

ORIGINAL ARTICLE

Mating frequency and genetic relatedness of workers in the hornet *Vespa analis* (Hymenoptera: Vespidae)

Jun-ichi TAKAHASHI,¹ Shin'ichi AKIMOTO,¹ Jun NAKAMURA² and Eisuke HASEGAWA³

¹Laboratory of Systematic Entomology and ³Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo and ²Honeybee Science Research Center, Tamagawa University, Machida, Tokyo, Japan

Abstract

Mating frequency of *Vespa analis* queens and the genetic relatedness of their workers was analyzed by DNA microsatellite genotyping. Of 20 colonies studied, 18 had a queen inseminated by a single male and two had queens each inseminated by two males. The estimated effective number of matings was 1.05 ± 0.037 (mean \pm SE), with 75–85% of the offspring of the two multiply mated queens sired by a single male. The pedigree relatedness between nestmate workers averaged over the 20 colonies was estimated to be 0.74 ± 0.008 , almost identical to the predicted value of 0.75 for colonies headed by a singly mated queen. Multiple matrilines; that is, the presence of workers not related to the current queens, were detected in six colonies, suggesting that queen replacement occurred via usurpation of the founding queens in these six colonies. These results demonstrate that the kin structure of *V. analis* is similar to that reported in other vespid species.

Key words: hornet, kinship, microsatellites, paternity frequency, relatedness.

INTRODUCTION

Multiple mating by eusocial Hymenoptera queens is a central issue of kin selection and social evolution (Crozier & Page 1985; Crozier & Pamilo 1996). The haplo-diploidy in Hymenoptera leads to a higher relatedness among female offspring than between the female and male offspring in these colonies (Hamilton 1964). However, if a single founding queen has multiple matings, the relatedness among female offspring is reduced. This is a theoretical problem in the evolution of the sociality, as low relatedness would not seem to favor worker sterility. Therefore, the evolution and maintenance of multiple mating in social Hymenoptera is a field of particular interest. Several hypotheses to explain such multiple mating have been proposed in

connection with kin selection and gene diversity (Strassmann 2001).

DNA microsatellite genotyping has been used to investigate the genetic structure of social insect colonies, mating frequency and nestmate relatedness, because of the abundance of polymorphism at microsatellite loci. For monogynous colonies, DNA microsatellite genotyping has revealed a large variation in queen mating frequency from one to 69 mates per queen (Boomsma & Ratnieks 1996; Crozier & Pamilo 1996; Strassmann 2001; Tarpy & Nielsen 2002). Although DNA microsatellite markers have been developed for the Vespinae (Thorén 1998; Daly *et al.* 2002; Hasegawa & Takahashi 2002), only a small number of markers are available, so that data on paternity distribution remain limited (Strassmann 2001). This is especially true for *Vespa*, in which effective mating frequency has been estimated with DNA microsatellite markers in only three of the 23 species. Previous studies have found average effective mating frequencies of 1.11 (Foster *et al.* 1999) or 1.13 (J. Takahashi, unpubl. data, 1999) in *Vespa crabro* Linnaeus, 1.00 in *Vespa ducalis* Smith (Takahashi *et al.*

Correspondence: Mr Jun-ichi Takahashi, Laboratory of Entomology, Department of Agriculture, Graduate School of Agriculture, Tamagawa University, Machida, Tokyo, 194-8610 Japan. Email: tkhsj1am@agr.tamagawa.ac.jp

Received 20 December 2002; accepted 21 March 2003.

2002) and 1.04 in *Vespa mandarinia* Smith (J. Takahashi, unpubl. data, 2000).

The genus *Vespa* is an excellent group to study the evolution of paternity frequency because the members have a similar life cycle and social structure (Carpenter 1987; Matsuura & Yamane 1990; Archer 1991; Foster & Ratnieks 2001). *Vespa analis* is a common and widespread hornet of the mainland and islands of South-East Asia (Matsuura & Yamane 1990; Archer 1998). Males of *V. analis* Fabricius are often observed flying on a certain course along lines of trees, suggesting that they wait for new queens at such places to copulate (Matsuura 1995). However, the mating behavior of *Vespa* species is little known except for *V. mandarinia*, where males aggregate around the entrance holes of nests and attempt to mate with queens that emerge from the nest (Matsuura & Yamane 1990). Intraspecific usurpation is often observed in *V. analis* colonies, possibly as a consequence of competition over nesting sites, although its frequency has not been estimated (Archer 1998).

The aim of this study was first, to confirm and quantify the results of previous field observations on *V. analis* through DNA microsatellite genotyping and second, to assess queen mating frequency, genetic relatedness among workers, and rate of successful usurpation.

MATERIALS AND METHODS

Sample collection

During September 1998, 20 colonies of *V. analis* were collected from a forest near Joetsu, Niigata, Japan. All nests were located within a 20 km² area and collected at night by blocking the nest entrance and adding 5 mL diethyl ether to the nest. For microsatellite analysis, 20 adult workers per colony were collected and stored in 99% ethanol at -20°C.

DNA extraction and microsatellite genotyping

DNA was extracted from individual hornets by incubating ground thoracic muscle in 300 µL of 5% Chelex resin (Bio-Rad Laboratories, Hercules, CA, USA) and 2 µL of proteinase K (0.8 mg/mL) for 2 h at 55°C and then boiling for 3 min at 98°C (Walsh *et al.* 1991). PCR amplification was carried out in a total volume of 10 µL, which contained 1.5 µL (approximately 10 ng) template DNA, 2.5 pmol of each microsatellite primer, 400 mM of dNTP, 1 µL of 10× reaction buffer, 1.5 mM MgCl₂ and 0.05 units of Taq DNA polymerase (TAKARA BIO, Otsu, Shiga, Japan). Temperature cycles were as follows:

3 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 52–58°C and 30 s at 72°C.

DNA microsatellite genotyping in the *V. analis* colonies was conducted by using four loci identified by Hasegawa and Takahashi (2002). The PCR products were applied to 8% polyacrylamide sequence gels and visualized by silver staining (Tegelström 1986).

Data analysis

We estimated the regression relatedness (b), inbreeding coefficient (F), and allele frequencies using the Relatedness 4.2 computer program (Goodnight & Queller 1994). Colonies were equally weighted, and the standard errors and t -test were calculated by jack-knifing over colonies (Queller & Goodnight 1989). The average coefficient of pedigree relatedness (g_{uw}) was inferred from worker genotypes over the four loci for each colony:

$$g_{uw} = 0.25 + 0.5 \sum_{i=1}^k p_i^2 \quad (1)$$

where P_i is the relative frequency of the i th paternity and k is the number of fathering males for each colony (Laidlaw & Page 1984). The effective mating frequency (M_e) was calculated according to Starr (1984):

$$M_e = 1 / \sum_{i=1}^k P_i^2 \quad (2)$$

where P_i is the proportional contribution of the i th fathering male, and k is the number of fathering males for each colony. Estimates of queen mating frequencies are affected by two sources of errors: non-sampling and non-detection (Boomsma & Ratnieks 1996). The risk of overlooking one or more rare paternal genotypes is high when the number of workers analyzed is small. We assumed that this risk was reduced to acceptable levels by the analysis of 20 workers from each colony. In any event, rare paternal genotype males have only a small effect on the effective mating frequency.

We used the procedure of Foster *et al.* (1999) to estimate the non-detection error probability. Patriline are indistinguishable when the genetic marker loci have only low levels of polymorphism. The probability of two patrilines in a population having identical genotypes at all analyzed loci is:

$$d_p = \prod (\sum q_i^2)^j \quad (3)$$

where q_i is the allele frequencies at the i th locus and j is the number of loci (Foster *et al.* 1999). However, these

Table 1 Number of alleles (n), allele frequency and expected heterozygosity (H_E) of four microsatellite loci in *Vespa analis*

| Locus | n | Allele frequency | | | | H_E |
|-------|-----|------------------|-------|-------|-------|-------|
| | | a | b | c | d | |
| VMA-1 | 4 | 0.132 | 0.385 | 0.251 | 0.232 | 0.65 |
| VMA-3 | 3 | 0.306 | 0.280 | 0.414 | – | 0.60 |
| VMA-7 | 4 | 0.244 | 0.138 | 0.455 | 0.163 | 0.60 |
| VMA-8 | 3 | 0.317 | 0.407 | 0.276 | – | 0.50 |
| Mean | 3.5 | | | | | 0.62 |

estimates assume that alleles of paternal and maternal origin can be distinguished.

RESULTS

Analysis of *Vespa analis* microsatellite loci

High allelic variation was observed in the 20 colonies (Table 1). The VMA-1, VMA-3, VMA-7 and VMA-8 loci had allelic numbers of four, three, four and three, respectively, with no allele reaching a frequency of 0.5 at any locus. The mean expected heterozygosity for all loci was 0.62.

Mating structure

Mother queens were obtained from all 20 colonies. In 18 of the 20 colonies, the queens were inseminated by a single male. In each of the two other colonies the queen was inseminated by two males (Table 2). In the two double-mated queens, the ratio of paternity of the two males was calculated as 15:5 and 17:3. The overall average effective number of matings was 1.05 ± 0.037 (mean \pm SE). The inbreeding coefficient was not significantly different from zero ($F = 0.0034$) over the four loci, suggesting that mating is random in this population. Non-detection errors at the population and colony levels were 0.0031, ranging from 0.0098 to 0.11. The detection error of second paternity was only 3%, suggesting that this did not affect our results. Therefore, colonies of *V. analis* are typically headed by a monandrous (single-mated) queen.

Genetic relatedness of nestmate workers

When averaged over the 20 colonies, the regression relatedness among nestmate workers was 0.695, very close to the expected value of 0.75 for full sisters. The pedigree relatedness among nestmate workers for the 20 colonies was 0.74 ± 0.008 (mean \pm SE), which agrees well with the estimated value of regression relatedness. These results support our finding that monandry is typical in *V. analis* (Table 2).

Table 2 Mating number, relatedness between workers and proportional contribution of male mates for 20 colonies of *Vespa analis*

| Nest no. | Mating number | | Pedigree relatedness | Proportional contribution of male mates |
|--------------------|---------------|-----------|----------------------|---|
| | Observed | Effective | | |
| 98113 [†] | 1 | 1.00 | 0.75 | – |
| 98114 | 2 | 1.60 | 0.63 | 15:5 |
| 98116 | 1 | 1.00 | 0.75 | – |
| 98119 | 2 | 1.34 | 0.63 | 17:3 |
| 98120 | 1 | 1.00 | 0.75 | – |
| 98122 [†] | 1 | 1.00 | 0.75 | – |
| 98123 | 1 | 1.00 | 0.75 | – |
| 98124 [†] | 1 | 1.00 | 0.75 | – |
| 98125 | 1 | 1.00 | 0.75 | – |
| 98127 | 1 | 1.00 | 0.75 | – |
| 98144 [†] | 1 | 1.00 | 0.75 | – |
| 98145 | 1 | 1.00 | 0.75 | – |
| 98146 | 1 | 1.00 | 0.75 | – |
| 98147 | 1 | 1.00 | 0.75 | – |
| 98148 | 1 | 1.00 | 0.75 | – |
| 98149 [†] | 1 | 1.00 | 0.75 | – |
| 98150 | 1 | 1.00 | 0.75 | – |
| 98151 [†] | 1 | 1.00 | 0.75 | – |
| 98152 | 1 | 1.00 | 0.75 | – |
| 98156 | 1 | 1.00 | 0.75 | – |
| Mean | 1.1 | 1.05 | 0.74 | |
| SE | 0.07 | 0.037 | 0.008 | |

[†]Relatedness not calculated between unrelated workers.

Unrelated workers of *Vespa analis*

Multiple matriline were detected in six of the 20 colonies, where one to three of the 20 adult workers analyzed were not compatible with the queen's genotype (Table 2).

DISCUSSION

Our research shows that *V. analis* queens are normally inseminated by a single male, and that colonies are headed by a single queen. These findings agree well with previous field observations of monogyny as typical of temperate hornets, including *V. analis* (Matsuura & Yamane 1990). Multiple mating of queens has been observed (J. Takahashi, unpubl. data, 1998), but this appears to be exceptional. The presence of a small number of multiply mated queens is consistent with previous observations. Furthermore, the effective mating frequency in this population remains very low at 1.05, as paternity among the workers was very skewed between the two mating partners. The mean effective number of matings in *V. crabro*, *V. ducalis* and *V. mandarinia* was

1.11, 1.00 and 1.02, respectively (Foster *et al.* 1999; J. Takahashi, unpubl. data, 2000; Takahashi *et al.* 2002). Thus, these four species are similar in the frequency of multiple paternity.

The pedigree relatedness among nestmate workers in *V. analis* (0.74) is higher than in *V. crabro* (0.69–0.70) (Foster *et al.* 1999, 2000; J. Takahashi, unpubl. data, 1991) and is similar to that of *V. ducalis* (0.75) and *V. mandarinia* (0.74) (J. Takahashi, unpubl. data, 2000; Takahashi *et al.* 2002). However, the average genetic relatedness of *V. analis* nestmate workers is not significantly different from that in other *Vespa* species.

Multiple matrilineal lines were detected in six colonies. These could arise from two causes: coexistence of workers produced by a founding queen and a usurper, and immigrant workers drifting between colonies. However, drifting of workers is expected to be rare, because workers at the nest entrance are very vigilant against intruders (Matsuura & Yamane 1990). In contrast, intraspecific queen usurpation in *V. analis* has often been observed in the field (Matsuura & Yamane 1990; Archer 1998) although its frequency has not been quantified. Using genetic methods, successful usurpation has now been detected in two of 33 *V. crabro* colonies in Britain (Foster *et al.* 1999, 2000), in three of 20 *V. ducalis* colonies in Japan (Takahashi *et al.* 2002) and in two of 20 *V. mandarinia* colonies in Japan (J. Takahashi, unpubl. data, 1999). The present analysis suggests that the rate of successful usurpation in this *V. analis* population (six of 20 *V. analis* colonies) is unusually high.

This study reveals that the kin structure of *V. analis* consists mainly of monogynous, single-mated queens that mate randomly. Therefore, Hamilton's (1964) kin selection theory predicts conflict between queens and workers over male production. The coefficient of genetic relatedness of workers to intracolony males is higher for their own sons ($r = 0.50$) and for their full sisters' sons ($r = 0.375$) than for the queen's sons ($r = 0.25$) or their half sisters' sons ($r = 0.125$). As a result, worker reproduction is expected to evolve in monogynous hymenopteran colonies, particularly in those with low paternity (Ratnieks 1988). In three monogynous taxa (*V. crabro*, *V. mandarinia* and *V. ducalis*) worker's sons were not detected (J. Takahashi, unpubl. data, 1999; Foster *et al.* 2000, Takahashi *et al.* 2002).

ACKNOWLEDGMENTS

We sincerely thank Professor Masami Sasaki and Dr Masato Ono of Tamagawa University for critical read-

ing of an early draft, and Dr Jun-ichi Kojima, Stephen J. Martin and David R. Tarpay for repeated help during the writing of this paper.

REFERENCES

- Archer ME (1991) The number of species that can be recognized within the genus *Vespa* (Hym., Vespidae). *Entomologist's Monthly Magazine* **127**, 161–164.
- Archer ME (1998) Taxonomy, distribution and nesting biology of *Vespa analis* F. (Hym., Vespidae). *Entomologist's Monthly Magazine* **134**, 215–222.
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society of London B* **351**, 947–975.
- Carpenter JM (1987) Phylogenetic relationships and classification of the Vespinae (Hymenoptera: Vespidae). *Systematic Entomology* **12**, 413–431.
- Crozier RH, Page RE (1985) On being the right size: male contributions and multiple mating in the social Hymenoptera. *Behavioral Ecology and Sociobiology* **18**, 105–115.
- Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies. Sex Allocation and Kin Selection*. University of Oxford Press, Oxford.
- Daly D, Archer ME, Watts PC *et al.* (2002) Polymorphic microsatellite loci for eusocial wasps (Hymenoptera: Vespidae). *Molecular Ecology Notes* **10**, 1046–1048.
- Foster KR, Ratnieks FLW (2001) Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behavioral Ecology and Sociobiology* **50**, 1–8.
- 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.
- Goodnight KF, Queller DC (1994) *Relatedness 4.2*. Goodnight Software, Houston, Texas.
- Hamilton WD (1964) The genetical theory of social behaviour. I, II. *Journal of Theoretical Biology* **7**, 1–52.
- Hasegawa E, Takahashi J (2002) Microsatellite loci for genetic research in the hornets *Vespa mandarinia* and related species. *Molecular Ecology Notes* **2**, 306–308.
- Laidlaw HH, Page RE (1984) Polyandry in honey bees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships. *Genetics* **108**, 985–997.
- Matsuura M (1995) [*Social Wasps of Japan in Color.*] Hokkaido University Press, Sapporo. (In Japanese.)
- Matsuura M, Yamane S (1990) *Biology of the Vespine Wasps*. Springer, Berlin.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* **46**, 258–275.

- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist* **132**, 217–236.
- Starr CK (1984) Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: Smith RL (ed.) *Sperm Competition and the Evolution of Animal Mating Systems*, pp 427–464. Academic Press, Orlando.
- Strassmann J (2001) The rarity of multiple mating by females in the social Hymenoptera. *Insectes Sociaux* **48**, 1–13.
- Takahashi J, Akimoto S, Hasegawa E, Nakamura J (2002) Queen mating frequencies and genetic relatedness between workers in the hornets *Vespa ducalis* (Hymenoptera: Vespidae). *Applied Entomology and Zoology* **37**, 481–486.
- Tarpy DR, Nielsen DI (2002) Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae). *Annals of the Entomological Society of America* **95**, 513–528.
- Tegelström H (1986) Mitochondrial DNA in natural populations: an improved routine for the screening of genetic variation based on sensitive silver staining. *Electrophoresis* **7**, 226–229.
- Thorén PA (1998) Mating structure and nest mate relatedness in primitively social Hymenoptera as revealed by microsatellites (PhD Thesis). Uppsala University, Sweden.
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513.