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Genetic relatedness and queen number in a neotropical swarm-founding wasp, *Polybia emaciata*

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GENETIC RELATEDNESS AND QUEEN NUMBER IN A NEOTROPICAL SWARM-FOUNDING WASP, POLYBIA EMACIATA

by

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ABSTRACT

GENETIC RELATEDNESS AND QUEEN NUMBER IN A NEOTROPICAL SWARM-FOUNDING WASP, POLYBIA EMACIATA

by

DEBBIE MORALEZ DELOACH

The maintenance of eusociality in neotropical, swarm-founding wasps is difficult to explain because brood relatedness is predicted to be very low due to the presence of multiple queens in each colony and because workers are not morphologically constrained from laying eggs. Analysis of 4 morphological measures revealed no physical reasons why workers could not lay eggs in Polybia emaciata. A large variance in queen number, high queen relatedness, and a significant negative correlation between mean queen number and number of eggs per queen strongly suggest that cyclical oligogyny is probably the most important, and possibly the only, mechanism maintaining high relatedness in P. emaciata. Preferential care by workers of closely related kin on a discrete section of a comb is another possible mechanism that requires further research. However, inbreeding and unequal egg-laying by active queens are not important in elevating relatedness P. emaciata.
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GENETIC RELATEDNESS AND QUEEN NUMBER IN A NEOTROPICAL
SWARM-FOUNDING WASP, POLYBIA EMACIATA

INTRODUCTION

Hamilton's Rule

Charles Darwin considered social insects with sterile worker castes one of the few serious challenges to his theory of evolution by natural selection (Darwin, 1859). Many years later, Hamilton (1964) very nearly defeated this challenge by showing that altruism may be selected if:

\[ rb - c > 0 \]  \hspace{1cm} (1)

where \( b \) and \( c \) are the benefit and cost, respectively, to the altruist of raising offspring other than its own and \( r \) is the relatedness of the altruist to the offspring. The cost of altruism is the loss of direct fitness that may have been gained had the altruist behaved selfishly and successfully reared its own offspring instead of helping others with theirs. Therefore, it represents direct fitness apparently sacrificed due to altruism. The benefit, on the other hand, is fitness gained by helping relatives, with which the altruist obviously shares some genes, rear their offspring. The altruist gains indirect fitness by passing on some genes through non-descendant reproductive offspring. The effect that the altruist has on the survival to reproduction of all non-descendant offspring that it helps raise, weighted by its relatedness to each of those offspring, equals this indirect fitness gain. The sum of direct fitness and indirect fitness is called inclusive fitness.

Relatedness between two individuals is determined by how many genes, identical by descent, the two share. Identical by descent means that shared
genes can be traced to a common ancestor. For a sexually reproducing diploid organism, each offspring gets 0.5 of their genes from each parent and so are related by 0.5 to each parent. Each meiotic event separating two related individuals reduces their relatedness along that line by 0.5 but each shared gene source (ie. parents, grandparents) must be considered separately. Therefore, full siblings share \((0.5)(0.5) = 0.25\) of their genes from their mother and \((0.5)(0.5) = 0.25\) from their father. They are thus related by \(0.25 + 0.25 = 0.5\). Half-siblings, on the other hand, share genes from only one parent and so are related by only \((0.5)(0.5) = 0.25\).

**Eusociality**

While honey bees, ants, and termites are the most widely recognized eusocial animals they are certainly not the only ones. Indeed, the insect order hymenoptera (bees, wasps, and ants) consists of species that represent every level of sociality from strictly solitary to highly eusocial (Wilson 1971). Most surprising is the ever increasing list of eusocial insects in orders other than hymenoptera and isoptera (termites). Eusociality has been described in some species of aphids (Hemiptera; Aoki, 1984; Itô, 1989), thrips (Thysanura; Crespi, 1990), and beetles (Coleoptera; Kent and Simpson, 1992). Further investigations into the natural histories of more insect species will likely enlarge the list. Also, eusociality is not confined to insects. Naked mole-rats of the order Rodentia are like mammalian termites. They are highly eusocial with a one reproducing queen and many workers and drones all living in a vast network of underground tunnels (Jarvis, 1981).
A species is eusocial if the following four conditions are met. First, individuals must live in groups, called colonies. Second, there should be generational overlap, colonies consist of individuals from more than one generation. Third, some individuals must forego direct reproduction, usually as members of a worker caste. Fourth, there must be some cooperation among colonymates in raising offspring. The degree of eusociality may vary within the above parameters and range from primitively eusocial to highly eusocial. A species need not be eusocial for its members to exhibit altruism and kin selection is not the only means of obtaining altruism. However, I restrict my discussion to apparently kin selected, eusocial species for the sake of brevity.

Primitively eusocial species, such as the females of several Polistes wasp species, typically consist of individuals with several, apparently viable reproductive options. They may stay with a group of related individuals and help or, if breeding positions are unavailable, they may leave and reproduce directly by founding a nest independently. If they elect to stay, they may never reproduce directly or they may help and, lay eggs if the opportunity arises. All individuals retain the physiological capacity to lay eggs.

Individuals of highly eusocial species lack these options. As in many ants, worker females are physiologically unable to lay eggs and so may reproduce only indirectly, by aiding kin. Frequently, this sterile caste is further subdivided into specialized sub-castes with individuals involved in nest defense (ie. aphids, Aoki, 1984; the ant Colobopsis truncatus, Hölldobler and Wilson, 1990), food storage (ie. honey pot ants, Creighton and Crandall, 1954), or food preparation (ie. the ant Solenopsis geminata, Wilson, 1978). Clearly, the indirect fitness advantages of these extreme forms of altruism must outweigh the
costs of loss of direct reproductive success for the altruists. However, an altruist may be credited with only a fraction of the fitness gained by its beneficiaries, that equivalent to his share of aid weighted by relatedness and summed over all beneficiaries (Hamilton, 1964; Grafen, 1984). This may amount to very little if many altruists (i.e. workers) aid in raising few reproductives (queens and drones). If the resultant benefit is low then the costs must be very low, the relatedness between altruist and beneficiaries high, or both.

Despite these apparent difficulties, eusociality is quite successful in certain insect orders (i.e. Hymenoptera, Isoptera) but less so in other animal taxa (Andersson, 1984). The question arises, why has eusociality evolved many times in certain orders of insects (Wilson, 1971; Andersson, 1984), in only one mammal, and not at all in other animals? How is Hamilton’s rule met and eusociality maintained in a population? To answer these questions one must examine the conditions that might lead to increased benefits, decreased cost, and increased relatedness such that altruism is selected in preference to selfish behavior.

Benefits, Costs, and Relatedness

The advantage of eusociality arises when individuals increase their inclusive fitness as members of multi-generational groups but decrease it as independents. Certain ecological constraints, better handled by groups, may make survival and reproduction very difficult or impossible for solitary individuals and may predispose them to live and reproduce in groups. Ecological constraints include procurement and maintenance of a rare or costly nest or territory and predator and parasitoid pressures.
If a rare or costly nest, nest site, or territory is required for successful reproduction then lone individuals may not be capable of procuring one within its own lifetime. However, a group of cooperating individuals may be able to obtain, build and maintain the necessary resource with great success. For instance, termites and Naked mole-rats build and maintain elaborate subterranean galleries that are maintained by a worker caste (Wilson, 1971; Jarvis, 1981). These nests enable these species to utilize a scarce or patchy food source from secure underground passages. Ambrosia beetles and the ant *Atta cephalotes* grow and harvest fungus within the safety of their tunnels and galleries (Kent and Simpson, 1992; Hölldobler and Wilson, 1990). Some eusocial species, such as honey pot ants store food within their nests to provide sustenance for the colony during food shortages (Wilson, 1971). Nests consisting of extensive tunnels, cells, and galleries are typically built up and maintained over many generations and may last many years, but only with the cooperation of many individuals.

Groups may foil parasitoids and predators better than solitary individuals (Alexander, 1974). Many colonymates of the wasp *Polybia occidentalis* may successfully repel marauding ants from their nests (Chadab, 1980). Nests of *Polistes annularis* with more foundresses survived to produce more young than did nests with smaller groups (Strassmann, 1989). However, Larger groups of *P. exclamans* and *P. bellicosus* were not better than smaller groups at repelling parasitoids and predators, respectively (Strassmann, 1981a, Gibo, 1978; Strassmann *et al.*, 1988). Also, groups may be better able to thwart and to survive predation events. Larger groups of the wasp *P. bellicosus* recover from nest destruction better than do smaller groups (Strassmann *et al.*, 1988)and
larger colonies of *P. exclamans* are more likely to have adults surviving until brood is independent than are smaller colonies (Strassmann, 1981a).

The effect that predation has had in fostering eusociality is further demonstrated by the weaponry possessed by individuals of eusocial species and by the evolution of soldier castes. All eusocial hymenoptera possess stings or are predisposed to biting or spraying noxious substances for defense but non-eusocial hymenoptera are not armed (Andersson, 1984). Gall-forming aphid and gall thrips soldiers possess enlarged forelegs for grappling with predators (Itô, 1989; Crespi, 1990). Foster (1990) demonstrated experimentally that only members of the soldier caste of the gall-forming aphid *Pemphigus spyrothecae* could successfully kill an introduced, natural predator.

The cost of altruism, loss of fitness attributed to direct reproduction, will be low if the probability of successfully rearing one’s own offspring is low. The most extreme example is the complete loss of reproductive ability. The soldier castes of the gall-forming aphid *Colophonita clematis* and of the gall thrips *Oncothrips tepperi* are composed of larvae who die before reaching maturity (Aoki, 1977 in Itô, 1989; Crespi, 1990). Likewise, workers of the ant genera *Pheidole* and *Solenopsis* do not possess ovaries so they cannot lay eggs (Hölldobler and Wilson, 1990). In these examples, the workers suffer no cost for behaving altruistically because they are physiologically constrained from producing any offspring in the first place.

A further advantage of group nesting among closely related females is that if an egg-laying female dies before her brood matures, the others on the nest will continue to rear the brood to maturity, even if she originally competed with other egg-layers for oviposition rights (Queller and Strassmann, 1988).
Therefore, the loss she suffered from not having the whole nest in which to lay her eggs is mediated by the insurance of having relatives present with a positive interest in her brood. Also, A newly emerged female may gain some indirect reproductive success by staying on her natal nest and helping rear related brood that are already partly grown. This option may be preferable to founding a new nest, laying eggs, and raising brood from scratch if there is a great risk of dying before her brood reaches maturity (Queller, 1989).

Both of these advantages, and many of the ecological constraints mentioned above, highlight another predisposition for eusociality, the burden of caring for brood with long developmental times. All eusocial species possess dependent brood. Adults must provide a safe, long-lasting nest with constant care-giving and a constant food supply if they are to realize any reproductive success.

Even if the benefits are high and the costs low, unless an altruist aids relatives, selection cannot act on his altruist alleles. If altruism is adaptive, it will be favored through kin selection acting on the same alleles present in the altruist's relatives if the altruist never reproduces directly. Therefore, the closer the relatedness, the more altruism is advantageous. An advantage to using relatedness to examine whether Hamilton's rule might be met is that relatedness, unlike benefits and costs, is easily measured. Since inclusive fitness calculation implies that both indirect and direct fitness are weighted by relatedness, we may use relatedness between parent and offspring to represent direct fitness and relatedness between altruist and beneficiaries to represent indirect fitness. However, the comparison is not direct since the altruist is responsible for only that fraction of fitness that his actions gained each
beneficiary. Nevertheless, examining relatedness is a powerful, practical, and very useful approach to studying the maintenance of eusociality as the following examples show.

Outbred, diploid organisms are related to their own offspring and to their siblings by 0.5. However, they would gain less than 0.5 of the fitness benefits from helping rear one sib but exactly 0.5 for rearing one offspring. Therefore, they are genetically predisposed to reproduce directly, if possible.

Clonal, inbred, and haplodiploid organisms are genetically predisposed to aid close relatives. Clonal individuals, such as aphids, are no different than individual cells of a single organism. What is good for one individual is equally good for its clone because they possess identical genotypes. Inbreeding causes relatedness to increase so that full-sibs are actually related higher than 0.5. Naked mole-rats and termites are inbred (Reeve et al., 1990; Reilly, 1987). Increased relatedness, together with ecological constraints on non-cooperative reproduction (dependence on costly yet safe nests that protect the inhabitants from predators and aid in finding food), provide a common base from which eusociality is favored in these two otherwise extremely different animals. The Hymenoptera, the social thrips, and the social scale *Aspidiotus osborni* are haplodiploid. Haplodiploidy causes full sisters to be related to each other by 0.75 but mothers to their daughters by 0.5. In this case, females are more closely related to their sisters than they are to their own daughters so inclusive fitness gained by rearing sisters may equal or surpass direct fitness gained by producing daughters instead. However, if the indirect fitness benefit gained by helping relatives rear their young is lower than fitness gained by rearing
daughters and females are physiologically capable of laying eggs they should do so.

Swarm-founding Wasps

Low relatednesses among the females of several species of neotropical, swarm-founding wasps (Queller et al., 1988) [tribe Epiponini (Carpenter, 1991)] are expected because each colony is founded by a swarm of closely-related queens and workers. Brood relatedness in colonies with many queens is expected to be very low so the benefit/cost ratio must subsequently increase if Hamilton’s rule is to be satisfied. Epiponine workers are morphologically capable of laying eggs (Jeanne, 1980; Richards, 1978; Strassmann et al., 1991) and are expected to do so if Hamilton’s rule is not met. Yet worker egg-laying is uncommon in queenright colonies (West-Eberhard, 1978, 1981; Forsyth, 1978) and young workers will become queens in colonies that lose their queens. One of the most interesting challenges of explaining the maintenance of eusociality in social insects now lies with the swarm-founding wasps. How can eusociality be maintained in swarm-founding wasps given the low brood relatedness expected when many queens are laying eggs and given the workers’ morphological ability to lay eggs themselves?

Relatedness may actually be higher than that expected based on queen number alone if one or a combination of several mechanisms are at work (Queller et al., 1988). First, inbred queens will produce more highly related brood than non-inbred queens (Hamilton 1964, 1972; Queller et al., 1988). Second, brood relatedness may increase if only one or two of the many queens in a colony lay most of the eggs thus resulting in a small effective queen number
(Forsyth, 1978; West-Eberhard, 1978, 1982). Third, intracolony groups of highly related kin may form if workers tend closely related subsets of brood (Page et al., 1989; Queller et al., 1988, in press). This may occur if each queen lays eggs on only one comb or on a section of one comb and workers subsequently rear closely related sisters on that comb. Queller et al. (in press) found that the relatedness of combmates was significantly greater than that of non-combmates for Polybia occidentalis. This finding does not mean that workers utilize or even recognize this relatedness difference but it does set the stage for it.

Finally, the colony cycle of swarm-founding wasps suggests a mechanism by which relatedness may be maintained above the expected value given the presence of multiple queens (Queller et al., in press; Strassmann et al., 1992). A colony is founded by a swarm of females, both queens and workers, from the same natal nest (Forsyth, 1981; Jeanne, 1991). Individuals in the first cohort of brood, consisting of workers only, are distantly related to one another because of the many queens that are laying eggs. As the colony ages, natural attrition of foundress queens leads to decreased queen number with a subsequent rise in brood relatedness. Prior to swarming, only one or a few queens remain and therefore brood is closely related (West-Eberhard, 1978). Daughter queens are produced at this time and they and other non-reproductive females (workers) leave as a closely-related founding swarm. Some members of this last cohort may choose to remain on the natal nest and the relatedness of females on the original nest is once again very close.

West-Eberhard (1978) first described this cycle, which she called cyclical monogyny, in Metapolybia aztecodies when she observed that daughter queens were not produced until queen number had dwindled to one old queen
on small colonies that were originally founded with many queens. Strassmann, et al. (1992) analyzed several demographic characteristics predicted by this cycle as well as genetic relatednesses to examine cyclical oligogyny in Polybia emaciata, an epiponine which constructs completely enclosed, hard, mud and paper nests. They determined that cyclical oligogyny not only occurs in P. emaciata but that it is also the best explanation for the maintenance of high brood relatedness in this species. In that study, high queen relatedness was predicted from brood relatedness and queen number but it was not determined directly. I obtained a genetically determined queen relatedness in my study.

Further insight may be gained into the relationship between queen number and brood relatedness by examining factors that may contribute to the deviation of brood relatedness from that predicted based on queen number from that actually found through genetic analyses. Brood relatedness can be predicted from the population mean of queen number alone. This prediction relies on several assumptions: 1) there is no egg-laying dominance among queen colony mates, 2) queens and workers are related to the same degree, 3) there is no variance among colonies in queen number, 4) there is no inbreeding, and 5) queens are not multiply mated. But, an unusual feature of swarm-founding wasps is that the actual, genetic brood relatedness is usually higher than that predicted from queen number alone (Strassmann et al., 1991, 1992; Queller et al., in press; Gastreich et al., in prep). Here I investigate the above assumptions for a large sample of Polybia emaciata. I also examine inbreeding, reproductive dominance, and cyclical oligogyny in light of the evidence gained through genetic analyses and demographic features of the population.
*Polybia emaciata*

*Polybia emaciata* is a locally abundant and very successful Epiponine. It builds enclosed nests which hang conspicuously from tree branches. It is a good species for study because an adequate number of nests can be quickly found and collected. When disturbed, the wasps respond to disturbance by retreating into their enclosed sturdy mud nest. Individuals of *P. emaciata* lack effective stings and Chadab (1980) postulated that Epiponine species with mud nests utilize retreat and entrance blocking as their primary means of anti-predator defense. This greatly facilitates collection because few wasps are lost when the nests are collected and the nests can withstand rougher handling than can the paper nests of other Epiponines. Previous and ongoing research on the maintenance of eusociality in this species (Queller *et al.*, 1988; Strassmann *et al.*, 1992, Queller, in press) and its congener *P. occidentalis*, which builds enclosed paper nests (Queller *et al.*, 1988; Queller *et al.*, 1993; Queller, in press), combined with the above species characteristics made this an ideal species with which to further examine the demographic and genetic factors involved in the maintenance of eusociality in neotropical, swarm-founding wasps.
METHODS

Collection

I collected 23 whole colonies of *P. emaciata* and their nests on the grounds of Fundación de Servicio para el Agricultura (FUSAGRI) in Cagua, state of Aragua, Venezuela (10°11'N, 67°27'W) on June 21, 1990, about a month into the rainy season. Since the nests are built at the ends of branches in trees, I used a device made of an insect net with a stiff, diamond-shaped wire collar placed flat over the net opening and up to 18 - 3 foot sections of handle to pluck the nests from the branches. The collar tore each nest free of the branches to which they were attached then the freed nests fell into the net and the net was flipped to close off exit of the captured wasps. Each colony, including the nest, was immediately bagged and placed on ice. Seven additional nests were not used in subsequent analyses because they fell past the collection net and split open as they hit the ground, resulting in a loss of many wasps.

I preserved and transported the wasps in either liquid nitrogen or Kahle's solution (30 ml. 95% ethanol, 12 ml. formaldehyde, 4 ml. glacial acetic acid, 60 ml. water; Borr or *et al.*, 1964). Adults were placed into up to two 5 ml. cryovials per colony and immediately stored in liquid nitrogen. Frozen samples were subsequently stored at -80°C. For 15 colonies there were too many wasps for the 2 cryovials so I preserved the remaining individuals in Kahle's solution.

I dismantled the nests, keeping the combs intact, and then photographed them with the combs numbered in order starting with the topmost comb, which is the oldest. The nests were then discarded and later the numbers of cells and pupae per comb were counted from the photographs.
Dissections

I determined the sex of adults by the presence of testes or ovaries. Each female with at least one mature egg in her ovaries was assigned queen status (Forsyth, 1978; Richards, 1978). I also recorded the number of mature eggs in the ovaries of every dissected queen. Females without mature eggs were assigned worker status.

Non-mature eggs in various stages of development were sometimes found in the ovaries of females. Mature eggs are full-sized, have a fully developed chorion, and are ready to be laid. To check whether my criterion for assigning queen status was robust, I examined a subset of the females, preserved in Kahle's solution, for insemination. Females with sperm in their spermathecae are inseminated. Full spermathecae are white whereas empty ones are transparent. Thus I was able to decide whether the mature egg criterion for assigning queen status was correct since all queens should be inseminated and females without mature eggs would not. Non-mature eggs of workers would likely be resorbed rather than laid.

To test for morphological caste differences between workers and queens, I made four morphological measures [number of hamuli (wing hooks) on the hindwing, head width, length of the first discoidal cell of the forewing, and first gastric segment width] of a subset of Kahle's-preserved females (n = 273) of known reproductive status from 15 nests. These morphological characteristics are known to differ between queen and worker castes in several polistine species (Richards, 1978). All dissections were made under Wild dissecting microscopes at 12 to 50X magnification.
Since females from the same nest are related and they share the experience of being larvae on the same nest, morphological measurements may be correlated among nestmates and therefore not independent. Therefore, I standardized all of the measurements for each morphological measure to eliminate variation between nests thus giving the same statistical weight to each measurement (Sullivan and Strassmann, 1984). I used the z-scores for subsequent analyses.

I used proportions per colony of dissected individuals of each caste to extrapolate total numbers of individuals in each caste from the number of undischsected individuals. From 34% to 100% of all colony members from each nest were dissected to obtain the caste proportions.

Electrophoresis and Relatedness

I obtained allozyme data from horizontal starch gel electrophoresis of *P. emaciata*. Clayton Tretiak buffer (.04 M Citric acid preserved with NaN₃) was used for both the running buffer, adjusted to pH 6.7 with N-(3 aminopropyl) morpholine, and the gel (1/20 concentration), adjusted to pH 7.0 with N-(3-aminopropyl) morpholine. Each wasp was ground for 10 seconds in a 0.5 ml. microfuge tube along with approximately 200 ml. of 2 mm. glass beads, 50 ml. grinding buffer (0.1 M Tris, 25 % glycerol, 0.001 M. EDTA, 1 ml./ml. Triton X-100, 0.25 mg./ml. NaN₃, 1.5 % ethanol, 1 ml./ml. β-mercaptoethanol, 1 mg./ml. NAD, 1 mg./ml. NADP, 1 ml./ml. BSA adjusted to pH 7.0 with HCl), and 100 ml. CCl₄. The resulting mixture was centrifuged for 10 minutes in a 4°C refrigerator and all samples were kept on ice between preparation and loading steps to retard protein degradation. Gels were loaded with 2 X 7 mm. filter paper wicks,
after each was soaked approximately 2/3 of the way up in a wasp sample, by placing them in a slit cut 2 inches from the top of the gel. Each gel, with a pan of ice on top, was run for 3 hours and 40 minutes at 38 mA and then for 20 minutes at 25 mA in a 4°C refrigerator. Gels were then sliced and stained with several specific enzyme stains (Murphy et al., 1990). (The exact protocol is available in both English and Spanish from J. E. Strassmann.) To screen the adult population for variable loci I initially ran 1 randomly selected individual from each of 29 colonies and stained for 21 enzymes.

I used the Queller and Goodnight (1989) method of relatedness calculation (Relatedness, Version 4.2) to obtain relatedness values and inbreeding coefficients for all females, for workers, and for queens. Colonies were weighted equally and standard errors were obtained by jackknifing over colonies (Sokal and Rohlf, 1981).

I determined the sex of all electrophoresed individuals as well as the reproductive status of females. Initially, I electrophoresed 12 randomly selected adults from each colony and then I electrophoresed all remaining frozen reproductives to increase the sample size. During the reproductive runs I checked the remaining workers from 22 colonies for intermediates, females that possessed one or more full-sized eggs lacking only a fully developed chorion. Fifteen of these 22 colonies had at least 1 intermediate. These intermediates were also electrophoresed. Since intermediates lacked mature eggs, I assigned them to the worker category although their real status remained ambiguous. I counted the remaining frozen workers but did not electrophorese them.
Demographics

The cyclical oligogyny hypothesis can be tested by examining certain predicted demographic correlations. Cyclical oligogyny predicts that at the beginning of the cycle, when queen number is high, there will be fewer eggs per queen, young queens, and low brood relatedness. Towards the end of the cycle, during production of reproductives, there should be low queen number along with many egg per queen, old queens, and high brood relatedness.

I compared the number of queens with the number of eggs per queen per nest to test one of these predictions. I did not include queen age in my analyses because I did not specifically assign queens to age categories. However, I did notice during dissections that females with many mature eggs appeared darker and more worn than those with few mature eggs, a condition that may be attributed to advanced age as well as to time off the nest due to foraging activity (Forsyth, 1978). Also, individual colony relatedness values are calculated on too few individuals to yield reliable estimates. The standard errors and the resulting confidence intervals are very large. Therefore, I did not attempt to test predictions involving relatedness of individual colonies.

Queen Number and Brood Relatedness

I examined the relationship between queen number and brood relatedness with an equation that can be used to estimate population brood relatedness from the population mean of queen number, given singly-mated queens and no inbreeding (Strassmann et al., 1991; Queller, in press),

\[ r_{pred} = \frac{3}{4}P + \frac{r_{queen}}{4} (1 - P) \]  
(2)
where $r_{pred}$ and $r_{queen}$ are the relatedness values for workers and queens, respectively, and

$$ P = \frac{1}{q^h_k} + q^h_k \sigma^2_P \text{ (within)} $$

(3)

where $P$ is the probability that 2 workers share the same mother and $q^h_k =$ harmonic mean of queen number, $\bar{q}^k =$ arithmetic mean of queen number, and $\sigma^2_P \text{ (within)} =$ within nest variance in egg-laying among queens (Queller et al., in press; Strassmann et al., 1991). Since $P$ is the reciprocal of queen number adjusted for unequal egg-laying among queens, brood relatedness can be calculated knowing only mean queen number if 1) workers and queens are related equally, 2) there is no reproductive dominance in egg-laying, 3) there is little variance in queen number among colonies, and 4) there is no multiple mating (Queller et al., in press; Strassmann et al., 1991; Wade, 1985). If brood relatedness derived from allozyme data ($r_{brood}$) is used as a baseline for comparison with brood relatedness derived from this formula ($r_{pred}$), the underlying assumptions may be examined in light of the difference between the two relatednesses.
RESULTS

Colony and Caste Characteristics

The numbers of workers, queens, males, and mature eggs per queen per colony and combs and cells per nest are shown in Table 1. The mean proportions and numbers (±SD) of queens, workers, and males for this population are given in Table 2. The mean number of queens per colony, $\bar{X} = 19.8$ (SD = 18.8), agrees closely with that found in a previous P. emaciata study ($\bar{X} = 23$, SD = 27; Strassmann et al., 1992).

Queen number was significantly correlated with number of combs but not with number of cells per nest (Figures 1 and 2). I found no correlation between either nest size measure and the number of eggs per queen (Figures 3 and 4). The means and ranges of the numbers of combs, cells, and pupae of 23 nests are given in Table 3. Male production occurs in P. emaciata when queen number is low (Figure 5) and when there are many eggs per queen (Figure 6).

Morphometrics of Queens and Workers

Workers’ heads were 1.3% larger than queens’ but the two castes were no different in width of first gastric segment, number of hamuli on the hindwing, and length of the first discoidal cell of the forewing (Table 4). Principal components analysis revealed no eigenvalues greater than 1.

Relatedness

This population of P. emaciata was polymorphic at 3 allozyme loci of those investigated (Table 5). Table 6 shows the population frequencies of the 3
TABLE 1. *P. emaciata* colony demographics in ascending order by queen number.

<table>
<thead>
<tr>
<th>Colony ID</th>
<th>Estimated Queen No.</th>
<th>Estimated Male No.</th>
<th>Estimated Worker No.</th>
<th>Total Counted Individuals</th>
<th>Percent Dissected</th>
<th>Eggs/Queen</th>
<th>Number of Combs</th>
<th>Number of Cells</th>
<th>Number of Pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>2</td>
<td>8</td>
<td>166</td>
<td>176</td>
<td>51</td>
<td>3.0</td>
<td>3</td>
<td>850</td>
<td>118</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>18</td>
<td>237</td>
<td>257</td>
<td>66</td>
<td>3.0</td>
<td>7</td>
<td>2748</td>
<td>111</td>
</tr>
<tr>
<td>36</td>
<td>2</td>
<td>28</td>
<td>238</td>
<td>268</td>
<td>65</td>
<td>6.0</td>
<td>3</td>
<td>1212</td>
<td>312</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>0</td>
<td>59</td>
<td>62</td>
<td>100</td>
<td>2.7</td>
<td>3</td>
<td>736</td>
<td>93</td>
</tr>
<tr>
<td>35</td>
<td>6</td>
<td>31</td>
<td>273</td>
<td>310</td>
<td>77</td>
<td>7.4</td>
<td>5</td>
<td>1646</td>
<td>151</td>
</tr>
<tr>
<td>43</td>
<td>6</td>
<td>40</td>
<td>200</td>
<td>246</td>
<td>70</td>
<td>4.5</td>
<td>5</td>
<td>1386</td>
<td>457</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>14</td>
<td>200</td>
<td>221</td>
<td>100</td>
<td>5.3</td>
<td>3</td>
<td>1126</td>
<td>216</td>
</tr>
<tr>
<td>41</td>
<td>8</td>
<td>22</td>
<td>338</td>
<td>368</td>
<td>73</td>
<td>7.8</td>
<td>3</td>
<td>1184</td>
<td>137</td>
</tr>
<tr>
<td>42</td>
<td>9</td>
<td>33</td>
<td>170</td>
<td>212</td>
<td>100</td>
<td>3.8</td>
<td>4</td>
<td>1464</td>
<td>51</td>
</tr>
<tr>
<td>24</td>
<td>13</td>
<td>0</td>
<td>164</td>
<td>177</td>
<td>53</td>
<td>1.1</td>
<td>3</td>
<td>666</td>
<td>175</td>
</tr>
<tr>
<td>34</td>
<td>14</td>
<td>49</td>
<td>436</td>
<td>499</td>
<td>76</td>
<td>5.1</td>
<td>3</td>
<td>1185</td>
<td>273</td>
</tr>
<tr>
<td>29</td>
<td>16</td>
<td>0</td>
<td>151</td>
<td>167</td>
<td>70</td>
<td>2.0</td>
<td>4</td>
<td>1173</td>
<td>163</td>
</tr>
<tr>
<td>18</td>
<td>19</td>
<td>0</td>
<td>113</td>
<td>132</td>
<td>86</td>
<td>1.3</td>
<td>2</td>
<td>776</td>
<td>213</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
<td>0</td>
<td>116</td>
<td>138</td>
<td>69</td>
<td>1.1</td>
<td>3</td>
<td>772</td>
<td>33</td>
</tr>
<tr>
<td>27</td>
<td>23</td>
<td>0</td>
<td>598</td>
<td>621</td>
<td>60</td>
<td>4.2</td>
<td>5</td>
<td>1796</td>
<td>291</td>
</tr>
<tr>
<td>33</td>
<td>24</td>
<td>0</td>
<td>154</td>
<td>178</td>
<td>63</td>
<td>2.0</td>
<td>6</td>
<td>2270</td>
<td>412</td>
</tr>
<tr>
<td>28</td>
<td>27</td>
<td>2</td>
<td>156</td>
<td>185</td>
<td>48</td>
<td>1.8</td>
<td>5</td>
<td>943</td>
<td>195</td>
</tr>
<tr>
<td>38</td>
<td>30</td>
<td>8</td>
<td>232</td>
<td>270</td>
<td>76</td>
<td>4.2</td>
<td>9</td>
<td>3265</td>
<td>322</td>
</tr>
<tr>
<td>23</td>
<td>32</td>
<td>0</td>
<td>168</td>
<td>200</td>
<td>51</td>
<td>1.1</td>
<td>4</td>
<td>1834</td>
<td>346</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>83</td>
<td>711</td>
<td>827</td>
<td>34</td>
<td>4.5</td>
<td>8</td>
<td>3567</td>
<td>153</td>
</tr>
<tr>
<td>39</td>
<td>33</td>
<td>0</td>
<td>286</td>
<td>319</td>
<td>100</td>
<td>1.7</td>
<td>4</td>
<td>1190</td>
<td>249</td>
</tr>
<tr>
<td>30</td>
<td>37</td>
<td>0</td>
<td>324</td>
<td>361</td>
<td>80</td>
<td>1.6</td>
<td>6</td>
<td>1495</td>
<td>276</td>
</tr>
<tr>
<td>21</td>
<td>88</td>
<td>0</td>
<td>318</td>
<td>406</td>
<td>83</td>
<td>1.4</td>
<td>5</td>
<td>2643</td>
<td>245</td>
</tr>
</tbody>
</table>
Table 2. Colony composition of *P. emaciata*. \( \bar{X} \pm SD, N = 23 \) colonies.

<table>
<thead>
<tr>
<th></th>
<th>Queens</th>
<th>Workers</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportions</td>
<td>0.081 (0.063)</td>
<td>0.869 (0.062)</td>
<td>0.05 (0.066)</td>
<td>100.0</td>
</tr>
<tr>
<td>Means</td>
<td>19.8 (18.8)</td>
<td>252.6 (154)</td>
<td>14.6 (21.3)</td>
<td>287 (172.5)</td>
</tr>
</tbody>
</table>
Figure 1. Larger nests, based on number of combs, have more queens than do smaller nests (Spearman correlation coefficient corrected for ties = 0.46, p < 0.05, N = 23 nests).
Figure 2. Larger nests, based on number of cells, do not have significantly more queens than do smaller nests (Spearman rank correlation coefficient = 0.35, p > 0.05, N = 23 nests).
Figure 3. Larger nests, based on number of combs, do not have queens with more eggs than do smaller nests (Spearman rank correlation coefficient corrected for ties = 0.062, p > 0.25, N = 23 nests).
Figure 4. Larger nests, based on number of cells, do not have queens with more eggs than do smaller nests (Spearman rank correlation coefficient corrected for ties = 0.221, p > 0.10, N = 23 nests).
Table 3. Nest characteristics of *P. emaciata*. N = 23 nests.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combs</td>
<td>4.48</td>
<td>1.78</td>
<td>2 - 9</td>
</tr>
<tr>
<td>Cells</td>
<td>1562.04</td>
<td>816.92</td>
<td>666 - 3567</td>
</tr>
<tr>
<td>Pupae</td>
<td>217.04</td>
<td>109.56</td>
<td>33 - 457</td>
</tr>
</tbody>
</table>
Figure 5. As queen number decreases male number increases (Spearman rank correlation coefficient corrected for ties = -0.444, p < 0.05, N = 23).
Figure 6. As number of males increases number of eggs per queen also increases (Spearman rank correlation coefficient corrected for ties = 0.79, p = 0.0002, N = 23 colonies).
Table 4. Queens and workers were not morphologically significantly different from one another (1-tailed Mann-Whitney U-tests) based on the number of hamuli on the hindwing, length of the first discoidal cell of the forewing, and first gastral segment width for queens and for workers in *P. emaciata*. Although workers had significantly larger heads than had queens the difference was very small, about 1 %, and there was much overlap of measurements between the two castes.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Queens</th>
<th>Workers</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number hamuli on hindwing</td>
<td>Mean</td>
<td>9.333</td>
<td>9.326</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.087</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>132</td>
<td>141</td>
</tr>
<tr>
<td>Head width</td>
<td>Mean</td>
<td>7.917</td>
<td>8.017</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.022</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>132</td>
<td>139</td>
</tr>
<tr>
<td>Length of first discoidal cell of forewing</td>
<td>Mean</td>
<td>12.630</td>
<td>12.535</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.045</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>132</td>
<td>141</td>
</tr>
<tr>
<td>First gastral segment width</td>
<td>Mean</td>
<td>7.117</td>
<td>7.074</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.031</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>132</td>
<td>141</td>
</tr>
</tbody>
</table>
Table 5. Enzymes for which 29 randomly chosen individuals from 30 nests of *P. emaciata* were screened for allozyme variability. Monomorphic, ambiguous, and smeary enzymes were not used for the subsequent study either because they offered no usable information or could not be reliably read.

<table>
<thead>
<tr>
<th>Enzyme*</th>
<th>No. of Loci</th>
<th>Variability</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACON</td>
<td>2</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>AK</td>
<td>1</td>
<td>variable</td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>G3PD</td>
<td>2</td>
<td>1 locus variable</td>
<td></td>
</tr>
<tr>
<td>G6PDH</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>GDH</td>
<td>2</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>GPI</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>HBDH</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>HEX</td>
<td>3 or 4</td>
<td>3 loci variable</td>
<td>All variable loci vary together. Score 1 locus only.</td>
</tr>
<tr>
<td>IDH</td>
<td>2</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>MDH</td>
<td>2</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>MPI</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>PEP</td>
<td>5</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>LA/LGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEP LP</td>
<td>1</td>
<td>Possibly variable</td>
<td></td>
</tr>
<tr>
<td>PGDH</td>
<td>1</td>
<td>smear</td>
<td></td>
</tr>
<tr>
<td>PGM</td>
<td>1</td>
<td>Possibly variable</td>
<td>Only variant weak.</td>
</tr>
<tr>
<td>SOD</td>
<td>2</td>
<td>monomorphic</td>
<td></td>
</tr>
<tr>
<td>XDH</td>
<td>1</td>
<td>monomorphic</td>
<td></td>
</tr>
</tbody>
</table>
*Note: Enzyme names and abbreviations are from Hillis and Moritz (1990) and are based on the International Union of Biochemistry recommendations for enzymes names. ACON, aconitase; AK, adenylate kinase; ES, esterase; G3PD, glycerol-3-phosphate dehydrogenase; G6PDH, Glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GPI, glucose-6-phosphate isomerase; HBDH, hydroxybutyrate dehydrogenase; HEX, hexokinase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; ME, malate decarboxylase; MPI, mannose phosphate isomerase; PEP LGG, leucylglycylglycine peptidase; PEP LA, leucylalanine peptidase; PEP LP, leucylproline peptidase; PGM, phosphoglucomutase; SOD, superoxide dismutase; XDH, xanthine dehydrogenase.
Table 6. Population allele frequencies of hexokinase (HEX), glycerol-3-phosphate dehydrogenase (G3PD), and adenylate kinase (AK) estimated from 725 individuals from 23 colonies of *P. emaciata*.

<table>
<thead>
<tr>
<th>Loci</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEX</td>
<td>0.861</td>
<td>0.119</td>
<td>0.021</td>
</tr>
<tr>
<td>G3PD</td>
<td>0.688</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td>AK</td>
<td>0.965</td>
<td>0.035</td>
<td></td>
</tr>
</tbody>
</table>
polymorphic loci: hexokinase (HEX) with 3 alleles, glycerol-3-phosphate dehydrogenase (G3PD) with 2 alleles, and adenylate kinase (AK) also with 2, found during the initial screen of a random sample of *P. emaciata* for allozyme variation. Relatednesses and inbreeding coefficients for queens, workers, and males are given in Table 7. The worker relatedness value supports the general trend for swarm-founding wasps that colony relatedness should be below 0.5, comparatively low among workers, and comparatively higher among queens than for all females (Queller et al., 1988, 1992; Strassmann et al., 1991, 1992; Gastreich et al., in prep). Using allozyme data, Strassmann et al. (1992) obtained an estimate of worker relatedness for *P. emaciata* ($r = 0.24$, SE = 0.076, 95% confidence interval: $0.074 < r < 0.404$) that is similar to mine but they were unable to obtain an allozyme estimate for queen relatedness. Instead, they inferred $r_{queen} = 0.71$ using mean queen number, egg-laying dominance among queens, and worker relatedness. This figure is higher than my point estimate of queen relatedness but within the 95% confidence interval.

**Intermediates**

All 131 queens and 4 intermediates were inseminated. Only 1 of 1661 females who had no ovarian development, workers, were inseminated. Only 4 of the 1795 females in this sample were intermediates (had at least 1 almost-mature but no mature egg). While this sample is too small for me to assume that all the other intermediates in this population were also inseminated I likewise cannot assume that they were not. Of the 22 colonies checked for intermediates, only 3.1 % of the females were intermediates, 89.4 % were workers and 7.5 % were queens.
Table 7. Relatednesses (r) and inbreeding coefficients (f) of a random sample of females chosen without regard to ovarian development, of a random sample of workers, and of a random sample of queens of *P. emaciata*. Standard errors (SE) were computed by jackknifing over colonies and 95 % confidence limits (95% C.I.) were obtained using those standard errors (Sokal and Rohlf, 1981, p. 795 - 799).

<table>
<thead>
<tr>
<th>Relatedness</th>
<th>Females, n = 271</th>
<th>Workers, n = 251</th>
<th>Queens, n = 193</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.228</td>
<td>0.203</td>
<td>0.552</td>
</tr>
<tr>
<td>SE</td>
<td>0.059</td>
<td>0.061</td>
<td>0.103</td>
</tr>
<tr>
<td>95 % C.I.</td>
<td>0.112 - 0.344</td>
<td>0.083 - 0.323</td>
<td>0.359 - 0.755</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inbreeding</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>0.089</td>
<td>0.075</td>
<td>0.088</td>
</tr>
<tr>
<td>SE</td>
<td>0.055</td>
<td>0.054</td>
<td>0.110</td>
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</tbody>
</table>
Further analyses indicated that intermediates may have been queens or at least original females from the founding swarm because the relatedness of intermediates \((r = 0.542, SE = \pm 0.163, n = 42)\) is the same as that of queens alone \((r = 0.549, SE = \pm 0.133, n = 106)\) in those 15 colonies from which allozyme data was obtained for intermediates. Also, there were more queens on colonies with intermediates than on colonies without them (2-tailed Mann-Whitney \(U = 91, P = 0.029, N = 22\) colonies) and I found males on only 1 of the 9 nests with intermediates but on 9 of the 13 nests without intermediates (G with Williams correction = 7.873, \(P < 0.05, N = 22\) colonies).

**Maintaining High Brood Relatedness**

Inbreeding is not a major factor contributing to high brood relatedness in *P. emaciata* because the inbreeding coefficient for queens \((f = 0.088, SE = \pm 0.1104)\) is low and not statistically different from zero.

Brood relatedness will increase if one or a few queens lay most of the eggs on a nest, but I found no evidence of significant unequal egg-laying among queens. First, to test whether larger queens lay more eggs than do smaller queens I used head width as a measure of overall size and found no correlation between the number of mature eggs in the ovaries of larger queens and the number of mature eggs in the ovaries of smaller queens (Figure 7, \(r_s\) corrected for ties = 0.003, \(P > 0.5, n = 126\)). Second, unequal egg-laying within a population can be estimated from per colony queen numbers and the numbers of mature eggs in the ovaries of each queen (Figures 8 - 27; Queller *et*
Figure 7. Queens with larger heads do not have more eggs than do queens with smaller heads. Head width is used as a measure of overall size ($r_s$ corrected for ties = 0.006, $p > 0.5$, $n = 127$ queens). The relative size of the circle indicates the relative number of individuals at that point.
Figure 8. Frequency distribution of number of eggs per queen on nest 14. n = 7 queens.
Figure 9. Frequency distribution of number of eggs per queen on nest 15. $n = 11$ queens.
Figure 10. Frequency distribution of number of eggs per queen on nest 18. $n = 16$ queens.
Figure 11. Frequency distribution of number of eggs per queen on nest 20. $n = 3$ queens.
Figure 12. Frequency distribution of number of eggs per queen on nest 21. n = 73 queens.
Figure 13. Frequency distribution of number of eggs per queen on nest 22. n = 15 queens.
Figure 14. Frequency distribution of number of eggs per queen on nest 23. n = 16 queens.
Figure 15. Frequency distribution of number of eggs per queen on nest 24. $n = 7$ queens.
Figure 16. Frequency distribution of number of eggs per queen on nest 27. \( n = 14 \) queens.
Figure 17. Frequency distribution of number of eggs per queen on nest 28. n = 13 queens.
Figure 18. Frequency distribution of number of eggs per queen on nest 29. n = 11 queens.
Figure 19. Frequency distribution of number of eggs per queen on nest 30. n = 30 queens.
Figure 20. Frequency distribution of number of eggs per queen on nest 33. n = 15 queens.
Figure 21. Frequency distribution of number of eggs per queen on nest 34. n = 11 queens.
Figure 22. Frequency distribution of number of eggs per queen on nest 35. $n = 5$ queens.
Figure 23. Frequency distribution of number of eggs per queen on nest 38. \( n = 26 \) queens.
Figure 24. Frequency distribution of number of eggs per queen on nest 39. \( n = 33 \) queens.
Figure 25. Frequency distribution of number of eggs per queen on nest 41. \( n = 6 \) queens.
Figure 26. Frequency distribution of number of eggs per queen on nest 42. n = 9 queens.
Figure 27. Frequency distribution of number of eggs per queen on nest 43. \( n = 4 \) queens.
al., in press). The estimate for within nest variance in egg-laying was very low
\[ q_k^2 \sigma^2_p \text{ (within)} = 0.0309. \]

Cyclical oligogyny predicts that at the beginning of the cycle there will be many queens, each with few eggs, and at the end of the cycle there will be few queens, each with many eggs. I found queen number and number of eggs per queen significantly negatively correlated (Figure 28) thus supporting the predictions.

Calculating Brood Relatedness using Queen Number

Brood relatedness estimated from mean queen number \( r_{pred} = .050 \) is considerably lower than brood relatedness obtained from allozyme data \( r_{brood} = 0.209 \). However, if workers are not related the same as queens, if there is a high variance in mean queen number between colonies, or if there is significant unequal egg-laying \( r_{pred} \) may be corrected to more closely estimate brood relatedness. First, allozyme data reveals a different \( r_{queen} \) than that of \( r_{brood} \) so the corrected \( r_{queen} \) should be used. Next, the harmonic mean of queen number is a better estimate of queen number because it is based on reciprocals which better reflect the division of egg-laying among females (Strassmann et al., 1991; Wade, 1985; Queller, in press). Lastly, within nest variance in egg-laying is calculated with number of queens per nest and number of eggs per queen data (Strassmann et al., 1991; Wade, 1985; Queller, in press). Table 8 shows the change in brood relatedness as the above corrections are implemented singly, in pairs, and all together. When all of the corrections are implemented, \( r_{pred} \) is actually higher than the point estimate of
Figure 28. As queen number decreases the number of eggs per queen increases (Spearman rank correlation coefficient corrected for ties = -0.499, p = 0.019, N = 23 colonies).
Table 8. When brood relatedness for *P. emaciata* is predicted using only queen number (\( r_{pred} = 0.050 \)) it is significantly lower than the estimated relatedness value obtained from allozyme data (\( r_{brood} = 0.203 \)). Corrections implemented singly (steps 1, 2, 3), in pairs (steps 4, 5, 6) and all together (step 7) result in increased estimates of brood relatedness using this method of calculation. Percentages are the % difference between \( r_{pred} \) and \( r_{brood} \) accounted for by the correction.

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Correction</th>
<th>( r_{pred} )</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ( r = r_q )</td>
<td>( r = 0.203 ) ( r_q = 0.552 )</td>
<td>0.169</td>
<td>77.8</td>
</tr>
<tr>
<td>2) ( \bar{q}_k^h = \bar{q}_k )</td>
<td>( \bar{q}_k^h = 6.7 ) ( \bar{q}_k = 19.8 )</td>
<td>0.142</td>
<td>60.4</td>
</tr>
<tr>
<td>3) ( q_k \sigma_p^2 \text{ (within)} = 0 )</td>
<td>( q_k \sigma_p^2 \text{ (within)} = 0.0309 )</td>
<td>0.079</td>
<td>19.3</td>
</tr>
<tr>
<td>4) 1) &amp; 2)</td>
<td>0.229</td>
<td>117.3</td>
<td></td>
</tr>
<tr>
<td>5) 1) &amp; 3)</td>
<td>0.188</td>
<td>90.1</td>
<td></td>
</tr>
<tr>
<td>6) 2) &amp; 3)</td>
<td>0.170</td>
<td>78.6</td>
<td></td>
</tr>
<tr>
<td>7) 1), 2), &amp; 3)</td>
<td>0.248</td>
<td>129.7</td>
<td></td>
</tr>
</tbody>
</table>
$r_{brood}$ but within the 95 % confidence interval (Figure 29). The difference between the two relatednesses could be due to multiple mating of queens since multiple mating would result in lower relatedness among the brood of each queen. Alternatively, the difference might also come from having included a few intermediates who are really like queens. However, since $r_{pred}$ lies within the 95% confidence interval of $r_{brood}$ no explanation for the difference is necessary.
Figure 29. Brood relatedness predicted from queen number alone was much smaller ($r_{\text{pred}} = 0.050$) than that obtained genetically ($r_{\text{brood}}$). Relatedness obtained after underlying assumptions were corrected (see Table 8) result in progressively more accurate estimates of brood relatedness. The correction for reproductive dominance yields little change whereas the corrections for harmonic mean of queen number and high queen relatedness yield substantial changes. When all corrections are made, $r_{\text{pred}}$ overshoots $r_{\text{brood}}$ but still remains within the 95% confidence interval of $r_{\text{brood}}$. 

![Relatedness diagram with legends](image)

- Point estimate of genetically determined worker relatedness
- No corrections
- Queen relatedness correction
- Harmonic mean of queen number correction
- Reproductive dominance correction
- Harmonic mean and queen relatedness corrections together
- Queen relatedness and reproductive dominance corrections together
- Harmonic mean and reproductive dominance corrections together
- All corrections together
DISCUSSION

Intermediates

Intermediates would be both inseminated and related to the same degree as queens if intermediates were former queens who lost in the dominance contests which occur near the beginning of the colony cycle when queen number is high and no reproducitives have yet been produced (Forsyth, 1978; West-Eberhard, 1978). My results are consistent with that hypothesis since intermediates were present at the beginning of the colony cycle, they were not present with males, which are thought to be produced later in the colony cycle, and they were related the same as queens. Queens with no mature eggs would most likely be among the losers in the inevitable dominance contests that occur in colonies with many queens. Even if they are laying eggs at the beginning of the colony cycle, loser queens would not lay eggs later when reproducitives are being produced. Strassmann et al. (1992) found inseminated workers that were the same age as the queens on P. emaciatea nests that had many queens. Indeed, queens that both lose the right to lay eggs and remain on the nest perform as workers and do not contribute to brood relatedness when gynes are produced. Therefore, they do not affect the various \( r_{pred} \) values.

Alternatively, these results are also consistent with the hypothesis that intermediates were queens that had just laid their last mature egg and they simply did not have the next egg ready because eggs laid per queen was small given the large number of queens on these colonies. In the 9 colonies with intermediates, 55 % of the queens had only 1 mature egg each in their ovaries. Including intermediates as workers does lead to one error since it inflates the
electrophoretic estimate of brood relatedness \( r_{brood} \) a little, but probably not much given the small number of intermediates.

It does not affect the estimate of brood relatedness based on queen number \( r_{pred} \) because the difference between the estimate with intermediates as workers and the estimate with intermediates as non-egg-laying queens is not significant (Queller, pers. comm.) Although mean queen number may be higher if intermediates were included as non-egg-laying queens, the variance of egg-laying among queens would be slightly higher also. Thus, these two new estimates would combine to yield a similar P value (probability that two workers share the same mother) as that obtained when intermediates are counted as workers. However, if intermediates are actually laying eggs, as their insemination status might indicate, then the P value might increase as the within-nest variance in egg-laying among queens becomes larger. This last option is not measurable since calculation of the variance in egg-laying among queens requires that each queen have at least one mature egg in her ovaries and intermediates have none.

If intermediates were indeed queens then some demographic data and the brood relatedness estimates obtained in this study may be in error. Future studies that test hypotheses requiring queen number in swarm-founding wasps should count, electrophorese, and check insemination status for all intermediates. However, although mean queen number and within-nest variance in egg-laying among queens may be different from that obtained and used in this study, in the absence of a method to obtain the variance measure using non-egg-laying queens, these measures are good enough to yield an accurate \( r_{pred} \) if intermediates are counted as workers.
Maintenance of Eusociality

Eusociality may be maintained if workers are physiologically constrained from egg-laying as are worker ants. I found no evidence of major morphological differences that could suggest that workers are unable to lay eggs. Richards (1978) found that P. emaciata queens had significantly more hamuli per mm. of hindwing length than did workers but otherwise castes were not well differentiated.

Eusociality may be maintained in P. emaciata by one or more of several mechanisms that would function to increase relatedness between the brood and the workers who care for them. This may occur if workers preferentially care for a closely related subset of brood, if queens are inbred, if there is a significant variance in within-colony egg-laying among queens, or through cyclical oligogyny.

Preferential care of closely related brood may occur if each queen lays all her eggs within one section of a single comb and then daughter workers preferentially care only for brood in that section. This hypothesis was not investigated in this study. However, Queller et al. (1993) found evidence for a modest amount of segregated egg-laying by queens in P. occidentalis but did not demonstrate whether workers subsequently preferentially aided only their sisters. Forsyth (1978) described segregated egg-laying for P. occidentalis but he suggested that this tactic functioned as an avoidance mechanism between queens in response to overt queen aggression. In order for foraging workers to preferentially care for closely related brood they would have to possess very fine levels of kin recognition because they do not directly feed the brood themselves. They would have to deliver their food only to sisters awaiting on or
within the nest. These sisters would then have to return to their mother's section and feed only their sister larvae (Jeanne 1986). There is some evidence for within colony kin discrimination in honey bees (Page et al., 1989 but see Carlin and Frumhoff, 1990) but none for wasps (Queller et al., 1990).

Hamilton (1964) suggested that inbreeding might keep brood relatedness high and therefore maintain eusociality. Large inbreeding coefficients have been measured for *Mischocyttarus immarginatus*, a primitively eusocial polistine wasp (Queller et al., 1992). However, no significant inbreeding has been found in any epiponine wasp including *P. emaciata* (Strassmann et al., 1991, 1992; Queller et al., 1988, in press; Gastreich et al., in prep).

A significant within-colony variance in egg-laying among queens (reproductive inequality) is expected given the many, competing queens on each colony (Forsyth, 1978; West-Eberhard, 1978). However, reproductive inequality does not substantially raise brood relatedness in *Polybia emaciata*. Forsyth (1978) suggested that unequal egg-laying (one or a few queens lay most of the eggs) occurred in *P. occidentalis* but a more recent study did not support that idea (Queller et al., 1993). Very low variances in reproductive success among queen colony mates have been found in other swarm-founding wasps also (Strassmann et al., 1991, 1992; Gastreich et al., in prep).

Maintenance of eusociality in *P. emaciata* may best be explained by cyclical oligogyny. This colony cycle is characterized by queen numbers that fluctuate from many queens at the beginning of the cycle to one or a few queens at the end when gynes are produced (West-Eberhard, 1978). The demographic data examined in this study agreed with that of Strassmann et al. (1992) in
support of the cyclical oligogyny hypothesis in *P. emaciata*. Evidence that
cyclical oligogyny functions to increase brood relatedness, above that expected
given the number of queens per colony, was gained by examining the
relationship between brood relatedness and queen number.

Brood relatedness for *P. emaciata* was significantly higher than that
expected based on mean queen number. Most of this difference was due to
high queen relatedness and a large variance in queen number between
colonies. Similar results have been obtained with the epiponines *P.
occurrentalis* (Queller et al., 1993), *Parachartergus colobopterus* (Strassmann et
al., 1991), and *Protopolybia exigua* (Gastreich et al., in prep). My queen
relatedness results support those of Strassmann et al.’s (1992) study of *Polybia
emaciata* in which they estimated $r_{\text{queen}}$ using equations 2 and 3 and their
electrophoretically determined $r_{\text{brood}}$ estimate. Their estimated queen
relatedness ($r_{\text{queen}} = 0.71$) was considerably higher than my
electrophoretically determined estimate ($r_{\text{queen}} = 0.552$) but falls within the 95%
confidence interval. Both estimates agree well with the prediction of the cyclical
oligogyny hypothesis that co-founding queens are the closely related brood of
one or a few queens.

Queen relatedness ($r_{\text{queen}} = 0.552$) was much higher than the
relatedness for all females ($r = 0.228$) in this population of *P. emaciata*. There
are several possible reasons why queens are so closely related. First, female
reproductives (gynes) may be produced by only 1 or a few queens. Second,
high queen relatedness may occur because swarming females form closely-
related subgroups to found nests. This alternative would require a high degree
of within-colony kin recognition which has not been found in wasps. However,
few detailed studies of within-colony kin discrimination in wasps have been
done (Queller et al., 1990).

If gynes are produced by only one or a few queens, gyne production may
occur if one or a few queens lay most of the eggs, resulting in reproductive
inequality among queen colonymates, or it may occur late in the colony cycle
when only one or a few queens remain laying eggs (West-Eberhard, 1978). I
have shown that reproductive inequality among current queens was not
significant in P. emaciata.

To investigate the first alternative, I used equations 2 and 3 to further
examine queen number at the time when gynes are produced. Assume that, at
equilibrium, relatedness in the old queen cohort is equal to relatedness in the
progeny queen cohort. Relatedness between swarms should not vary if the
population is at equilibrium. Therefore, with \( r_{\text{pred}} = r_{\text{queen}} \) and \( q_k \sigma_p^2 \) _P (within) =
0.0309, solving for \( \frac{1}{\bar{q}_k} \), I got the harmonic mean of queen number when gynes
are produced. An approximate error of the mean is calculated by substituting
first the upper and then the lower bounds of the 95% confidence interval of
\( r_{\text{queen}} \) into the equation. Solving the 3 equations for P. emaciata, \( \frac{1}{\bar{q}_k} = 1.55 
\)
with a range between 2.75 and 1.02 queens present when gynes are produced.
Similar results were obtained for the swarm-founders Parachartergus
colobopterus (\( \frac{1}{\bar{q}_k} = 1.2 \), range = 1 - 1.7), P. occidentalis (\( \frac{1}{\bar{q}_k} = 1.4 \), range 1 -
2.1), and Protopolybia exigua (\( \frac{1}{\bar{q}_k} = 0.88 \), range 0.7 - 1.1) by Hughes et al. (in
press), Queller et al. (1993) and Gastreich et al. (in prep), respectively. These
results are consistent with the prediction of cyclical oligogyny that gynes are not produced until queen number has dropped to one or a few queens.

A large variance in queen number between colonies also contributed significantly to brood relatedness being higher than expected based on mean queen number in *Polybia emaciata*. A large variance in queen number is expected if the colonies within a cyclically oligogynous population are at different stages in the colony cycle so that queen number varies widely between colonies at any given point in time.

Although the production of males is not specifically dealt with under the cyclical oligogyny hypothesis, it is interesting that male production occurs in *P. emaciata* when queen number is low yet when there are many eggs per queen. Another study of *P. emaciata* (Strassmann *et al.*, 1992) and one with *P. occidentalis* (Queller *et al.*, 1993) found similar results with those wasps. Males may be produced towards the end of the colony cycle if new colonies delay male production until a large worker force has been established but before gynes are produced (Queller *et al.*, in prep.). Then, male production would occur when queen number is low simply because many queens would have died by the time the worker force is numerous.

**Summary**

The maintenance of eusociality in swarm-founding wasps is difficult to explain because brood relatedness is predicted to be very low due to the presence of multiple queens in each colony and because workers are not morphologically constrained from laying eggs. However, brood relatedness is actually much higher than expected given the number of queens per colony.
High queen relatedness and a large population variance in queen number best explain why brood relatedness in *P. emaciata* is higher than expected based on queen number alone. Inbreeding and reproductive dominance were not important.

High queen relatedness may occur because gynes are not produced until queen number is very low or because swarming females preferentially sort into closely-related, within-colony kin groups to found new colonies. While preferential swarming was not investigated, the evidence presented here suggests that cyclical oligogyny is responsible for maintaining high brood relatedness because gynes are not produced until queen number is very low. It appears that cyclical oligogyny may be general for all epiponines since strong supporting evidence for it has been found for several species (West-Eberhard, 1978; Forsyth, 1978; Strassmann *et al.*, 1991, 1992; Queller *et al.*, 1992; Gastreich *et al.*, in prep.).

Cyclical oligogyny provides for the maintenance of eusociality in swarm-founding wasps by insuring that founding queens are closely related. Another possible mechanism that was not investigated here is that workers care for a closely-related subset of brood within the nest. This mechanism requires a high level of within-colony kin recognition that has not yet been demonstrated in wasps. However, it cannot be ruled out.
REFERENCES


