Molecular Ecology (1998) 7, 709–718

The genetic structure of swarms and the timing of their production in the queen cycles of neotropical wasps

JOAN E. STRASSMANN, KEITH F. GOODNIGHT, CEAL J. KLINGLER and DAVID C. QUELLER
Department of Ecology and Evolutionary Biology, Rice University, 6100 Main, Houston, TX 77005–1892, USA

Abstract

Kin selection theory has received some of its strongest support from analyses of within-colony conflicts between workers and queens in social insects. One of these conflicts involves the timing of queen production. In neotropical wasps, new queens are only produced by colonies with just one queen while males are produced by colonies with more queens, a pattern favoured by worker interests. We now show that new colonies, or swarms, have few queens and variable within-colony relatednesses which means that their production is not tied to new queen production. The queens in these swarms are seldom the mothers of the workers in the swarm. Therefore, either colonies producing swarms have very many queens, or queens joining daughter swarms are reproductive losers on the original colonies. As new colony production is not linked to queen production, it can occur at the ecologically optimum time, i.e. the rainy season. This disassociation between queen production and new colony production allows worker interests in sex ratios to prevail without hampering new colony production at the most favourable season, an uncoupling that may contribute to the ecological success of the Epiponini.

Keywords: eusociality, genetic relatedness, kin selection, microsatellites, split-sex ratios, Vespidae

Received 12 May 1997; revision received 10 November 1997; accepted 9 December 1997

Introduction

In most social insects, production of new queens is a prelude to production of daughter colonies; it is the role of the new queens to independently found new colonies (Wilson 1971). Where colony reproduction is by fissioning, also known as swarming, the same pattern often holds, for example in honeybees and army ants (Wilson 1971). However, the selective factors operating on the timing of new queen and new colony production may be different, and may not be the same for workers and queens. This is particularly true of the subject of this study, the Epiponini, a highly successful tribe of neotropical swarm-founding wasps characterized by large, multiple-queen colonies (Richards 1978; Jeanne 1991).

If new colony production occurs only shortly after new queen production in epiponine wasps, it may be constrained by other pressures operating on queen production. In epiponine wasps, new queens are produced in single-queen colonies while males are produced in colonies with more queens, a pattern consistent with worker sex-ratio interests (West-Eberhard 1978; Strassmann *et al.* 1991; Hughes *et al.* 1993; Queller *et al.* 1993a). Workers prefer this pattern because they are three times more closely related to their sisters than to their brothers on single-queen colonies and equally related to the nieces and nephews that make up most of the brood on multiple-queen colonies (Trivers & Hare 1976). When both single-queen and multiple-queen colonies occur in the same population, the single-queen colonies should specialize in queen production while the multiple-queen colonies should specialize in male production (Grafen 1986; Boomsma & Grafen 1990, 1991; Pamilo 1991; Boomsma 1993; Queller & Strassmann 1998).

At any given time, most colonies have many queens (Jeanne 1991; Hughes *et al.* 1993) and are therefore not at the stage where they will produce new queens (West-Eberhard 1978; Strassmann *et al.* 1991; Queller *et al.* 1993a,b; Gastreich *et al.* 1993). They appear to arrive at the queen-producing stage at haphazard times, whenever all but one old queen has died. If colonies are constrained to produce new colonies only after producing new queens, then many colonies would be unable to produce them at otherwise optimum times, such as the rainy season (Jeanne 1991; Strassmann *et al.* 1997). This limitation in

Correspondence: J. E. Strassmann. Tel.: +01-(713)-527-4922; Fax: +01-(713)-285-5232; E-mail: strassm@rice.edua

new colony production, imposed by the queen cycle, might be expected to reduce the ecological success of swarm-founding wasps. In fact, the 200 species in 21 genera comprising the Epiponini are ecologically dominant and highly successful (Richards 1978; Wilson 1990; Jeanne 1991). A resolution to this conflict could be the uncoupling of queen and new colony production, something that could be attained if new colonies were not dependent on large numbers of queens during their initial periods.

Whether new colony production rapidly follows new queen production in the Epiponini is uncertain. Forsyth (1978, 1981) states that swarming occurs after a new cohort of queens begins ovarian development in *Metapolybia azteca* and *Polybia occidentalis*. If this is the case, then swarms should contain large numbers of queens, but the swarms he tabulates have modest queen numbers, averaging eight and 11, respectively. In *Metapolybia aztecoides*, West-Eberhard (1982) found that swarming was one consequence of an emergence peak of females and ascribed swarming to heightened aggression among the many young potential queens, again implying that swarming is likely to be preceded by queen production.

The current study characterizes colony size, relatedness and queen numbers in new colonies in three species of epiponine wasps, *Parachartergus colobopterus*, *Polybia occidentalis* and *Protopolybia exigua*. We also investigate the relationship between new colony, queen and male production. For one of these species, *Pa. colobopterus*, we used DNA microsatellites to get precise estimates of both genetic relatedness and parentage within the swarms (or young, pre-emergence colonies) so that we could also evaluate exactly which queens and workers joined swarms. It is difficult to observe colonies in the process of producing new colonies or queens, but we can infer something about these colonies from the characteristics of their swarms and from genetic relatednesses. From relatedness among queens, among workers in new pre-emergence colonies, and among workers in male-producing colonies, we can infer numbers of reproductively active queens producing these classes of individuals.

Materials and methods

Field collecting

We located swarms in the process of establishing new nests of *Parachartergus colobopterus* on buildings of the Facultad de Agronomía, Universidad Central de Venezuela, Maracay, Venezuela (10°16' N 67°36' W, 450 m elevation). Swarms of *Pa. colobopterus* begin nest construction within approximately 1 h of landing on a new nest site. Within the first day or two, the workers build several combs and cover them with an envelope. Therefore, many of the swarm nests we collected had eggs. In no case had any adults hatched from the new nests, so all the adults we collected came from a parental nest elsewhere (Table 1).

We collected our 21 study swarms by placing a zip-shut plastic bag over the nest and coaxing all adults and any nest material into the bag. We revisited nest sites over the next 24 h and collected any wasps present. To maximize the representation of reproductive as opposed to absconding swarms in our sample, we collected during the rainy season when reproductive swarms are produced (Strassmann *et al.* 1997; our unpublished observations). We collected one swarm on 13 June 1991 (trip V16), four on 10 July 1991 (V17), 12 on 8 August 1991 (V18), one on 29 July 1992 (V19) and three on 27 July 1993 (V20, Table 1).

For comparison we used a sample of 20 mature colonies that had emergences that were collected in August 1988 from the same site (Strassmann *et al.* 1991). They comprise previously discussed colonies (Strassmann *et al.* 1991) except for the swarms that were included in that study.

In July 1988, we collected seven new and 28 mature colonies of *Polybia occidentalis* from trees and bushes on cattle ranches south of Calabozo, Venezuela (8°30'N, 67°30' W, 75 m elevation). The new colonies had nest numbers V2–118, V2–126, V2–130, V2–133, V2–134, V2–136 and V2–146 (Table 1 in Queller *et al.* 1993b). In July 1989, we collected seven new and 30 mature colonies of *Protopolybia exigua* built on orange tree leaves in Bejuma, Venezuela (10°11'N, 68° W, 500 m elevation). The new colonies had nest numbers V8–36, V8–38, V8–40, V8–41, V8–44, V8–46, and V8–54 (Table 1 in Gastreich *et al.* 1993).

Assessment of age and reproductive status

We counted the number of mature and nearly mature eggs in the ovaries and the length of the longest oocyte in micrometer units at 25× magnification. We determined whether the spermatheca contained sperm and, in the case of *Parachartergus colobopterus*, extracted the sperm for paternity analysis. For analysis of young colonies from *Pa. colobopterus*, we assigned females to five age and reproductive classes, four of workers and one of queens. (A) Old females with no ovarian development; (B) young females with no ovarian development; (C) females of any age with some ovarian development but no mature eggs and no sperm in their spermathecae; (D) females of any age with mature eggs in their ovaries and no sperm in their spermathecae; (E) females of any age with mature or nearly mature eggs in their ovaries and sperm in their spemathecae (queens; Table 1).

We dissected all 1280 individuals from the 21 swarms of *Pa. colobopterus*, and dissected random samples usually consisting of most of the wasps on the nest from the other species (Strassmann *et al.* 1991; Gastreich *et al.* 1993;

Table 1 Characteristics of the swarms of *Parachartergus colobopterus*. Individual colony relatednesses shows standard errors jackknifed over loci. Sample sizes of loci for relatednesses are 10 loci for V19–42 and six loci for the others

Swarm*	No. of queens	No. of unmated egg layers	No. of workers with some ovarian development	No. of old workers with no ovarian development	No. of young workers with no ovarian development	No. of cells	Most advanced brood stage†	R ± SE (N) workers with no ovarian development	R ± SE (N) workers with some ovarian development	R ± SE (N) queens
V18–18	9	0	1	15	159	87	Eggs	0.255 ± 0.066 (15)		0.802 ± 0.146 (9)
V18–37	6	0	2	50	27	< 20	Eggs	0.213 ± 0.137 (5)		0.896 ± 0.109 (3)
V18–24	5	0	2	23	20	< 20	Eggs	0.182 ± 0.056 (10)	0.335 ± 0.27(2)	0.493 ± 0.124 (5)
V19–42	4	0	9	113	20	285	Larvae	0.189 ± 0.043 (136)	0.288 ± 0.054 (6)	0.834 ± 0.044 (4)
V20–43	3	0	0	34	0	149	Larvae	0.086 ± 0.135 (5)		0.613 ± 0.164 (3)
V20–48	2	1	8	64	53	96	Eggs	0.110 ± 0.075 (10)	0.121 ± 0.081 (9)	–0.242 ± 0.209 (2)
V17–14	2	2	22	21	44	181	Eggs	0.401 ± 0.155 (6)	0.412 ± 0.110 (13)	1 ± 0 (2)
V18–4	1	0	1	20	65	52	Eggs	0.188 ± 0.14 (5)		
V20–37	1	2	12	45	5	247	Pupae	0.175 ± 0.078 (10)	0.225 ± 0.117 (14)	
V16–51	1	0	5	1	45	7	Eggs	0.226 ± 0.102 (5)	0.238 ± 0.157 (5)	
V17–18	1	1	1	9	25	26	Eggs	0.097 ± 0.087 (10)	0.406 ± 0.307 (2)	
V18–15	1	2	12	12	10	43	Larvae	0.799 ± 0.111 (6)	0.780 ± 0.119 (7)	
V18–28	1	0	2	24	0	< 20	Eggs	0.015 ± 0.071 (5)		
V18–3	1	0	2	23	1	37	Larvae	0.161 ± 0.066 (10)	0.633 ± 0.220 (2)	
V17–8	1	3	0	10	13	20	Eggs	0.095 ± 0.077 (10)	0.009 ± 0.125 (3)	
V18–9	1	0	1	7	13	9	Eggs	0.093 ± 0.055 (10)		
V18–16	1	1	3	7	4	21	Larvae	0.283 ± 0.172 (4)	0.561 ± 0.168 (4)	
V18–33	0	0	9	42	35	< 20	Eggs	0.272 ± 0.109 (5)	0.471 ± 0.096 (5)	
V17–39	0	0	14	15	7	9	Empty	0.440 ± 0.108 (5)	0.549 ± 0.107 (5)	
V18–20	0	1	2	4	6	< 20	Eggs	0.048 ± 0.049 (6)	0.092 ± 0.125 (5)	
V18–10	0	1	2	2	2	98	Larvae	0.378 ± 0.475 (2)	0.083 ± 0.120 (3)	

* The first part of the ID reflects collection date (see text) .

† In most cases the larvae were first instar.

Queller *et al.* 1993b). We assigned individuals to one of four age categories based on degree of darkening of the sternite (Forsyth 1980; Strassmann 1991). This measure is less accurate for queens than it is for foragers, as queens of epiponine wasps do not darken as quickly because queens remain inside the nest.

Assessment of genetic relatedness

To estimate genetic relatednesses from mature colonies of *P. colobopterus*, we used allozyme variation at glyceraldehyde-3-phosphate dehydrogenase (two loci), 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, β-hydroxybutyrate dehydrogenase, phosphoglucose isomerase, and peptidase (leucylglycylglycine) as described previously (Strassmann *et al.* 1991). For relatedness among colony members of *Po. occidentalis* we used allozyme variation at adenylate kinase, isocitrate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, peptidase (leucylglycylglycine) and three phosphoglucomutase loci (Queller *et al.* 1993b). For relatedness among colony members of *Pr. exigua* we used allozyme variation at phosphoglucomutase and peptidase (leucylalanine; Gastreich *et al.* 1993).

We assayed genetic relatedness in the young colonies of *Pa. colobopterus* using DNA microsatellites as our polymorphic genetic markers (Strassmann *et al.* 1996a,b). We used 10 polymorphic trinucleotide microsatellite loci for assessing relatedness and parentage (Queller *et al.* 1993b). They were Paco41TAG, Paco3304CAT, Paco3155TAG, Paco3436AAT, Paco3457AAT, and Paco3417AAT, Paco3434AAT, Paco3107TAG, Paco3117TAG, and Paco3305CAT (Strassmann *et al.* 1996a). The last four loci were only used for swarm V19–42 from which all individuals were genotyped. In this study, these loci had between three and 17 alleles each and the most common allele varied in frequency from 0.20 to 0.75 (Table 2). Methods were standard for our laboratory (Strassmann *et al.* 1996b). They involved extracting the DNA from genomic samples, conducting a separate polymerase chain reaction (PCR) reaction for each primer pair in a 10 μL reaction, using ^{35}S as a label, running the PCR product on a denaturing sequencing gel with an M13 sequencing reaction as a size standard, exposing the gel to autoradiographic film,

Table 2 Allele lengths and representation in the sample for the 10 microsatellites in *Parachartergus colobopterus*. Frequency is calculated from a sample of 57 colonies including the 21 swarms and is weighted by colony. When frequencies do not add up to 1 it is because of rounding

Locus	Allele length	Frequency	Locus	Allele length	Frequency	Locus	Allele length	Frequency
PACO41TAG	139	0.22	PACO3117TAG	197	0.04	PACO3434AAT	106	< 0.01
	142	0.01		200	< 0.01		109	0.64
	145	0.23		203	0.43		112	0.36
	148	0.01		206	0.04	PACO3436AAT	114	0.05
	151	0.10		209	0.47		117	< 0.01
	154	< 0.01		212	0.02		120	0.22
	157	0.40	PACO3155TAG	136	0.48		123	0.03
	160	0.01		139	0.03		126	0.33
	163	0.02		164	< 0.01		129	0.34
PACO3107TAG	119	< 0.01		167	0.02		132	0.03
	131	< 0.01		170	< 0.01	PACO3417AAT	119	0.39
	134	0.12		173	0.33		122	0.04
	137	< 0.01		176	0.10		128	0.05
	140	0.06		179	0.04		134	0.06
	143	0.20		182	< 0.01		137	0.13
	146	0.03	PACO3304CAT	199	0.58		140	0.27
	149	< 0.01		202	0.15		143	0.05
	152	0.18		205	0.26		146	0.01
	155	0.05		208	0.02		170	< 0.01
	158	0.14		214	< 0.01	PACO3457AAT	171	0.03
	161	0.01	PACO3305CAT	248	0.18		183	0.21
	164	0.11		251	< 0.01		192	0.22
	167	0.03		260	0.75		195	0.01
	170	0.03		263	0.05		198	0.10
	173	0.01		266	0.02		201	0.41
	176	< 0.01		272	< 0.01		204	0.02
	179	0.01		287	< 0.01		207	0.01

and scoring the size polymorphisms (specific protocols are in Strassmann *et al.* 1996b).

We genotyped all individuals from one swarm of *Pa. colobopterus.* From the other 20 swarms we chose a subset which included all females with mature eggs in their ovaries, whether inseminated or not, and a sample of five individuals from as many of the other four categories as were available. In all, we performed DNA microsatellite analyses on 405 individuals and 38 sperm samples from inseminated females' spermathecae, plus one wasp each from 36 additional colonies to improve background allele frequency estimates.

For estimating relatednesses we used the Macintosh computer program RELATEDNESS 4.2b (Queller & Goodnight 1989; Goodnight & Queller 1995). We weighted colonies equally, estimating standard errors by jackknifing over colonies when reporting average relatednesses and over loci for relatednesses within specific swarms. For determining whether a given queen could have been the mother of any of the workers from the microsatellite data we used the Macintosh computer program KINSHIP 1.0 (Goodnight & Queller 1996), which estimates the likelihood ratio for two relatedness hypotheses; this program was also used to indicate which pairs of individuals cannot be full sisters. We also evaluated the genotypes of full sister groups obtained from these likelihood tests to verify that they fitted pedigree requirements for full sister groups. Full sisters had to share at least one allele at any given locus (from the shared haploid father), and the sistership could have only two other alleles at that locus (from the mother). Queens mate only once (Goodnight *et al.* 1996).

Effective queen number

We used worker relatedness as a common currency to compare queen numbers in colonies producing males, queens and swarms. We estimated effective numbers of queens on colonies producing queens, males and new swarms from relatednesses as follows (Queller 1993).

$$\text{Effective no. of queens} = \frac{3 - r_{\text{queen}}}{4r_{\text{worker}} - r_{\text{queen}}} \qquad \text{(eqn 1)}$$

In this equation r_{queen} is relatedness among queens and r_{worker} is relatedness among workers. We assume that it is differences in numbers of queens reproducing and not the relatedness among the queens that varies among colony types, so we used the same value for queen relatedness within species for all colony types and varied the value of worker relatedness according to what it was for each colony type. This increased the precision of our estimates as queen numbers from subsamples are often very small. The statistical tests for differences among colony types were also performed on worker relatedness alone, not varying the queen relatedness (Table 3). To do this we had to assume that workers in swarms are a random sample of workers from the parental colony as we were using them to make inferences about their natal colony. This inference is supported by our findings that workers are generally not the daughters of the queens in the swarms (this study) and that there is no subdividing by relatedness at swarming in a sample for which we had mother colonies and their swarms (Solís *et al.* 1998). We also assumed that relatedness among queens is equivalent to relatedness among workers on colonies producing queens (i.e. that queens are a random sample of females of the same age on nests producing queens). We used relatedness among workers on nests producing males as the males themselves are haploid. The lower relatedness among workers, the more queens that contributed to producing them.

Parentage in swarms of Pa. colobopterus

The workers in a swarm were produced on the parental nest, so they were not necessarily the daughters of the swarm queens. We performed maternity analyses for the 18 queens from nine swarms that had sufficient genetic data (at least three loci scored for both maternal genotype and sperm). A worker was excluded as a daughter if, at any locus, the worker's two alleles could not have been obtained from the sperm allele and one of the maternal alleles.

We also attempted to determine whether the swarm queens were reproductive losers from the parental nest, a possibility suggested by a prior analysis of colony V19–42 (Solís *et al.* 1998). If swarm queens are losers, the number contributing to the swarm workers (S_c) as a fraction of the total number of swarm queens assessed for maternity (S_t) should be less than the number of queens from the parental colony contributing to the swarm workers (P_c) as a fraction of the total queens on the parental colony (P_t):

$$\frac{S_c}{S_t} < \frac{P_c}{P_t} . \qquad \text{(eqn 2)}$$

S_c and S_t were determined from the maternity analysis and dissections of swarm queens. P_c was estimated by grouping the workers into the minimum number of full-sister groups (each of which would correspond to one mother as the species is singly mated; Goodnight *et al.* 1996) and subtracting the number of swarm queens already identified as mothers. We lack an estimate of the fourth parameter, P_t, the total number of queens on parental colonies producing swarms. If this number is very large, then any observed S_c/S_t, no matter how low, could be the result a random draw from the parental queens. Therefore, we performed simulations to determine the minimum P_t consistent with swarm queens being losers, examining values of P_t for each colony, starting with the number of known queens,

Table 3 A. Relatednesses among workers on colonies of different types (see text). B. Statistical comparisons of relatedness among workers between colonies of different types. Tests are two-tailed

	Parachartergus colobopterus		*Polybia occidentalis*		*Protopolybia exigua*	
A. Colony type	Effective no. of queens	Genetic relatedness among workers (no. of colonies, no. of individuals)	Effective no. of queens	Genetic relatedness among workers (no. of colonies, no. of individuals)	Effective no. of queens	Genetic relatedness among workers (no. of colonies, no. of individuals)
All post emergence	5.0	0.283 ± 0.127 (20, 297)	3.6	0.314 ± 0.062 (29, 325)	2.8	0.400 ± 0.088 (30, 285)
Male producing	4.4	0.298 ± 0.147 (8, 116)	4.8	0.271 ± 0.066 (13, 159)	1.9	0.489 ± 0.133 (13, 107)
Queen producing	1.2	0.661 ± 0.086 (19, 156)	1.4	0.583 ± 0.081 (27, 216)	0.9	0.824 ± 0.070 (29, 223)
Swarm producing	9.6	0.226 ± 0.037 (21, 366)	> 9.3*	0.129 ± 0.082 (7,84)	4.2	0.335 ± 0.170 (7, 70)

B. Comparison	*t* statistic	d.f.	Significance	*t* statistic	d.f.	Significance	*t* statistic	d.f.	Significance
Swarm producing to post emergence	–0.44	39	NS	–1.39	34	NS	– 0.32	35	NS
Swarm producing to male producing	–0.67	27	NS	–1.31	18	NS	– 0.70	18	NS
Swarm producing to queen producing	–4.81	38	< 0.001	–2.74	32	< 0.05	– 2.97	34	< 0.01
Male producing to queen producing	–2.23	25	< 0.05	–2.48	38	< 0.05	– 2.45	40	< 0.05

*Cannot estimate with worker relatedness because it is less than 0.25 queen relatedness. To obtain this estimate, we used worker relatedness plus 1 standard error for worker relatedness.

$S_c + S_n + P_c$, and increasing by units of 1. For each hypothetical value of P_t, we ran 2500 simulations. For each simulation, each colony's S_t swarm queens were randomly drawn, with a probability of P_c/P_t of being a queen who contributes to the swarm worker pool. If queens do join swarms randomly, a point estimate of P_t is the simulated P_t at which the 2500 simulated values of S_c fall equally on either side of the observed value. Swarm queens can be viewed as reproductive losers (less likely to have contributed to the swarm workers than average) if P_t is less than the simulated threshold where fewer than 5% of the simulations yielded a simulated S_c lower than the observed value.

Results

Numbers of queens and workers in swarms and mature colonies

Numbers of queens in new colonies were low. In *Parachartergus colobopterus*, new colonies had an average of two queens, ranging from 0 to 9 (Table 1; Fig. 1). The new colonies of *Polybia occidentalis* averaged 12 queens, ranging from eight to 18. The new colonies of *Protopolybia exigua* averaged 13 queens, ranging from 0 (very young new colony V8–36) to 34.

Numbers of workers in new colonies varied among the species. In *Pa. colobopterus*, new colonies averaged 58 ± 10 workers, ranging from seven to 175 workers (Fig. 1, Table 1). New colonies of *Po. occidentalis* averaged 370 ± 49 workers, ranging from 118 to 515 (Table 1 in Queller *et al.* 1993b). New colonies of *Pr. exigua* averaged 129 ± 28 workers, ranging from 37 to 241 (Table 1 in Gastreich *et al.* 1993). Workers in swarms did not form any one class with respect to age or degree of ovarian development (Table 1; Gastreich *et al.* 1993; Queller *et al.* 1993b). One new colony of each species had males (*P. colobopterus:* V18–10, one male; *Po. occidentalis:* V2–118, 28 males; *Pr. exigua:* V8–40, 10 males). Young colonies had significantly fewer workers than mature colonies only in *P.a colobopterus* (Fig. 1).

Stage in the queen cycle of colonies producing swarms and colonies producing new queens

In all three species, relatedness-based estimates of queen number were lowest on queen-producing colonies and highest on swarm-producing colonies (Table 3, Fig. 2). All differences between queen-producing colonies and other types of colonies were significant (Table 3). The threshold queen number for male production is lower than that for swarm production but colonies producing swarms can also produce males. Colonies produce swarms when they have many laying queens, and new queens when there is only one queen.

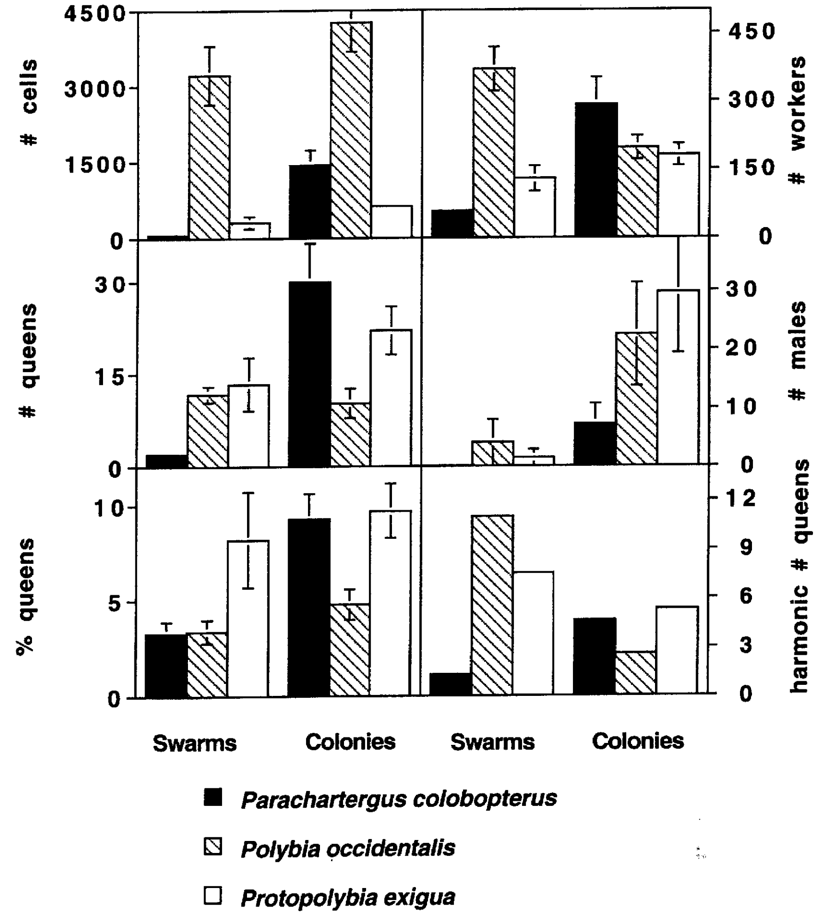

Fig. 1 Characteristics of swarms and colonies. Means and standard errors are shown. Where the SE is not shown, it is too small to be visible. Harmonic mean of queen number is also shown because this is used in relatedness estimation and is the effective number of queens.

The same pattern holds within individual colonies. In paired comparisons of relatedness, queens on swarms were significantly more closely related to each other than workers from the same young colony were to each other (combining all ovarian development classes within workers; difference between relatednesses = 0.398 ± 0.17, seven colonies). Swarm queens and swarm workers were clearly produced at different points in the queen cycle, with workers being produced after new queens became reproductive on the parental colony.

Relatedness among workers varied among the swarms, indicating that their parental colonies are at different stages in the queen cycle and have variable, but high, numbers of queens (Table 1). It is unlikely that this variation can be explained by sampling variance alone as the 95% confidence intervals of different colonies do not always overlap. For example, the lowest value for relatedness among females with no ovarian development comes from swarm V18–28 and is 0.015 with an upper limit of the 95% confidence interval of 0.19. The highest value for relatedness among workers comes from swarm V18–15 with an average relatedness of 0.799 and a lower limit of the 95% confidence interval of 0.53. These values and those between them indicate that workers are a mix of full sisters (r = 0.75) and lower relationships, usually cousins (r = 0.1875), whose exact proportions depend on the numbers of queens in the parental colony.

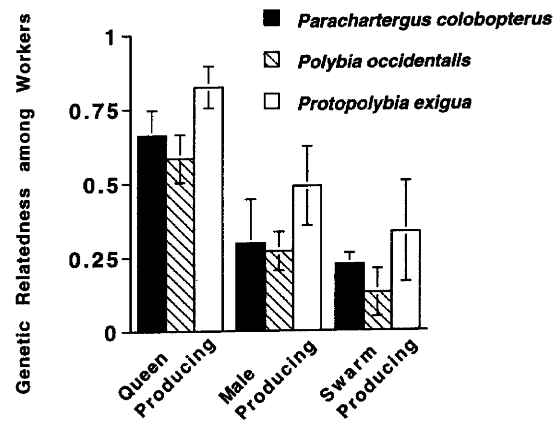

Fig. 2 Genetic relatedness (and 95% confidence intervals) among workers for colonies producing queens, swarms and males. Relatedness is lowest on swarm-producing colonies, indicating that they have the most queens. See Table 3 for sample sizes.

The estimates of queen number for queen-producing and swarm-producing colonies are based on relatedness, rather than actual queen counts, because such colonies are hard to identify (new queens are not morphologically different from workers, and swarming happens quickly). However, in *Pa. colobopterus*, wherever it was possible to compare, estimates based on dissections and on genetic relatednesses were very similar (Fig. 3), supporting the use of the genetic method for estimating queen numbers where the dissection method cannot be used. Even so, as queen number becomes large, the impact on relatedness of either additional queens or of reproductive variance among queens becomes small. Therefore the match of queen number estimates from dissections and relatedness is less meaningful when queen number is large.

The small number of queens in many swarms, one or zero for 14 swarms (Table 1), suggests that these swarms may soon requeen. Workers with some ovarian development are present (Table 1), but their low relatedness suggests they are not future queens. We compared relatednesses among workers with and without ovarian development to see if the former were more like workers or queens on relatedness grounds. Paired comparisons of relatedness between workers with and without developed ovaries indicated that there was not a significant difference between these values (difference between these relatednesses = – 0.091 ± 0.052, 15 new colonies). As expected, queens were not more closely related to workers with no ovarian development ($r = 0.350$) than they were to workers with some ovarian development ($r = 0.295$, difference between relatednesses 0.055 ± 0.047, 12 colonies).

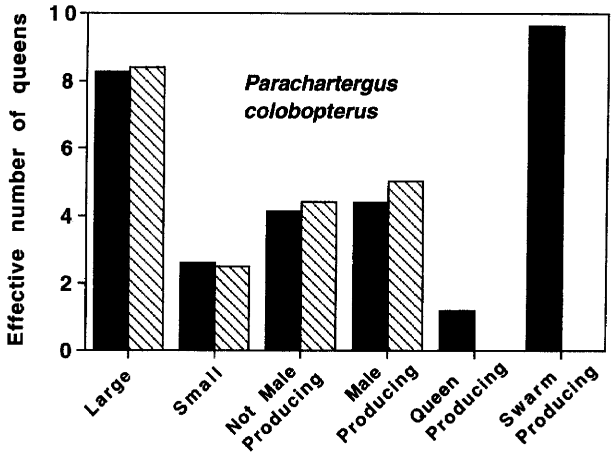

Fig. 3 In *Parachartergus colobopterus*, the figure shows estimates of effective queen number for colonies in different stages based on relatedness estimates (solid bars) and dissections (striped bars). We have no dissection-based estimates of effective queen numbers for queen producing or swarm producing colonies. See Table 3 for significance of differences. Comparisons not shown in Table 3 are not significantly different.

Table 4 Data for assessing the probability swarm queens are losers on the natal colony in *Parachartergus colobopterus*. Of the samples with both queen and sperm genotypes, S_c is the number of swarm queens that are the mother of a worker, S_t is the total number of swarm queens and P_c is the minimum number of mothers contributing to the workers in the swarm. Note that all workers were genotyped only for colony V19–42. Only samples were genotyped for the other colonies so these numbers do not reflect all sibships among the workers. See text for methods

Colony	S_c	S_t	P_c
V19–42	1	4	62
V20–48	0	2	9
V18–18	0	2	8
V17–18	0	1	7
V18–9	0	1	6
V18–24	1	5	5
V20–43	0	1	5
V18–16	0	1	4
V18–4	0	1	3

Maternity in swarms of Pa. colobopterus

Of the 18 swarm queens with good genetic data, only two could have been the mother of any of the workers in their swarm (one worker each; Table 4). This seems particularly clear for nest V19–42 where all 142 workers were genotyped and only one was the daughter of a queen. In this new colony offspring were excluded from being the daughters of all queens by an average of 5.2 microsatellite loci. All but four progeny were excluded by more than one locus. We considered whether or not null alleles at any of the loci could obscure parentage and found only one case where a null allele at one locus could result in an assignment of that worker to a queen in the swarm (in microsatellites, a null allele is usually the result of multiple base pair deletions or substitutions in the primer sites, causing amplification to fail).

Table 4 shows the numbers necessary for the simulations. The simulations yielded a point estimate of $P_t = 101$ queens on parental nest. That is, if queens join swarms randomly, this is the estimate most consistent with having observed only two swarm queens with progeny. However, the number of queens indicating significant departures from randomness is 43. If $P_t < 43$, our empirical observation of two contributing swarm queens is statistically unlikely, and swarm queens can be viewed as reproductive losers from the parental colony.

Discussion

The low worker relatedness of swarms indicates that they come from colonies with large numbers of laying queens. In contrast, colonies producing queens have very low numbers of queens and high worker relatedness (Tables 1 and 3, Figs 1 and 2), as predicted from worker interests concerning sex investment (Queller *et al.* 1993a). Therefore, queen production is not directly tied to production of new colonies, as it is in most social insects. Instead, colonies requeen only after they arrive at the single-queen stage, and the cohort of new queens typically reproduces in the old colony, lowering relatedness, before swarms are produced. Swarming may take place later, at various points in the queen-reduction cycle, presumably if conditions are appropriate.

The answer to our central question is therefore clear. The dissociation between queen cycle and the colony cycle permits worker interests to be satisfied with respect to the timing of queen and male production, without sacrificing the ability to initiate new colonies at appropriate times. This study also revealed some additional aspects of the colony size, queen number, and queen reproduction in parental colonies and swarms.

Swarms have fewer workers, queens, and males than have a random sample of mature colonies in *Parachartergus colobopterus,* but do not differ from mature colonies in these respects in *Polybia occidentalis* or *Protopolybia exigua* (Fig. 1). Perhaps swarms have fewer individuals in *Pa. colobopterus* and not in the other two species because the stable, perennial nature of *Pa. colobopterus* colonies induces them to send out smaller swarms (Strassmann *et al.* 1997). Colonies of this species have very low mortality rates and last on average 347 days and as long as 4.5 years (Strassmann *et al.* 1997). *Pa. colobopterus* also has low rates of absconding. Even after nests are destroyed and most females collected, the remaining wasps typically rebuild at the same location (Strassmann *et al.* 1991). By contrast, nests of both *Po. occidentalis* and *Pr. exigua* are much less permanent. Our surveys of *Po. occidentalis* indicate that nests often failed within 3–6 months (J. E. Strassmann and D. C. Queller, unpublished), and *Pr. exigua* is likely to be similar as nests are attached to leaves and will fall when the leaves fall. This pattern of small swarms from perennial nests and large ones from colonies with more ephemeral nests was also described by Richards & Richards (1951). However, other studies have found that swarms are more frequently small relative to the parental colony even in species suffering heavy nest predation and having multiple nests per season (Naumann 1970; West-Eberhard 1978; Forsyth 1981; Jeanne 1991).

The low number of queens in swarms of *Pa. colobopterus* is striking. Two-thirds of the new colonies had one or zero queens when they were collected. We suspect that swarms often requeen rather rapidly after colony initiation, which would result in young colonies with many queens shortly after initiation when the colonies are still in the rainy season and growing rapidly. This queen production following swarming would also help explain the observed rainy-season dip in relatedness among workers (Strassmann *et al.* 1997). As queens are normally highly related, we further suspect that the new queen cohorts must come from the high-relatedness brood of the new colony, rather than from among the high-relatedness workers. However, our collections clearly indicated that colonies at all stages of both nest development and queen cycle can be found at a single date (Strassmann *et al.* 1991; Gastreich *et al.* 1993; Queller *et al.* 1993b).

In *Pa. colobopterus,* seven of the nine swarm queens with sufficient genetic information had no daughters in the genotyped swarm progeny, suggesting that swarm queens may be reproductive losers on the parental colonies. Our simulations show that this number is expected if there are 101 queens on parental colonies. However, seven out of nine nonreproducing queens can be said to be statistically unlikely only if there are fewer than 43 queens on parental colonies. We do not have enough information to settle this question because colonies are hard to find in the process of swarming. Colonies with as many as 101 queens are very rare but those with at least 43 queens are not (with zero and five representatives, respectively, in our 17 postemergence colonies; Strassmann *et al.* 1991). Our worker relatedness estimate from swarm workers yields an estimate of 9.7 queens on parental colonies (Table 3), much lower than the threshold of 43 indicating loser queens, but there are two problems with this comparison. First, the 9.7 is an estimate of effective queen number, representing a harmonic mean over colonies, and assuming all queens reproduce equally, so it is not strictly comparable to a real queen count. Second, the 9.7 estimate is very sensitive to small errors in worker relatedness, so a much higher value is possible. Thus, as we cannot exclude the possibility that swarms are produced by a subset of colonies with high queen numbers, it seems premature to conclude that swarm queens are losers.

We now understand more about the colony cycle of *P. colobopterus* than perhaps any other epiponine wasp. But there are still important elements that we have not worked out. For example, we do not know if workers or queens produce males. We do not know what drives the decrease in numbers of queens, what the timing of that decrease is, or what determines the winners.

Acknowledgements

We thank Colin Hughes, Jorge Negrón-Sotomayor, and Karin Gastreich for help with field work. We thank Juan Castillo A., Simonetta Holley de Castillo, Maria Antonietta Castillo Holley, Tomás Blohm, Tom Swain and Frederico Arp for facilitating access to study areas, help with field logistics and being delightful hosts in Venezuela. We thank the Universidad Central de Venezuela in Maracay for permitting us to work in their grounds. This research was supported by National Science Foundation grants BSR-9021514, IBN92–10051, and IBN95–07515.

References

Boomsma JJ (1993) Sex ratio variation in polygynous ants. In: *Queen Number and Sociality in Insects* (ed. Keller L), pp. 86–109. Oxford University Press, Oxford, UK.

Boomsma JJ, Grafen A (1990) Intraspecific variation in ant sex ratios and the Trivers–Hare hypothesis. *Evolution*, **44**, 1026–1034.

Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. *Journal of evolutionary Biology*, **3**, 383–407.

Forsyth AB (1978) Studies on the behavioral ecology of polygynous social wasps. PhD Thesis. Harvard University, Cambridge, MA.

Forsyth A (1980) Worker control of queen density in hymenopteran societies. *American Natur*, **116**, 895–898.

Forsyth AB (1981) Swarming activity of polybiine social wasps (Hymenoptera: Vespidae: Polybiini). *Biotropica*, **13**, 93–99.

Gastreich KR, Strassmann JE, Queller DC (1993) Determinants of high genetic relatedness in the swarm-founding wasp, *Protopolybia exigua*. *Ethology Ecology and Evolution*, **5**, 529–539.

Goodnight KF, Queller DC (1995) Relatedness 4.2b. Goodnight Software. Available at URL http://www.rice.edu/wasps.

Goodnight KF, Queller DC (1996) Kinship 1.0. Goodnight Software. Available at URL http://www.rice.edu/wasps.

Goodnight KF, Strassmann JE, Klingler CJ, Queller DC (1996) Single mating and its implications for kinship structure in a multiple-queen wasp, *Parachartergus colobopterus*. *Ethology Ecology and Evolution*, **8**, 191–198.

Grafen A (1986) Split sex ratios and the evolutionary origins of eusociality. *Journal of Theoretical Biology*, **122**, 95–121.

Hughes CR, Queller DC, Strassmann JE, Solís CR, Negrón-Sotomayor JA, Gastreich KR (1993) The maintenenace of high genetic relatedness in multi-queen colonies of social wasps. In: *Queen Number and Sociality in Insects* (ed. Keller L), pp. 153–170. Oxford University Press, Oxford, UK.

Jeanne RL (1991) The swarm-founding Vespidae. In: *The Social Biology of Wasps* (eds Ross K, Matthews R), pp. 191–231. Cornell University Press, Ithaca NY.

Naumann MG (1970) The nesting behavior of *Protopolybia pumila* in Panama (Hymenoptera: Vespidae), PhD Thesis. University of Kansas, Kansas City, KA.

Pamilo P (1991) Evolution of colony characteristics in social insects: I. Sex allocation. *American Naturalist*, **137**, 83–107.

Queller DC (1993) Genetic relatedness and its components in polygynous colonies of social insects. In: *Queen Number and Sociality in Insects* (ed. Keller L), pp. 132–152. Oxford University Press, Oxford, UK.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Queller DC, Strassmann JE (1998) Kin selection in social insects. *Bioscience*, **48**, 165–175.

Queller DC, Negrón-Sotomayor J, Strassmann JE, Hughes CR (1993b) Queen number and genetic relatedness in a neotropical wasp *Polybia occidentalis*. *Behavioral Ecology*, **4**, 7–13.

Queller DC, Strassmann JE, Solís CR, Hughes CR, DeLoach DM (1993a) A selfish strategy of social insect workers that promotes social cohesion. *Nature*, **365**, 639–641.

Richards OW (1978) *The Social Wasps of the Americas excluding the Vespinae*. London: British Museum (Natural History).

Richards OW, Richards MJ (1951) Observations on the social wasps of South America. *Transactions of the Entomological Society of London*, **102**, 1–170.

Solís CR, Hughes CR, Klingler CJ, Queller DC (1998) Lack of kin discrimination during wasp colony fission. *Behavioural Ecology*, **9**, in press.

Strassmann JE, Queller DC, Solís CR, Hughes CR (1991) Relatedness and queen number in the neotropical wasp, *Parachartergus colobopterus*. *Animal Behaviour*, **42**, 461–470.

Strassmann JE, Solís CR, Barefield K, Queller DC (1996a) Trinucleotide microsatellite loci in a swarm-founding neotropical wasp, *Parachartergus colobopterus* and their usefulness in other social wasps. *Molecular Ecology*, **5**, 459–461.

Strassmann JE, Solís CR, Peters JM, Queller DC (1996b) Strategies for finding and using highly polymorphic DNA microsatellite loci for studies of genetic relatedness and pedigrees. In: *Molecular Zoology: Advances, Strategies and Protocols* (eds Ferraris JD, Palumbi SR), pp, 163–180, 528–549. Wiley–Liss, Inc., New York, NY.

Strassmann JE, Solís CR, Hughes CR, Goodnight KF, Queller DC (1997) Colony life history and demography of a swarm-founding social wasp. *Behavioral Ecology Sociobiology*, **40**, 71–77.

Trivers RL, Hare. H (1976) Haplodiploidy and the evolution of the social insects. *Science*, **191**, 249–263.

West-Eberhard MJ (1978) Temporary queens in *Metapolybia* wasps: nonreproductive helpers without altrusim? *Science*, **200**, 441–443.

West-Eberhard MJ (1982) The nature and evolution of swarming in tropical social wasps (Vespidae, Polistinae, Polybiini). In: *Social Insects in the Tropics, Vol. 1* (ed. Jaisson, P), pp. 97–128. University of Paris XIII Press, Paris.

Wilson EO (1971) *The Insect Societies*. Harvard University Press, Cambridge, MA.

Wilson EO (1990) *Success and Dominance in Ecosystems: the Case of the Social Insects*. Ecology Institute, Oldendorf/Luhe.

The research program run jointly by Joan Strassmann and David Queller investigates questions of within-family conflicts of interest using social insects as model systems for understanding changes in levels of selection. We contrast studies of small-colony social insects where queens appear to be in control, with studies of large-colony species where workers appear to be in control. This paper is one of our first applications of DNA microsatellite loci to insect societies. Keith Goodnight, a postdoctoral fellow, wrote the Macintosh computer programs for this project and, with Ceal Klingler, a masters student, ran the microsatellite genotypes.
