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Relatedness and queen number in the Neotropical wasp, *Parachartergus colobopterus*

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Abstract. The maintenance of sociality is most difficult to explain under circumstances where non-reproducing helpers are physiologically capable of reproducing and distantly related to the brood they rear. The Neotropical swarm-founding wasps are likely to fulfil these conditions because most taxa lack morphological differences between workers and queens, and they have many queens per nest, which is expected to substantially lower worker to brood relatedness. No morphological differences between workers and queens in *Parachartergus colobopterus* were detected. Colonies contained an average of 27 queens. However, relatedness among nestmates in *P. colobopterus* was higher ($r=0{\cdot}31$) than would be expected on the basis of queen number alone because the queens themselves are very closely related ($r=0{\cdot}67$) and because of large variation in numbers of queens among colonies. This makes the harmonic mean of queen number (five queens), which is the appropriate measure for investigating the impact of queen number on relatedness, much lower than the arithmetic mean. Reproductive dominance of one or a few queens within colonies was not a factor that greatly increased relatedness among workers. Taken together, these results support the cyclical oligogyny hypothesis for the maintenance of sociality in Neotropical social wasps.

The Neotropical swarm-founding wasps represent a challenge for kin selection theory because colonies contain many queens. Large numbers of queens are expected to reduce relatedness between workers and the brood they rear (Hamilton 1972; Queller et al. 1988). Because morphological differences among workers and queens are not present in most Neotropical wasps (Richards 1978; Jeanne 1980; Carpenter, personal communication), it is unlikely that these workers are morphologically limited to rearing the progeny of others. Unless there are large ecological advantages to remaining in the colony, kin selection theory would predict that workers would choose to reproduce themselves rather than continuing to rear distant relatives. Nevertheless, eusociality is maintained in this group. In fact, colonies may have tens of thousands of workers and many queens (Richards 1978; Jeanne 1991).

Colonies begin with a swarm containing many workers and queens that all originate from the same natal nest (West-Eberhard 1978). The swarm leaves the natal nest site and relocates to a new site, usually by following scouts which often lay down chemical trails (Jeanne 1981). At the new site, workers begin nest construction. The queens lay eggs in the cells and workers care for the developing brood. In most species more and more combs are added as the colonies grow. Typically, production of males and swarming to produce daughter nests occur only after the colony has produced several generations of workers. Because these are tropical animals, the timing of the colony cycle with respect to season is complex. Colonies may be initiated synchronously, perhaps at the start of the wet season, or they may be initiated all year round. They may endure perennially or they may be abandoned after swarm production (Jeanne 1991).

Because of the difficulties of observing individuals within enclosed nests, little is known about the behavioural aspects of the colony cycle (but see Jeanne 1991 and references therein). West-Eberhard (1978) conducted detailed behavioural observations of a colony of *Metapolybia aztecoides* by opening the nest envelope repeatedly. The colony was initiated by a swarm containing a large number of queens. As the colony aged, the queens

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died until only one remained. The remaining queen became the sole egg layer and produced a generation of daughters. Some of these females left their natal colony in a swarm and began a new colony. Since the queens in this swarm developed only after queen number had been reduced, they were very closely related. This type of colony cycle, which we will refer to as 'cyclical oligogyny', will maintain reasonably high levels of worker to brood relatedness in taxa that regularly have many queens because these queens are so closely related. However, it is unclear how widely distributed cyclical oligogyny is among tropical wasp taxa since the detailed behavioural or genetic studies necessary to document its presence have not been conducted on many species (Forsyth 1978; West-Eberhard 1978).

The extent to which the presence of multiple queens reduces relatedness among colony workers can be calculated precisely (see Appendix). Assuming no inbreeding, single mating and no covariance between number of queens in a colony and relatedness among those queens, the relationship between queen number and the relatedness is the following

$$r = \tfrac{3}{4}P + (r_{\text{queen}}/4)(1 - P) \quad (1)$$

where

$$P = (1/q_k^h) + \bar{q}_k\sigma^2_{\text{p(within)}} \quad (2)$$

where r_{queen} is relatedness among queens, r is relatedness among their progeny and P is the proportion of full siblings (see Appendix). In equation (2), q_k^h is the harmonic mean number of queens, $\bar{q}_k$ is the arithmetic mean number of queens and $\sigma^2_{\text{p(within)}}$ is the within-group variance in egg laying among queens. The first term of equation (1) represents the contribution to overall relatedness of full sisters and the second term represents the contribution of progeny of different queens related by r_{queen}. If queens are inbred, then r among progeny will be higher than predicted by equation (1). If queens mate multiply, r will be lower than predicted by equation (1).

If three conditions are met we can solve for r with no information other than average queen number. First, if relatedness among queens is not different from relatedness among their progeny, then $r = r_{\text{queen}}$. This assumption will be met if reproducing colonies are a random subset of all colonies with respect to relatedness, if the queens of new colonies are a random set of progeny from their old colony, and if these reproductive females do not sort preferentially onto new colonies. Second, if there is no variance in queen numbers between colonies then $q_k^h = \bar{q}_k$. The harmonic mean of queen number is used in equation (1) rather than the arithmetic mean because the harmonic mean more accurately reflects the effect of number of queens on relatedness (see Appendix; Wade 1985), but these will be equal if there is no variance among colonies in queen number. Third, if there is no variance within colonies in reproductive success of queens (i.e. they lay equal numbers of eggs that develop into equivalent castes of adults), then $\sigma^2_{\text{p(within)}} = 0$. This will be the case if there is no dominance among queens.

Solving equation (1) for r, if these three assumptions are met, shows that relatedness among nestmates is expected to be 0·75, 0·19, 0·10 and 0·05 for 1, 5, 10 and 20 queens, respectively. Thus, it is clear that relatedness declines rapidly with increasing queen number. On the other hand, if these conditions are not met, we can decompose the contribution of each factor to higher levels of r than are expected from queen number alone.

We investigated the relationship between relatedness and queen number in *Parachartergus colobopterus*. We chose this species because it exhibited the lowest value of nestmate relatedness of any of the three species studied to date (Queller et al. 1988). Furthermore, it is a locally abundant and docile wasp, which makes it an easy subject to study (Strassmann et al. 1990).

METHODS

We collected 21 entire colonies of *P. colobopterus*, and samples from three additional colonies in August 1988, from buildings (classrooms and farm structures) on the campus of Universidad Central de Venezuela, Facultad de Agronomía, Maracay, Venezuela (Table I). Much of the campus has native vegetation because it borders undisturbed land that extends up to Henri Pittier National Park on several sides. Most of the nests were located close to the park. The vegetation is a scrubby dry forest on the inland side of the coastal range at an elevation of 445 m. August is the middle of the rainy season in Maracay, which usually lasts from June to October.

We used two methods to collect colonies. In the first we taped a net around the nest, and the wasps were then coaxed into it by removing the outer

Table I. Colonies of *P. colobopterus* collected in August 1988

Nest	Nest age*	No. dissected	No. combs	No. cells	No. queens†	No. workers†	No. males†
V4-16	1	48	1	63	10	45	0
V4-21	1	61	1	127	22	72	0
V4-54	1	63	1	48	1	98	0
V4-55	1	63	9	466	29	71	0
V4-14	2	161	13	3235	64	661	48
V4-25	2	50	7	869	40	192	0
V4-3	2	164	11	1666	54	424	0
V4-4	2	164	12	3019	88	848	0
V4-56	2	63	7	570	28	184	0
V4-6	2	150	8	1022	37	349	0
V4-7	2	64	10	1378	50	287	0
V4-10	3	54	4	429	5	80	0
V4-11	3	64	4	345	15	83	0
V4-17	3	19	2	170	1	37	0
V4-19	3	43	5	285	11	66	0
V4-20	3	157	16	2321	55	565	3
V4-26	3	152	11	1728	18	608	26
V4-33	3	63	7	609	1	157	9
V4-43	3	63	8	274	1	68	0
V4-32	4	53	14	4373	13	180	4
V4-57	4	76	15	2082	33	178	33

*The youngest nest age is 1; see text for explanation of age categories.
†See text for estimation techniques for these numbers.

envelope of the nest. Alternatively, one person held a net underneath the nest and another person sliced the entire nest off the wall and into the net using a long knife. Both methods were very effective; nearly all wasps at the site were captured. Since these collections were made during the day, some foragers were off the nest. To obtain an estimate of the numbers of uncollected individuals, we returned to all nest sites between 2–24 h after nest collection and counted the returned foragers. We preserved most of the collected wasps in Kahle's solution (Borror et al. 1964), which allowed us to measure ovarian development, presence or absence of sperm in the spermatheca and various size measures. We also preserved a random sample of about 100 wasps per nest in liquid nitrogen to conduct electrophoresis in addition to ovarian measures.

Numbers of queens, workers and males were determined by dissecting a sample of individuals from each colony. In no case did we dissect fewer than 20% of all collected wasps from a colony and on average we dissected 64% of wasps per colony (Table I). To estimate the number of queens (and males) in a colony, we assumed that the proportion of queens (and males) in the sample not dissected was the same as it was in the dissected sample. Males are superficially similar in appearance to females but could be identified by presence of testes. A queen was identified as any female that had at least one mature egg in her ovaries. We estimated the share of egg laying obtained by a given queen by counting the number of mature eggs in her ovaries. This technique indicated that a number of females were laying eggs simultaneously, a finding corroborated by behavioural observations on several nests. In these observations we also noted that there was no egg guarding, at least in young colonies. All females with undeveloped or slightly developed ovaries were assigned to the worker category. We also collected 58 foragers as they flew back to the colony and dissected them and found that none of them had developed ovaries. We were therefore justified in assuming that foragers not present when the colony was collected were workers. This assumption did not change the results of any statistical analyses, partly because the number of uncollected wasps was small ($\bar{X} \pm \text{SE} = 62 \pm 11$) relative to the total number of adults.

To investigate caste differences, we measured seven morphological traits on wasp parts taped to strips of herbarium paper. We took measurements through a dissecting microscope (Wild M5A)

Table II. Enzymes scored and allele percentages in the Maracay population of *P. colobopterus*

Enzyme	Allele percentages		
	1	2	3
Peptidase (leucylglycylglycine) (PEPLGG)	84·0	16·0	
6-Phosphogluconate dehydrogenase (PGD)	96·3	3·6	0·1
Phosphoglucose isomerase (PGI)	97·6	2·4	
Glyceraldehyde-3-phosphate dehydrogenase 1 (G3PD1)	98·1	1·9	
Glyceraldehyde-3-phosphate dehydrogenase 2 (G3PD2)	98·5	1·5	
Isocitrate dehydrogenase (IDH)	99·6	0·3	0·1
Beta-hydroxybutyrate dehydrogenase (HBDH)	99·9	0·1	

equipped with a graticule at 25× and 50×. We measured head width, antennal scape length, foreleg tibia length, length of the first discoidal cell in the forewing, the 2 mcu vein in the forewing and the distance between the cua and the 1 rm veins in the hind wing, and we counted the number of hamuli (wing-hooks) on the hind-wing (see Spradbery 1973, plate II, for wing cell and vein identifications). One sample of head widths was measured with a computer-aided tree-ring measuring screw.

We determined the relatedness of females using the method of Queller & Goodnight (1989) on allozyme data. We were able to resolve seven polymorphic loci in this population of *P. colobopterus* (Table II) using standard horizontal starch gel electrophoresis. Individuals were placed in 0·5-ml microfuge tubes containing approximately 200 µl of 2-mm glass beads, 50 µl of grinding buffer (0·1 M Tris–Cl, pH 7·0, 25% glycerol, 1·5% ethanol, 0·1% Triton X-100, 0·1% β mercaptoethanol, 1 mg/ml NAD, NADP and bovine serum albumin, preserved with 0·25 mg/ml sodium azide) and 100 µl of carbon tetrachloride. Samples were then ground for 10 s in a mini-bead beater (Biospec Products) and spun in a microcentrifuge for 10 min at 4°C. Samples were kept on ice whenever they were not being ground or centrifuged. Filter paper wicks (Whatman 3 mm) were soaked in the aqueous (upper) phase then inserted into a slit made across a 12% Connaught starch gel. Buffer trays contained an amine citrate buffer modified from Clayton & Tretiak (1972): 0·04 M citric acid adjusted to pH 6·7 with *N*-3-(aminopropyl) morpholine. Gels were made up with a 1/20th concentration of the same buffer but the buffer/starch slurry was adjusted to pH 7·0 with *N*-3-(aminopropyl) morpholine before cooking. Gels were run for 3·5–4 h at 35 mA in a 4°C refrigerator with trays of ice on top of them. They were then sliced and stained according to standard protocols (e.g. Murphy et al. 1990; a detailed protocol is available from the authors).

We divided the 21 colonies into four age categories (Table I). (1) Four colonies were new swarms whose nests had not yet had any emergences of adults from the nest cells. (2) Seven colonies were young, with rapidly growing nests and many emergences. This type of colony could be distinguished from older ones by the clear age pattern apparent in the combs (Richards 1978). The youngest combs (which are added at the bottom of the nest) had no emergences; slightly older ones had emergences only in their centres; the oldest combs had emergences towards the edges and pupae in the centres of combs where cells were being used for the second time. (3) Eight nests were no longer adding combs (all combs had multiple emergences) but were still using most of the cells in the existing nest. These had no pattern to brood distribution in the nest. (4) Two nests were old and declining; most of the combs were no longer being used to rear brood. For most analyses all nests were used. For those analyses where we investigated changes with colony age, we considered each age class separately.

RESULTS

Colony Characteristics

The 21 colonies averaged (±SE) 1194±264 cells distributed among an average of 7·9±1·0 combs (Table I). There was an average of 250±52 workers and 27±6 queens per colony. The harmonic mean of queen number was 4·9. Six of the colonies had an average of 21±7 males each. The other colonies had no males.

Table III. Morphological measures of workers and queens in *P. colobopterus*

Variable	Queens			Workers			Kruskall–Wallis
	$\bar{X}$	SE	N	$\bar{X}$	SE	N	P
Head width (mm)	2·27	0·110	17	2·25	0·005	104	0·08
Antennal scape (mm)	0·86	0·005	17	0·84	0·003	104	0·16
Foreleg tibia (mm)	1·82	0·015	16	1·81	0·006	91	0·67
1st discoidal cell (mm)	3·25	0·300	17	3·23	0·100	102	0·32
2mcu vein* (mm)	0·69	0·100	17	0·69	0·003	101	0·65
Cua to lrm vein* (mm)	1·52	0·011	17	1·51	0·006	103	0·34
Number of hamuli	6·35	0·209	17	6·53	0·067	104	0·49
Principal component 1	20·65	0·135	16	20·52	0·055	86	0·29
Principal component 2	4·11	0·217	16	4·29	0·066	86	0·58

*See text for definitions of these veins.

Morphological and Ovarian Characteristics of Workers and Queens

One indication that the presence of a mature egg is an appropriate criterion to distinguish queens from workers is a correspondence between queen status and insemination, since we expect all queens to have mated so they can lay eggs that become females (females arise from fertilized eggs) as well as males (males arise from unfertilized eggs). Of the 113 queens that were examined for insemination, all but one were inseminated. We examined 678 workers and found that only one was inseminated. Thus, our criterion for deciding queen status is a robust one. We dissected 233 queens from 24 nests and found that on averge ($\pm$SE) they had $5{\cdot}4 \pm 0{\cdot}3$ (range = 1–25) mature eggs. Of these, 24% had one egg, 34% had 2–5 eggs, and 42% had 6–25 eggs in their ovaries.

We further analysed the data to determine whether larger queens have more developed ovaries, but we found no correlation between head width and number of mature eggs in either of two sets of queens whose heads were measured using two different techniques (sample 1, Spearman $r = 0{\cdot}11$, $N = 17$, $P > 0{\cdot}6$; sample 2, Spearman $r = 0{\cdot}17$, $N = 25$, $P > 0{\cdot}4$).

Workers and queens did not differ significantly in any of the morphological characters measured (Table III). These measures were combined in a principal components analysis to investigate shape differences. Two principal components had eigenvalues greater than one (Table IV). When workers and queens were compared for values of these principal components, they did not differ significantly (Table III). We detected no morphological differences between workers and queens in *P. colobopterus*.

Relatedness

The relatedness of female nestmates (a random sample of workers and queens) was calculated to be 0·31 from allozyme data (Table V). The relatedness of queens in the same colony was 0·67 (95% confidence interval: $0{\cdot}49 < r < 0{\cdot}84$; Table V). The relatedness of workers in the same colony was 0·31 (95% confidence interval: $0{\cdot}07 < r < 0{\cdot}54$). The difference between queen relatedness and worker relatedness was 0·37 (95% confidence interval: $0{\cdot}22 <$ difference $< 0{\cdot}51$, paired jackknife on the difference between relatedness values). We found no evidence of inbreeding (Table V).

Comparison of Relatedness from Queen Number and Allozyme Data

Our best estimate of relatedness from equation (1) was obtained by substituting the measured values for relatedness among queens, the harmonic mean of queen number and the variance in within-colony reproduction into equations (1) and (2). Substituting 0·667 for r_{queen}, 4·9 for q_k^h and 0·0007 for $\sigma^2_{p(\text{within})}$, we obtain $r = 0{\cdot}30$. This is very similar to the allozyme measure of $r = 0{\cdot}31$. Below we investigate the contributions of queen relatedness, queen number and queen dominance to this value for nestmate relatedness.

Determinants of Relatedness

We can investigate the relative contributions of three factors to raising average relatedness among

Table IV. Morphometric principal components for females of *P. colobopterus*

Variable	Principal component 1	Principal component 2
Head width (mm)	0·44	0·14
Antennal scape (mm)	0·35	0·10
Foreleg tibia (mm)	0·42	−0·22
1st discoidal cell (mm)	0·48	−0·12
2mcu vein* (mm)	0·32	0·26
Cua to lrm vein* (mm)	0·42	−0·05
Number of hamuli	−0·006	0·92
Eigenvalue	3·36	1·08
Percentage of variance explained	48	15

*See text for definition of these veins.

Table V. Nestmate relatedness of workers and queens in *P. colobopterus*

	Number of colonies	Number of individuals	Inbreeding $f \pm$ SE	Relatedness $r \pm$ SE
Random sample	24	375	$-0{\cdot}012 \pm 0{\cdot}031$	$0{\cdot}314 \pm 0{\cdot}109$
Workers	24	334	$-0{\cdot}035 \pm 0{\cdot}028$	$0{\cdot}306 \pm 0{\cdot}114$
Queens	19	156	$0{\cdot}243 \pm 0{\cdot}124$	$0{\cdot}668 \pm 0{\cdot}083$

Table VI. Determinants of relatedness among workers

Source of variation	Predicted relatedness	Percentage of best estimate
No corrections	0·037	12
Queen relatedness correction only	0·190	63
Harmonic mean correction only	0·220	73
Queen fertility correction only	0·057	19
Harmonic mean and queen relatedness corrections only	0·290	97
All corrections	0·300	100

workers: (1) relatedness among queens of the same colony, (2) variation in numbers of queens per colony and (3) unequal within-colony reproductive success among queens. We do this by examining the effect of each factor in turn on a default estimation of relatedness among workers that originally ignores these three factors. For this default estimate we assume first, that relatedness among queens is not different from relatedness among their progeny, so $r = r_{queen}$. Second, we assume that there is no variance in queen numbers among colonies so $\tilde{q}_k^h = \tilde{q}$. Third, we assume that there is no variance within colonies in reproductive success of queens (i.e. they lay equal numbers of eggs that develop into equivalent castes of adults), so $\sigma^2_{p(within)} = 0$. Substituting these values into equation (1) gives a relatedness value among workers of $r = 0{\cdot}037$ (Table VI). Clearly this is much lower than the allozyme value of 0·31.

How much do the three components of equation (1), mentioned above, contribute to elevating relatedness among workers to 0·30 (Table VI)? If we use the measured value of 0·667 for relatedness among queens, and do not change the other factors, then $r = 0{\cdot}19$. If we use the harmonic mean of queen

number of 4·9, which takes variation in queen numbers among colonies into account, and do not change the other factors, then $r=0·22$. These two factors combined yield $r=0·29$. If we use the measured variance in reproductive success among queens of the same colony of 0·0007, and do not change the other factors, then $r=0·057$. All three corrections yield $r=0·30$.

Queen Number and Colony Age

We did not find systematic demographic evidence for cyclical oligogyny in *P. colobopterus*. Colony age was not correlated with number of queens (Spearman $r=-0·25$, $N=21$ colonies, $P>0·2$) or percentage of queens (Spearman $r=-0·41$, $N=21$ colonies, $P>0·06$). If anything, queen number is best explained by nest size as measured by number of cells (Spearman $r=0·71$, $N=21$ colonies, $P<0·002$) (Table I). However, there were four colonies with only one queen, and three of these were in the third oldest age category. Older colonies were more likely to have males (Spearman $r=0·50$, $N=21$ colonies, $P<0·03$).

DISCUSSION

Two factors proved to be significant in elevating the relatedness of female colony members in *P. colobopterus*: (1) high relatedness of queens approaching that for full sisters and (2) high variance in numbers of queens per colony. Both these factors may be explained by cyclical oligogyny where colonies are founded by many queens, but only produce daughter swarms when queen number is reduced in the mother colony. As the colony matures, many of the queens die until only one or a few remain. At this point the brood in the colony is derived from few queens and its members are therefore closely related. Only then does the cyclically oligogynous colony produce daughter swarms. Thus, the pattern observed by West-Eberhard (1978) in *Metappolybia aztecoides* may be general.

However our demographic data did not reveal a clear relationship between oligogyny and colony age. Three of four single queen colonies were mature but not declining and were among the smallest colonies of that stage. Our measure of colony age is rough, however, and our sample sizes of colonies in each age category are small (though larger than other studies), so we might have missed a pattern that exists. Taken alone, the presence of colonies with only one or two queens is not evidence for the cyclical oligogyny hypothesis because occasional small colonies with few queens can be explained in many other ways and because this hypothesis also holds that this is when swarms are produced.

An alternative explanation for high relatedness among queens is that reproductive swarms are comprised of a closely related subset of females from the natal nest. Such sorting would require within-colony discrimination on the basis of relatedness. There is no evidence on this point for epiponine wasps and the small amount of evidence from other groups is mixed (Page et al. 1989; Carlin & Frumhoff 1990). In *Polistes annularis*, females that emerged from the same natal nest do not sort on to new nests with closer relatives from among their natal nestmates (Queller et al. 1990).

A careful study of *P. colobopterus* at Hato Masaguaral failed to find evidence that females discriminate colony mates from other females (Gastreich et al. 1990). More casual observations of inter-colony transfers in Maracay suggest that females of *P. colobopterus* do sometimes make this kind of discrimination (Queller & Strassmann, personal observations). These findings do not support, but also do not exclude, the possibility of finer levels of kin discrimination of the sort suggested above. Clearly, further work is necessary to identify how high levels of relatedness among queens are maintained.

We did not find that within-colony variation in egg laying was important in maintaining high levels of relatedness. Our study might be criticized because we used numbers of mature oocytes in the ovary as a measure of reproductive dominance rather than behavioural observations, but brief behavioural observations supported the view that many females lay eggs on young colonies (Strassmann, personal observations). Furthermore, no egg eating was observed and the absence of egg guarding further suggests that it is absent or rare.

If workers are morphologically constrained from reproducing, they will be more likely to become helpers even when they are distantly related to the brood. However, we were unable to find morphological differences between workers and queens.

We found surprisingly high values of nestmate relatedness in *P. colobopterus*: $0·31 \pm 0·11$. We also found that relatedness in *P. colobopterus* at Hato Masaguaral, about 200 km south of Maracay, was

much lower in April 1988 ($r = 0{\cdot}11 \pm 0{\cdot}05$; Queller et al. 1988). These data are not sufficient to determine whether the difference in relatedness is caused by the difference in location or by seasonal differences between April and August (towards the end of the dry season and the middle of the wet season), or by other habitat differences. There have been so few repeated measures of relatedness in natural populations that it is hard to predict how variable relatedness will prove to be (but see Crozier et al. 1987; McCauley et al. 1988; Strassmann et al. 1989).

The value of the relatedness of workers ($0{\cdot}31 \pm 0{\cdot}11$) is not much lower than that found in primitively eusocial wasps from Venezuela (Strassmann et al. 1989). For example, *Polistes versicolor* and *Polistes canadensis* had nestmate relatedness values of $0{\cdot}37 \pm 0{\cdot}08$ and $0{\cdot}34 \pm 0{\cdot}10$ respectively (Strassmann et al. 1989). In *Polistes* sociality can be explained by the benefits of group nesting when relatedness falls in this range (e.g. Queller 1989; Strassmann & Queller 1989), and this may also prove to be true in the epiponines when relatedness among nestmates is this high. It is interesting, however, to note that the factors that increase relatedness among colony mates in *Polistes* are not the same as those for the epiponine wasps. Colonies of *Polistes* are begun by few queens as compared to epiponine wasps and a dominance hierarchy in which only one of these queens lays most of the eggs is quickly established (West-Eberhard 1969; Strassmann 1981). Thus, the within-colony variance in reproductive success would be high, which is not the case for *P. colobopterus*.

There is some indication that the patterns relatedness and queen number that we have observed in *P. colobopterus* occur in other epiponine wasps. We found that relatedness among queens was elevated in *Polybia occidentalis*, *Polybia emaciata* and in *Protopolybia exigua* (Queller et al., unpublished data) and West-Eberhard (1990) found that relatedness among queens was elevated in *Agelaia multipicta*. The general finding of elevated relatedness among epiponine wasps, even in the presence of many queens, means that we have come quite a way towards explaining the maintenance of sociality in wasps with multi-queen colonies.

APPENDIX

This derivation follows the general approach of Wade (1985) with the added feature of relatedness among queens. The relatedness among the progeny of a group of singly mated haplodiploid queens is

$$r = \tfrac{3}{4}P + (r_{\text{queen}}/4)(1 - P) \tag{A1}$$

where P is the probability that two colony-mates share the same mother. We assume that relatedness among the queens, r_{queen}, does not covary with the probability of sharing a mother. Let p_{jk} be the fractional contribution of the jth queen of the kth colony to the colony's progeny. Then P can be written as

$$P = (1/g) \sum_{k=1}^{g} \sum_{j=1}^{q_k} p_{jk}^2 \tag{A2}$$

where g is the number of groups (colonies) and q_k is the number of queens in the kth colony. Now p can be written as the group mean value plus the deviation from the group mean: $p_{jk} = p_{\cdot k} + (p_{jk} - p_{\cdot k})$. Substitution into equation (A2) yields

$$P = (1/g) \sum_{k=1}^{g} \sum_{j=1}^{q_k} p_{\cdot k}^2 + (1/g) \sum_{k=1}^{g} \sum_{j=1}^{q_k} (p_{jk} - p_{\cdot k})^2$$

The cross-product terms do not appear because the sum of the deviations from the group mean is zero for each group. The mean fractional contribution to a group, $p_{\cdot k}$, is equal to $1/q_k$. Substituting this in the first term and then multiplying and dividing by the mean queen number in the second term gives

$$(1/g) \sum_{k=1}^{g} (1/\bar{q}_k) + \bar{q}_k \left[\sum_{k=1}^{g} \sum_{j=1}^{q_k} (p_{jk} - p_{\cdot k})^2 \right] \Big/ (g\bar{q}_k)$$

The first term is now seen to be the reciprocal of the harmonic mean queen number. The quotient in the second term is a within-group variance of the p-values, that is, the squared sum of the deviations from the group means divided by the number of sums taken. Incorporating these features into the notation gives

$$P = (1/q_k^h) + \bar{q}_k \sigma^2_{p(\text{within})}$$

which is equation (2) of the main text.

In our data set, the fractional contribution of each queen was estimated from the number of mature eggs found in the ovaries. Because of the large numbers of wasps we left a fraction of each colony, x_k, undissected. This means there are additional queens that we did not count. If we assume that the undissected portion of each colony is similar to the dissection portion, we can still estimate equation (1) by using

$$P = (1/g) \sum_{k=1}^{g} \sum_{j=1}^{q_k} p_{jk}^2/(1 + x_k)$$

Here the queen number, q_k, and the fractional contributions, p_{jk}, are still calculated from the sample as before. But the probability of sharing a mother must be adjusted to account for the fact that there are expected to be k additional queens for every one counted, and that these contribute no full sisters to the progeny of the counted queens. Substituting into equation (A1) and following the same steps as above yields

$$r = \frac{1}{1 - \bar{x}_k}\left[\frac{3}{4}\left(\frac{1}{q_k^h} + \bar{q}_k \sigma^2_{p(\text{within})}\right) + \frac{r_{\text{queen}}}{4}\left(1 + \bar{x}_k - \frac{1}{q_k^h} - \bar{q}_k \sigma^2_{p(\text{within})}\right)\right]$$

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