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Genetic Relatedness in Colonies of Tropical Wasps with Multiple Queens

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The evolution of worker behavior in the social insects is usually explained by kin selection: although workers do not produce offspring, they do reproduce their genes by aiding the reproduction of relatives. The most difficult case for kin selection theory would be species in which workers are fully capable of reproducing but instead opt to rear brood of low relatedness. These conditions are perhaps best fulfilled by the swarm-founding wasps because they have little caste differentiation and their colonies usually have multiple queens, which should lower relatedness. Estimates of within-colony relatedness for three species in this group confirm that it is sometimes (but not always) very low. Inbreeding is negligible in these species, so the hypothesis that inbreeding may raise relatedness is not supported. The maintenance of worker behavior in such species is a significant challenge for kin selection theory.

THE SWARM-FOUNDING WASPS OF the neo-tropics are a monophyletic group including some 23 genera (*1*). All are social, with even new colonies being founded by swarms of females rather than lone individuals (*2*). Though they are a very successful group (*3*), they have been the subject of few detailed studies (*4*), probably because of their tropical distribution and because most species enclose their nests in an envelope. They are nevertheless a critical group for understanding social evolution.

As in other social Hymenoptera (ants, bees, and wasps), most females function as workers, rearing the young of others instead of rearing their own young. Hamilton showed that this reproductive sacrifice could be favored by kin selection if, by rearing the young of relatives, workers transmit more copies of their genes to future generations than they would by producing offspring themselves (*5*). Hamilton considered the swarm-founding wasps to present "the most testing difficulty" facing his views on the evolution of insect sociality (*6*). The difficulty arises from the polygynous habits of this group; colonies generally have multiple queens, sometimes even numbering in the hundreds (*7–10*). Polygyny poses a problem because egg laying by multiple queens should lower relatedness within colonies, thus diminishing the genetic payoff available to workers. Why should workers continue to rear relatives of a very low degree instead of producing offspring of their own?

At least four explanations are possible. First, workers may be physically unable to reproduce on their own. This seems likely for workers in polygynous ant species (*11*) because ant workers have morphological specializations that may prevent them from successfully reproducing. But it is less plausible for the swarm-founding wasps because most species show little or no morphological differentiation between workers and queens (*4*). A second possibility is that, although workers may be physically able to reproduce, the ecological advantages of group living may be so large as to outweigh the genetic loss of not raising their own offspring. Third, workers may somehow be able to pick out and aid their closest relatives among the brood. Hamilton himself argued for a fourth hypothesis: that inbreeding may increase relatedness and thereby compensate for the relatedness-lowering effect of polygyny (*6*). Inbreeding seemed plausible for these wasps because of the limited dispersal range of swarms and because tropical habitats may permit nonseasonal, asynchronous rearing of reproductives, which makes mates harder to find outside the natal colony. In addition, some circumstantial evidence suggests that mating may take place on the natal nest (*7*).

Although the problem posed by polygyny has been widely recognized (*12*), neither relatedness nor inbreeding has ever been measured for any swarm-founding wasp. We report estimates for three species (*13*). They were collected in April 1988 at Hato Masaguaral, some 40 km south of Calabozo in the Venezuelan Llanos. We collected *Parachartergus colobopterus* along a 4-km stretch of concrete electrical pylons, *Polybia sericea* from shrubs in a small (60 ha) area of savannah, and *Polybia occidentalis* from trees in various areas spread over 2500 ha of

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Table 1. Coefficients of inbreeding, f (*16*), and within-colony relatedness, r (*15*), for females of three species. Standard errors were estimated by jackknifing over colonies.

Species	Number of colonies	Number of individuals	Enzymes scored	Allele percentages	Inbreeding ($f \pm$ SE)	Relatedness ($r \pm$ SE)
Polybia occidentalis	21	197	All polymorphic		0.044 ± 0.063	0.339 ± 0.047
			Esterase	57, 43	−0.019 ± 0.096	0.303 ± 0.090
			Mannose phosphate isomerase	81, 19	0.175 ± 0.097	0.302 ± 0.062
			Isocitrate dehydrogenase	95, 4, 1	−0.019 ± 0.015	0.645 ± 0.276
			Hexokinase	98, 2	−0.009 ± 0.008	0.301 ± 0.308
Polybia sericea	18	198	All polymorphic		0.081 ± 0.047	0.276 ± 0.069
			Mannose phosphate isomerase	58, 42	0.123 ± 0.067	0.276 ± 0.081
			Malate dehydrogenase	94, 6	−0.053 ± 0.028	0.357 ± 0.132
			Phosphoglucomutase	98, 2	−0.010 ± 0.007	0.123 ± 0.140
			Adenylate kinase	99, 1	−0.004 ± 0.006	0.139 ± 0.146
Parachartergus colobopterus	15	161	All polymorphic		−0.045 ± 0.084	0.113 ± 0.051
			Peptidase	82, 18	−0.086 ± 0.096	0.101 ± 0.066
			Hexokinase	96, 4	0.112 ± 0.149	0.152 ± 0.033

savannah. April is the end of the long dry season. We opened a number of nests and found that *Polybia occidentalis* colonies were actively raising brood, as were some colonies of *Parachartergus colobopterus*. *Polybia sericea* colonies were not. Up to 20 adult females were collected from each colony for electrophoresis, either by netting or by holding a plastic bag over the nest entrance and prodding the nest to provoke flight of its inhabitants. The collected wasps were transported alive in plastic bags on ice until they could be frozen at −70°C. Starch gel electrophoresis (*14*) revealed between two and four protein polymorphisms for each species. These data were used to generate estimates of both genetic relatedness within colonies (*15*) and inbreeding coefficients (*16*).

Inbreeding does not appear to play the role hypothesized for it by Hamilton. In all three species the inbreeding coefficient is small and less than 2 standard errors from zero (Table 1), so that the hypothesis that mating is random cannot be rejected.

Relatedness of female nestmates tended to be low, ranging from 0.11 to 0.34 (Table 1), consistent with the colonies being polygynous. How many egg-laying queens are there in these colonies? An estimate can be obtained as follows. Assume that the wasps on a nest are the progeny of n egg layers, each mated to a different male, and each contributing equally to the brood. Then the relatedness of an average female to her female nestmates will be approximately

$$r = \frac{1}{n}\frac{3}{4} + \frac{n-1}{n}\frac{r'}{4}$$

where r' is the relatedness among the n mothers. That is, one out of n females will be a sister related by three-quarters and the remainder will have a different mother and will be related by one-quarter the amount that their mothers are. Since new nests are founded by swarms of female nestmates, the egg-laying mothers should be related to the same degree as their progeny: $r = r'$. Solving for n under this constraint yields $n = (3 - r)/3r$. Substitution of the r values from Table 1 suggests an average of 2.62 equally contributing queens in *Polybia occidentalis*, 3.29 in *Polybia sericea*, and 8.49 in *Parachartergus colobopterus*. For a variety of reasons these should be viewed as rough estimates. We assumed single matings, equal contributions by all egg layers, that the mothers themselves constitute a negligible fraction of the colony, and that an equilibrium would occur at which $r = r'$ (*17*). Nevertheless, at least for the two *Polybia* species, it appears quite unlikely that colonies with dozens or hundreds of egg-laying queens could be common, so that the significance of morphological and behavioral evidence for such colonies (*18*) may need reevaluation.

However many queens are responsible, the relatedness values themselves are the results of greatest interest. On the one hand, the two *Polybia* species show rather low relatedness, but the values are about as high as those for two species of *Polistes*, wasps that generally have a single dominant queen at any given time (five other *Polistes* species show much higher r values) (*19*). To the extent that these two *Polybia* species are typical, Hamilton may have overestimated the difficulty that his kin selection theory would have in accounting for worker altruism: relatively small efficiencies of group living could make up for not rearing offspring related by one-half. For *Parachartergus colobopterus*, however, Hamilton's concern was justified. Aside from several polygynous ants (*11*), it has the lowest relatedness reported for any eusocial insect. Special circumstances must apply if kin selection is to explain the maintenance of worker behavior in species like this one. Either workers must be able to preferentially aid close relatives within the brood, something that has never been documented, or the advantages of group living over solitary reproduction must be very large to compensate for the workers' reduced relatedness to the brood they rear. A satisfactory explanation of the maintenance of worker behavior in *Parachartergus colobopterus* must await investigation of these possibilities.

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Nitrogen Fixation by Anaerobic Cellulolytic Bacteria

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Four strains of anaerobic nitrogen-fixing, cellulose-fermenting bacteria were isolated in pure culture from freshwater mud and soil. Nitrogenase activity was demonstrated in these strains and also in several previously described anaerobic cellulolytic bacteria isolated from various natural environments. These are the first anaerobic bacteria known to use cellulose as an energy source for nitrogen fixation. Because cellulose is a plant polysaccharide that abounds in nature, these results raise the possibility that nitrogen-fixing, cellulose-fermenting bacteria may be widespread and thus play a major role in carbon and nitrogen cycling.

THE REDUCTION OF ATMOSPHERIC dinitrogen (N_2) to ammonia (NH_3) by bacteria (biological nitrogen fixation) is a key transformation in the cyclic turnover of this element in natural environments. It is estimated that nitrogen fixation by symbiotic and free-living (nonsymbiotic) bacteria accounts for approximately 60% of the 2×10^8 metric tons of nitrogen fixed annually on our planet by biological and nonbiological processes (*1*).

Free-living, nitrogen-fixing bacteria are widely distributed in nature and add substantial amounts of combined nitrogen to the environments they inhabit (*2*). For example, Bormann and co-workers (*3*) reported that more than half of the nitrogen added to a northern hardwood forest ecosystem each year may derive from the activity of free-living, nitrogen-fixing bacteria in the soil, with the remaining nitrogen added mainly through precipitation.

Nitrogen fixation by free-living heterotrophic bacteria in natural ecosystems may be limited by the availability of oxidizable growth substrates (*4*) that serve as energy sources for the reduction of N_2 to NH_3 and for other growth processes. Surprisingly, even though cellulose occurs abundantly in natural environments where nonsymbiotic nitrogen fixation has been observed, it has not been determined whether this plant polysaccharide is widely used as an energy source by nitrogen-fixing bacteria. Photosynthesis yields annually up to 1.5×10^{11} tons of dry plant material worldwide, almost half of which consists of cellulose (*5*). The degradation of this vast amount of cellulose is carried out almost exclusively by microorganisms. Recently, Waterbury and co-workers (*6*) showed that cellulose serves as a growth substrate for a nitrogen-fixing aerobic bacterium that exists in a symbiotic relation with shipworms. Their findings demonstrate that these two complex physiological processes, nitrogen fixation and cellulose degradation, can be performed by a single bacterium. The objective of the present study was to determine whether free-living anaerobic cellulolytic bacteria that are widespread in terrestrial environments fix nitrogen when they utilize cellulose as the fermentable substrate for growth.

We isolated four strains of anaerobic cellulolytic bacteria from forest soil and freshwater mud, using a procedure that selected for nitrogen-fixing strains. We prepared enrichment cultures by serially diluting soil or mud samples into anaerobic culture tubes containing a liquid growth medium, designated MW-C (*7*), which lacked a source of combined nitrogen, included cellulose as the fermentable substrate, and was maintained in an N_2 atmosphere. After 7 to 14 days of incubation at 30°C, enrichment cultures showed significant disappearance of cellulose. Spent medium and remaining cellulose fibers from enrichment cultures were serially diluted into melted (40° to 45°C) cellulose soft agar medium in tubes. The contents of these tubes were poured onto plates of agar medium within an anaerobic chamber (*7*). After 2 to 4 weeks of incubation at 30°C, colonies surrounded by zones of clearing appeared in the otherwise opaque medium. These colonies were transferred by streaking

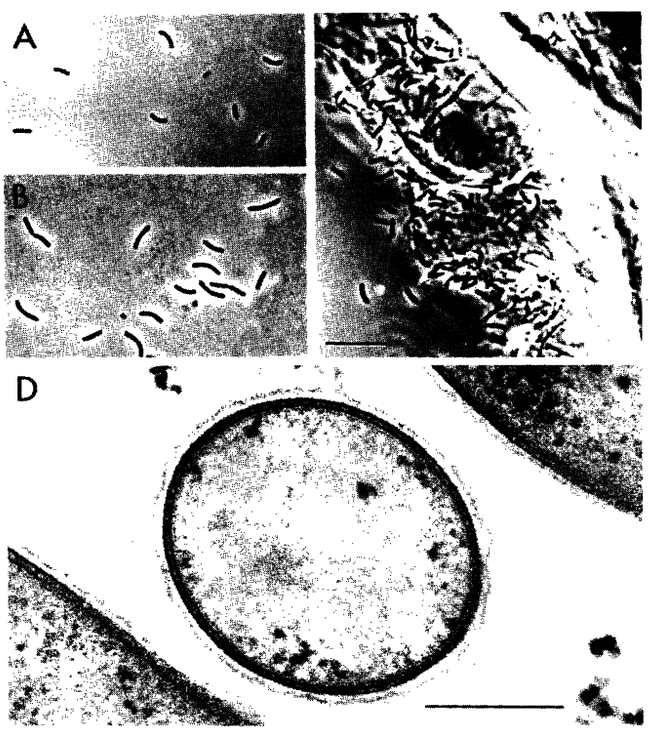

Fig. 1. (**A** through **C**) Phase-contrast photomicrographs of nitrogen-fixing cellulolytic isolates (wet-mount preparations), strains (A) B1B, (B) B3B, and (C) B1C. All phase-contrast micrographs are at the same magnification. Scale bar, 10 μm. (**D**) Transmission electron micrograph of a thin section of strain B1A cells stained with uranyl acetate. Scale bar, 0.2 μm. All cells were cultured to late-exponential phase in cellobiose-containing medium MJOU-CB (*15*). Strain B1C cells (C) are entangled in cellulose fibers introduced into the culture along with the inoculum [a 7-day culture in cellulose medium MJOU-C (*15*)].

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