Kin selection and the problem of sperm utilization in social insects

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The evolution of social behaviour has posed a special problem for natural selection theory since Darwin. Sociality entails at least a partial loss of personal reproduction by some individuals, a seeming contradiction to the expectation that it be maximized by natural selection. The theory of kin selection[1,2] seeks to explain social evolution by reference to high degrees of genetic relatedness among cooperating individuals. In the most diverse and conspicuous of social organisms, the social Hymenoptera (ants, bees, wasps), kin selection theory has been especially attractive because the male-haploid genetic system of the group leads to potentially high relatedness among female nestmates[3-5]. Multiple mating by queens poses a serious difficulty for kin selection, however, in that it reduces nestmate relatedness unless sperm from different males are used non-randomly[6-10]. This possibility has been investigated in only one highly social insect, the honey bee *Apis mellifera*, where the absence of suitable genetic markers has necessitated the use of artificial insemination[10,11]. In this first study of long-term sperm utilization in natural populations of highly social insects I show that queens of two social wasp species use sperm from different males in relatively constant proportions through time, resulting in consistently low relatedness among female nestmates. I also show that workers do not supplement their inclusive fitness by reproducing directly in colonies in which the mother queen is present.

Colonies of the two species (20 colonies for *Paravespula maculifrons* and 17 colonies for *Vespula squamosa*) were collected in the southeastern U.S.A. during August–November of 1984 and 1985 and allozyme genotypes of colony members were determined electrophoretically. The mother queen was captured in 28 of the 37 colonies (76%); in the remainder her genotype was inferred from progeny genotypes. Genotype distributions indicate multiple insemination of at least 35 (94.6%) of the queens (90% of *P. maculifrons* and all *V. squamosa*). This is indicated by the presence of more than one or more than two genotypic classes among the female progeny of queens homozygous or heterozygous, respectively, at a marker locus. The effect of multiple insemination is to reduce relatedness among workers and, more importantly, relatedness between workers and the new queens they rear from the value of 0.75 that would be found in colonies headed by singly-mated queens to 0.320 for *P. maculifrons* and 0.403 for *V. squamosa* (Table 1).

The minimum number of matings per queen was determined from the distribution of multi-locus genotypes for her daughters; the modal minimum number is two matings for *P. maculifrons* and three for *V. squamosa* (Fig. 1). In conjunction with estimates for the harmonic mean numbers and insemination-effective numbers of matings derived from relatedness values (Table 1), it is evident that queens of the two species typically mate with 2–7 males before entering winter dormancy.

The brief period of multiple mating in the fall and the prolonged storage of sperm by queens for use the following year make it possible to study the effects of non-random usage of sperm from different males for fertilizations in a natural context. Such effects would be significant in that they increase relatedness among nestmates present at a given time and thus restore conditions favourable to the action of kin selection[8,10]. Any male achieving a disproportionate representation of his sperm in late-season fertilizations would secure a fitness advantage, as most eggs fertilized at this time are reared as queens.

Sperm utilization patterns in the wasps were studied by sampling foraging workers from eight colonies (five *V. squamosa*,
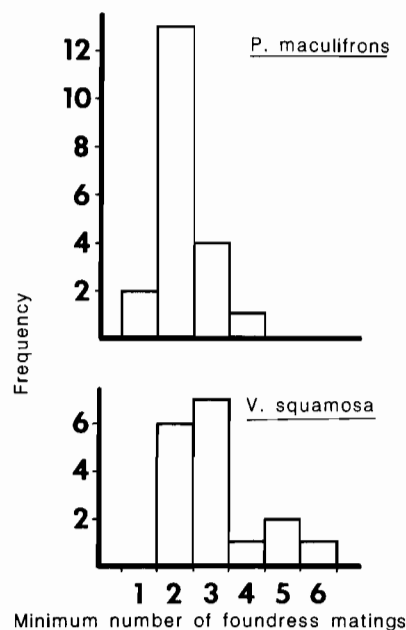

Fig. 1 Minimum number of matings by queens of two social wasp species, as determined from the diversity and distribution of multilocus genotypes among female progeny. The ability to detect greater numbers of matings is constrained by the number of polymorphic genetic markers available for each species and the level of polymorphism (number and frequency of alleles) at each marker.

Table 1 Relatedness and number of matings for two species of social wasps

Species	No. colonies	$r(\pm\text{s.e.})$	$\bar{H}$	m_1	m_2
P. maculifrons	20	0.320 ± 0.060	7.14	5.43	5.36
V. squamosa	17	0.403 ± 0.073	3.27	2.13	1.96

Average intra-colony relatedness among females (r), harmonic mean number of matings by queens ($\bar{H}$), insemination-effective number of matings (m_1) and short-term effective number of matings (m_2) are given. Relatedness was estimated from the distributions of genetic markers in colonies[23], and represents the mean for the three and two most polymorphic loci in *P. maculifrons* and *V. squamosa*, respectively. Harmonic mean numbers of matings were calculated from relatedness values[10,24], and assume equal and constant proportions of fertilizations by each male. These values indicate the maximum number of matings consistent with given relatedness values. These estimates for numbers of matings were adjusted downwards (effective numbers of matings) by taking into account variance in male contributions to fertilizations evident from point samples (m_1) and over time (m_2), and assuming that each unique sperm haplotype is contributed by a single male. Differences between m_1 and m_2 reflect the importance of intraspermathecal events (for example, incomplete sperm mixing or differential viability) leading to changes in proportions of fertilizations by each male and concomitant changes in nestmate relatedness through time[10,11]. Genetic markers were generated using starch gel electrophoresis[25]. Six polymorphic loci were found for *P. maculifrons* (*Pgm-1,3; Hbdh; Mpi; Agp-1,2*) and four for *V. squamosa* (*Pgm-1; Est-1,2; Hbdh*). Mendelian inheritance of the enzyme products was confirmed by the predicted banding patterns among presumed progeny, banding patterns in heterozygotes consistent with known quaternary structures, and single bands for (haploid) males. A mean of 50.3 ± 13.7 (s.d.) workers, 82.0 ± 39.0 males and 37.9 ± 18.2 daughter queens were studied per colony. Genotype frequency distributions at polymorphic loci did not differ significantly between workers and queens in 27 of 28 (96.4%) comparisons (χ^2 test, all $p > 0.05$), consistent with a trophic mode of caste determination in vespines[26]. Relatedness between workers and queens is thus essentially identical to relatedness among workers, and genotypes for the two castes were pooled for the relatedness estimates.
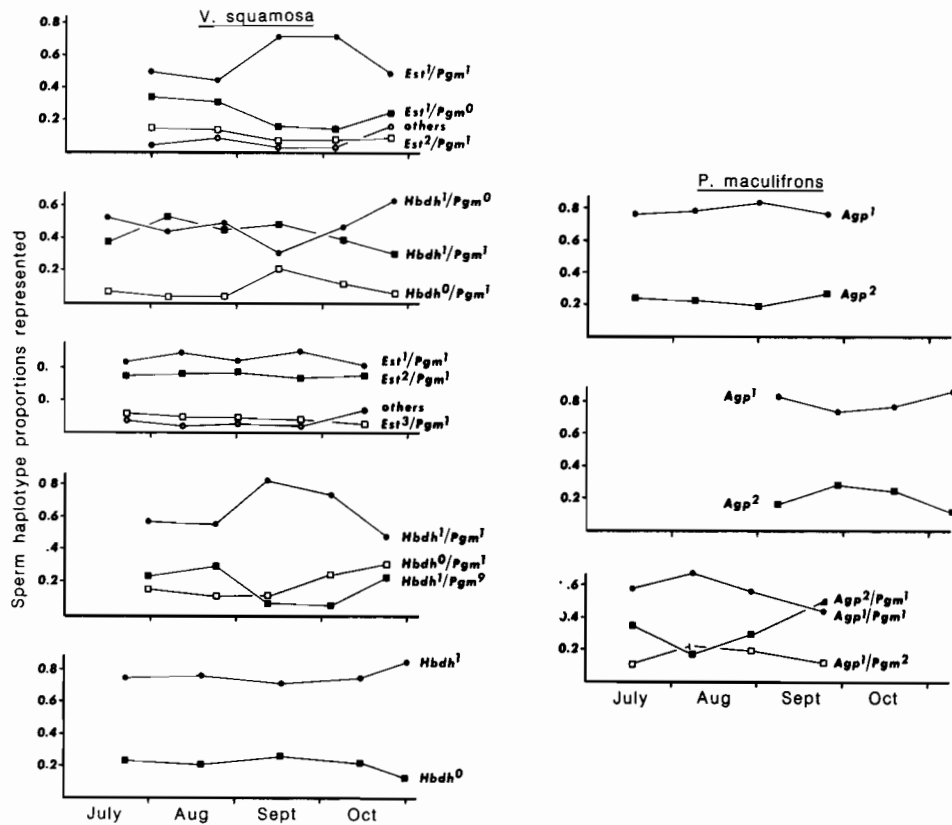
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Fig. 2 Temporal sperm use patterns for queens of five colonies of *V. squamosa* and three colonies of *P. maculifrons*. Sperm haplotype proportions represented by each sample point were determined from worker genotype distributions (50 workers collected per sample) and genotypes of mother queens (collected at the final samples). Haplotypes are labelled with the diagnostic alleles as superscripts of informative marker loci. Numerical designations of alleles refer to the relative mobilities of the enzyme products in electrophoretic gels. New adult queens of the two species first appear in nests from late September to mid-October in the study area[27,28].

three *P. maculifrons*) at regular intervals during the latter part of the season. Haplotypes of sperm used for fertilizations were inferred from worker and mother queen genotypes. Typical adult worker lifespans are shorter than the sample intervals[12], so the samples of workers did not overlap in age. Use of sperm from different males appears remarkably constant in several of the study colonies (Fig. 2), with few changes in the ordering of haplotype fertilization proportions between the beginning of sampling and season's end evident in any of the colonies. All but the rarest haplotypes are represented at every sample. Thus sperm from different ejaculates probably mix freely in the queens' spermathecae (sperm storage organs) and are used randomly for fertilizations. Variation in male contributions to fertilizations through time does not alter relatedness patterns in these social wasp colonies significantly, since the differences in estimates of effective numbers of matings (m_1 and m_2) for each of the species are small (Table 1). Thus workers present throughout the season have similar relatedness to and a common genetic interest in the new queens reared in autumn, and individual males cannot gain a fitness advantage by having their sperm used preferentially for the production of these queens.

Theoretical models[13-15] indicate that under circumstances of low inclusive fitness effects among workers due to multiple mating of queens, conditions favourable to social evolution may be maintained by the retention of a measure of personal fitness among the workers, that is, by their directly producing some of the colony males (from unfertilized eggs). Although the ability of workers to produce such males in the presence of the queen is poorly known for most social Hymenoptera[16], several authors have speculated that many or all of the males in queenright vespine colonies may be worker offspring[17]. Twenty-three study colonies with males also contained workers and mother queens with genotypes appropriate for determining parentage of the males. In these colonies queens were homozygotes while all or a substantial proportion (>10%) of workers were heterozygous at one or more marker loci. Of 2,347 males studied from such queenright colonies, none were found to possess genotypes inconsistent with their being progeny of the queen. On the other hand, in three *V. squamosa* colonies that had evidently been

queenless for some time (that is showing irregular brood patterns, extra eggs in cells and discoloration of worker gastral terga), substantial numbers of males were found with genotypes indicating that they were offspring of workers rather than the queen. Thus it appears unlikely that workers in these species reproduce directly when the queen is present in the nest.

I have shown here that workers in these wasp societies have relatively low inclusive fitness effects due to multiple mating by the mother queens, and also that the workers' personal fitness is essentially zero because they cannot reproduce directly in queenright colonies. Together with findings from other highly social groups[10,18], these results demonstrate that neither high levels of nestmate relatedness nor direct worker reproduction are necessary for the maintenance of advanced eusociality in Hymenoptera. Alternative explanations that emphasize constrained options of morphologically specialized workers; maternal manipulation, or factors extrinsic to the colony may help to explain the evolutionary stability of sociality in these advanced forms[19-21]. It is worth considering that sociality may evolve under conditions of single mating (with kin selection playing an important role), with a later switch to multiple mating occurring after worker differentiation (see refs. 7, 10, 22 for discussions of factors leading to multiple mating in social Hymenoptera). In this view the origin of sociality was mediated by kin selection whereas its subsequent elaboration has been, to some extent, uncoupled from the necessity for close kinship, although still constrained by the specialized morphologies and behaviours of the participants. Close analogies may exist in other social organisms exhibiting diverse social structures.

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