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Genetic structure of the first brood of workers and mating frequency of queens in a Japanese paper wasp, *Polistes chinensis antennalis*

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Genotypes of a queen and the first brood of workers of 20 colonies of *Polistes chinensis antennalis* were electrophoretically analyzed. We found allelic polymorphisms in two loci which encode two enzymes, i.e., creatine kinase (CK) and isocitrate dehydrogenase (IDH). The genotype array of the first brood of workers in five colonies could not be explained by monoandry of the queen. Two to four suggested multiple matings of the queen, and one to three suggested replacement of the queen. Although multiple matings of some queens is apparent, the mean estimate of relatedness of the first brood of workers from the genotype frequency data, 0.648, is ambiguous, because it is not significantly different from 0.75 (one singly-mated queen) nor 0.545 (one doubly-mated queen).

KEY WORD: electrophoresis, genetic structure, mating frequency, *Polistes chinensis antennalis*

INTRODUCTION

After the development of kin selection theory by HAMILTON (1964), the mating frequency of queens has been a focus of theoretical and empirical studies of social insects. At first the high genetic relatedness among daughters of a singly-mated queen was considered to be a main cause of the evolution of social life in the Hymenoptera (HAMILTON 1972). However, recent studies revealed relatively low genetic relatedness among colony members (STRASSMANN et al. 1989, GADAGKAR 1991, GADAGKAR et al. 1991). STARR (1984), WOYCIECHOWSKI & LOMNICKI (1987), and RATNIEKS (1988) correlated the mating frequency of queens with worker reproduction. They predicted that workers favour no reproduction of other workers under the condition of low genetic relatedness caused by a queen's multiple matings.

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Polistes chinensis antennalis is a primitively eusocial wasp, and has a peculiar reproductive system, i.e., nearly all males produced in queen-right colonies originate from eggs laid by unmated workers (MIYANO 1983). TSUCHIDA (1994) recently revealed monoandry of the queen and high genetic relatedness of colony members in another Japanese polistine wasp, *Polistes jadwigae*, in which workers do not lay eggs in queen-right colonies (MIYANO 1991). The genetic structure of *P. chinensis antennalis* was investigated in the present study which sought to determine the genetic structure of the first brood of workers by electrophoretic technique.

MATERIALS AND METHODS

Study organisms

P. chinensis antennalis is a primitively eusocial wasp, and has an annual life cycle in Japan (MIYANO 1983). Hibernated gynes haplometrotically initiate their nests in the spring. The first brood of workers usually appear in early summer. After emergence of a number of workers, males and gynes appear in middle to late summer. Copulations take place in late autumn. Only gynes survive the winter.

In June of 1994 we collected 20 queens (15 in Chiba City and 5 in Funabashi City in Chiba Prefecture, central Japan) with their nests, which were about to produce the first brood of workers, i.e., they had several cocoons, but no workers. Before collection we observed queens on the nests for a few minutes to confirm that they were legitimate queens, not alien transient ones. Queens were individually put in a small vial and stored in the freezer at -60°C until electrophoretic analysis. Each nest was kept in a small cage and checked for worker emergence every day. Emerging workers were treated in the same way as queens. Not only cocoons but also some 5th instar larvae at the time of collection eclosed as adults in 20 days. They were all females, i.e., workers.

Electrophoresis

In *P. chinensis antennalis*, three loci encoding creatine kinase (CK; EC 2.7.3.2), isocitrate dehydrogenase (IDH; EC 1.1.1.42) and malate dehydrogenase (MDH; EC 1.1.1.37) are polymorphic, and these allelic polymorphisms can be detected electrophoretically (E. HASEGAWA et al. in prep.). The genotype of individuals was scored following horizontal electrophoresis in 14% starch gels using a pH 8.4 Tris-Citrate gel buffer with a pH 7.1 Tris-Citrate electrode buffer (see SEPPÄ 1992). The thorax of each individual was squashed by a disposable plastic pestle in a microcentrifuge tube of 1.5 ml volume with 50 μl of distilled water. Each sample was centrifuged at 12000 rpm, 4°C for 5 min, and then, 5 μl of supernatant was applied to a gel wick (4×7 mm, 2M filter paper). The wicks were applied to a 14% starch gel of 8 mm thickness. The proteins were electrophoresed at 350 v for 3.5 hr. After the electrophoresis, we sliced each gel into three thin layers and the protein bands were revealed by specific histochemical staining (MURPHY et al. 1990).

Genetic analysis

In order to determine the genetic relationship within colony members, we conducted two analyses on the genotype data. First, we compared a genotype of the colony queen with a genotype array of workers in her colony. Second, average relatedness between nestmate workers was calculated using the identity by descent method (QUELLER & GOODNIGHT 1989) at each

locus by using a computer program (Relatedness 4.2, provided by K.F. Goodnight). We also calculated the inbreeding coefficient (F) for both queens and workers. In the calculation for the workers' data, we resampled a single genotype 5000 times from each colony and calculated the F value for all 5000 data sets and obtained a population representative F value by averaging the 5000 F values. This procedure ensures independence of the data from strongly family structured social insect colonies (see ROSS & MATTHEWS 1989).

RESULTS

Queen-worker relationship

Among the three loci examined, the locus that encodes MDH showed no polymorphism in this population. The other two loci (CK and IDH) had two alleles each. The locus IDH lacked the fastest allele among the three observed in the Kanagawa population (E. HASEGAWA et al. in prep.).

Table 1 shows genotypes of the colony queens and genotype arrays of their workers at the two polymorphic loci. In 15 of 20 colonies, the observed genotype array of the workers did not contradict the possible monoandry of their queen. In

Table 1.

Genotype of colony queen and genotype array of the first brood of workers on two polymorphic loci. Asterisks indicate colonies in which a discrepancy between the queen genotype and workers' genotype array is observed under the assumption of monoandry of the queen. Mean number \pm SE of the first brood of workers is 11.9 ± 1.0 .

Colony	Genotype of queen		Genotype array of workers						
			CK			IDH			
	CK	IDH	SS	SF	FF	SS	SM	MM	
* B1	SF	MM		3	5			4	4
* B2	SF	SM		10	8	2		10	6
B3	FF	MM		8					8
B4	SF	MM		3	7				10
B5	FF	MM			11				11
* B6	FF	MM		10	4				14
B7	FF	MM		4					4
B8	SF	MM		8	8				16
B9	FF	SM			16	7		9	
B10	SF	MM		2	5				7
* B11	SF	MM	1	10	3				14
B12	FF	MM		12					12
B13	FF	MM			20				20
B14	SF	MM		3	4				7
B15	SF	MM		4	6				10
C7	FF	MM		16					16
C8	FF	MM			19				19
* C9	SF	MM			9				9
C10	FF	MM			8				8
C11	FF	MM			11			11	

addition, in colonies having two genotypes among workers, the ratio of the two genotypes was not significantly different from 1:1 (χ^2 test, for all colonies, $P > 0.05$). However, these results do not always imply that in these colonies the queen is the true mother of the workers and that she mated with a single male, because, for example, double matings with the same genotype males and queen replacement between the same genotype females can not be detected by this analysis. In the other five colonies it is clear that a single mating of the queen can not explain the genotype arrays of the workers. In colony C9, the queen had the genotype SF at locus CK but all nine workers were FF at the same locus. Of course, we can assume that the C9 queen mated with an F male and all the haploid eggs produced by the queen had F genotypes. However, the probability of the occurrence of this event is significantly low [$P = (1/2)^9 < 0.002$] when the genotypes of haploid eggs are determined by chance (this is likely to occur in the other colonies; see above). Thus, this case suggests a replacement of the colony queen (FF to FS female) before nest collection. In another two colonies (B2 and B11) the genotype array of the workers, which includes three genotypes, can only be explained by double matings of the queen, if we assume that all workers were originated from eggs laid by the original queen even if a queen replacement had occurred. This assumption is consistent with the fact that C9 might have no offspring from the replacement queen. The apportionment rate of paternity of the most successful male is calculated as 0.75 (6/8 and 3/4) in both colonies. The genotype array of the other two colonies (B1 and B6) can be explained by either double matings of the queen or queen replacement.

Relatedness between workers

Table 2 shows inbreeding coefficients among queens and among workers at the two polymorphic loci. In the workers' data, F values for both loci deviated considerably from zero, but these deviations were not statistically significant (χ^2 test, for all values, $P > 0.05$). Therefore we did not correct the relatedness estimates for the F values. The estimated relatednesses are shown in Table 3. The average relatedness over both loci was 0.648, which is a little lower than the value of 0.75 expected under the assumption that they are descendants of a singly mated queen, and a little higher than the value of 0.545 expected under the assumption of double matings of all queens, 0.75 as a apportionment rate of paternity of most successful male, and 11.9 (see Table 1) as the mean number of first brood of workers (for the method of calculation of the expected value, see PAMILO 1993). But the difference is not significant in either case ($t = 0.87$, $P > 0.05$ and $t = 0.88$, $P > 0.05$, respectively).

Table 2.
Inbreeding coefficient (F) of queens and the first brood of workers.

Locus	Queen	Worker (mean \pm SE)
CK	- 0.290	- 0.252 \pm 0.001
IDH	- 0.052	0.187 \pm 0.004

Table 3.

Genetic relatedness between the first brood of workers estimated from the genotype frequency data.

Locus	Relatedness \pm SE
CK	0.536 \pm 0.141
IDH	0.778 \pm 0.129
Total	0.648 \pm 0.117

Table 4.

Queen condition (single mating, double mating, or replacement) and the pedigree value of relatedness between the first brood of workers. The inter-colony average was calculated in two ways which assume four or two doubly mated queens, respectively.

Colony	Queen condition	Average relatedness
B1	Double mating/Replacement	0.464/0.75
B2	Double mating	0.551
B6	Double mating/Replacement	0.530/0.75
B11	Double mating	0.548
C9	Replacement	0.75
Other 15 colonies	Single mating	0.75
Inter-colony average \pm SE		0.705 \pm 0.021/0.730 \pm 0.014

We also calculated the pedigree value of relatedness from the Table 1, although this method has some limitations (PAMILO 1993). Calculated relatednesses are shown in Table 4. Both average values, each of which was calculated on the assumption of four or two doubly mated queens, are near to the value estimated from the genotype frequency data above.

DISCUSSION

The genotype array of the first brood of workers in five out of 20 colonies of *P. chinensis antennalis* can not be explained by a singly mated queen. Although multi-foundress nests have been reported for this species (YAMANE 1973, HOSHIKAWA 1979, KOJIMA & HAGIWARA 1990), their frequency is very low (1.5% in HOSHIKAWA 1979 or lower, S. MIYANO pers. obs.). In fact we found no case of multiple queens on a nest in this study. Therefore we can exclude an effect of polygyny on genetic composition and relatedness of colony members from consideration.

Queens of two to four colonies were considered to be mated with at least two males. Copulations take place in sunny places in this species (SUZUKI 1995). Males gather and fly about over sun-lit objects such as stones, crowns of small trees and man-made structures. When a female alights on such sun-lit object, a male pounces on her and copulation takes place. On one occasion, a female accepted two males

successively (S. MIYANO pers. obs.). Multiple matings do take place in some females of this species.

One to three queens were considered to be replacement queens whose offspring did not yet appear as adults. Queen replacement may be more frequent than one to three out of 20, because replacements between, for example, FF female inseminated by S male and SS female with F male as well as between two females with the same genotype can not be detected by this analysis. Queen replacement was also confirmed by observation of individually marked queens in this species (KASUYA 1982). We assumed in the data analysis that the first brood of workers did not include offspring of a replacement queen even if queen replacement occurred, and that they were full or half sisters. This is reasonable, because the first brood of workers grow from a few more than 10 eggs (the mean number \pm SE of first brood of workers in this study was 11.9 ± 1.0) which are laid during the first few days after nest initiation, when queen replacement is considered to be rare. In *Polistes riparius*, which belongs to the same subgenus *Polistes* as the *P. chinensis antennalis*, almost all queen replacement occurs in the later substages of a pre-emergence stage (MAKINO 1985, 1989a, 1989b; MAKINO & SAYAMA 1991).

Although multiple matings of some queens are apparent, the mean estimate of relatedness of the first brood of workers from the genotype frequency data, 0.648, is ambiguous, because it is not significantly different from 0.75 (one singly-mated queen) nor 0.545 (one doubly-mated queen). TSUCHIDA (1994) showed a high average relatedness (0.729) among female offspring, and monoandry of queens in another Japanese paper wasp, *P. jadvigae*, which has a similar life cycle to that of *P. chinensis antennalis*, and whose workers do not lay eggs in a queen-right condition (MIYANO 1991). In *P. chinensis antennalis*, however, workers lay male-producing eggs in queen-right colonies (MIYANO 1983). Why are two species so different in terms of their worker reproduction? To answer this question we need more empirical studies. First of all we need to re-examine the frequency of multiple matings of queens on the basis of a larger number of samples in *P. chinensis antennalis*. Queen replacement should somewhat lower the relatedness of colony members at later nesting stages, and might exert some influence on worker reproduction in *P. chinensis antennalis*. There may be a variation of reproductive pattern among colonies according to each queen's mating frequency in both species.

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