THE ROYAL SOCIETY biology letters

Queen and worker policing in monogynous and monandrous colonies of a primitively eusocial wasp

T. Saigo and K. Tsuchida*

Laboratory of Entomology, Faculty of Applied Biological Sciences, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan
** Author for correspondence (tsuchida@cc.gifu-u.ac.jp).*

Recd 19.06.04; *Accptd* 16.08.04; *Published online* 17.11.04

Kin-selection theory predicts that a worker prefers to produce her own sons in a colony with monandry and monogyny because relatedness to her sons (0.5) and nephews (0.375) exceeds that to brothers (0.25). In spite of this prediction, recent studies reveal that workers police each other (mutual-worker egg removal) even in monandrous and monogynous colonies. We conducted field and laboratory studies to evaluate queen and worker policing in queen-right colonies of the primitively eusocial wasp *Polistes chinensis antennalis*. Genetic studies using microsatellite markers, as well as extensive observations of natural colonies, revealed that both queen and workers removed both queen- and worker-laid eggs in monogynous and monandrous colonies. The queen's eggs survived to hatching more successfully than those of the workers (88.5% versus 1.4%). We discuss the likely factors to explain these worker-policing behaviours.

Keywords: *Polistes chinensis antennalis*; worker policing; queen policing; male parentage

1. INTRODUCTION

According to kin-selection theory (Hamilton 1964), in monogynous and monandrous hymenopteran social insect colonies without inbreeding, a worker prefers to rear her sons or nephews over her brothers. Kin selection also predicts that mutual-worker policing (mutual-worker egg removal) evolves in polygynous or polyandrous colonies (Ratnieks 1988). Mutual-worker policing could be favoured by a collective-worker force when a mother queen mates with several males, such that workers could be more related to their brothers than to nephews. Indeed, the lack of worker-produced males has been attributed to worker policing in hymenopteran social insects (Ratnieks 1993; Monnin & Peeters 1999; Foster & Ratnieks 2000). However, recent studies show that worker policing is observed even in monogynous and monandrous colonies (Kikuta & Tsuji 1999), where workers do not gain any genetic benefits. Alternatively, if worker policing increases colony productivity, then policing is theoretically selected for (Ratnieks 1988).

Worker-policing behaviours have been observed in advanced eusocial species such as honeybees and yellowjackets (Ratnieks & Visscher 1989; Foster & Ratnieks 2000), or ponerine ants where worker egg productions are experimentally induced (e.g. Kikuta & Tsuji 1999). To advance the understanding of worker policing, it is crucial to study the magnitude of worker policing under natural conditions using primitively eusocial species. A Japanese paper wasp *Polistes chinensis antennalis* provides an excellent opportunity to test worker-policing theory under a relatedness framework in a primitively eusocial stage, because both monogyny and monandry generally occur and worker reproduction is frequent in the queen-right colonies in which a queen is alive (Tsuchida *et al.* 2003*a*). We evaluate the survivorships of queen- and worker-derived eggs using highly polymorphic microsatellite markers, coupled with extensive behavioural observation, and discuss the potential factors that may explain for explaining worker-policing behaviour.

2. MATERIAL AND METHODS

In 2001 and 2002, all wasps from five wild queen-right colonies were individually marked, and the cells in which the wasps oviposited were recorded for a total of 72.5 h. Our study period (16 June–31 July) corresponded to the production stage of new reproductive females and males. Approximately every 2 days, each newly emerged wasp was marked with individual paint dots, and every cell's position and its content (egg, larva and pupa) were recorded. Within 2 days of egg hatching the resulting larvae were collected and preserved in 100% ethanol.

Samples of each individual's DNA were prepared from the legs of adult wasps ($n = 102$) and the whole body of larvae ($n = 122$), and genotyped for 13 microsatellite loci (Tsuchida *et al.* 2003*b*). We screened between 14 and 25 adult females per colony. In addition, we found that 27 immature individuals in our genotyped larvae were females, resulting in 20–37 females per colony being screened. When 20 and 37 females are genotyped, non-sampling probabilities (see Foster *et al.* 1999) of 10% paternity become 0.12 and 0.02, respectively. These values seem to be acceptable levels for our pedigree constructions. We investigated the mother–offspring relationship between the genotype of a larva and that of its putative mother whose oviposition we observed. The compatibilities were examined by using the exclusion method: if the genotype of a larva was incompatible with that of a putative mother at any one of the 13 loci, then we judged the larva to have been replaced. Otherwise, we judged that it had not been replaced. We confirmed that, from our cell records, all larvae of the five colonies survived during our study period. Neither queens nor workers ate their own eggs. Thus, it is safe to conclude that cannibalism took place only during the egg stage. We successfully tested the mother–offspring compatibilities between 45 putative mothers and 98 larvae. The production of diploid offspring by mated workers is eliminated, because early males that are possible mating partners for workers are rare and diploid (Tsuchida *et al.* 2002, 2004). To our knowledge, thelytokous parthenogenesis has not previously been reported in this species.

3. RESULTS

The total number of workers in the five colonies was 39.4 ± 20.8 (s.d.) at the end of our observations. Adult males were not present in the colonies. Non-detection error (Boomsma & Ratnieks 1996) owing to low allelic variability was negligible for every colony ($p < 0.001$). None of the adult genotypes of the colonies was inconsistent with monogyny and monandry. On average, 15.2% of the marked workers in the colonies were observed to oviposit (15.2 ± 5.7 s.d.), whereas the remaining workers did not. Workers' eggs were removed more frequently than queen's eggs, and the survival rates significantly differed between the castes in each colony (figure 1*b*,*c*,*d*) except for colonies

DOI 10.1098/rsbl.2004.0238

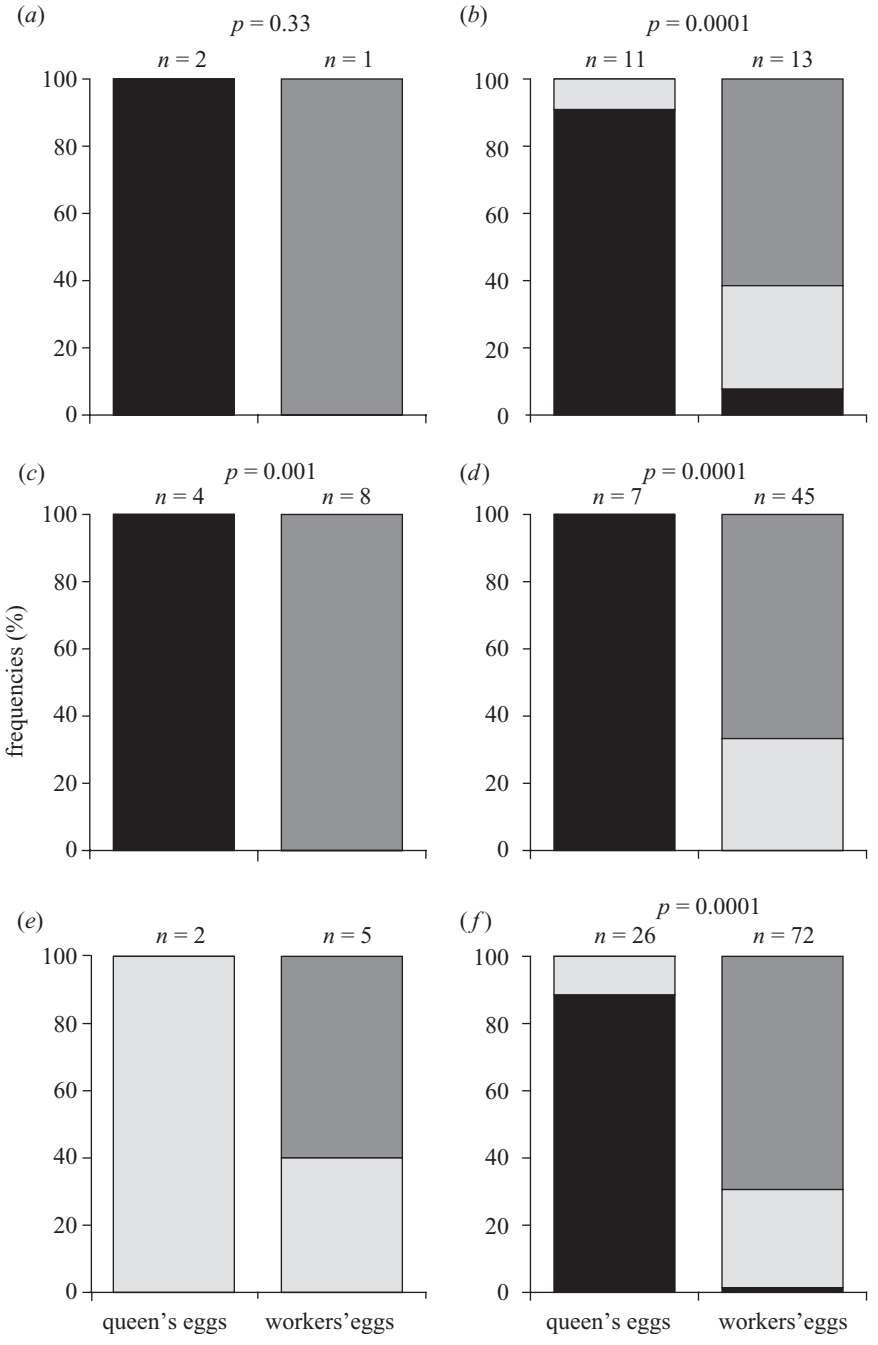

Figure 1. Frequencies of worker and queen policing. The differences in survival rate of queen's and workers' eggs (un-policed eggs) were highly significant, except for colony 0008 and 0073, using a Fisher's exact probability test. Workers produced only males. See table 1 for the sex and number of queen-produced eggs. (*a*) Colony 0008; (*b*) colony 1015; (*c*) colony 0071; (*d*) colony 1019; (*e*) colony 0073; (*f*) total. Dark grey, eggs replaced by queen; light grey, eggs replaced by workers; black, survived eggs.

0008 and 0073 (figure 1*a*,*e*; Fisher's exact probability test). Overall, the queen's eggs survived to hatching more frequently than the workers' eggs (88.5% versus 1.4%, Fisher's combined probability test, $\chi^2_8 = 56.07$, $p < 0.001$). In four out of the five colonies, the queen replaced the workers' eggs with her eggs. In three out of the five colonies, workers replaced eggs produced by other workers with their own eggs. In two colonies (0073 and 1015), workers removed queen's egg(s), but in 1019, workers removed only workers' eggs, which significantly deviated from random elimination (binominal test, $p < 0.0001$). In total, 69.4% of workers' eggs (50/72) were replaced by queen's eggs (queen policing) and 29.2% of the eggs (21/72) were replaced by workers' eggs (worker policing) (figure 1). We sexed the queens' eggs (table 1) by assuming that males were those that were not heterozygous at any of the 13 loci. The probability of females with homozygosities at all loci was extremely low ($p < 0.0001$). Queens produced both males and females, and the sex ratios seemed either to be unbiased or male-biased; the ratios of colonies 0008 and 1019 were unbiased, and those of 0071 and 1015 were male-biased (binominal test). The sex ratio for the pooled data was male-biased, as

Table 1. Sex ratio (proportion of males) among individuals with and without queens policing and the number of worker-policed eggs.

	number of offspring of queen									
	without policing		with queen policing		number of worker-policed individuals[a]					
colony	male	female	male	female	queen eggs	worker eggs	sex ratio	total no. of larvae (%)[b]	observation time (min)	number of cells
0008	1	1	1	0	0	0	0.67 n.s.	3 (100)	300	173
0071	4	0	8	0	0	0	1**	12 (80)	780	507
0073	—	—	3	0	2	2	1 n.s.	7 (78)	870	435
1015	8	2	6	2	1	4	0.78*	24 (73)	780	385
1019	4	3	11	19	0	15**	0.41 n.s.	52 (84)	1620	382
pool	17	6	29	21	3	21**	0.63c*	98 (80)	—	—

[a] Number of worker-policed individuals.
[b] Number of larvae (%) are those where we successfully constructed mother–offspring relationships using microsatellite genotypings.
[c] We calculate Fisher's combined probability, which revealed a significant difference from parity ($p < 0.01$).
* $p < 0.05$; ** $p < 0.005$; n.s., not significant.

shown by a binomial test ($p = 0.03$) and Fisher's combined probability test ($\chi^2_8 = 20.39$, $p < 0.005$). These results indicate that queens did not oviposit only female eggs. Queens contributed 77.6±22.8% of the male production of the colonies, which was marginally higher than the workers' contribution (22.4±22.8%; Wilcoxon test, $p = 0.06$).

4. DISCUSSION

Our microsatellite genotyping clearly demonstrated that most of a queen's eggs survived to hatching (88.5%), whereas most workers' eggs did not (1.4%). Queens' eggs were also policed in three cases. If we assume that all three queens' eggs that were replaced later by workers were males, the survival rate of queens' male eggs would be 85% (17/20), and the above-mentioned trend unchanged. Both queen and workers replaced the workers' eggs. In total, queens contributed 2.4 times (69.4/29.2) more replacing than did workers. However, the rate of worker policing here could be an underestimated value, because it was the rate at which fellow workers policed the eggs at least once; our observed worker-policing behaviours could be snapshots of continuous mutual-egg removal. We observed that different workers sequentially conducted oophagy and oviposited in the same cells. In addition, workers produce three to four times more eggs than a queen in queen-right colonies with 100–500 cells (Tsuchida *et al.* 2003*a*). We observed 26 oviposition by queens and 72 by workers, and confirmed 50 replacements by queens and 24 by workers, indicating 76 egg productions by queens and 96 by workers during the study period (figure 1). These results suggest that our estimated frequency of worker policing seems to be a minimum value: 'the tip of an iceberg'.

Worker policing is theoretically favoured under two conditions. First, worker policing should be favoured under polyandry, because workers are still more closely related to their own sons, but with an effective queen-mating frequency above two, their average relatedness to their nephews is lower than to their brothers. We have detected worker policing in three out of the five monogynous and monandrous colonies, suggesting that the traditional explanation of relatedness benefits for workers is unlikely.

Second, when worker reproduction imposes a cost on colony productivity, worker policing is selected for (Ratnieks 1988), even under monogyny and monandry. One piece of crucial evidence to contradict the colony-efficiency hypothesis is that non-reproducing workers seemed not to conduct oophagy; if colony efficiency promotes worker policing, every worker should remove eggs laid by fellow workers. In *P. chinensis antennalis*, reproducing workers check the cells and subsequently insert their abdominal tips when they oviposit. We did not detect any apparent oophagies by any workers except upon oviposition, suggesting that non-reproducing workers never eat eggs and selfishness of reproducing workers is likely to cause mutual-egg removal. Connected with this selfishness, size constraints limiting ovipositing in the nests may be another probable explanation for the mutual-egg removal. Workers produce more eggs than a queen, and the difference in the egg productivities between the castes became more pronounced as colony size increased (Tsuchida *et al.* 2003*a*), suggesting that reproducing workers compete with each other and they should remove their nest-mate-workers' eggs upon their oviposition under the condition of limited oviposition place.

The ability of an adult to recognize the sex of an immature is a prerequisite to manipulating the sex ratio for maximizing inclusive fitness. However, to our knowledge, no report has confirmed that adults can discriminate sex at the egg stage (Passera & Aron 1996). This lack of evidence for egg sexing leads us to suggest that adults of social insects can discriminate the sex of an immature only after egg hatching. Moreover, many researchers have reported that a queen's eggs are chemically marked (e.g. Endler *et al.* 2004). These findings suggest that adults of social insects might recognize the odour marking on eggs, signalling the identity of queen-laid eggs, but might not discriminate the sex of eggs (Nonacs & Carlin 1990). If a queen marks her eggs and workers can recognize the odour, upon every replacement, they should favour the removal of eggs destined to be nephews, avoiding the high cost of destroying queen-laid female eggs (sexual-deception hypothesis; Nonacs & Carlin 1990). In one of our study colonies (1019), workers removed only fellow workers' eggs. This result suggests that, albeit that variation between colonies

could exist, workers potentially have an ability to discriminate between queen's eggs and workers' eggs. It has been shown that there is higher survivorship of eggs oviposited by dominant individuals than by subordinate ones, suggesting the existence of an egg-marking pheromone (e.g. Ratnieks & Visscher 1989; Endler *et al.* 2004). Alternatively, Pirk *et al.* (2004) reported that inferior viability of workers' eggs is one factor responsible for worker policing in the honeybee. Further studies are needed to evaluate the proximate mechanisms, particularly for the difference in egg nature between queen and worker eggs, for a deeper understanding of worker policing.

Acknowledgements

The authors thank E. Kasuya, B. Kranz, K. Kudô and K. Tsuji for comments on the manuscript. They also express their thanks to K. Takeuchi for fieldwork assistance. Comments of three anonymous reviewers were helpful to improving this manuscript. This study was supported in part by grants-in-aid from the Japan Ministry of Education, Science and Culture (11440228, 11833001 and 12304048).

Boomsma, J. J. & Ratnieks, F. L. W. 1996 Paternity in eusocial Hymenoptera. *Phil. Trans. R. Soc. Lond.* B **351**, 947–975.

Endler, A., Liebig, J., Schmitt, T., Parker, J. E., Jones, G. R., Schreier, P. & Hölldobler, B. 2004 Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. *Proc. Natl Acad. Sci. USA* **101**, 2945–2950.

Foster, K. R. & Ratnieks, F. L. W. 2000 Facultative worker policing in a wasp. *Nature* **407**, 692–693.

Foster, K. R., Seppä, P., Ratnieks, F. L. W. & Thoren, P. A. 1999 Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behav. Ecol. Sociobiol.* **46**, 252–257.

Hamilton, W. D. 1964 The genetical evolution of social behavior I. II. *J. Theor. Biol.* **7**, 15–72.

Kikuta, N. & Tsuji, K. 1999 Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. *Behav. Ecol. Sociobiol.* **46**, 180–189.

Monnin, T. & Peeters, C. 1999 Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behav. Ecol.* **10**, 323–332.

Nonacs, P. & Carlin, N. F. 1990 When can ants discriminate the sex of brood? A new aspect of queen–worker conflict. *Proc. Natl Acad. Sci. USA* **87**, 9670–9673.

Passera, L. & Aron, S. 1996 Early sex discrimination and male brood elimination by workers of the Argentine ant. *Proc. R. Soc. Lond.* B **263**, 1041–1046.

Pirk, C. W. W., Neumann, P., Hepburn, R., Moritz, R. F. A. & Tautz, J. 2004 Egg viability and worker policing in honey bees. *Proc. Natl Acad. Sci. USA* **101**, 8649–8651.

Ratnieks, F. L. W. 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**, 217–236.

Ratnieks, F. L. W. 1993 Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behav. Ecol. Sociobiol.* **32**, 191–198.

Ratnieks, F. L. W. & Visscher, P. K. 1989 Worker policing in the honeybee. *Nature* **342**, 796–797.

Tsuchida, K., Nagata, N. & Kojima, J. 2002 Diploid males and sex determination in a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera, Vespidae). *Insect. Soc.* **49**, 120–124.

Tsuchida, K., Saigo, T., Nagata, N., Tsujita, S., Takeuchi, K. & Miyano, S. 2003*a* Queen–worker conflict over male production and sex allocation in a primitively eusocial wasp. *Evolution* **57**, 2365–2373.

Tsuchida, K., Saigo, T., Tsujita, S., Takeuchi, K., Ito, N. & Sugiyama, M. 2003*b* Polymorphic microsatellite loci for the Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). *Mol. Ecol. Notes* **3**, 384–386.

Tsuchida, K., Saigo, T., Tsujita, S. & Takeuchi, K. 2004 Early male production is not linked to a reproductive strategy in the Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). *J. Ethol.* **22**, 119–121.