

POPULATION GENETIC STRUCTURE AND SOCIAL EVOLUTION
IN THE SPHECID WASP *MICROSTIGMUS COMES*

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Recent studies of social insects have often sought to examine the conditions under which sterile-worker castes may evolve and be maintained. Theoretical studies attempt to specify the opportunities for and constraints on selection for worker altruism, whereas empirical studies examine whether particular features of the natural history conform to theoretically predicted favorable states. Perhaps the most influential theoretical contribution in this area has been the concept of kin selection (Hamilton 1964*a,b*, 1972; Maynard Smith 1964), in which heritable altruistic traits such as sterility may evolve in the context of the kin group (West-Eberhard 1975; Crozier 1979; Michod 1982). According to kin-selection theory, the evolution of altruism is opposed by individual-level selection within relatively homogeneous (or kin) groups, but such traits may spread to fixation if they lead to differential contributions to the population gamete pool among kin groups, with altruist-containing groups faring better than others (Wade 1979, 1980, 1985*a*; Uyenoyama and Feldman 1980; Michod 1982). A fundamental corollary of the theory, then, is that the classical concept of individual fitness be expanded to include the effects of one's behavior on the reproduction of genetically related individuals (inclusive fitness).

Kin-selection theory provides an explicit population-genetics framework for empirical studies by specifying the patterns of family and local genetic structure most favorable to social evolution. This framework may be further extended to consider the effects on selection of genetic structure at increasingly higher levels of population organization (Wade 1979, 1985*a*; Michod 1982; Pollock 1983; McCauley et al. 1988; see also Wright 1978; D. Wilson 1980; Selander and Whittam 1983; Grafen 1984). Such higher-level structure, which may arise from habitat fragmentation or properties of the dispersal or breeding systems, grades into kinship structure at increasingly local scales. The central importance of population genetic structure in this context is that it signals the existence of nonrandom associations of genotypes (Michod 1982), and this genetic variation among groups, coupled with their differential reproduction, makes plausible the operation of kin (or interdemic) selection at a hierarchy of levels.

Studies of local and colony-level genetic structure (relatedness) in social insects have been undertaken at an increasing rate as a means of assessing the potential

significance of kin selection in promoting worker altruism (Crozier 1980; Crozier et al. 1987; Reilly 1987; Schwarz 1987; Ross 1988; Kukuk 1989). Interest in such studies has been fueled by the advent of molecular methods for generating genetic markers as well as by the development of robust statistical procedures for describing genetic structure. In addition, there is growing skepticism about whether attempts to measure the benefit and cost components of inclusive-fitness effects from short-term ecological studies can provide realistic assessments of the potential for kin selection to mediate social evolution, particularly in the absence of relatedness data (Gadagkar 1985; but see Grafen 1984).

Regrettably, most available studies of genetic structure in social Hymenoptera fall short of providing results that are fully applicable to the problem of the origin of eusociality. For instance, only a few studies have considered primitively eusocial or parasocial taxa (Metcalf and Whitt 1977; Metcalf 1980; Lester and Selander 1981; Schwarz 1986, 1987; Crozier et al. 1987; McCorquodale 1988; Kukuk 1989; C. R. Hughes, D. C. Queller, J. E. Strassmann, and S. K. Davis, MS), which, by virtue of their presumed close resemblance to the stem groups giving rise to eusocial forms, must be the focus of attempts to elucidate social origins. Furthermore, these studies have been restricted to a limited subset of the diverse species of wasps and bees representing such relevant groups. Of the above studies, only those of Crozier et al. (1987), Schwarz (1987), McCorquodale (1988), and Kukuk (1989) have considered the effects of hierarchical genetic structure above the level of the nest. Finally, population sex-investment ratios, which significantly influence the opportunity for kin selection to act within the constraints of given genetic structures (Trivers and Hare 1976; Charnov 1978; Wade 1979), have been estimated in conjunction with genetic studies of socially intermediate forms in only three cases (Metcalf 1980; Schwarz 1987, 1988; Kukuk 1989). Thus, pertinent empirical genetic data bearing on the role of kin selection in the inception of hymenopteran eusociality are rare, and it would appear premature to discount the importance of kin selection in this regard (for other views, see Lester and Selander 1981; Crozier 1982; Andersson 1984; Stubblefield and Charnov 1986).

This paper reports the results of an investigation of colony-level and local genetic structure in the sphecid wasp *Microstigmus comes*. This species is a member of a socially transitional group (subfamily Pemphredoninae) characterized by some species exhibiting advanced parasocial or eusocial behaviors, in contrast to the remainder of the Sphecidae, in which solitary behavior is the rule (Evans 1966; Matthews 1968a, 1970; Akre 1982; Matthews and Naumann 1988). Because the social pemphredonines are only distantly related to the remaining lineages of aculeate Hymenoptera with eusocial representatives (Brothers 1975), they clearly have developed eusociality convergently and thereby represent ideal candidates for comparative studies of social evolution in the Hymenoptera. Furthermore, because the level of social complexity in *M. comes* is consistent with its ancestors having recently crossed the threshold of eusociality, this species may be presumed to resemble in many ways an incipient social stem group (Matthews 1968a; Ross and Matthews 1989).

The results from the genetic analyses, in conjunction with sex-allocation data,

are used here to provide an empirical basis for interpreting the role of kin selection in the social evolution of this unique group of wasps. The scope of the analysis is subsequently expanded by reference to genetic studies of other primitively social forms in order to develop broader conclusions regarding the likely importance of kin selection in this context. Finally, because the genetic data also shed light on colony composition and social organization in *M. comes* and because various models of the "routes to eusociality" in Hymenoptera make contrasting predictions regarding these attributes in socially intermediate forms (Wheeler 1923; Lin and Michener 1972; West-Eberhard 1978), the data are also used to assess these "process" theories of social evolution.

NATURAL HISTORY OF *MICROSTIGMUS COMES*

This small wasp (mean weight 0.79 mg) is restricted to humid forests of the Neotropics, where it builds its nests on the undersurface of fronds of the palm *Chryosophila guagara*. Nests are constructed with material scraped from the frond surface, as well as silk of glandular origin, and are initiated either by single females or by as many as six females working cooperatively (Matthews and Starr 1984; Matthews 1990). Nest initiation and other aspects of colony development do not appear to be seasonal or synchronous in the study population (Matthews 1968*a*; Matthews and Starr 1984). Nest-mate females cooperate in brood rearing by mass-provisioning cells with prey (Collembola); a single egg is laid in each cell after it is provisioned. Cells are constructed and provisioned sequentially, leading to the presence of a graded series of brood stages in nests with several cells (Matthews 1968*a,b*). An absence of larval wastes in cells indicates that adults provide some parental care beyond initial prey provisioning. The cooperative brood care displayed by this species, in conjunction with the apparent occurrence of routinely sterile females in multigenerational groups of up to 13 females, suggests that *Microstigmus comes* is fully eusocial (Ross and Matthews 1989; Matthews 1990).

METHODS

Collection of Nests

Nests were collected in March 1987 at Corcovado National Park on the Osa Peninsula in southern Costa Rica. Nests ($N = 147$) were scouted and their positions mapped during the daylight hours; all collections were made after dark to increase the probability that all nest inhabitants were present. Collected nests were distributed among three disjunct subpopulations distinguished by local topography and vegetation and corresponding to different segments of the park trail system (fig. 1). We collected 67 nests from the Anacardium Trail subpopulation, 40 from the Jack's Trail subpopulation, and 38 from the Ollas Trail subpopulation (fig. 2); 2 additional nests were not associated with any of these subpopulations.

Following nest collection, all resident wasps were immediately removed and frozen in a liquid-nitrogen-cooled cryogenic vessel. A total of 611 adult females

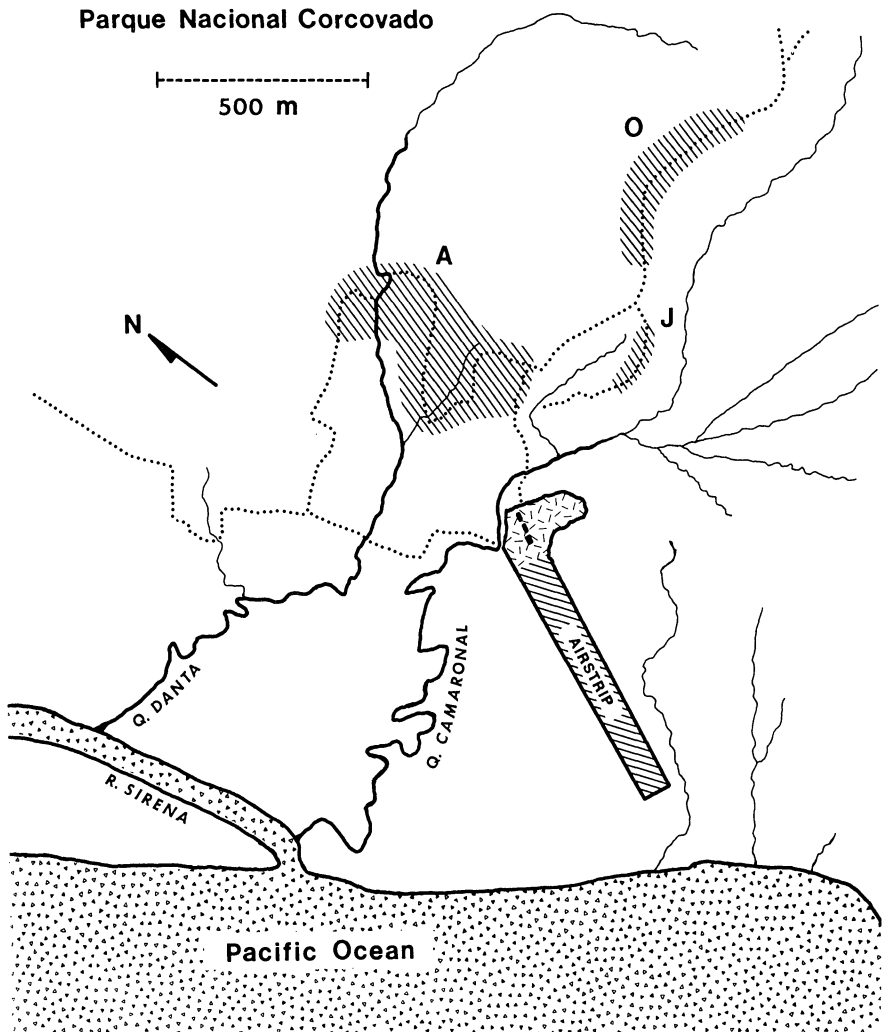


FIG. 1.—Study population of *Microstigmus comes* in Corcovado National Park, Osa Peninsula, Costa Rica. The three sampled subpopulations are indicated by hatching. The Anacardium Trail (A) subpopulation occupies a low-lying area of primary forest, whereas the other two subpopulations occur at higher elevations on ridges. The Jack's Trail (J) subpopulation is in an area of second-growth vegetation, and the Ollas Trail (O) subpopulation is in primary forest. Distributions of collected nests within each subpopulation are shown in figure 2.

($\bar{x} = 4.2/\text{nest}$; range, 1–13) and 154 males (from 51 of the nests, $\bar{x} = 3.0/\text{nest}$; range, 1–10) were collected. The nests were dissected and their composition and state of development recorded; pupae and prepupae were kept with the nest fragments in small vials in the laboratory to continue their development. Newly emerged adults derived from these immatures ($N = 147$ females and 22 males from a total of 89 nests) were provided with a water-soaked raisin upon emergence

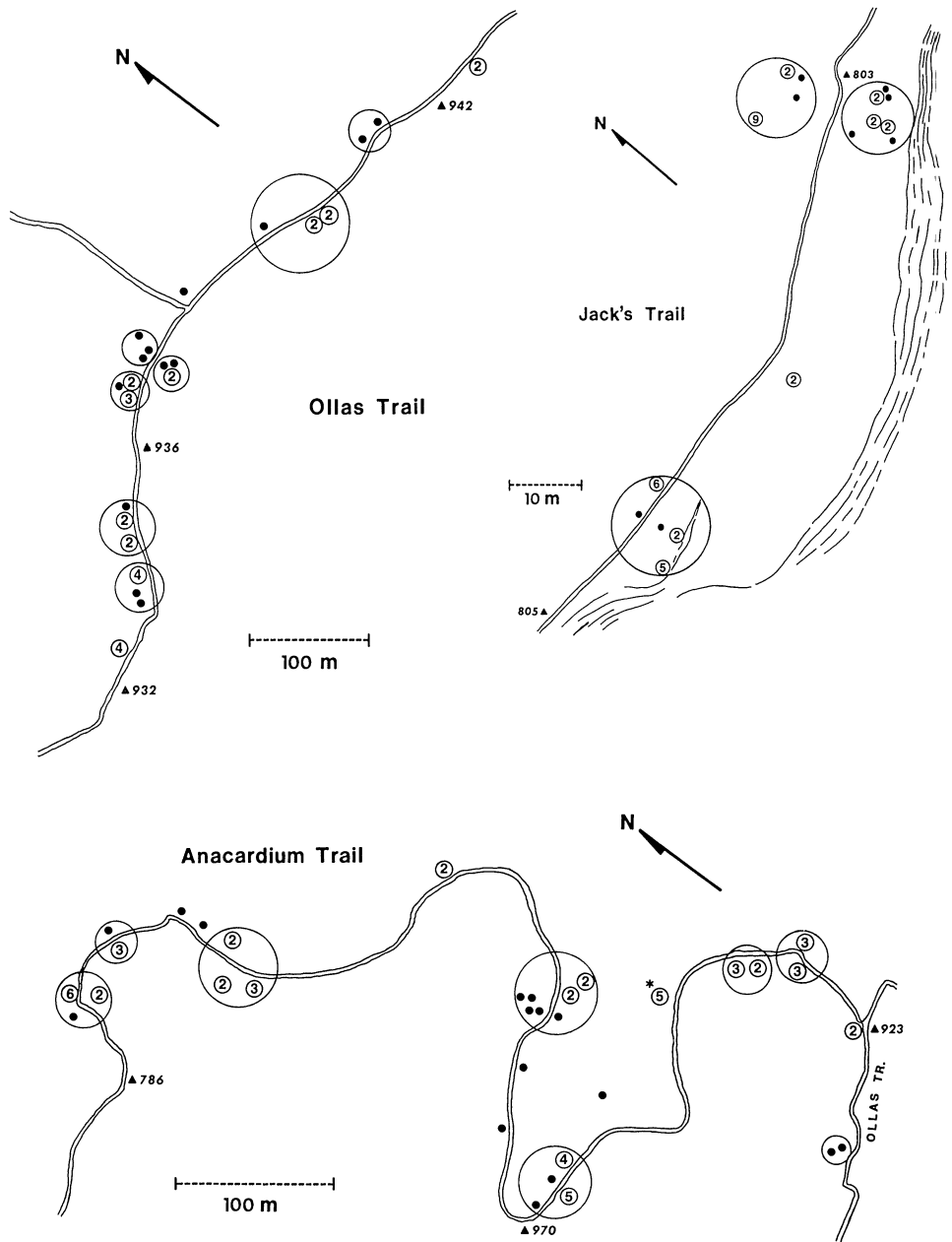


FIG. 2.—Locations of collected nests of *Microstigmus comes* in three subpopulations. Dots, Palms from which only one nest was collected; small circles, palms from which more than one nest was collected (the number of nests is indicated in the circle); large circles, groups of palms representing “demes” for the relatedness analyses of microgeographic structure; triangles, location and numbering of trail markers; asterisk, the palm in the Anacardium Trail subpopulation on which two nests contained individuals bearing the rare *Dia-4^S* allele.

and were held for 3 days to mature. Field-collected wasps and those emerging in the laboratory were stored in an ultra-low-temperature freezer at -60°C until electrophoresis.

Electrophoretic Analyses

Electrophoresis was conducted on 14% horizontal starch gels using standard methods (see Harris and Hopkinson 1976; Steiner and Joslyn 1979; Ross et al. 1987). The enzyme products of three presumptive Mendelian loci were found to be variable and thus informative for the purposes of the present study. These loci (with abbreviation and buffer system used; see Ross et al. 1985, 1987) code for peptidase (*Pep*, "M" buffer, phenylalanyl-proline as substrate), diaphorase (*Dia-4*, "4" buffer), and glucosephosphate isomerase (*Gpi*, "C" buffer). The head plus thorax of the wasps was used as the tissue source for all three enzymes by homogenizing these body parts in 30 μl of an extraction buffer (Tris/HCl), centrifuging for 2 min, and dividing the supernatant into three aliquots for the three different gel-buffer systems. Before homogenization, each wasp was blotted dry, and the whole body, as well as only the head plus the thorax, was weighed to a precision of 0.01 mg for 540 of the 765 adult wasps (71%) and all newly emerged wasps. Gels stained for scoring *Dia-4* and *Pep* were run for 2 h, whereas those stained for scoring *Gpi* were run for 12 h to achieve sufficient separation of the electromorphs. Intensity of staining (and thus level of expression) for *Dia-4* and *Gpi* was noticeably diminished in young wasps and could therefore be used in conjunction with light integumental pigmentation to indicate which wasps were likely to have recently emerged in a nest.

Pep and *Gpi* are diallelic in the study population, whereas three alleles were discovered for *Dia-4*. Mendelian inheritance of the products of the loci was confirmed by (1) the presence of a single staining band for (haploid) males, (2) the occurrence of predicted genotypes among presumed progeny of individual females, (3) the presence of banding patterns in heterozygotes consistent with previously published quaternary enzyme structures (Harris and Hopkinson 1976), and (4) the occurrence of genotypes at frequencies close to those expected under Hardy-Weinberg equilibrium (see below).

The possible association of alleles at all pair-wise combinations of loci was studied using Nass' (1959) contingency-table χ^2 analysis, which accommodates the relatively small expected values generated for cells involving genotypes with rare alleles. Only one diploid genotype per nest was used in this analysis. Values for χ^2 were 3.26, 0.89, and 3.99, corresponding to probability values of $P > 0.50$, $P > 0.50$, and $P > 0.10$. Thus, there is no indication of a significant association of alleles between any pairs of loci, and they are regarded as independent sources of information.

Data Analyses

Analysis of population genetic structure in social Hymenoptera is somewhat more complicated than for most other organisms because of the effects of pronounced family structure as well as uncertainties regarding the identity of the Mendelian population (Crozier 1980; Pamilo 1983; Crozier et al. 1987; Ross 1988).

Family structure (or significant kinship structure at higher levels) means that sampled genomes may not in many cases be independent, since they may be linked by recent pedigree connections (Pamilo 1983). A sampling regimen failing to account for such structure violates a basic assumption in testing for Hardy-Weinberg equilibrium in populations—that is, the assumption that a large number of unrelated genomes have been sampled (Robertson and Hill 1984)—thus posing difficulties when methods based on deviations from Hardy-Weinberg expectations are employed to study genetic structure. In the case of relatedness estimation, the inclusion of nonindependent genotypes means that relatedness components from lower levels of structure contribute to the estimates for higher levels. An inability to identify which females constitute the Mendelian (reproductive) population is a further obstacle because of resulting uncertainties in determining which individuals should be included and which sample sizes should be specified for estimating population-genetics parameters (see, e.g., Crozier et al. 1987). This latter problem is particularly acute in primitively social forms lacking clearly defined reproductive castes.

One approach to ameliorate these problems is to estimate the number of independent haploid genomes at the most fundamental level of structure (the nest) and to use this estimate to specify the maximum number of sampled genomes per nest that may be used in analyses of genetic structure at higher levels (Pamilo 1983; Ross et al. 1987). The number of independent haploid genomes represented in a nest is reflected in the distribution of within-nest genetic variability (Pamilo 1983), which is measured by the genetic relatedness of nest mates. For instance, in nests headed by single, once-mated queens, only three haploid genomes are represented (two from the queen and one from her haploid mate), genetic variability within the nest is low (only one or two genotypes at each locus), and female nest-mate relatedness is high (0.75). Additional functional queens or the use of sperm from different mates by single queens leads to an increase in the number of independent genomes, an increase in within-nest genetic variability, and a corresponding decrease in nest-mate relatedness. In general, the number of independent haploid genomes can be approximated by $2.25/r$, where r is female nest-mate relatedness (Pamilo 1983).

Relatedness for nest-mate females of *Microstigmus comes* is high (mean $r = 0.67$; see below), so that relatively few independent haploid genomes (3.4) are represented in an average nest. Therefore, all population-genetics parameters (other than nest-mate relatedness) have been estimated in this study using only the first one or two females sampled from each nest (first two or first four haploid genomes).

Nest-mate relatedness was in most instances estimated using the genotypic correlation procedure of Stanton (1960), as modified by Pamilo (1984, eq. 4; see also Crozier et al. 1984). Genotypes of all females in a nest were used, and estimates were generated both by weighting nests equally and by weighting them by the number of female inhabitants. Relatedness values and variance estimates were generated using a jackknife procedure over nests (Crozier et al. 1984; Pamilo 1984). Only the loci *Pep* and *Gpi* were sufficiently polymorphic (i.e., the frequency of the common allele was less than 0.9) to be used in these relatedness

estimates (see Pamilo 1984; Wilkinson and McCracken 1985). For the purpose of estimating relatedness of females in multiple-foundress nests, the identity-by-descent method of Queller and Goodnight (1989) was employed because of its superior performance when limited numbers of groups are available and because of its applicability to groups containing only two individuals. Estimates of the values and their variances were again obtained by jackknifing over groups.

Because of their social habits and specificity to one plant species for nesting sites, natural populations of *M. comes* appear to exhibit hierarchical spatial subdivision. Specifically, individuals reside in discrete nests, groups of nests occur on individual host palms, and palms often occur in distinct clusters separated by some distance from other such clusters (see, e.g., Matthews 1968*b*). This hierarchical structure is reflected in the dispersion of nests sampled for this study (fig. 2) and serves to define biologically appropriate levels for the analysis of local genetic structure. The objective of such analyses is to determine whether significant genetic discontinuities delimiting groups of importance for the action of natural selection correspond to recognizable discontinuities in the two-dimensional spatial structure of populations.

Relatedness of females at two levels of spatial structure above the nest, that of the host palm and of the "deme," was ascertained using the genotypic-correlation method with the markers *Pep* and *Gpi* and jackknifing over the relevant groups. All estimates were generated both by weighting groups equally and by weighting groups by the number of constituent genotypes. The level of the host palm represents the neighborhood of nests located on an individual palm, whereas the level of the deme represents the neighborhood of nests located in an individual cluster of palms (fig. 2). We identified 18 such demes by visually inspecting the nest-location maps; the demes occur at a spatial scale of 10–50 m (i.e., nests in a deme are located within 10–50 m of one another). Because these two levels of structure above the nest probably represent spatial scales within the dispersal distance of typical wasps, they correspond to the "microgeographic" scale of population structure (Pamilo 1983).

Larger-scale genetic structure was assessed at the level of the three sampled subpopulations using Wright's (1951) hierarchical *F* statistics. Estimators for these parameters, which correct for small and unequal sample sizes as well as small numbers of subpopulations, have been derived by Weir and Cockerham (1984). Because these estimators can be extended to situations in which there are two levels of hierarchical genetic structure (Weir and Cockerham 1984, p. 1365), they were used in this study to assess structure simultaneously at the levels of the subpopulation and the deme. (Some demes defined for this analysis represent composites of the demes identified for the relatedness analysis at the same level because of a need for larger sample sizes for *F*-statistics analysis.) Because of the few polymorphic loci available, estimates for the parameters and the sample variances were calculated using a jackknife routine over subpopulations or demes rather than over loci (see Weir and Cockerham 1984). Confidence limits (95%) for these estimates (as well as for those derived from the relatedness analyses) were generated from the jackknife variances based on the *t* distribution. Conceptually, the parameters F_{IT} and F_{IS} represent correlations of genes within individuals in the

TABLE 1

OBSERVED AND EXPECTED GENOTYPE FREQUENCIES AND ALLELE FREQUENCIES IN *MICROSTIGMUS COMES*

POPULATION	<i>Pep</i>			<i>Dia-4</i>					<i>Gpi</i>								
	F/F	F/S	S/S	<i>p_S</i>	<i>P_F</i>	M/M	M/F	M/S	<i>p_M</i>	<i>P_F</i>	<i>p_S</i>	F/F	F/S	S/S	<i>P_S</i>	<i>P_F</i>	
ONE FEMALE SAMPLED PER NEST																	
Anacardium Trail																	
Observed	10	28	29	.642	.358	60	5	2	.948	.037	.015	4	29	34	.724	.276	
Expected	8.6	30.8	27.6			60.2	4.7	1.9				5.1	26.8	35.1			
Jack's Trail																	
Observed	9	19	11	.526	.474	31	9	0	.888	.112	0	2	14	24	.775	.225	
Expected	8.8	19.4	10.8			31.5	8	0				2	14	24			
Ollas Trail																	
Observed	9	19	10	.513	.487	34	4	0	.947	.053	0	4	14	20	.711	.289	
Expected	9	19	10			34.1	3.8	0				3.2	15.6	19.2			
TOTAL																	
Observed	28	66	52	.582	.418	127	18	2	.932	.061	.007	11	58	78	.728	.272	
Expected	25.5	71	49.5			127.7	16.7	1.9				10.9	58.2	77.9			
TWO FEMALES SAMPLED PER NEST																	
Anacardium Trail																	
Observed	21	51	54	.631	.369	110	14	2	.936	.056	.008	6	56	64	.730	.270	
Expected	17.2	58.7	50.2			110.4	13.2	1.9				9.2	49.7	67.1			
Jack's Trail																	
Observed	19	34	21	.514	.486	58	17	0	.887	.113	0	3	23	49	.807	.193	
Expected	17.5	37	19.6			59	15	0				2.8	23.4	48.8			
Ollas Trail																	
Observed	16	36	20	.528	.472	63	9	0	.937	.063	0	6	28	38	.722	.278	
Expected	16	35.9	20.1			63.2	8.5	0				5.6	28.9	37.5			
TOTAL																	
Observed	56	122	98	.576	.424	235	40	2	.924	.072	.004	17	109	151	.742	.258	
Expected	49.6	134.8	91.6			236.5	36.9	2.1				18.4	106.1	152.5			

NOTE.—For *Dia-4*, only the genotypic classes that are represented by sampled individuals are displayed. χ^2 tests for conformity of observed genotype frequencies to Hardy-Weinberg expectations yield values with all $P > 0.10$ (except for *Pep* in the total population with two females sampled per nest, $P > 0.05$). Subscripts denote allelic designations for each locus.

total population and within subpopulations, respectively, and may also be viewed as measures of the departure from Hardy-Weinberg genotype proportions in the total population (F_{IT}) and in the subpopulations (F_{IS}) (Nei 1977). The correlation of genes between individuals within a deme or subpopulation, F_{ST} , represents the extent of genetic differentiation among demes (F_{ST1}) and among subpopulations (F_{ST2}) (Nei 1977; Weir and Cockerham 1984).

Frequencies of observed single-locus genotypes in the sampled population and subpopulations of *M. comes* were tested for conformity to Hardy-Weinberg expectations using classical χ^2 goodness-of-fit tests for the loci *Pep* and *Gpi*. Because of extreme allele frequencies at *Dia-4* (see below), the two rare alleles of this locus were collapsed and Emigh's (1980) continuity-corrected χ^2 test was applied (see Hernández and Weir 1989).

RESULTS

Allele frequencies, genotype frequencies observed, and genotype frequencies expected under Hardy-Weinberg equilibrium are presented for the three subpopulations and total population in table 1. The nature of polymorphism exhibited by each of the marker loci varies considerably, with *Pep* characterized by the most similar allele frequencies and *Dia-4* by the most extreme allele frequencies. Indeed, two of the three alleles at this latter locus were sufficiently rare that they were encountered in females only in heterozygous combination with the common allele.

In general, there is remarkably close agreement between observed and expected genotype frequencies. Sampling one rather than two females per nest yields genotype frequencies closer to Hardy-Weinberg expectations in 10 of 12 cases (83%), as might be predicted considering that the latter procedure oversamples relative to the number of independent genomes per nest. Nonetheless, all χ^2 values return probability estimates of greater than 0.05. The data, therefore, do not permit rejection of the null hypothesis that the three subpopulations and the total population are in Hardy-Weinberg equilibrium. This suggests that the underlying assumptions for this model, including absence of selection on the marker loci and lack of significant local inbreeding, may be met.

Microgeographic and Larger-Scale Genetic Structure

Estimates for hierarchical F statistics at the levels of the subpopulation and deme are presented in table 2. The results are similar whether one or two females were sampled per nest. Values for F_{IT} and F_{IS} are generally close to zero, as expected given the close match of observed genotype frequencies to Hardy-Weinberg proportions, but they indicate slight excesses of heterozygotes for *Dia-4* at the subpopulation and population levels and slight deficiencies of heterozygotes for *Pep* at the population level (see also table 1). The varying values of F_{IS} among the individual loci, as well as the lack of significant positive values for the combined loci, mean that the null hypothesis of random mating within the subpopulations cannot be rejected. Values for F_{ST} at both the deme and subpopulation levels are uniformly quite low, although significant positive values of F_{ST1} characterize

TABLE 2
ESTIMATES FOR HIERARCHICAL F STATISTICS AT TWO LEVELS OF STRUCTURE
(SUBPOPULATION AND DEME) IN *MICROSTIGMUS COMES*

F Statistic	<i>Pep</i>	<i>Dia-4</i>	<i>Gpi</i>	All loci
One female sampled per nest				
F_{IT} (total population)	0.063* (0.024)	-0.068* (0.007)	0.002 (0.040)	0.023 (0.016)
F_{IS} (subpopulations)	0.055 (0.021)	-0.074* (0.022)	0.004 (0.047)	0.018 (0.011)
F_{ST1} (demes) ^a	0.024* (0.012)	0.009 (0.006)	0.030* (0.009)	0.024* (0.006)
F_{ST2} (subpopulations)	0.007 (0.009)	0.003 (0.009)	-0.010 (0.020)	0.000 (0.007)
Two females sampled per nest				
F_{IT} (total population)	0.083* (0.016)	-0.084* (0.005)	-0.041 (0.025)	0.012 (0.014)
F_{IS} (subpopulations)	0.083 (0.028)	-0.082* (0.019)	-0.035 (0.048)	0.014 (0.005)
F_{ST1} (demes) ^a	0.017 (0.009)	0.004 (0.005)	0.051* (0.011)	0.028* (0.006)
F_{ST2} (subpopulations)	0.014* (0.004)	0.000 (0.003)	0.000 (0.018)	0.007 (0.007)

NOTE.—Standard errors of the estimates are in parentheses.

^a $N = 12$ demes.

* Differs from zero at the $P < 0.05$ level.

two of the three loci individually when one female was sampled per nest, as well as all loci in combination under either sampling protocol. These data suggest that although no meaningful genetic differentiation occurs among the three subpopulations, a low level of differentiation may exist at the level of the deme.

Genetic structure at this latter level was further investigated by determining relatedness from genotypic correlations. Relatedness was calculated, in the first instance, by including one or two female genotypes per nest for all nests on each palm making up a deme. Because of the potential for relatedness at the next lower level (individual palms) to inflate the estimate of intrademic relatedness, this latter value was in the second instance calculated using genotypic data from no more than two nests per palm. In general, intrademic relatedness values are low (table 3), although five of the eight estimates are significantly greater than zero. Four of these five significant estimates result from including two females per nest in the data set, a procedure likely to inflate values slightly. The relatedness data are quite concordant with the results from F -statistics analysis in suggesting that at most only modest differentiation exists at the level of the deme.

Genetic structure at the level of the palm was assessed by estimating relatedness for groups comprising one or two females sampled from each nest on single palms. To avoid using groups of only two individuals for the genotypic correlations (inclusion of which may lead to highly variable results; see Pamilo and Crozier 1982; Wilkinson and McCracken 1985), only palms with three or more nests were considered when one female genotype was sampled per nest. Relatedness values for this level are in some instances higher than for intrademic

TABLE 3

ESTIMATES OF RELATEDNESS AT THE LEVELS OF THE DEME (CLUSTER OF PALMS)
AND PALM IN *MICROSTIGMUS COMES*

Level	Unweighted	Weighted
Deme ($N = 18$ demes)		
One female sampled per nest		
All nests included	0.087 (0.049)	0.108* (0.041)
≤2 nests per palm included	0.086 (0.061)	0.056 (0.050)
Two females sampled per nest		
All nests included	0.124* (0.032)	0.116* (0.027)
≤2 nests per palm included	0.128* (0.036)	0.092* (0.031)
Palm		
One female sampled per nest ($N = 15$ palms)	0.097 (0.112)	0.169 (0.091)
Two females sampled per nest ($N = 37$ palms)	0.143* (0.060)	0.168* (0.050)

NOTE.—“Unweighted” indicates that the groups are weighted equally, whereas “weighted” indicates that they are weighted by the number of genotypes sampled in each group. Standard errors of the estimates are in parentheses.

* Differs from zero at the $P < 0.05$ level.

relatedness, but the only estimates significantly greater than zero are based on two sampled females per nest (table 3). Bearing in mind the necessary caveats regarding the two-female samples and the possible effects of small sample sizes (Wilkinson and McCracken 1985; Crozier et al. 1987; Queller and Goodnight 1989), we may interpret the data as suggesting that genetic differentiation of wasps on different palms is of approximately the same magnitude as that occurring among demes. The observed distribution of the rare *Dia-4^S* allele also suggests that some significant structure at the level of the palm may exist. This allele was found in only 2 of the 147 study colonies (population frequency $p_S = 0.007$), yet these two colonies were collected from the same palm in the *Anacardium* Trail subpopulation (fig. 2).

Colony Genetic Structure

The genotypic composition of *Microstigmus comes* colonies is detailed elsewhere (Ross and Matthews 1989). Colonies appear invariably to comprise family groups of differing levels of complexity. Of the colonies with a sufficient number of inhabitants for detailed study, two-thirds exhibited multilocus genotype arrays consistent with their being the simplest type of family group, that is, a singly mated mother and her offspring or a group of siblings (full sisters [= supersisters] in the case of females). In almost one-half of the large study colonies, a putative mother of the remaining inhabitants of the colony could be identified on the basis of her genotype and large size (Ross and Matthews 1989), suggesting that matrilineal monogyny may be common in *M. comes*.

TABLE 4
ESTIMATES OF FEMALE NEST-MATE RELATEDNESS IN *MICROSTIGMUS COMES*

NEST TYPE	NO. OF NESTS	UNWEIGHTED			WEIGHTED		
		<i>Pep</i>	<i>Gpi</i>	Both Loci	<i>Pep</i>	<i>Gpi</i>	Both Loci
RESIDENT ADULT FEMALES ONLY							
≥ 3 wasps	97	0.605 (0.044)	0.663 (0.044)	0.633 (0.032)	0.615 (0.043)	0.682 (0.049)	0.647 (0.035)
≥ 4 wasps	72	0.652 (0.045)	0.712 (0.045)	0.681 (0.033)	0.639 (0.045)	0.707 (0.052)	0.672 (0.037)
≥ 5 wasps	56	0.614 (0.052)	0.714 (0.062)	0.664 (0.042)	0.616 (0.051)	0.707 (0.064)	0.662 (0.044)
ADULT PLUS EMERGED FEMALES							
≥ 3 wasps	111	0.625 (0.040)	0.658 (0.042)	0.640 (0.030)	0.646 (0.037)	0.687 (0.047)	0.665 (0.031)
≥ 4 wasps	88	0.656 (0.039)	0.700 (0.041)	0.677 (0.029)	0.661 (0.039)	0.707 (0.048)	0.682 (0.033)
≥ 5 wasps	64	0.663 (0.043)	0.730 (0.053)	0.695 (0.035)	0.666 (0.043)	0.724 (0.057)	0.693 (0.037)

NOTE.—“Unweighted” indicates that the nests are weighted equally, whereas “weighted” indicates that they are weighted by the number of inhabitants. Estimates based only on genotypes of resident adults and estimates based on genotypes of these adults plus newly emerged wasps (the latter derived from pupae in the nests) are presented separately, as are estimates from nests with at least three, four, or five females. The values are not corrected for higher-level genetic structure and thus represent global rather than local estimates. Standard errors of the estimates are in parentheses.

In accord with this significant family structure, female nest-mate relatedness is high in the study population, with a consensus estimate of 0.67 (this is the arithmetic mean of values derived under several different criteria; see table 4; see also Ross and Matthews 1989). This estimate does not, however, take into account genetic structure at higher levels, the effect of which is to increase genetic variance among colonies and thereby inflate the global estimate relative to a local estimate (Crozier et al. 1984; Pamilo 1984). Since there appears to be some significant structure at the level of the palm and/or deme in *M. comes*, it seems prudent to adjust estimates of nest-mate relatedness to derive local values (see Pamilo 1984, eq. 17). Assuming that the combined effects of structure at these two higher levels do not exceed the extent of structure indicated by the highest relatedness value at either level, a reasonable course may be to correct the nest-mate estimates using the range of relatedness values in table 3. The resulting adjusted estimates for nest-mate relatedness (both loci combined) range from 0.56 to 0.70, with a consensus estimate of 0.63.

Relatedness in Multiple-Foundress Groups

Ten nests were identified, on the basis of their external appearance and the presence of few cells relative to the number of resident females, as having been recently established by multiple-foundress groups (see Matthews 1968*b*; Matthews and Starr 1984). The multilocus genotypes of females in such groups hint that they may have been close kin; indeed, in only one instance were three

genotypes at a locus represented among co-foundresses. Furthermore, in two of the nests co-foundresses shared the rare *Dia-4^F* allele. The estimated relatedness (\pm SE) for the females of these multiple-foundress associations is 0.42 ± 0.16 or 0.51 ± 0.16 , depending on whether groups are weighted equally or by the number of members (identity-by-descent method).

Sex-Investment Ratios

Samples obtained for the genetic analyses provide potentially useful information on the patterns of sex allocation in this population of *M. comes*. The usefulness of such data is limited to some extent by the lack of long-term sampling over several seasons and the lack of samples from several populations, and analysis assumes the absence of both differential mortality between the sexes and differential dispersal from the sampled units when adults are considered (e.g., Alexander and Sherman 1977; Metcalf 1980; Strassmann 1984; Boomsma 1989). These deficiencies notwithstanding, an apparent female bias in the sex ratio of *M. comes* has previously been reported (Matthews 1968b), and it is therefore of interest to quantify sex ratios from the larger data set available here.

Among the 765 adults collected, females outnumbered males by a ratio of 3.97:1. If only the wasps that emerged after colony collection are considered ($N = 169$), this female bias is increased to 6.68:1. A more meaningful measure of population sex allocation considers the relative investment in the two sexes (see, e.g., Crozier 1979; Metcalf 1980; Charnov 1982), which may be usefully approximated by adjusting the numerical values by the relative weights of the two sexes (Trivers and Hare 1976; Nonacs 1986; see also Boomsma 1989). Mean wet weights of both the head plus thorax and the whole body of adult females exceeded those of males by a factor of 1.07. (The weight differences are both significant at $P < 0.02$, Mann-Whitney test; for distributions of head-plus-thorax weights, see fig. 3.) Applying this conversion factor to the numerical data leads to a female-biased sex-investment ratio of 4.25:1 for the adults. Because the weights of wasps emerging after colony collection were significantly lower than those of field-collected adults (perhaps because of some influence of artificial rearing), relative investment was not calculated from the numerical data for these individuals.

In eusocial species, it is legitimate to estimate sex-investment ratios only by comparing *reproductive* females with males. In *M. comes*, potential reproductives appear to constitute a somewhat distinct subgroup of higher-weight females (Ross and Matthews 1989; see fig. 3). To estimate population-wide relative investment in these, a conservative threshold weight of 0.60 mg was established to identify which females may be considered potential reproductives (fig. 3). Females above this weight account for 33.7% of the total adult female population, and considering that their mean weight (head plus thorax) is 1.33 times the mean weight of males, their production represents a population sex-investment ratio of 1.78:1 (females:males). Because this estimate is based on a conservative criterion for identifying potential reproductive females, it should be regarded as a minimum value for the extent of female-biased investment.

The overall female bias in numerical sex ratio appears to be related to the pattern of male production in colonies, whereby males are typically produced

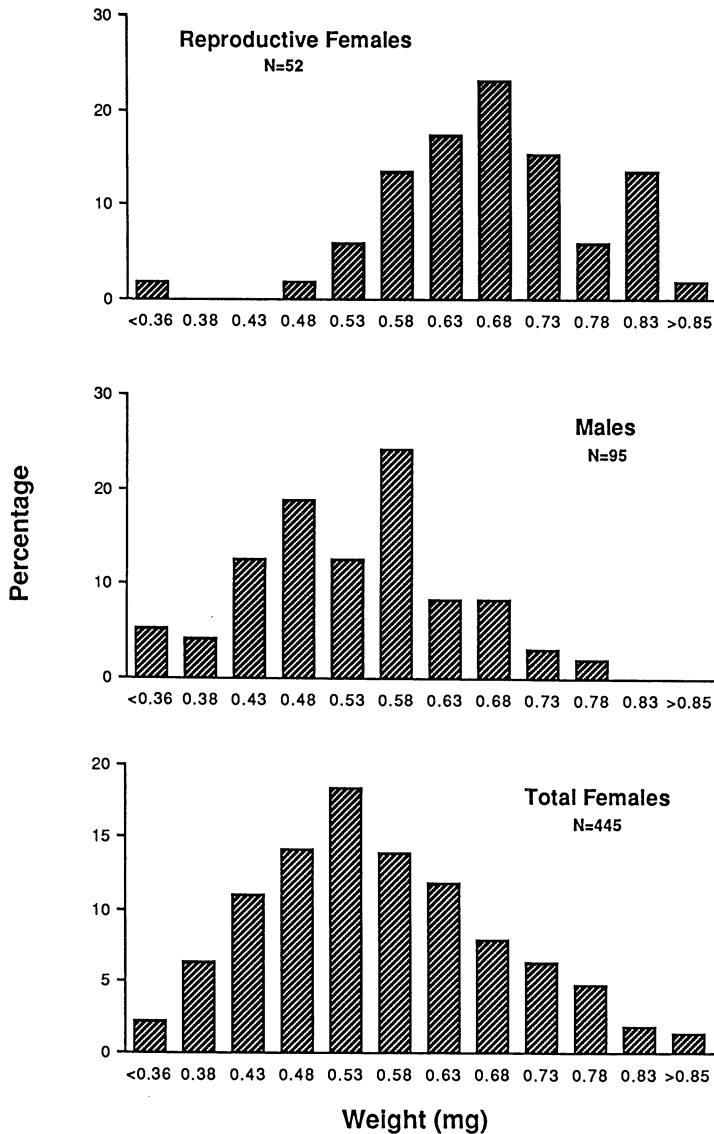


FIG. 3.—Distributions of head-plus-thorax weights for the total sampled female population of *Microstigmus comes*, the male population, and reproductive females (see also Ross and Matthews 1989). Numbers under the bars indicate interval midpoints.

rather late in colony development. Of the 51 nests collected with males, only 5 (9.8%) had four or fewer cells, and 4 of these were clearly newly founded nests that could not have produced the resident males (see below). Moreover, all collected nests with more than nine cells ($N = 21$) contained 1 or more adult males. Of the 22 males emerging after nest collection, 21 (95%) were produced by nests with eight or more cells. If five cells is considered the usual threshold nest

size for male production, and assuming that our collections are representative in terms of colony size, only 43% of the nests in a population would be capable of producing males at any given time (see also Matthews 1968b).

Affinities of Males

Collected adult males had multilocus (haploid) genotypes that were consistent with these being the offspring and/or siblings of resident females. Particularly useful here are nests in which all females were homozygous for the same allele at one or both of the strongly polymorphic loci. In all such cases ($N = 25$, only nests with more than three females considered), all adult males were found to bear the same allele for which nest-mate females were homozygous ($N = 90$ haploid genotypes examined). In 21 apparently matrilineal families, the resident adult males possessed multilocus genotypes consistent with their being sons of the putative mother female (see also Ross and Matthews 1989). The relatedness (\pm SE) of males to nest-mate females, estimated for all nests with four or more males ($N = 15$), is 0.21 ± 0.08 (nests weighted equally) or 0.20 ± 0.07 (nests weighted by the number of inhabitants). From these genotypic data and the observation that males are rarely found on nests with fewer than five cells, it would appear that males collected in nests were almost always individuals produced by those nests and, therefore, that males are unlikely to take up long-term residence in foreign nests.

The exceptions to the above generalization are represented by four newly founded nests that clearly could not have produced the resident males. These cases are significant because they suggest that males may occasionally be present at the time of nest initiation. The arrays of genotypes in these young nests hint that the males may have been closely related to the foundresses; however, the sample sizes are too small for a formal analysis.

DISCUSSION

This study provides a description of population and colony-level genetic structure in a eusocial wasp species which, by virtue of its distant relationship to other eusocial groups and primitive level of sociality, has commonly been regarded as holding important clues to the origin of eusociality in Hymenoptera (e.g., E. Wilson 1971; Akre 1982). The results presented here are of value in two related respects when applied to the problem of hymenopteran social evolution. First, they are used to assess various models of the "routes to eusociality." These are theories of evolutionary process that hypothesize the sequence of intermediate stages in the evolution of eusocial behavior by reference to colony composition and social organization in extant putatively intermediate forms (Lin and Michener 1972; West-Eberhard 1978; Brockmann 1984; Fletcher and Ross 1985). Second, the results are used to infer the likely strength of kin (or interdemic) selection in promoting the origin of eusociality in the recent ancestors of *Microstigmus comes*. Pursuit of these two goals is complementary in the sense that particular modes of selection are viewed as more or less characteristic of the various routes to eusociality (Pollock 1983; Andersson 1984; Fletcher and Ross 1985).

Our results reveal that the most striking degree of population genetic structure occurs at the level of the nest in *M. comes*, as the manifestation of family groups inhabiting individual nests. Data on the genotypic composition of colonies indicate that in many instances these are simple family groups consisting of a singly mated mother and her progeny. Indeed, observed patterns of nest-mate relatedness are concordant with values generated under a population model in which all colonies represent such simple families (Ross and Matthews 1989). From these data, we conclude that a significant proportion of the colonies in the study population may be characterized as matrifilial monogynous groups.

Such matrifilial societies have been viewed as an appropriate setting in which the fundamental step in the origin of eusociality, the development of sterile workers, has occurred according to the "subsocial" model of hymenopteran social evolution (Wheeler 1923; Hamilton 1964*b*; E. Wilson 1971; Pollock 1983; see also West-Eberhard 1978). This model is appealing largely because such a structuring of populations into simple families presumably is favorable to a role for both maternal manipulation of offspring and kin selection (Alexander 1974; Craig 1979, 1983). Assuming that only minor differences separate *M. comes* from its ancestors in which eusociality evolved, colony composition in the study population may be interpreted as offering some support to the subsocial hypothesis with respect to this lineage of eusocial Hymenoptera.

Two additional models of the routes to sociality suggest that polygynous groups—that is, groups with multiple reproductive females—constitute the most likely setting in which worker sterility originated. In the "semisocial" ("mutualism") model, such groups include unrelated individuals (Lin and Michener 1972), whereas associating individuals are proposed to be relatives in the "polygynous family" model of wasp social evolution (West-Eberhard 1978). The inability of the semisocial model to describe social organization in *M. comes* is evident, and at first sight, the polygynous-family model would also appear to be less applicable to this species than is the subsocial model. Some uncertainties remain, however.

Foremost among these is the ambiguous treatment of long-term versus short-term monogyny in the polygynous-family model and the difficulty of clearly distinguishing between the two on the basis of our data. Short-term monogyny (also termed serial polygyny) refers to a sequential replacement of single reproductive females in a nest, each of which monopolizes egg laying for some period of time. West-Eberhard (1978) considered short-term monogyny analogous to function polygyny under some conditions, in that both may result in decreased relatedness (increased genetic variability) among nest mates relative to long-term monogyny. However, the magnitude of this effect under short-term monogyny depends on such factors as brood developmental periods and duration of reproductive dominance by single females. On the continuum of reproductive structures of colonies, ranging from long-term monogyny to true functional polygyny, short-term monogyny should be regarded as equivalent to polygyny only when its effects on nest-mate relatedness are similar. The high nest-mate relatedness estimated for *M. comes* may be taken as evidence that the effect of any short-term monogyny or polygyny on the genotypic composition of colonies is relatively minor.

Stable matrilineal monogyny may not occur invariably in *M. comes* because approximately one-third of the large nests studied exhibited genotypic arrays inconsistent with simple family structure (a single once-mated queen and her offspring). Given the high nest-mate relatedness estimated even for this subset of colonies ($r = 0.63$; Ross and Matthews 1989), it is likely that these represent families characterized by effectively low levels of multiple mating by single mothers, short-term monogyny, or true polygyny. Distinguishing between multiple maternity (polygyny or short-term monogyny) and monogyny with multiple mating is not possible without isolation experiments (e.g., Pamilo 1982; Ross and Fletcher 1985) or the development of additional genetic markers, but clarification of the relative importance of these two factors in increasing the genotypic diversity of a colony is necessary to fully characterize social organization in this species. A demonstration of some level of multiple mating in *M. comes* would lend further support to the claim that matrilineal monogyny is predominant, whereas invariant monandry would implicate polygyny as a factor influencing the genetic structure of colonies.

It may be argued that higher-level structure (population "viscosity" of Hamilton 1964*b*) resulting from limited dispersal may be of sufficient magnitude to generate the family structure envisioned in the polygynous-family model (West-Eberhard 1978; Pollock 1983). The nature and extent of such structure required for the operation of this model remain to be described explicitly, but for the purposes of this discussion it is sufficient to note that higher-level genetic structure in *M. comes* (at the level of the palm and/or deme) contributes only minimally to high nest-mate relatedness and thus cannot be considered an important factor influencing family structure.

A necessary caveat for a discussion of the hypothetical routes to eusociality regards the appropriateness of *M. comes* for testing predictions of the models. It is conceivable that this species has proceeded too far toward routine worker sterility to be considered a legitimate socially intermediate group (see Ross and Matthews 1989). In this view, simple family structure may represent a secondary development acquired following the origin of facultative sterility in an ancestral group with a different social organization (see West-Eberhard 1978; Fletcher and Ross 1985). These remarks may, of course, pertain to other species used to test or even construct these models; they point to the advantage of basing such evolutionary-process theories on well-established phylogenetic hypotheses. Unfortunately, in the pemphredonine wasps, as in many other groups of interest, insufficient knowledge of phylogenetic relationships and comparative social biology precludes strict adoption of this method (but see Carpenter 1990).

How does the population genetic structure of *M. comes* constrain or favor the action of kin selection in promoting the evolution of worker altruism? This structure, which features families of close kin inhabiting nests likely to be embedded in a network of distantly related kin, appears to correspond well to the type of population structure conceived of in family-structured models of kin selection (Abugov and Michod 1981; Wade and Breden 1981; Michod 1982; Pollock 1983; Wade 1985*b*). In these models, kin selection is partitioned into a within-family component, in which selection invariably acts against altruists, and a between-

family component, in which selection may favor a global increase in the altruist genotype. The relative strengths of the within- and between-family components are influenced by population genetic structure, such that any factors increasing genetic homogeneity within families or genetic differentiation among families favorably affect the probability of origin and rate of spread of worker altruism (Wade 1985*b*). For *M. comes*, colonies are relatively genetically homogeneous within, whereas concomitant genetic variance among colonies is high. Comparatively little of this variance is the result of structure at levels higher than the nest; rather, it is the product of significant genetic affinities among nest mates due, largely, to their common descent from a single mother. Demonstration of significant differential contributions among families to the population pool of reproductives, an admittedly formidable task, would appear to be a final requirement to build a compelling case for a major role for kin selection in the social evolution of this wasp.

Viewed from the somewhat different perspective of inclusive-fitness models, significant genetic structure at the nest level translates to high genetic relatedness, or a large proportion of shared genes identical by descent, between female brood-care providers and the reproductive females they help rear. If abstention from direct reproduction by brood-care providers (coded for by a hypothetical allele for altruism) has a positive effect on the inclusive fitness of these individuals, then Hamilton's rule is satisfied and this trait spreads through kin selection (Hamilton 1964*a*; Michod 1982; Grafen 1984). Following Hamilton's rule and incorporating the nest-mate relatedness values estimated from this study, the fitness benefits to female reproductives reared must be, on the average, 1.6 times the fitness costs to brood-care providers for kin-directed altruistic behavior to be favored in *M. comes*.

In the social Hymenoptera, additional complexities not addressed in the above analyses are introduced by the asymmetries of relatedness associated with a male-haploid genetic system. Specifically, population genetic structure in *M. comes* has been described by considering only female genotypes, such that no consideration has been given to the effect of producing males of generally lower relatedness to brood-care providers on the distribution of within-colony and between-colony genetic variance. With reference to simple families, the rearing of brothers rather than of sexual sisters by worker females dilutes their average relatedness to the sexual brood in proportion to the sex ratio (Trivers and Hare 1976; Charnov 1978), thus potentially disrupting a population genetic structure conducive to kin selection (see Craig 1979, 1980; Wade 1979; Stubblefield and Charnov 1986). In general, a sex-investment ratio of ($r_{F(F)}/r_{M(F)}$):1 (females:males)—where $r_{F(F)}$ is the relatedness of female reproductives to brood-care providers and $r_{M(F)}$ is the relatedness of males to brood-care providers—may be expected to preserve values of nest-mate relatedness favorable to the operation of kin selection (Oster and Wilson 1978; Crozier 1979; Pamilo and Rosengren 1983). With the consensus values of $r_{F(F)} = 0.67$ or 0.63 and $r_{M(F)} = 0.20$ for *M. comes* and the assumption that the reproductive value of males and females is equal, a population sex-investment ratio of 3.1:1–3.4:1 would appear to be optimal for the operation of kin

selection. The sex-investment ratios estimated for *M. comes* in this study are invariably female-biased and bracket the predicted optimal values. Thus, female brood-care providers may capitalize on their differential relatedness to male and female reproductives to bolster their inclusive fitness.

The apparent occurrence of modest but significant microgeographic genetic structure above the level of the nest presumably results from some limits to the vagility of *M. comes*, since the low and insignificant values of F_{IS} at the subpopulation level and all lower levels (data not shown) are not consistent with local inbreeding playing a major role in generating this structure. Even though hierarchical microgeographic structure is detectable, its magnitude is such that the level of gene flow at local scales is no doubt considerable, and thus, the opportunity for selection to act at local levels is quite limited relative to selection acting at the level of the nest. This finding of weak higher-level structure is of relevance to kin selection operating at the colony level in that dispersal from the natal nesting area is presumably sufficient to ensure interfamilial competition in the population breeding pool (see Pollock 1983).

The importance of local inbreeding in the evolution of eusociality via kin selection has been shown by population-genetics models to be variable and sensitive to the initial assumptions of the models (e.g., Wade and Breden 1981; Michod 1982; Uyenoyama 1984). The absence of evidence for significant inbreeding in *M. comes* means that any of its potential effects on the opportunity for kin selection to promote worker altruism are probably negligible. The lack of evidence for inbreeding suggests that males may disperse before mating, although the absence of incongruent male genotypes in nests indicates that they are unlikely to take up long-term residence in foreign nests to secure matings. Prenuptial male dispersal reduces the probability that related males compete for potential mates; such dispersal would thus be inconsistent with local mate competition (Hamilton 1967; Alexander and Sherman 1977) as the cause of the female-biased sex ratios observed in *M. comes*.

Nests of *M. comes* may be reestablished at a site following their destruction during such natural events as storms (Matthews and Starr 1984). The high relatedness of females in multiple-foundress associations could be explained solely on the basis of nest-site fidelity if these groups invariably occupy such reconstructed nests, but presumably some proportion of new multiple-foundress nests represents initiations by related females at new sites some distance from the natal nests (Matthews and Starr 1984). In this case, it seems likely that kin and/or nest-mate recognition may operate in these wasps. Such a capability is significant because it provides a means of generating nonrandom associations of genotypes in incipient colonies, and this genetic structure positively influences the opportunity for kin selection to act in these groups.

Few previous studies of primitively social or parasocial Hymenoptera are available in which robust statistical procedures were used to estimate nest-mate relatedness and describe microgeographic structure. In the primitively social halictine bee *Lasioglossum zephyrum*, Crozier et al. (1987) and Kukuk (1989) reported that modest differentiation is likely to occur at macrogeographic and/or

microgeographic scales and that average female nest-mate relatedness is often greater than 0.50. Schwarz (1987) reported the absence of significant structure at the level of the "aggregation" (group of nests) in the parasocial allodapine bee *Exoneura bicolor* but found rather high female nest-mate relatedness in two colony types differing in social organization ($r = 0.49, 0.60$). Female-biased population sex ratios have been reported for both species (Batra 1966; Schwarz 1988; Kukuk 1989). Assuming that these two bee species and *M. comes* are representative of transitional taxa constituting appropriate foci for questions of social origins, it would appear that all possess population genetic structures and patterns of sex allocation at least minimally favorable to a significant role for kin selection. Indeed, a survey of the currently available genetic data for other parasocial or primitively social forms (most in the wasp genus *Polistes*) reveals that nest-mate relatedness may often be of sufficient magnitude to suggest the involvement of kin selection in the social evolution of these species as well (Metcalf and Whitt 1977; Metcalf 1980; Lester and Selander 1981; McCorquodale 1988; Hughes et al., MS).

It may thus be concluded, given the empirical genetic data at hand, that kin selection appears to have been important in facilitating the transition from parasocial to eusocial behaviors in several lineages of Hymenoptera. This statement must not be construed in simplistic fashion as excluding a role for factors such as mutualism or preadaptations relating to nesting behavior (Crozier 1979, 1982; Andersson 1984; Stubblefield and Charnov 1986); it merely emphasizes that a retreat from kin-selection theory as a major explanatory concept for the origin of eusociality owing to perceived difficulties in the genetic mechanism is not currently justified. It is clear that a significant expansion of the data base concerning the genetic structure of populations of primitively social Hymenoptera is necessary in order to test the generality of the findings of this study.

SUMMARY

Genetic structure and sex allocation were studied in a natural population of the primitively eusocial sphecid wasp *Microstigma comes*. No higher-level genetic structure was discernible at the level of the subpopulation, and microgeographic structure at the level of the local deme or individual host palm was, at most, modest. In contrast, genetic structure at the level of the individual nest was striking, with the average genetic relatedness among female nest mates estimated to be 0.62–0.67. This high relatedness results from the fact that colonies comprise relatively simple families, which are, in many instances, matrilineal monogynous societies. High relatedness among female nest mates and the absence of conspicuous higher-level structure, in conjunction with the observed female-biased sex-investment ratios, are compatible with the interpretation that kin selection has played a major role in the origin of eusocial behavior in the recent ancestors of this wasp. The inferred colony composition and social organization further suggest that this social evolution may have occurred via a subsocial route, with simple matrilineal families rather than polygynous groups serving as the principal setting for the development of worker altruism.

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