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# Single mating and its implications for kinship structure in a multiple-queen wasp, *Parachartergus colobopterus*

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The number of matings by social insect queens is an important determinant of the kinship structure of their colonies, and it is therefore expected to affect a variety of kin-selected worker strategies. Here we report mate number data for *Parachartergus colobopterus*, the first such data for any member of the polygynous epiponine wasps. Mate number is assessed by microsatellite genotyping of the sperm stored in queen spermathecae. Queens of this species mate only once. This finding is consistent with a prediction of several theories positing that multiple mating functions to increase genetic diversity and is therefore less necessary in multiple-queen species. The finding of single mating, together with earlier data, allows estimation of effective queen numbers for all colonies, 4.40 queens, for male-producing colonies, 4.16 queens, and for queen-producing colonies, 1.18.

KEY WORDS: Epiponini, wasp, mate number, *Parachartergus*, social insects.



## INTRODUCTION

The number of times a female mates has special importance in social insect biology. Mate number affects the genetic relatedness of the colony progeny and therefore influences the evolution of progeny strategies through kin selection. High

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relatedness, which follows from low mate number, helps select for helping behavior (HAMILTON 1964a, 1964b). But even after the choice to work has been made, the effect of mate number on relatedness has other consequences for social structure. By altering worker relatedness to sisters, it changes the workers' optimal sex ratio (TRIVERS & HARE 1976, BOOMSMA & GRAFEN 1991, PAMILO 1991a). Because it changes relatedness among workers, it also affects whether workers should allow other workers to lay male haploid eggs (STARR 1984, WOYCIECHOWSKI & LOMNICKI 1987, RATNIECKS 1988). Similarly it affects the conditions under which daughter queens should be allowed to reproduce (NONACS 1988, PAMILO 1991b).

The increase in within-colony genetic variation with mate number can have other potentially important effects: increased resistance to disease, more complete expression of genetically influenced caste systems, increased tolerance of variable environmental conditions, or altered consequences of diploid male production. These effects are among the forces that could select for multiple mating in social insects (CROZIER & PAGE 1985, PAGE 1986, SHERMAN et al. 1988). (These effects of mate number all assume that insemination has occurred. Nevertheless, throughout this paper we will use the term "mate number", with its implicit assumption, instead of the more unwieldy "insemination number").

Mate number has never been determined for any member of the vespid wasp tribe Epiponini (as delimited by CARPENTER 1993). Though it is an ecologically important group of about 200 species (RICHARDS 1978), its tropical distribution has made it less-studied than other groups. Moreover, it is much harder to use genetic markers to estimate mate number in epiponines because colonies normally have many queens and the progeny of single queens cannot easily be reared in isolation, as can usually be done in ants.

Determinations of epiponine mate numbers would be valuable for several reasons. First, it would bear on all of the hypotheses that attribute multiple mating to the need for increased genetic variation in colonies. Under these hypotheses, polygynous colonies would be predicted to be singly mated, because they would already have an unusually large amount of genetic variation within colonies (KELLER & REEVE 1994). In agreement with this prediction, polygynous ant species tend to have fewer mates than monogynous species (KELLER & REEVE 1994). As one of the most consistently polygynous taxa of social insects, epiponine wasps could provide important information on this relationship.

There are other reasons for studying mate numbers in epiponines. Allozyme studies of epiponines have revealed much about the life cycles and social structure of several species (STRASSMANN et al. 1991; HUGHES et al. 1993; GASTREICH et al. 1993; QUELLER et al. 1993a, 1993c) but this information is incomplete without knowing mate numbers. For example, genetic relatedness from several species at first seemed unexpectedly high (given the large number of queens), but these studies showed that it was actually very close to what would predicted from queen number, variation in queen fertility, and relatedness among queens, assuming single mating. An accurate assessment of the single mating assumption is therefore necessary to cement this conclusion. Similarly, studies of relatedness in four epiponines (*Parachartergus colobopterus*, *Protopolybia exigua*, *Polybia occidentalis*, *Polybia emaciata*) showed that a colony's queen number cycles between high and low values, and that queen number is significantly lower when new queens are reared than when males are reared (QUELLER et al. 1993c; an observational study of *Metapolybia aztecoides* showed the same pattern WEST-EBERHARD 1978). This result, which was predicted if workers control production of sexuals (BOOMSMA & GRAFEN 1991, PAMI-

LO 1991a), is robust, but the actual numbers of queens expected at various stages of the colony cycle cannot be estimated without mate number. In particular, it is of interest to know how few queens are present when new queens are reared. It is thought that epiponines may requeen only after colonies reduce to a single queen, and perhaps after her death (WEST-EBERHARD 1973, 1978, 1981; QUELLER et al. 1993c).

Finally, a finding of significant multiple mating could have major implications for caste determination in epiponines. Relatedness among colony-mate queens is very high (0.55 to 0.82 in four species; QUELLER et al. 1993c), consistent with them being the progeny of one or a very few queens who mated once. If queens typically used the sperm of several males, they could not produce a cohort of new queens that highly related, unless there were some mechanism whereby the daughters in one patriline come to predominate among the brood that will become new queens. In other words, the difference between workers and queens would need to have a strong genetic component, contrary to what is generally true in social insects.

In this study, we investigated mate number for the epiponine wasp, *Parachartergus colobopterus*, by microsatellite genotyping of the sperm stored in queens' spermathecae. This technique has been used with success on an ant, *Myrmica tahoensis* (EVANS 1993), and on a wasp, *Polistes annularis* (PETERS et al. 1995). Microsatellites consist of tandem repeats of short nucleotide motifs. These loci are frequently highly variable and different alleles at a locus vary in the number of repeats. The loci were amplified via the polymerase chain reaction from primers flanking the tandem repeat region, and alleles are easily distinguished after separation in polyacrylamide gels (QUELLER et al. 1993b). Only tiny amounts of target DNA are required, so the sperm stored in a spermatheca can be genotyped. Since males are haploid, the consistent presence of only a single allele in the sperm would indicate single mating.

## METHODS

Twenty-one colonies of *Parachartergus colobopterus* were collected on the campus of the Universidad Central de Venezuela in Maracay, Venezuela during the rainy seasons (May to August) of 1991 to 1993. The colonies used in this study were young, collected after they had begun building nests but before the emergence of any brood on the new nest. Collections were made by quickly drawing a zip-loc bag over the new nest and closing it before the wasps could escape, collecting both wasps and nest material. Nest sites were revisited after a few hours and on the following day to collect any wasps absent from the nest at the time of collection.

The collected adults were stored at – 80 °C until they could be dissected and genotyped. By dissection, it is possible to identify mated females by the presence of sperm in the spermatheca, and to separate the sperm from the female tissue (see PETERS et al. 1995, STRASSMANN et al. in press b).

Thirty-eight of the wasps collected had mated. Most of these females, and the sperm from their spermathecae, were amplified for six microsatellite loci using the polymerase chain reaction. The microsatellite loci used are shown in Table 1. These loci were found by screening a partial genomic library (for methods see STRASSMANN et al. in press b). The primer sequences for these loci are described elsewhere, together with others from this species (STRASSMANN et al. in press a). Four of the 38 sperm samples were scored at three additional loci, also shown in Table 1. Methods for PCR amplification of both sperm and adults follow PETERS et al. (1995), but using the locus-specific annealing temperatures given in Table 1.

Table 1.

Microsatellite loci used in this study (for primer sequences see STRASSMANN et al. in press a).

| Locus name * | Observed heterozygosity ** | Annealing temperature (°C) |
|---|---|---|
| PACO41TAG | 0.74 | 45 |
| PACO3155TAG | 0.71 | 50 |
| PACO3304CAT | 0.58 | 45 |
| PACO3417AAT | 0.66 | 55 |
| PACO3436AAT | 0.67 | 55 |
| PACO3457AAT | 0.69 | 50 |
| PACO3107bTAG † | 0.94 | 50 |
| PACO3117TAG † | 0.53 | 48 |
| PACO3434AAT † | 0.50 | 45 |

* The triplet repeat motif for each locus is given by the last three letters in its name. ** Observed heterozygosities are based on 18-36 individuals, each from a different colony. † Genotyped only for 4 sperm samples in this study.

Complete protocols are given in STRASSMANN et al. (in press b). Briefly, PCR reactions were run in 10 µl samples containing about 1 ng of DNA, with 0.5 µM primer, 0.025 U/µl Taq polymerase and 0.1 µM dNTP's, with $^{35}$S-labeled ATP as a visualizing agent. Sperm samples were double-amplified (PETERS et al. 1995). The amplified DNA was run out on denaturing polyacrylamide gels and scored by exposing X-ray film to the radioactivity of the incorporated $^{35}$S. All samples were scored twice, by two different observers, and any discrepancies were resolved by re-examining the autorad.

During dissection of spermathecae, a few samples appeared to be contaminated with tissue from the female. These were noted; such contamination would be expected to produce bands in the sperm sample matching those in the female.

Occasionally a sample failed to amplify at some loci. The first 12 sperm samples were rerun for missing loci until the entire sample was used up. The remaining samples were only run once each, and loci failing to amplify were simply scored as missing. Samples from the adult females amplified more consistently, although they also occasionally had missing loci. Only loci for which both sperm and female amplified, allowing comparison of the two, are counted below.

## RESULTS

No sample, at any locus, showed more than a single band not present in the female from which the sperm sample came.

Thirty-two different samples amplified for at least one locus, for a total of 138 sperm/female genotype scores. Four sperm samples were successfully scored at all 9 loci. Of the samples amplified at 6 loci, 6 were successful at all loci, 10 at 5 loci, 1 at 4 loci, 4 at 3 loci, 2 at 2 loci and 5 at only 1 locus. Twenty-six of the 32 samples showed only a single band across all amplified loci; in 22 of these the band was distinct from any allele in the female.

The remaining six sperm samples showed additional bands consistent with alleles found in the female. Bands resulting from female contamination are often fainter than the non-maternal allele assumed to come from the sperm. For 4 of these samples, probable contamination of the sperm sample by female tissue was

noted during dissection. If the presence of additional bands in the sperm sample is caused by contamination by DNA from the female, then such samples should show a consistent pattern of female alleles present at all amplified loci. This was the case with the exception of 6 genotypes in 4 of the contaminated samples. These six cases (out of a total 28 scored for these 4 samples) were scored for only one allele despite the presence of supernumerary alleles matching the female at other loci. However five of these cases turned out on closer examination to be consistent with maternal contamination, either showing very faint maternal bands that had not been scored, or possibly possessing such bands hidden beneath stutter bands of the sperm allele [stutter bands would typically cover only one other allelic position, and the missing maternal band(s) had to match this position for this explanation to be invoked]. One sperm sample remained puzzling; five loci showed clear maternal contamination, but one locus showed a clear single band and no evident contamination.

If multiple alleles in the sperm sample were the result of multiple mating, they would not consistently match the female's genotype. These results, showing never more than a single band not from the adult female, therefore strongly indicate single mating in this species.

## DISCUSSION

We found no evidence for multiple mating in *Parachartergus colobopterus*. Queens appear to be singly mated, or at least to store the sperm of only one male. Although the spermathecae of six females showed multiple bands, these were always consistent with contamination from the maternal genotype. Given the high heterozygosities of these microsatellite loci (Table 1), the odds are very small that these extra bands would always match maternal alleles if they were in fact due to multiple matings.

It is possible that some very small amount of a second male's sperm could be missed. But the single sperm bands on our autorads were often quite dark, and even a small fraction of a second male's sperm should have shown up as a fainter band. Sensitivity studies would need to be conducted to determine exactly what fraction could be detected. But in terms of the overall genetic composition of the colony, it does not matter very much if we might have missed second matings that contribute a tiny fraction of sperm. For all practical purposes, the progeny of each female will be full sisters.

Studies of mate number in social wasps are reviewed by ROSS & CARPENTER (1991). There are only a few other studies of mate number using genetic markers. Allozyme studies of the single-queen vespines, *Vespula maculifrons* and *Vespula squamosa*, show them to be multiply mated (ROSS 1986). Studies of *Polistes metricus* and *Polistes variatus* attribute within-colony genetic variation to multiple mating, with one male producing most of the progeny (METCALF & WHITT 1977, METCALF 1980). However it is not clear that the progeny not fitting the singly mated single-queen hypothesis could not have been laid by a second female. The colonies were thought to have a single queen (foundress) but it is hard to rule out the possibility that another foundress had been present at some time. *Polistes annularis* is known to be singly mated (though two out of 40 females mated twice), based on the same sperm-scoring method used in this study (PETERS et al. 1995).

In ants, polygynous species are more often singly mated than monogynous species, supporting the view that mate number is adjusted in part because of its

effects on within-colony genetic variability (KELLER & REEVE 1994). Though the data for vespids are very limited, they are so far roughly consistent with this correlation. We now have the first data for a strongly polygynous species (*P. colobopterus*) and its single mating shows the predicted difference with the two single-queen multiply-mated vespine species (ROSS 1986). *Polistes annularis* is harder to classify in this respect, since many foundresses contribute during the earliest part of the season, but within 2 months there are fewer, often only one (PETERS et al. 1995).

The finding of single mating in *Parachartergus colobopterus* allows us to sharpen some conclusions drawn from earlier studies. An allozyme study showed that, if we assume single mating, the worker relatedness (r = 0.306) could be predicted very accurately from queen number, relative queen fertility, and the relatedness among queens (STRASSMANN et al. 1991). The mating assumption underlying that prediction is now validated, and the success of the prediction suggests we have an accurate understanding of the genetic structure of *P. colobopterus* colonies. It also tends to suggest that similar studies of other epiponine species (QUELLER et al. 1993a, GASTREICH et al. 1993) are also reliable. These studies also successfully predicted worker relatedness using the assumption of single mating, so the confirmation of the method in *P. colobopterus* suggests that these other species may also be singly mated (however, this needs to be confirmed for each species, because the confidence intervals for worker relatedness are broad enough to also be consistent with some degree of multiple mating).

Now that mate number is known, we can use relatedness estimates at various stages of the colony cycle to reliably estimate queen numbers. Under single mating, the relatedness of diploid daughters can be written as

$$r_{\text{daughters}} = \frac{3}{4}\left(\frac{1}{\bar{f}_h}\right) + \frac{r_{\text{queen}}}{4}\left(1 - \frac{1}{\bar{f}_h}\right) \qquad (1)$$

where $\bar{f}_h$ is the harmonic mean of queen number (QUELLER 1993). This is best viewed as an effective queen number; it is the number of equally fecund queens that would produce the observed daughter relatedness. The true queen number will be somewhat higher to the extent that the queens have unequal reproduction. However, this effect is probably small in *P. colobopterus*, because within-colony variation in the number of mature eggs in queen ovaries is modest (STRASSMANN et al. 1991).

Substitution of values for $r_{\text{queen}}$ and $r_{\text{daughters}}$ allows solving for the effective queen number, $\bar{f}_h$. Relatedness among queens is 0.661 and relatedness among workers is 0.306 (STRASSMANN et al. 1991), so the estimated harmonic mean of queen number is 4.40. For colonies producing males, the relatedness of workers is 0.298 (QUELLER et al. 1993c), so estimated queen number in these colonies is 4.16, not appreciably different. However, queen number in queen producing colonies is much lower. These colonies are not identified directly, since queen production is a rare and hard-to-observe event. Instead, queen relatedness is used recursively in (1), once as the relatedness among queen mothers, and once as relatedness among new queen daughters. This yields an estimate of 1.18 queens in colonies that produce new queens. This is consistent with cyclical monogyny (WEST-EBERHARD 1973, 1978, 1981): new queens are usually produced on colonies that have been reduced to a single old queen, or even with new queens developing after the death of the last remaining old queen. The results make more concrete the previous finding that queen number is lower on colonies producing new queens than on colonies producing males (QUELLER et al. 1993c), a result consistent with worker control of sexual investment (BOOMSMA & GRAFEN 1991, PAMILO 1991a).

The fact that *P. colobopterus* mates once will facilitate future detailed genetic work on the species. Microsatellite markers scored from mothers and their sperm can be used to assign the maternity of progeny (EVANS 1993, PETERS et al. 1995) and this method is quite powerful when there is only a single mate. Of course, this will be less effective when there is maternal contamination of the sperm samples. The presence of some maternal contamination in this study, when we did not experience it in an earlier study of a different species (PETERS et al. 1995), suggests that the sperm isolation methods should be perfected on non-critical samples for each new species studied. But even without parental genotypes, single mating may allow for fine-scaled genetic work. This is because each full sister group constitutes the full reproductive output of a single mother, and each full sister group forms a very distinct class (they have only two genotypes at each locus, which must share a common allele). These kinds of methods should permit studies of reproductive competition among mothers and within-colony kin discrimination among progeny.

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