

POPULATION STRUCTURE, LOCAL MATE COMPETITION, AND SEX-ALLOCATION PATTERN IN THE ANT *MESSOR ACICULATUS*

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Abstract.—Population-genetic structure and sex-allocation ratios were investigated for the ant *Messor aciculatus*, a species that conducts mass nuptial flights. An electrophoretic survey on two polymorphic loci revealed excessive homozygosities in two populations. Because inbreeding inside nests does not occur, the heterozygote deficiency may result from population subdivision rather than assortative inbreeding during nuptial flights. Assuming no inbreeding, a simulation based on the observed genotype distribution in the study site suggested that, on average, a breeding swarm consists of alates from only 1.7 colonies. This population genetic structure seems to cause local mate competition (LMC), a factor that can shift population sex ratio toward females. The sex-allocation ratio to males in the population (0.166 ± 0.030 ; mean \pm SE) was significantly female biased and lower than the expected optima for queens (0.5) and for workers (0.25) without LMC. Sex-ratio variability among colonies was explained by a pattern of constant male investment, which is predictable assuming LMC. Thus, the study provides the first evidence of LMC in ants with mass nuptial flights and contradicts previous assumptions about breeding structure in swarming ants. The results suggest that LMC can affect sex-allocation patterns for ant colonies and populations.

Key words.—Ant, local mate competition, population structure, sex-allocation ratio, sex-ratio variability.

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In the past two decades, a great deal of attention has focused on measuring sex allocation in eusocial Hymenoptera (Trivers and Hare 1976; Ward 1983; Nonacs 1986a; Bourke et al. 1988; Boomsma 1989; Bourke 1989; Ross and Matthews 1989; Herbers 1990; Hasegawa 1994) to test predictions regarding the relative power of queens and workers in controlling sex ratio (Pamilo 1991). Several studies have analyzed the allocation ratios expected, depending on whether the workers or queen were in control (Trivers and Hare 1976; Ward 1983; Herbers 1984; Nonacs 1986a; Boomsma 1989; Ross and Matthews 1989; Hasegawa 1994). Sex-allocation ratios have been found to be female biased in many species (see Nonacs 1986a; Boomsma 1989), which is in quantitative agreement with the queen-worker conflict hypothesis (Trivers and Hare 1976).

An alternative hypothesis for sex-ratio bias was proposed by Alexander and Sherman (1977). This hypothesis is contingent upon competition for mates among related males (local mate competition [LMC]) in breeding populations. However, LMC has been thought unlikely in ants, because mass nuptial flights result in large breeding populations and thus the opportunity for mating competition among related males is diminished (for discussions, see Nonacs 1986a and Boomsma 1989). Moreover, several genetic analyses at the level of entire populations have shown no sign of LMC in eusocial Hymenoptera (Ward 1983; Bourke et al. 1988; Ross and Matthews 1989; Hasegawa 1994). Thus, female biased sex ratios in ants have been regarded as consistent with worker manipulation (Nonacs 1986a; Boomsma 1989). However, extreme variability also is found in allocation ratios among colonies (see Pamilo and Rosengren 1983; Herbers 1984; Nonacs 1986a; Boomsma 1989).

Several hypotheses have been proposed to explain sex-ratio variation (Yamaguchi 1985; Nonacs 1986b; Frank 1987; Boomsma and Grafen 1990, 1991). Yamaguchi (1985)

showed that LMC should lead to constant male investment among mothers with variable amount of resources for reproduction. Frank (1987) argued that this constant male investment should cause sex-ratio variability among ant colonies. Assuming that LMC is unlikely in ants, sex-ratio variability in ants has been explained either by the “relative-relatedness asymmetry hypothesis” (RRA) (Boomsma and Grafen 1990, 1991) or the “resource-availability hypothesis” (RAH) (Nonacs 1986a). RRA predicts that if there is variability in the degree of genetic asymmetries among colony members, then workers—given that they can recognize asymmetry but cannot discriminate their full siblings—produce the sex to whom they are more closely related than the population average (Boomsma and Grafen 1990, 1991). Nonacs (1986a) argued that because female larvae of eusocial Hymenoptera can develop into either workers or sexual alates, colonies with abundant resources should produce many female reproductives. Accordingly, colonies with poor resources should produce mostly males and workers (the latter are not included in female sexual investment). Only a few empirical tests for these hypotheses exist (Backus and Herbers 1993; Queller et al. 1993b; Sundström 1994).

Here we provide the first evidence of LMC in ants that conduct mass nuptial flights. This evidence contradicts a previous assumption that such competition does not occur in mating swarms of ants. We also present the first empirical data that support the existence of mating competition among related males as the main cause of the sex-ratio variability in an ant species.

MATERIALS AND METHODS

Messor aciculatus is a harvester ant that conducts mass nuptial flights during the spring in Japan (Taki 1976, Onoyama 1980). Because the mode of nuptial flights is an important determinant of population structure, in 1992 we ob-

served and recorded the nuptial flight characteristics of *M. aciculatus* in the Ecology Park of Natural History Museum and Institute, Chiba, Japan.

In late March 1992, 38 of 66 nests, within an area (100 × 40 m) inside the Ecology Park, were selected randomly. The nest entrance of each colony was covered with a mesh frame (30 × 50 cm), and all alates that appeared on the ground were collected from April to late May. At appearance, 54 queens were selected randomly and dissected to confirm insemination. Dry weights of 10 males and 10 females were weighed for each colony. The sex-allocation ratio of each colony was estimated from the total dry weights of two sexes in that colony, because dry weight is a good estimator of the production cost (Danforth 1990, but see also Boomsma 1989). Thirty-two dealated queens found walking on the ground after a nuptial flight were collected from the Kasori population, about 4 km northeast from the Ecology Park. In November 1993, workers were collected from both populations. A single worker was collected from each of 55 colonies in the Kasori population. In the Ecology Park, 5 to 10 workers were sampled from each of 66 colonies, including the colonies sampled in 1992.

An electrophoretic survey on polymorphic loci was conducted on alate females and workers. One locus, encoding glycerol-3-phosphate dehydrogenase (*G3pdh*), has two alleles (*F* and *S*) in the Ecology Park population; an additional locus, encoding phosphogluconate dehydrogenase (*Pgdh*), is also polymorphic with two alleles in the Kasori population. Genotypes were scored following horizontal electrophoresis in 14% starch gels using a pH 8.4 Tris-Citrate gel buffer with a pH 7.1 Tris-Citrate electrode buffer (following Seppä 1992). Protein bands were visualized by specific histochemical staining (Murphy et al. 1990). At the Ecology Park, individuals were genotyped only for *G3pdh*.

Population genetic structure was detected by measuring heterozygote deficiency. Deviations from Hardy-Weinberg equilibrium are expressed as a deficiency of the observed heterozygotes (H_{obs}) to the expected value (H_{exp}), that is, $F = (H_{\text{exp}} - H_{\text{obs}})/H_{\text{exp}}$. For the Ecology Park population, genotype and allele frequencies were calculated from the means of 50 random draws (with replacement) of single genotypes from each nest. This procedure ensures the statistical independence of sampled genotypes in strongly family-structured social insect populations (see Crozier et al. 1987; Ross and Matthews 1989; Ross 1992). For the Kasori population, dealate queens were not collected in association with nests and are assumed each to have originated from a different nest. The statistical significance of heterozygote deficiency was examined with a χ^2 test (Weir 1990).

For social insects, sample size for genotype analyses is often problematic. Because the female progeny of a colony are strongly related to each other, an individual cannot be regarded as an independent genotype from the population. Thus, we used colony number as the sample size for calculating statistics. This assumption provides the most conservative estimate for sample size, because the true sample size must lie between the number of colonies and the number of examined individuals.

We estimated the degree of population subdivision and inbreeding using a simulation based on Wright's hierarchical

F-statistics (Wright 1951). In subdivided populations, the total deficiency of heterozygosity (F_{IT}) can be divided into two components, F_{ST} and F_{IS} , which are related by the equation $(1 - F_{\text{IT}}) = (1 - F_{\text{IS}})(1 - F_{\text{ST}})$ (Wright 1951). The fixation index (F_{ST}) represents the reduction in heterozygosity in the total population caused by variation of gene frequencies among subpopulations. Accordingly, F_{ST} reflects the degree of genetic differentiation among subpopulations. By contrast, F_{IS} measures heterozygote deficiency resulting from assortative inbreeding within a subpopulation.

To simulate the degree of population subdivision and inbreeding, the number of alates with the observed genotype frequency were each allotted to their own nest and located on a map of the Ecology Park. The alates were assumed to disperse from their natal nest by a distance d . In the simulations, d was changed from 0 to 20 m with an interval of 0.1 m. Alates of a colony were assumed to mate with alates from colonies within a radius of d from their natal nest, that is, each circle was regarded as a mating swarm. Thus, if the circle having a radius d of a colony contained no other colonies, alates of this colony mated with only nest mates. As the value of d became large, multiple colonies were contained within a circle. When the same colony was included within several circles, an equal number of the colony's alates was assumed to disperse to each circle. For example, if colony A was included within the circles of colony B and C, but the circles of B and C did not contain each other, half of A's alates were assumed to mate with B's alates and the other half mated with C's alates. When several circles contained the same colonies, such coupled circles were regarded as a single, large breeding swarm. Thus, when d exceeds the maximum distance between the most distant pair of colonies on the map, there is only a single breeding swarm, that is, no population subdivision. For each d , F_{ST} , and F_{IS} in the total population and the average number of colonies in each swarm were calculated (see Hartl and Clark 1989, pp. 297–298). Because the simulation was based on the actual frequencies of genotypes and alleles, F_{IT} was always fixed at the observed *F*-value. Thus, the simulation gave us an estimate of the degree of population subdivision and inbreeding consistent with the observed *F*-values.

RESULTS

Mode of Nuptial Flights

From April to late May 1992, three mass flights were observed on April 6, 20, and 22 in the Ecology Park. All days were clear, with almost no wind. On April 6 around 10:30 A.M., many males and alate females appeared on the ground and walked around the nest entrances. About 30 min later, alates climbed to grass blades near the nest and flew to the sky. Alates flew above the nest until out of sight. About 10 min after the start of the nuptial flight, we found the first dealate queen on the ground within the observed site. The number of dealate queens rapidly increased within the observed site; however, we could not find any dealate queen within another site at a distance ca. 50 m from the observed site. Both sites have similar environmental features characterized by bare ground with coarse vegetation, and we could not detect physical differences of the two sites. Because there

TABLE 1. Observed and expected population genotype and allele frequencies and the inbreeding coefficient (F) at the two loci in the two populations of *Messor acciculatus*. *G3pdh* and *Pgdh* encode the enzyme glycerol-3-phosphate dehydrogenase (EC1.1.1.8) and phosphogluconate dehydrogenase (EC 1.1.1.44), respectively. N , number of nests; n , number of individuals examined. Genotype proportions expected under Hardy-Weinberg equilibrium are shown in parentheses. Deviations from Hardy-Weinberg equilibrium are expressed as deficiency of the observed heterozygotes (H_{obs}) to the expected value (H_{exp}), that is, $F = (H_{exp} - H_{obs})/H_{exp}$. Significance of the deviations are examined by a χ^2 test, and 5% significance levels are shown by asterisks.

	Genotype frequencies observed (expected)			Allele frequencies		Inbreeding coefficient (F)
	SS	SF	FF	S	F	
Ecology Park						
<i>G3pdh</i>						
Queens ($N = 29$, $n = 404$)	0.712 (0.677)	0.221 (0.291)	0.067 (0.031)	0.823	0.177	0.243
Workers ($N = 66$, $n = 364$)	0.656 (0.612)	0.253 (0.340)	0.091 (0.047)	0.782	0.218	0.256*
Kasori						
<i>G3pdh</i>						
Queens ($n = 32$)	0.781 (0.738)	0.156 (0.242)	0.063 (0.020)	0.859	0.141	0.354*
Workers ($N = 55$, $n = 55$)	0.691 (0.640)	0.218 (0.320)	0.091 (0.040)	0.800	0.200	0.318*
<i>Pgdh</i>						
Queens ($n = 32$)	0.031 (0.012)	0.156 (0.194)	0.813 (0.794)	0.109	0.891	0.193

was almost no wind, the dispersal distance of alate females seemed short (at least less than 50 m). Some dealate queens were attacked by *Messor aciculatus* workers. Adoption of dealate queens by *M. aciculatus* nests was not observed. In the Ecology Park (ca. 2.3 ha), many nests released alates simultaneously in the same day (e.g., 75.9% of the covered nests). The flight continued until about 2:00 P.M. Thereafter, alates that did not participate in the flight returned to their nest. On April 20 and 22, the second and the third nuptial flights occurred, similar to the previous day, but the number of colonies participating in the flight were fewer than in the first flight (44.8% and 10.3% of the covered nests, respectively). In the Kasori population, a nuptial flight occurred on the same day because we collected 32 dealate queens from the ground on April 20, 1992.

Population Structure

During the study period, 29 of 38 colonies released alates. A total of 3519 alates were collected from these 29 colonies. In the electrophoretic analyses, 404 and 32 queens, and 364 and 55 workers, were genotyped for the Ecology Park and the Kasori populations, respectively. The genotype distributions on the *G3pdh* locus revealed that inbreeding coefficients (F) are positive for all four data categories and significantly deviated from zero for workers in the Ecology Park population and for both queens and workers in the Kasori population (table 1). The *Pgdh* locus in the Kasori population also showed an excessive homozygosity for queens, although the value was not statistically significant (table 1).

To examine a possible linkage for a specific pair of alleles on the two loci, we compared allele frequencies at a locus, depending on zygotic conditions at the other locus. The 32 dealate queens at the Kasori population were classified into two groups based on her genotype at a locus. One consisted of the homozygotes for the abundant allele, the other consisted of heterozygotes and homozygotes of the rare allele. Then, allele frequencies at the other locus for the two groups were compared with a χ^2 test. If there is linkage, allele frequencies should show a considerable difference between the two groups. However, no differences exist between two

groups for both loci (for *Pgdh* grouped by *G3pdh*, $\chi^2 = 0.265$, $P > 0.10$; for *G3pdh* grouped by *Pgdh*, $\chi^2 = 0.401$, $P > 0.10$). Thus, any specific linkage of alleles seems unlikely.

In the Ecology Park, because no alate females were inseminated at appearance on the ground ($N = 54$), and alates never conducted matings near the nests or in a rearing cage (E.H. and T.Y. pers. obs.), matings among nest mates within the colony did not occur. Moreover, assortative inbreeding during nuptial flights seems unlikely. Thus, the positive F -values observed seem attributable only to population subdivision resulting from low dispersal distances of alates from their natal nest.

Figure 1 shows how F_{ST} - and F_{IS} -values depend on the average number of colonies that form a single swarm. As mentioned above, the observed positive F -value (0.243) seems to reflect only population subdivision. Hence, we searched for parameter values at which the observed F could be explained by only F_{ST} , that is, $F_{ST} = 0.243$ with $F_{IS} = 0$. As a result, when no inbreeding in the mating swarms is assumed, we concluded that, on the average, alates from about 1.7 colonies comprise a breeding swarm.

Sex-Allocation Patterns

Sex-allocation ratios in the 29 colonies were estimated for the Ecology Park population. The population average sex allocation to males was 0.166 ± 0.030 (mean \pm SE; for data weighting, see Boomsma 1988) and significantly less than 0.5 or 0.25 (one sample t -test, $P < 0.05$). Figure 2 shows the observed distribution of sex-allocation ratio to males in the Ecology Park population. Considerable variation existed in this ratio among colonies. The relationship between sex-allocation ratio and the total sex investment in each colony is shown in figure 3. The observed sex-allocation pattern fits well the expected line for colony investment, assuming a constant supply of reproductive resources to males and the remainder to females. The coefficient of determination (r^2) was 0.659 in the linear regression using log-transformed data. Thus, a large proportion of the variance in sex-allocation ratio can be attributed to the variance in total sex investment.

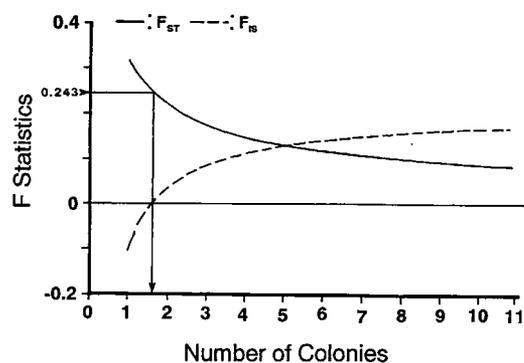


FIG. 1. Relationship between the number of colonies forming one breeding swarm and the resulting Wright's fixation index (F_{ST}) across 29 colonies of *Messor aciculatus* within the study area.

DISCUSSION

Deviations from the Hardy-Weinberg equilibrium were observed in two populations of *Messor aciculatus*, with excessive homozygosities at two polymorphic loci. Although F -values for *G3pdh* in queens within the Ecology Park population and for *Pgdh* for queens in the Kasori population were not significant, positive F -values were observed in worker samples. In addition, the larger sample size for workers ($N = 55$ and 66 , respectively) resulted in statistical significance for the positive F -values on *G3pdh* (table 1). This suggests that positive F -values in queens are not due to chance. Either or both population subdivision or inbreeding could result in positive F -values. Therefore, the study populations could be structured genetically by either a population subdivision or inbreeding. There exists, however, another possibility that may account for the observed heterozygote deficiency in the populations. Keller and Ross (1993) showed that homozygotes of an allele at a locus were eliminated selectively by workers in the polygynous form of the ant *Solenopsis invicta*. If similar differential mortality for genotypes occurred in *M. aciculatus*, the reduction in heterozygosities could not be attributed to population genetic structures. Our data showed that there is no evidence of linkage between the loci *G3pdh* and *Pgdh*, and an excessive homozygosity also observed for *Pgdh*. It is difficult for us to assume that selection to heterozygosity occurred simultaneously on the two independent loci. Thus, the observed reduction in heterozygosities is more likely to result from population subdivision or inbreeding or both.

The dissection of queens showed that no individual was inseminated within her nest. Accordingly, if positive F was caused by assortative inbreeding, alates must have mated with kin during nuptial flights. There have been several reports on inbreeding avoidance in social Hymenoptera (Smith 1983; Ryan and Gamboa 1986; Keller and Passera 1993), but no evidence of such assortative inbreeding within swarms. Thus, it is very difficult to assume that alates of *M. aciculatus* selectively mate with kin within the mating swarms. Consequently, an alternative explanation is that the observed heterozygote deficiencies result from population subdivision owing to low dispersal of alates, an effect that exists despite mass nuptial flights.

When no inbreeding within mating swarms was assumed,

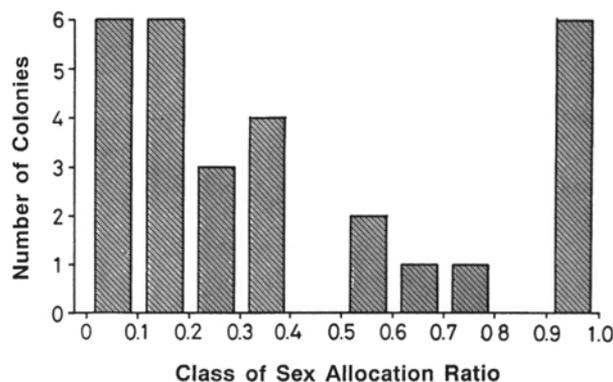


FIG. 2. Number of colonies belonging to different classes of sex-allocation ratio to males in *Messor aciculatus*.

our simulation showed that a single swarm consists of alates from only a few colonies. Local mate competition (LMC) requires inbreeding or small breeding populations (Hamilton 1967). The obtained estimate of colony numbers (1.7) in a swarm seems sufficient to lead to LMC (Hamilton 1967; Taylor and Bulmer 1980). Even if assortative inbreeding had occurred within the mating swarms, LMC can still be expected. Thus, in spite of mass nuptial flights, LMC is likely to occur in the study populations, which is contrary to the widely held assumption that such competition is unlikely in ant species with mass nuptial flights (for discussions, see Nonacs 1986b and Boomsma 1989).

Under LMC, the optimal sex-allocation ratio is biased toward females and is higher than both the optima for queens (0.5) or for workers (0.25) without LMC (Hamilton 1967; Alexander and Sherman 1977). The observed allocation ratio was significantly biased toward females, relative to these theoretical predictions. Thus, we suggest that even if workers do control sex ratio, the occurrence of LMC has further shifted sex-allocation ratio toward females. Taylor and Bulmer (1980) presented a formula for calculating male investment by mothers (= queens) in haplodiploid populations under

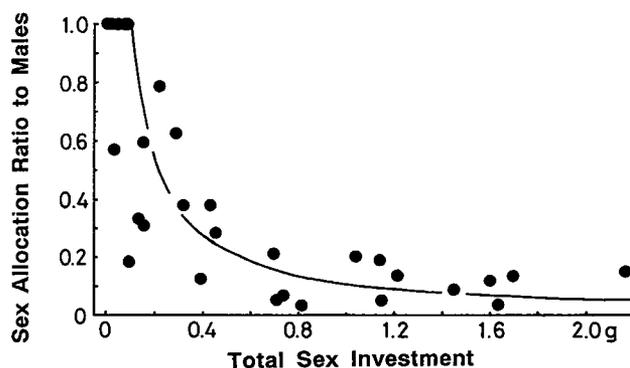


FIG. 3. Sex-allocation ratios plotted on the total sex investment in each colony of *Messor aciculatus* in the Ecology Park. The line represents the expected relationship in which a colony is assumed to invest a constant amount of reproductive resource to males and the rest to females. The threshold investment to males was assumed to be 108.6 mg, which was the average of all colonies. All statistical analyses on sex-allocation data in the text were made after arcsine transformation.

LMC. Using their formula, a mating aggregation of 1.7 mothers predicts a male investment level of 0.170. This is quite close to the observed male investment (0.166) in the Ecology Park population. However, our study did not determine queen numbers in a colony and cannot assess the relative controlling power of queens and workers on sex ratio. Whether queens control the sex ratio is an interesting subject for future research.

A theoretical model (Yamaguchi 1985; Frank 1987) predicts that when LMC occurs, each colony should invest a constant amount of reproductive resource to males and the rest to females, resulting in constant male investment among colonies. The observed sex-allocation pattern of *M. aciculatus* colonies suggests that a constant male investment is indeed the case in the Ecology Park population. The constant-male hypothesis also predicts that large sex investment in a colony results in more female-biased sex allocation. Variation in the total sex investment, accordingly, causes sex-ratio variability among colonies (Frank 1987). A large proportion of the observed variability of sex-allocation ratio in *M. aciculatus* can be attributed to the variance in total sex investment. Thus, sex-ratio variability in *M. aciculatus* seems to be caused by the constant male investment that is predicted by LMC.

The constant-male hypothesis may not hold if males mate only once because unmated females would be expected to appear with increasing female investment. Single mating of males occurs in some ant species (e.g., Hasegawa 1994), but the mating frequency of males is not known for *M. aciculatus*. In *M. aciculatus*, an alate female is about 13 times larger than a male (Hasegawa and Yamaguchi unpubl. data). This size difference will result in a male-biased numerical sex ratio even when the investment sex ratio is female biased. In the Ecology Park population, the most productive colony showed a male-biased numerical sex ratio (Hasegawa and Yamaguchi unpubl. data). Therefore, even if a male can mate only once, most colonies would not have unmated females because the numerical sex ratios are always male biased.

Our data do not rule out other explanations that have been proposed for variation in sex-allocation ratios among colonies, such as the resource availability hypothesis (RAH) by Nonacs (1986b) and the relative relatedness asymmetry (RRA) hypothesis by Boomsma and Grafen (1990, 1991). However, a constant male production with the genetic structure in the population is not predicted by such hypotheses. Thus, although several studies have provided evidence for RRA hypothesis (Mueller 1991; Queller et al. 1993b; Sundström 1994), our study has presented the first empirical data in which sex-ratio variability in an ant species is well accounted for by the constant-male hypothesis.

Our observations suggest that the dispersal distances of alate females are rather short, that is, less than 50 m. A significant heterozygote deficiency was detected within the narrow study area (ca. 2.3 ha). It would be interesting to examine this population structure in more detail. Unfortunately, we could only use a single genetic marker in the Ecology Park population, and individual workers of *M. aciculatus* were too small to resolve products of multiple loci on single gels. We are now developing microsatellite locus markers of genomic DNA as a powerful tool for genetic studies (for microsatellite markers, see Queller et al. 1993a).

Accordingly, use of multiple genetic markers should bring many new insights into field population genetics of *M. aciculatus*.

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