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Evidence for multiple mating in the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera : Vespidae)

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Abstract. Asymmetries in genetic relatedness created by haplodiploidy have been considered to be crucially important for the evolution of worker behaviour in Hymenoptera. Multiple mating by the queens destroys this asymmetry and should make kin selection less powerful. The number of males that social insect queens mate with is thus of considerable theoretical interest especially in primitively eusocial species. The results presented here provide evidence for multiple mating by foundresses of the primitively eusocial wasp *Ropalidia marginata* (Lep.)

Keywords. Haplodiploidy; kin selection; social Hymenoptera; multiple mating; electrophoresis; *Ropalidia marginata* (Lep.).

1. Introduction

In the order Hymenoptera, females store sperm derived from their mates in an organ called the spermatheca. Subsequently this sperm is used to fertilise eggs for the production of female offspring. Male offspring are normally produced from unfertilised eggs. Consequently, male Hymenopterans are haploid while females are diploid. Such haplodiploidy leads to asymmetries in genetic relatedness so that full sisters have a coefficient of genetic relatedness of 0·75 compared to a value of 0·5 between mother and daughter. This asymmetry is expected to favour the evolution of worker behaviour in female Hymenopterans because a female can gain more inclusive fitness by caring for full sisters than by rearing its own daughters (Hamilton 1964a, b; for reviews see Wilson 1971; Hamilton 1972; West-Eberhard 1975; Gadagkar 1985a). The asymmetry in genetic relatedness breaks down, however, if Hymenopteran queens mate with more than one unrelated male. Multiple mating results in different genetic lines of half sisters who would be related to each other by a coefficient of relatedness of 0·25 (for a more detailed treatment see Starr 1984, Joshi and Gadagkar 1985, Page 1986).

The number of males that queens of social insect colonies mate with is thus of considerable theoretical interest. Multiple mating (in the well-known case of the

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honey bee for instance) has in the past been assumed not to significantly affect worker brood relatedness by virtue of the presumed propensity of sperm of each male to remain clumped leading to non-random sperm usuage (see for instance, Orlove 1975). This assumption seems inconsistent with a number of lines of evidence (Adams *et al* 1977; Crozier and Bruckener 1981). The recent application of electrophoretic techniques for studying the segregation of isozyme patterns has more definitively demonstrated sperm mixing leading to simultaneous use of sperm from two or more males in the honey bee (Page and Metcalf 1982; Laidlaw and Page 1984). Besides, multiple mating and sperm mixing are now clearly evident in several species of ants (Pamilo 1982; Fletcher and Ross 1985). From the point of view of the evolution of worker behaviour, however, multiple mating in primitively eusocial insects would be of greater significance (for details see Gadagkar 1985b). The only electrophoretic study of mating patterns in primitively eusocial insects is that of Metcalf and Whitt (1977), who showed that in the primitively eusocial wasp *Polistes metricus*, foundresses mate at least twice but use sperm from the two mates in a 9:1 ratio. Here, we report evidence of multiple mating in another primitively eusocial wasp *Ropalidia marginata* (Lep.).

2. Materials and methods

2.1 *Experimental materials and rearing techniques*

Ropalidia marginata is a very common social wasp in peninsular India whose ecology, behaviour and social organisation are being intensively investigated (Gadagkar 1980; Gadagkar *et al* 1982; Gadagkar and Joshi 1983). In nature, colonies are initiated by one or more foundresses. Small pre-emergence colonies were collected in and around Bangalore (13° 00′ N and 77° 32′ E). The nest and brood were discarded and the adults were individually identified with unique spots of coloured paint before being housed in a wood and wire mesh cage of dimension 15 × 15 × 15 inches. Water, honey and *Corcyra cephalonica* larvae were provided *ad libitum*. A small piece of soft wood provided in the cage was readily used to build a new nest. Continuous observation was made to identify the wasp which laid the first egg. This was termed the primary egg layer. Immediately after the first egg was laid all the wasps except the primary egg layer were removed to another cage. The wasps transferred to the second cage often built another nest and the new egg layer was again identified so that all the remaining non-egg layers could again be removed. This egg layer in the second cage was termed the secondary egg layer. Each egg layer (primary or secondary) was thus allowed to tend its own nest and produce offspring unaided by any other wasp, after it laid the first egg.

This ensured that all wasps emerging from a cage were the offspring of the same female. This technique, we believe, removes any doubt about the maternity of the experimental animals that might remain if single foundress nests and offspring produced in the wild are simply collected and electrophoresed, as for instance in the *Polistes* study (Metcalf and Whitt 1977). Offspring (which were all females during this initial phase of the nesting cycle) produced by the egg layers were removed and used for electrophoresis from time to time leaving one or two behind

to assist the egg layer in brood care. The egg layers were themselves removed and electrophoresed when it appeared that they might die.

Male and female wasps from other randomly collected nests and left overs from other experimental nests were used for the initial standardization of the electrophoretic and staining techniques. These initial experiments were also used to assess the number of loci and the number of alleles segregating at each locus in our experimental system.

2.2 *Electrophoretic and staining techniques*

Single wasps were homogenised in 100 microlitres of 0·1 M phosphate buffer, pH 7·1, and centrifuged at 12,000 rpm for 4 mins in a Beckman Microfuge. The supernatant was electrophoresed on vertical polyacrylamide (7·5%) slab gels using standard methodology (Shaw and Prasad 1970), except that Tris-HCl buffer (pH 7·1) was used as gel buffer. Gels were stained for non-specific esterases as described by Shaw and Prasad (1970).

3. Results and discussion

The initial standardisation experiments revealed that 3 non-specific esterase loci were being detected on our gels because all males showed three distinct bands. These are designated as *a*, *b* and *c*. Females occasionally showed double bands in the *b* and *c* region but never in the *a* region, while males never showed any double bands. This suggests that the locus *a* is monomorphic and at least two alleles segregate at each of the *b* and *c* loci. These double bands were designated as b^f, b^s, c^f and c^s, to represent fast and slow moving bands, respectively. Genotypic and allele frequencies from the analysis of 39 females and 18 males show that the slow moving bands at both the loci have a much higher frequency as compared to their fast moving counterparts (tables 1 and 2).

The 39 females and 18 males analysed here do not represent a random gametic population since several animals including workers from a small number of nests were analysed. Besides, the sample sizes are as yet inadequate to make any inferences regarding the genetic structure of the population. The information that

Table 1. Genotypic frequencies of esterases in *R. marginata*

Genotype	Frequency
Females (sample size = 39)	
$b^s b^s c^s c^s$	0.564
$b^s b^s c^f c^s$	0.308
$b^s b^s c^f c^f$	0.051
$b^f b^s c^s c^s$	0.051
$b^f b^s c^f c^s$	0.026
Male (sample size = 18)	
$b^s c^s$	0.889
$b^f c^s$	0.111

Table 2. Allele frequencies at esterase loci in *R. marginata*

Alleles	Frequency*
b^s	0.95
b^f	0.05
c^s	0.82
c^f	0.18

* Calculated from the genotypes of 39 females and 18 males. Allele frequencies are calculated by counting the number of times an allele is seen (counted as 2 for homozygous females) and dividing by 96 (39 diploid females × 2 + 18 haploid males = 96), which is the maximum number of times any allele could have been seen.

two loci are dimorphic, however, permits us to determine if the egg layers mate multiply. A single locus with at least two alleles would be sufficient for this purpose. When a homozygous female produces both homozygous and heterozygous daughters, multiple mating is clearly indicated. Similarly, if a heterozygous mother produces homozygotes for both alleles amongst her female progeny, she must have mated with at least two males. We use this logic to infer the minimum number of matings as well as the genotypes of the fathers in five laboratory single foundress colonies (table 3) established by animals caught from the wild as described above (see §2). The five foundresses, shown in table 3, must have mated with a minimum of 1, 3, 2, 3, and 1 male, respectively. In other words, at least three foundresses have produced daughters of more than one genetic line among the first 10 or 12 offspring.

It must be pointed out that the frequencies of multiple mating reported here are minimum estimates. The actual frequencies may be higher. Our methodology cannot detect multiple mating if two mates have the same genotype or if sperm from any one mate is used preferentially as may happen, in spite of sperm mixing, in the small sample sizes of progeny studied by us. These factors can be corrected for and the true frequency of multiple mating can be estimated (Pamilo 1982) if one knows the frequencies of different alleles in the population. The frequencies presented by us in table 2 cannot be used as they do not represent a random Mendelian population. Pamilo (1982) has used allele frequencies inferred from the queens and their expected mates. In our case, clearly, the sample sizes are too small to do this. We therefore prefer to defer estimation of actual multiple mating frequencies.

In any case, our data clearly suggest that multiple mating occurs and is also followed by considerable mixing of sperms from different males. We infer from this that intra-nest or worker-brood genetic relatedness will often be considerably below 0·75 even amongst the females. This should reduce the efficacy of kin selection for the evolution of worker behaviour in this species unless workers dis-

Table 3. Evidence for multiple mating in *R. marginata*

Experiment code number	Primary/ secondary egg layer*	Genotype of mother	Genotype of daughters (number of individuals)	Minimum number of matings	Inferred genotype of father/s
M6	Secondary	$b^s b^s c^s c^s$	$b^s b^s c^s c^s$ (4)	1	$b^s c^s$
M11	Primary	$b^s b^s c^s c^s$	$b^s b^s c^s c^s$ (6) $b^f b^s c^s c^s$ (3) $b^f b^s c^f c^s$ (1)	3	$b^s c^s$ $b^f c^s$ $b^f c^f$
M13	Primary	$b^s b^s c^s c^s$	$b^s b^s c^s c^s$ (5) $b^f b^s c^f c^s$ (1)	2	$b^s c^s$ $b^f c^f$
M15	Primary	$b^s b^s c^s c^s$	$b^s b^s c^s c^s$ (7) $b^f b^s c^s c^s$ (2) $b^s b^s c^f c^s$ (3)	3	$b^s c^s$ $b^f c^s$ $b^s c^f$
M23	Primary	$b^f b^s c^s c^s$	$b^s b^s c^s c^s$ (1) $b^f b^s c^s c^s$ (2)	1	$b^s c^s$

* A primary egg layer is the female who starts laying eggs when all the animals present at the time of nest collection are housed together in a laboratory cage. When all but the primary egg layer are removed and put into a fresh cage another female begins to lay eggs. This is called the secondary egg layer.

criminate against half-sisters and preferentially care for their own full-sisters (see Gadagkar 1985).

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Note added in proof: Kenneth G Ross (Kin selection and the problem of sperm utilization in social insects. *Nature* 1986, 323: 798–800) has recently provided evidence for multiple mating followed by use of sperm from different males in relatively constant proportions through time in 2 species of highly eusocial wasps, *Paravespula maculifrons* and *Vespula squamosa*.