), conflict over male production is of particular importance. Even though workers in most species are unable to mate, they usually possess functional ovaries and are able to lay unfertilized, male-destined eggs (Wilson 1971; Trivers and Hare 1976; Bourke 1988). This results in a conflict of interest between the queen and individual workers, with each preferring to produce the colony's males (Trivers and Hare 1976; Woyciechowski and Lomnicki 1987; Ratnieks 1988; Wenseleers et al. 2004b).

Policing, in the context of conflict over male parentage, is the process whereby individual workers are prevented from reproducing. Policing can be performed by the queen or by other workers, and has been observed to occur via one of

two mechanisms: aggression toward reproductive workers or destruction of worker-laid eggs (reviewed in Wenseleers et al. 2004b; Ratnieks et al. 2006). Kin selection theory (Hamilton 1964) predicts that the queen is selected to carry out such policing because she is more related to sons ($r = 1/2$) than to her daughter workers' sons (grandsons, $r = 1/4$; Trivers and Hare 1976). The workers, on relatedness grounds alone, are selected to police when queens mate with multiple males. This is because when the queen mates with multiple males, workers are on average more closely related to queen's sons (brothers, $r = 0.25$) than to other workers' sons (full- and half-nephews, $r < 0.25$; Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988).

Current data partially support these relatedness predictions (reviewed in Wenseleers et al. 2004b; Hammond and Keller 2004). Worker policing occurs in *Apis* honeybees (Ratnieks and Visscher 1989; Ratnieks 1993, 2000; Halling et al. 2001; Oldroyd et al. 2001) and in the common wasp *Vespula vulgaris* (Foster and Ratnieks 2001a), in which queens are multiply mated (Estoup et al. 1994; Palmer and Oldroyd 2000; Foster and Ratnieks 2001a). Worker policing also occurs in the saxon wasp *Dolichovespula saxonica* when colonies are headed by a multiple-mated queen but not when headed by a single-mated queen (Foster and Ratnieks 2000). In further agreement with the theory, little or no worker policing, but sometimes queen policing, occurs in taxa in which the queen is normally single mated (Strassmann 2001), such as stingless bees (Bego 1990), some *Polistes* and *Dolichovespula* wasps (Greene 1979; Fletcher and Ross 1985; Reeve 1991; Foster and Ratnieks 2001b; Saigo and Tsuchida 2004; Wenseleers et al. 2005), *Dinoponera* and *Diacamma* ants (Nakata and Tsuji 1996; Monnin and Peeters 1997; Kikuta and Tsuji 1999), bumblebees (e.g., Free et al. 1969; Pomeroy 1979)
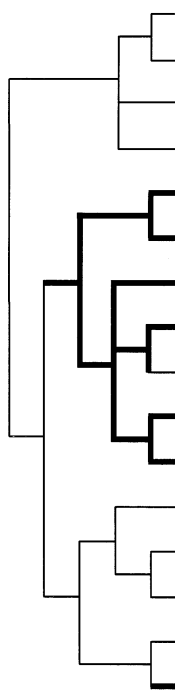
Phylogeny	Species	type of policing	effective paternity	% males workers' sons	% workers with active ovaries	mean colony size	N_c	N_a	Ref.
	Vespa crabro flavofasciata	NA	1.13	0	1	125	20	231	1
	Vespa crabro gribodi	W	1.11	0	2	550	14	176	2,3
	Vespa ducalis	NA	1.00	0	0	44	20	257	4
	Vespa mandarinia	NA	1.03	0	0	221	20	321	5
	Dolichovespula maculata	**Q+W**	**1.00**	**21**	NA	181	10	153	6,7
	Dolichovespula media	**Q**	**1.08**	**7**	6	74	8	148	6
	Dolichovespula arenaria	**Q**	**1.09**	**17**	6	378	9	236	7,8
	***Dolichovespula saxonica* (S)**	**Q**	**1.12**	**48**	14	69	6	110	6,9,10
	Dolichovespula saxonica (M)	Q+W	1.99	4	8	69	3	48	6,9,10
	Dolichovespula norwegica	**Q+W**	**1.08**	**3**	8	59	8	152	6,11
	Dolichovespula sylvestris	**Q+W**	**1.15**	**10**	9	76	10	154	6,12
	Vespula germanica	NA	2.35	0.6	0.6	1,000	12	64	13,14
	Vespula maculifrons	NA	7.14	0	2	1,000	15	172	13,15
	Vespula vulgaris	W	1.90	0	1	1,000	9	171	16
	Vespula squamosa	NA	3.27	0	NA	1,000	7	170	15
	Vespula rufa	**Q+W**	**1.50**	**11**	9	57	13	296	this study

FIG. 1. Data on queen mating frequency (effective paternity) and the percentage of males that are queen's versus workers' sons in the Vespinae (*Vespa, Dolichovespula, Vespula*) wasps. The type of policing is queen or worker policing, or both. The percentage of males that are workers' sons is for queenright colonies. Species with significant worker reproduction in queenright colonies are shown in bold, and are drawn with thick lines on the phylogeny; species with effective paternities not significantly different from 2 or greater are shown in a box. The percentage of workers with active ovaries is for queenright colonies. Active ovaries contain at least one greater than half-size egg. The phylogeny is from Carpenter (1987) and Carpenter and Perera (2002). Mean colony sizes are from Matsuura and Yamane (1990); Greene (1991); Foster et al. (2001); Hammond and Keller (2004); Takahashi et al. (2002, 2004a,b); and T. Wenseleers, unpublished data (for *D. norwegica*). N_c and N_a are the number of colonies and the number of assignable males assayed for male parentage, respectively; NA, not available. References: 1: Takahashi et al. (2004b); 2: Foster et al. (1999); 3: Foster et al. (2000); 4: Takahashi et al. (2002); 5: Takahashi et al. (2004a); 6: Foster et al. (2001); 7: Greene (1979); 8: F. L. W. Ratnieks and J. J. Boomsma, unpublished data; 9: Foster and Ratnieks (2000); 10: Foster and Ratnieks (2001b); 11: presence of queen and worker policing: T. Wenseleers, A. Tofilski, F. S. Nascimento and F. L. W. Ratnieks, unpublished data; 12: Wenseleers et al. (2005); 13: Goodisman et al. (2002); exact estimate of the proportion of worker produced males uncertain; worker reproduction was absent in 11 of 12 queenright colonies; one colony may have contained worker-produced males. However, the regression relatedness among workers in this colony was only 0.13 and below the permitted value for single-queen nests, 0.25. In addition, the actual worker genotypes strongly suggested that two matrilines were present, leaving only 1 of 40 males in this colony as definitely worker produced (note that this was overlooked in Hammond and Keller [2004], leading them to report a higher value for the percentage of males that are workers' sons). 14: Ross (1985); 15: Ross (1986); numbers of assignable males calculated from numbers of informative loci provided by K. Ross; 16: Foster and Ratnieks (2001a).

and *Lasioglossum* sweat bees (Michener and Brothers 1974). On the other hand, worker policing has also been shown in several ants (e.g., Iwanishi et al. 2003; Endler et al. 2004) and in the British hornet *Vespa crabro* (Foster et al. 2002), where queens usually mate only once (Foster et al. 2002; Iwanishi et al. 2003; Endler et al. 2004). This has been explained on the basis that worker policing could have additional benefits (Ratnieks 1988; Foster and Ratnieks 2001c). For example, policing of reproductive workers could increase colony efficiency (Ratnieks 1988), or the removal of worker-laid eggs, which are all male, could help the workers cause a female-biased sex allocation ratio (the worker optimum; Trivers and Hare 1976; Foster and Ratnieks 2001c).

Although all the above studies provide some support for the relatedness predictions of worker policing theory, there are still too few phylogenetically independent datapoints to make this trend statistically significant overall (Hammond and Keller 2004). To increase the number of available contrasts, we present an additional test of worker policing theory in the highly eusocial red wasp, *Vespula rufa*. *Vespula rufa* is thought to be of particular interest for studies of worker policing (Foster and Ratnieks 2001b) because *V. rufa*–group species, including *V. rufa* itself, have small colonies (typically 50–150 workers; Greene 1991; Archer 1997) compared to *V. squamosa* or *V. vulgaris*–group species (several thousand workers; Greene 1991; Fig. 1), which may be linked to a reduced queen mating frequency (Foster and Ratnieks 2001b). In *Vespula* species with high queen mating frequencies, few or none of the adult males are worker derived (Ross 1986; Foster and Ratnieks 2001a; Goodisman et al. 2002;

Fig. 1), presumably as a result of effective worker policing, which has been shown directly in one species, *V. vulgaris* (Foster and Ratnieks 2001a). If *V. rufa* were shown to have low queen mating frequencies (<2), kin selection theory predicts that worker policing should be reduced in comparison to other *Vespula*, and that it might have increased queen policing. Here we show that this prediction is upheld, providing significant novel support for relatedness-based theories of conflict in insect societies.

MATERIALS AND METHODS

Study Organism

Sixteen colonies of the red wasp, *Vespula rufa*, were collected between 2002 and 2004 (Table 1) in July at Cronshaw Chairs, an abandoned quarry in Whalley, Lancashire, United Kingdom. A further seven colonies were collected by M. E. Archer between 1971 and 1979 in the same locality (Table 1), and samples of these were stored on alcohol as part of a bionomic study. Overall, 20 of the 23 colonies (87%) were queenright, that is, had a laying queen, and were used for genetic analysis. The three queenless colonies had irregular brood patterns, with multiple eggs per cell as is typical in queenless nests of Vespinae wasps (Spradbery 1973; Ross 1986). Nests were mainly found at a shallow depth beneath bilberry (*Vaccinium myrtillus*) and heather (*Calluna vulgaris*) bushes. Nests have a short annual cycle, being founded in spring by a single overwintered and inseminated queen and dying out by the end of August after the production of young queens and males (Edwards 1980; Archer 1997). Nests generally consist of one comb of small worker cells and up to three combs of larger cells used for rearing males and queens, surrounded by multiple layers of envelope (Archer 1997). The collected nests were small, with the queenright colonies containing a mean of only 57 workers (SD = 20). Nevertheless, mature colonies of this species have been reported to contain up to 282 workers (Archer 1997).

Queen Mating Frequency and Worker Relatedness

From each of the 20 queenright nests, 15–40 queens or workers (mean = 25.2 ± 8.6 SD) were analyzed at four highly polymorphic microsatellite loci: RUFA03, RUFA15 (Thorén et al. 1995), LIST2019, and LIST2013 (Daly et al. 2002), using the protocol of Foster et al. (1999). Polymerase chain reaction amplification products were run on an ABI 3730 48-capillary automatic DNA sequencer (Applied Biosystems, Foster City, CA) and alleles were called using GeneMapper software supplied with the sequencer.

Genotypes of the mother queens were determined by direct genotyping, derived from male offspring genotypes (see below) or, when no males could be sampled, were reconstructed from worker genotypes using the program Matesoft (Moilanen et al. 2004; Table 1). Once the mother queen's genotypes had been established, Matesoft was used to determine the number of patrilines (queen's female offspring of the same father) contributing to each colony and to assign individual workers to particular patrilines. Individual patriline assignments were then used to calculate the effective paternity of each colony (Starr 1984), $M_e = 1/\Sigma_i p_i^2$, where p_i is the pro-

portional contribution of the *i*th male mate. This is the effective number of males the queen mates with, correcting for unequal paternity contribution. The corresponding pedigree worker-worker relatedness was calculated for each colony as $R = 1/4 + 1/2M_e$ (Pamilo 1991). Arithmetic mean relatedness and the corresponding harmonic mean paternity were calculated as population summary values. Harmonic mean is used because relatedness and paternity are inversely related to each other (Boomsma and Ratnieks 1996).

Two types of errors potentially confound effective paternity estimates: nondetection and nonsampling (Boomsma and Ratnieks 1996). The nondetection error is the probability that father males have identical multilocus genotypes, and hence cannot be distinguished; nonsampling error is the probability that one or more patrilines are actually present but are not sampled. However, the magnitude of both of these errors was found to be negligible. From the equations in Foster and Ratnieks (2001a), the nondetection error was estimated at 0.0003, and the mean nonsampling probabilities of males with 50% or 33% paternity were only 0.000005 and 0.0005, respectively, per colony. Hence, nonsampling did not significantly affect paternity estimates (Nielsen et al. 2003). The inequality in paternity contributions across father males in 14 multiple paternity nests was evaluated using the paternity skew index $S = (M_t - M_e)/(M_t - 1)$ (Pamilo and Crozier 1996), where M_t is the total number of male mates and M_e the effective number of mates, for the given worker array. This index rises from 0 to 1 as paternity becomes more unequal. For six double-paternity nests we also calculated the proportional contribution of the majority male. As a result of sampling error, unequal paternity is expected even when the fathers have equal total numbers of offspring (Foster and Ratnieks 2001a). Therefore, the paternity skew and majority male contribution for each colony were compared to those expected from a simulated binomial or multinomial distribution given a particular sample size using a paired *t*-test. With the sampling of 15–40 workers, equal paternity should result in skews of 0.03–0.16 and majority male contributions of 0.56–0.61, respectively.

To test for random mating, we calculated the relatedness between the queen and her mates using the reconstructed parental genotypes and the program Relatedness 5.04, which employs the algorithms of Queller and Goodnight (1989). The null hypothesis was that if mating is random, the queen and her mates should be unrelated to each other. The Relatedness program was also used to calculate allele frequencies. To do this, colonies were weighted equally.

Male Parentage

To determine the proportion of offspring males that were the queen's and the workers' sons, a total of 342 (*N*) males from 13 queenright colonies were genotyped (mean 26.3 ± 13.3 SD per colony) using the same four loci. Loci are informative only if the paternal and maternal alleles of the workers differ; that is, a worker's son can be distinguished from a queen's sons when he inherits a paternal allele not present in the queen (Foster et al. 2000). The proportion of worker-produced males for each nest was estimated as $N_w/(P_j N_j)$, where N_j and N_w are the number of detected worker's

TABLE 1. Collection dates, paternity and pedigree relatedness of 20 queenright *Vespula rufa* colonies. For multiple and double paternity nests, paternity skew and the majority male contribution are given.

Nest number	Date collected	Number of workers genotyped	Number of males genotyped	Number of fathers detected	Effective paternity	Paternity skew	Majority male contribution	Pedigree sister-sister relatedness
1[a]	Aug. 1971	17	0	3	2.51	0.24		0.45
2[b]	Aug. 1976	23	0	1	1.00			0.75
3[a]	Aug. 1978	40	8	2	2.00	0.00	0.53	0.50
4[a]	Aug. 1978	39	21	4	2.25	0.58		0.47
5[a]	Aug. 1978	19	0	1	1.00			0.75
6[a]	Aug. 1979	21	15	1	1.00			0.75
7[b]	Aug. 1979	19	0	1	1.00			0.75
8[a]	July 2002	26	17	2	1.90	0.10	0.62	0.51
9[a]	July 2002	15	8	2	1.47	0.53	0.80	0.59
10[b]	July 2002	25	0	3	1.90	0.55		0.51
11[a]	July 2002	21	49	4	3.08	0.31		0.41
12m1[a,c]	July 2002	4	0	1	1.00			0.75
12m2[b,c]	July 2002	12	0	3	2.00	0.50		0.50
13[a]	July 2003	24	19	4	3.03	0.32		0.41
14[a]	July 2003	31	42	3	2.24	0.38		0.47
15[b]	July 2003	25	0	3	2.04	0.48		0.50
16[a]	July 2003	33	27	2	1.50	0.50	0.79	0.58
17[a]	July 2003	18	29	2	1.80	0.20	0.67	0.53
18[a]	July 2003	37	28	1	1.00			0.75
19[a]	July 2003	15	36	1	1.00			0.75
20[a]	July 2004	40	43	2	1.72	0.28	0.70	0.54
Mean		$\bar{x} = 25.20$	$\bar{x} = 26.31^d$	$\bar{x}_h = 1.69^e$	$\bar{x}_h = 1.50^e$	$\bar{x} = 0.36^f$	$\bar{x} = 0.68^f$	$\bar{x} = 0.58^f$
(95% confidence interval)				(1.37,2.22)	(1.28,1.82)	(0.25,0.46)	(0.57,0.79)	(0.52,0.64)

[a] Maternal genotype known from direct genotyping or from male offspring genotypes. [b] Most parsimonious maternal genotype reconstructed from the worker genotypes using the Matesoft program (Moilanen et al. 2004). [c] Two matrilines detected within the same colony. [d] For colonies from which males could be sampled. [e] Harmonic mean. [f] Arithmetic mean.

sons and the total number of males analyzed for the jth nest and P_j is the power of detecting a worker's son in the jth nest (Foster and Ratnieks 2001a). From equation (2) in Foster and Ratnieks (2001a), the power for detecting workers' sons was found to be high, 88%, leading to an effective number of assignable males of $P \times N = 0.88 \times 342 = 296$.

To establish how many workers were capable of laying eggs in each colony, we dissected a total of 240 workers, taken from 12 mature queenright nests, using a binocular microscope with a reticle eyepiece. The size of the largest egg present in each worker's ovaries was placed into one of three categories: <50%, 50–90%, and >90% the size of a mature, newly laid egg.

Behavioral Observations of Worker Reproduction and Policing

Ten *V. rufa* colonies were transferred to 15 cm (w) × 17 cm (d) × 20 cm (h) wooden observation boxes maintained outside the Laboratory of Apiculture and Social Insects, University of Sheffield, Sheffield, United Kingdom. Colonies were allowed to fly freely and forage naturally. Of these 10 nests, two successfully re-established (colonies 13 and 20, Table 1) and were used to observe natural rates of worker egg laying and policing following the protocol of Wenseleers et al. (2005). Briefly, the lower part of the nest envelope was removed and the lower sexual comb glued onto a piece of thin wire. Any eggs or larvae were removed so that all cells were available for egg laying. The comb was then put back into its original position using children's modeling clay to hold the wire (for details see Wenseleers et al. 2005). Subsequently, observations on egg laying and policing were made for 24 h per trial using a small infrared night vision camera (model MS37S, Maplin Electronics, Barnsley, U.K.) placed at the bottom of each nest box and connected to a computer using an EZMaker framegrabber (Avermedia, Milpitas, CA) and VirtualDub (http://www.virtualdub.org/) software. This allowed time-lapse (five frames per second) digital video recording onto the computer hard disk. Queen and worker egg laying was observed for the first 8 h of every trial, and the fate of all eggs laid was tracked for up to 24 h from the start of the trial. Since the actual eggs were not always visible on the video, we carefully removed and checked the comb by eye once an hour to look for the appearance of new eggs or the absence of eggs laid earlier. The video recordings were then analyzed in detail to determine how many eggs were queen- and worker-laid and whether these eggs were policed by either the queen or by a worker (Wenseleers et al. 2005). This procedure was replicated six times for each colony, resulting in a total observation time of 48 h per colony, and data from these separate trials were pooled. Observations were carried out between 9 and 23 July 2003 (for colony 13) and between 15 and 23 July 2004 (for colony 20), which was the peak period of egg laying in sexual cells.

Results
Allelic Diversity

Genetic variation at the four microsatellite loci studied was very high, with 26, 19, 9, and 11 detected alleles and expected heterozygosities of 0.94, 0.90, 0.63, and 0.85 at loci RUFA03, RUFA15, LIST2019, and LIST2013, respectively.

Paternity and Worker Relatedness

The mean pedigree relatedness was 0.58, which is significantly greater than 0.5 (single-sample t-test, $t = 2.94$; one-sided $P = 0.004$; Table 1). The corresponding harmonic mean effective paternity was 1.50, the lowest yet recorded in *Vespula* (Fig. 1). One factor contributing to this low effective paternity was the unequal paternity found in multiple paternity nests (Table 1): the mean paternity skew in 14 multiple paternity nests was 0.36 (Table 1), significantly greater than the value expected if all fathers contributed equally, 0.03–0.16 with $n = 15$–40 (paired t-test, $t = 6.08$, $P = 0.00004$). The majority male contribution in six double paternity nests was 68%, which was also significantly higher than the value expected with equal paternity, 0.56–0.61 with $n = 15$–40 (paired t-test, $t = 2.61$, $P = 0.047$).

In one colony, two matrilines were detected, indicating queen takeover (the current queen was represented by 4/16 workers, and the remaining workers were the offspring of the superseded queen). The two matrilines were treated separately in the calculation of paternity statistics (Table 1). The relatedness between the queen and her mates was −0.08, and not significantly different from zero (single sample t-test, $t = -1.52$, $P = 0.14$), indicating that mating was random.

Male Parentage

Workers' sons were found in 6 of 13 queenright colonies from which males could be sampled, with an estimated 18%, 6%, 51%, 12%, 10%, and 8% of all males being workers' sons in each of these six colonies. In total, 33 males were assigned to workers, hence 11.1% (33/296; 95% confidence interval: 8.1–14.9%) of the sampled males were workers' sons. Sampling error may explain why no workers' sons were detected in some nests, as a positive correlation was found between the number of males analyzed per nests and the proportion of males that were worker produced (Spearman $R = 0.71$, $P = 0.006$). Worker production of males was not less common in low-paternity nests, as shown by the nonsignificant relationship between intracolony relatedness and the proportion of workers' sons (Spearman $R = -0.28$, $P = 0.36$). This indicates that worker reproduction and policing are not facultatively adjusted by colony members based on the relatedness structure of the colony they find themselves in, but that these behaviors are shaped evolutionarily in the context of population averages.

Worker Ovary Activation

At least one worker with active ovaries was found among the 20 workers dissected in each of the 12 queenright colonies studied ($n = 240$ workers in total); 13 workers (5.4%) had a more than 90% full-sized oocyte, and a further 9 (3.8%) had more than a 50% full-sized oocyte. Hence, 22 of 240 (9.2%; 95% confidence interval: 6.2–13.1%) workers had active ovaries and were capable of laying eggs. Given a mean colony size of 57 workers, each colony was expected to contain an average of five egg-laying workers.

TABLE 2. Number of eggs laid by the queen and the workers during the first 8 h of six trials in two *Vespula rufa* colonies, number 13 and 20 in Table 1, and the number of eggs that remained after 24 h.

Colony	Eggs laid by:	During first 8 h of 6 trials[a]	No. remaining after 24 h (%)	Percentage of policing by the queen	Observed percentage of eggs that are workers' sons	Inferred percentage of males workers' sons[b]	
						At egg laying	After 24 h
13	queen	76	74 (97%)				
	workers	15	3 (20%)	8/12 = 67%	17%	44%	14%
20	queen	128	125 (98%)				
	workers	13	6 (46%)	3/7 = 43%	9%	29%	16%
Total	queen	204	199 (98%)				
	workers	28	9 (32%)	11/19 = 58%	12%	36%	15%

[a] Total observation time per colony: 48 h. [b] Calculated given that queens lay 24.8% male eggs, an estimate that is based on the fact that a large-celled comb contains 27.1% males ($n = 15$ colonies, six from this study and nine from Archer 1997), of which 11% are workers' sons.

Behavioral Observations of Worker Reproduction and Policing

The two observation colonies contained 88 and 64 workers and had effective paternities of 3.03 and 1.72, respectively. Despite the variation in paternity, both colonies behaved alike. In both colonies, a significant percentage of the eggs were worker laid (17% and 9%, mean 12%; 95% confidence interval: 9–17%; Table 2). Furthermore, whereas 199 of 204 (98%; 95% confidence interval: 95–99%) queen-laid eggs survived for one day after laying, only 9 of 28 (32%; 95% confidence interval: 19–49%) worker-laid eggs survived. Although this difference in survival is significant (Fisher exact test, $P < 10^{-16}$), the survival of worker-laid eggs is much higher than in *V. vulgaris*, in which 97% of all worker-laid eggs were removed within one hour and none (of 120) remained after one day (Foster and Ratnieks 2001a). Hence, the policing of worker-laid eggs is less effective in *rufa* than in *vulgaris*. The fact that the *V. vulgaris* study used transferred worker-laid eggs from a queenless colony (Foster and Ratnieks 2001a), as opposed to naturally laid eggs in this study, is unlikely to account for this great difference, since most of the transferred queen-laid eggs, 80 of 120 (67%), successfully survived for one day (Foster and Ratnieks 2001a). In further contrast to *V. vulgaris*, 11 of 19 (58%, 95% confidence interval: 38–76%) of the worker-laid eggs that were removed from their cell were policed by the queen. In *V. vulgaris*, videotaping showed that all policing was carried out by workers (Foster and Ratnieks 2001a). A video clip of worker egg-laying and queen policing in *V. rufa* is available as online supplementary data to this paper (available online at http://dx.doi.org/10.1554/04-532.1.s1).

There is good agreement between the inferred percentage of male eggs that survive for one day (15%; Table 2) and the average percentage of adult males that are workers' sons in queenright nests (11%). This suggests that most eggs are policed within 24 h of being laid.

Aggressive interactions between the queen and workers and among the workers themselves were infrequently observed. During a total of 96 hours of observation, we observed two cases, once in colony 13 and once in colony 20, of the queen preventing a worker from laying an egg by pushing the worker off the cell. In one further case (in colony 20), the queen attacked a worker that had recently laid an egg. In five cases, three times in colony 13 and twice in colony 20, workers were observed to fight among themselves, and in

one case the worker that initiated the fight was a reproductive worker and had recently oviposited. In four cases, twice in colony 13 and twice in colony 20, a worker attacked the queen, but whether the attacking workers were egg-laying workers could not be determined.

DISCUSSION

Vespula rufa was found to have an effective paternity of 1.50, much lower than that of closely related *Vespula* in which paternity is either not significantly different from 2.0 (*V. vulgaris*; Foster and Ratnieks 2001a) or higher (2.4, 3.3, and 7.1 in *V. germanica*, *V. squamosa*, and *V. maculifrons*, Goodisman et al. 2002; Ross 1986, Fig. 1). One contributory factor to this low paternity was the fact that when queens were multiply mated, paternity contributions were frequently very unequal (Table 1), similar to what has been observed in *Dolichovespula* (Foster et al. 2001), but unlike large-colony *Vespula*, in which fathering males contribute more or less equally (Foster and Ratnieks 2001a). The observed low paternity allowed for a test of worker policing theory, with greater worker policing being expected in the high paternity *Vespula* than in *V. rufa* (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988). Previous results, complemented by our new data on *V. rufa*, support this prediction. Worker policing has been shown in *V. vulgaris* (Foster and Ratnieks 2001a) and is strongly suspected in *V. germanica*, *V. squamosa*, and *V. maculifrons* given that few or none of the adult males are workers' sons (Ross 1986; Goodisman et al. 2002; Fig. 1), despite the presence of a significant number of egg-laying workers (0.6–2.0% in queenright colonies; Fig. 1). By contrast, in *V. rufa*, we found that a much larger proportion of workers had active ovaries (9%), that 12% of the eggs were worker laid, and that a significant proportion, 11%, of the adult males were workers' sons. Furthermore, observations confirmed that worker policing was much less effective in *V. rufa* than in *V. vulgaris* (Table 2) and that most (58%) of the worker-laid eggs that were removed from cells were policed by the queen (Table 2) rather than by workers. We also observed three instances of queen aggression against egg-laying workers. All these observations demonstrate severe queen-worker conflict, as expected when paternity is less than 2 (Starr 1984; Ratnieks 1988).

With its low paternity, high levels of worker reproduction and queen policing, *Vespula rufa* resembles *Dolichovespula* more than other *Vespula*. In *Dolichovespula*, paternity is close

to 1, many workers (6–12%) have active ovaries, and a high proportion of the male eggs and adults are workers' sons (Foster and Ratnieks 2000; Wenseleers et al. 2005; Fig. 1). Furthermore, as in *V. rufa*, queen policing plays an important role in limiting the success of reproductive workers (Greene 1979; Foster and Ratnieks 2001b; Foster et al. 2001; Wenseleers et al. 2005; Fig. 1), although worker policing also occurs in some species (Foster and Ratnieks 2000; Wenseleers et al. 2005; Fig.1). That both *V. rufa* and *Dolichovespula* have small colonies of only several hundred workers (Greene 1991) probably helps the queen to keep control over the colony (Bourke 1999). Undoubtedly, effective queen policing would be much harder in the large-colony *Vespula* in which colonies can have several thousand workers (Edwards 1980) and possibilities to evade queen policing would seem much greater (Bourke 1999).

In *Vespula*, worker oviposition in queenright colonies has previously only been observed in two other small-colony species of the *V. rufa* species group, the blackjacket *V. consobrina* (Akre et al. 1982) and the forest yellowjacket *V. acadica* (Reed and Akre 1983). In the latter, the queen was also reported to attack egg-laying workers (Reed and Akre 1983). On the other hand, no worker laying was seen in three colonies of *Vespula atropilosa* (Akre et al. 1976), another member of the *rufa* species group, and egg-laying workers in queenright colonies were observed to be killed by other workers (Landolt et al. 1977). Whether differences in relatedness explain this variation remains unknown, but future work could attempt to test this.

Overall, our study is important in that it provides significant additional support for the postulated role of relatedness in shaping male parentage conflict in social insects (Ratnieks 1988). Comparing species within *Vespula* and *Dolichovespula*, there is a good match between theory and data (Fig. 1). That is, in the high paternity *Vespula* and in multiple paternity colonies of *D. saxonica*, effective worker policing virtually eliminates worker production of sons (Foster and Ratnieks 2000, 2001a). By contrast, in single paternity colonies of *D. saxonica* and in the low paternity *Dolichovespula sylvestris*, *D. norwegica*, and *V. rufa*, a significant proportion of the males are workers' sons (Foster and Ratnieks 2000; Foster et al. 2001; Wenseleers et al. 2005), because in these worker policing is much less pronounced and often replaced by less effective queen policing (Wenseleers et al. 2005; Fig. 1). Interestingly, a small amount of worker policing does occur in *Dolichovespula sylvestris*, *D. norwegica*, and *V. rufa* (Wenseleers et al. 2005; Fig. 1). However, at least in *D. sylvestris*, there is some evidence that this is carried out by ovipositing workers, who eat each other's eggs to free up cells to lay into, but spare the queen's eggs (Wenseleers et al. 2005). Hence, the benefits of direct reproduction may well explain why worker policing occurs in these species even though their mating frequency is close to 1. On the other hand, the complete absence of workers' sons in hornets, *Vespa* (Foster et al. 2002; Takahashi et al. 2002, 2004a,b) shows that relatedness is not the only factor, and that other factors such as colony efficiency, sex-ratio costs or a queen-worker arms race may play additional roles in shaping male parentage conflict (Ratnieks 1988; Foster and Ratnieks 2001c; Foster et al. 2002). This mirrors the conclusion reached by Hammond and Keller (2004), who analyzed male parentage patterns across 50 species of ants, bees, and wasps and found that relatedness alone does not explain all the variation seen in the proportion of males that are worker produced. Even so, from our study it is clear that relatedness is important. In the future, comparative analyses such as those performed by Hammond and Keller (2004) could help to reinforce this point, and thereby provide an important test of kin selection theory (Hamilton 1964).

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