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Aggression and worker control of caste fate in a multiple-queen wasp, *Parachartergus colobopterus*

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Although famously cooperative, social insect colonies harbour considerable potential for genetic conflict among colonymates. This conflict may be expressed behaviourally as aggression by workers. We investigated aggression in 34 colonies of the wasp *Parachartergus colobopterus*, by evaluating the characteristics of both instigators and victims of aggressive interactions. We estimated genetic relatedness and queen number using DNA microsatellites and found that workers and emerging females should be most in conflict over the caste of the latter when there are many queens on the nest. We found that aggressive interactions are more likely to involve older workers attacking either males or younger workers, and that victim and aggressor females have more ovarian development than randomly sampled colonymates. Moreover, mated females with low levels of ovarian development relative to active queens were also more likely to be aggressors and victims than were randomly sampled females. Aggression among females supports the hypothesis that older workers use aggression towards younger females as a means of policing the development of emerging females into queens. Workers also may use aggression to suppress the reproduction of some mated females. Our findings thus support the hypothesis that genetic conflicts of interest motivate worker aggression in swarm-founding wasp colonies.

Insect societies typically show high degrees of cooperation, with a reproductive division of labour so great that they are often viewed as superorganisms (Seeley 1989; Wilson & Sober 1989; Moritz & Southwick 1992; Ratnieks & Reeve 1992). Kin selection theory has served as a framework for providing insight into the evolution of this cooperation (Hamilton 1964; Bourke & Franks 1995; Crozier & Pamilo 1996; Bourke 1997; Queller & Strassmann 1998). It has also provided a framework for understanding potential conflicts within these societies, because colonymates are genetically distinct from one another and thus have different genetic interests concerning reproduction (Hamilton 1964; Trivers & Hare 1976; Ratnieks 1988; Pamilo 1991; Ratnieks & Reeve 1992; Queller & Strassmann 1998; Keller & Chapuisat 1999; Keller & Reeve 1999). Conflicts of interest between colonymates can manifest themselves in a variety of ways including oophagy and direct aggression (e.g. Ratnieks & Visscher 1989; Gobin et al. 1999). Our study focuses on whether genetic conflicts of interest motivate behavioural aggression in the well-studied swarm-founding Neotropical wasp, *Parachartergus colobopterus*, of the tribe Epiponini (Strassmann et al. 1991, 1997, 2002; Goodnight et al. 1996; Herman et al. 2000).

Epiponine wasp societies have many complex features: they have an advanced division of labour, task partitioning, alarm and trail pheromones, and large colony sizes (Jeanne 1980, 1991, in press; Zucchi et al. 1995). Yet, in contrast with other highly eusocial insects, many epiponine wasps have weak caste differentiation (Bourke 1999; O'Donnell 1998; Jeanne, in press). *Parachartergus colobopterus* colonies typically have large numbers of workers and a varying number of singly mated queens, with little or no morphological caste differences between workers and queens (Strassmann et al. 1991, 1997, 1998; Goodnight et al. 1996). As in some other epiponine societies (*Metapolybia azecoides*, *Synoeca surinama*: West-Eberhard 1978, 1981), emerging *P. colobopterus* females are totipotent. They can become either workers or queens (Strassmann et al. 2002). This leads to potential conflict over caste determination because individuals gain more from becoming queens than do colonymates (Strassmann 1989; Bourke & Ratnieks 1999; Ratnieks 2001; Reuter & Keller 2001).

In accord with worker collective interests, new *P. colobopterus* queens are produced only when queen number is low (Strassmann et al. 1991, 2002; Queller et al. 1993). This raises the question of how totipotent

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females emerging on nests with many queens are kept from becoming queens themselves when it may be in their interest to do so. Females attempting to become a queen could be forced to become workers instead (West-Eberhard 1978, 1981; Herman et al. 2000; Strassmann et al. 2002), an option not possible when there are fixed caste differences, as in *Melipona* stingless bees, where workers slaughter excess queens (Imperatriz-Fonseca & Zucchi 1995).

Eusocial wasp males seldom work or forage; yet they do depend on the colony for support. Consequently, the presence of males on a nest can impose substantial costs to social insect colonies (O'Donnell 1999). Moreover, workers are more related to female larvae than to adult males, and thus should favour investing in the former over the latter (Hamilton 1972; Trivers & Hare 1976; Starks & Poe 1997). Workers may use aggression towards males to minimize the colony costs they impose and discourage investment in males.

This study examines aggression on a large sample of *P. colobopterus* colonies. We predicted that males and young females would be the targets of aggression because of their genetic conflicts with workers.

METHODS

Sampling

We observed 34 *P. colobopterus* colonies on the campus of the Universidad Central de Venezuela at Maracay, Venezuela (10°16′N, 67°36′W, altitude 445 m) over 5 days (6–10 August 1999) during the rainy season. To sample and observe the entire nest, we removed the surrounding envelope of each colony. Removal of the envelope does not disturb the nest comb because the envelope and the comb are independently attached to the substrate. Thus, we could observe all areas of the nest for activity and sample wasps from any part of the nest. Following envelope removal, we gave the wasps at least 10 min to resume normal activity before starting observations.

We were interested in quantifying two aspects of aggressive behaviour: frequency of aggression, and who participates in aggressive interactions. To quantify the aggression level of each colony, we counted all aggressive interactions that occurred on the exposed combs for 30 min (Table 1). We conducted all observations during clear weather between 0900 and 1600 hours, when the wasps were most active. Our time frame of 30 min to characterize aggressive activity of a colony is based on hundreds of hours of observing these wasps (Strassmann et al. 1991, 1997, 1998, 2002; Queller et al. 1993; Herman et al. 2000) and on a preliminary study from the previous year in which we watched fewer colonies for periods of 3 h or more across days (data not shown). Furthermore, using a time frame of 30 min allowed us to include all 34 colonies, which we felt was the best strategy for assessing the underlying role of aggression in reproductive regulation. We observed a mean ± SD of 14 ± 13 aggressive acts per colony (range 0–57), which is sufficient for detecting differences between colonies (Table 1). An aggressive interaction involved one wasp working its mandibles over another in a chewing, snapping or biting action, often with the aggressor on top of the victim. Aggressive interactions also often included the aggressor curving her gaster and exposing her sting to the victim. The victim sometimes responded by curling over on her side and remaining motionless, even after we pulled the actor off (Strassmann et al. 1997). Sometimes the aggressor dragged the victim to another part of the nest. Each such aggressive interaction typically lasted several seconds, and may be similar to an exaggerated form of biting behaviour of *Polybia* wasps (O'Donnell & Jeanne 1995; O'Donnell 2001a). Aggressive interactions had a clear beginning and most probably would have ended with the victim running away or flying off the nest had we not removed one party (see below). These aggressive acts were quite stereotyped and did not vary greatly in intensity, making them a clear class for sampling. We distinguished aggression from social grooming by noting that the latter involves one wasp slowly and gently working her mouthparts and mandibles over another wasp's body while antennating her and never involves snapping or biting, the exposure of the actor's sting, or the pulling of the recipient to another part of the nest (Strassmann et al. 1997). Also, social grooming does not induce curling in the recipient. We distinguished aggression from solicitation for trophyllaxis by noting that the key feature of the latter always involves bites directed towards the mandibles of another wasp and seldom extends to generalized aggression with bites directed at the rest of the body (Strassmann et al. 1997).

We collected only one of the participants because we could not accurately collect both participants in a single interaction. Although collecting only one participant meant that we had very accurate samples of aggressors and victims, we could not associate interactants with a particular task (i.e. to determine whether subcategories of aggressors attacked subcategories of victims). This did not prove to be a problem because of the strong overall patterns we found (see Results) and because we were most interested in comparing victims and aggressors with a random sample that, by definition, could not be paired with particular acts. The random sample consisted of 12 individuals plucked at random from all areas of the nest comb and the substrate surface behind the nest structure. We used this random sample to estimate average relatedness and to provide a baseline for ovarian development and mating status. We stored all individuals and comb parts in 100% ethanol, which is sufficient for accurate assessment of ovarian development and insemination status and for preserving DNA for microsatellite genotyping (Strassmann et al. 2002).

For each colony, we counted the number of adults on the nest and the number of combs (Table 1). Counts of adults were necessarily approximate because they were done on active colonies with live wasps. To gauge colony productivity, we inventoried the number of total cells, empty cells, pupae, larvae and eggs in the middle comb of each nest, which we removed, knowing that the wasps would rapidly replace it (Table 1). These measures allowed us to complete this study without destroying a single colony.

Table 1. Colony characteristics

	Number of						Aggressors		Victims			
Colony	Combs	Adults	Cells in the central comb	% Empty cells	Total aggressive acts/h	% Aggressive acts involving females	Unmated females	Mated females	Unmated females	Mated females	Males	Average colony relatedness
1	21	97	130	51	0	—	0	0	0	0	0	0.22
2	7	300	323	3	0	—	0	0	0	0	0	0.25
3	8	500	382	13	28	100	6	0	6	0	0	0.16
4	9	395	806	15	8	100	1	0	2	0	0	0.06
5	9	500	466	35	66	86	11	0	7	0	3	0.21
6		520	532	10	12	100	3	0	3	0	0	0.15
7	5	65	62	24	4	100	0	0	1	0	0	0.21
8	5	110	86	10	6	100	1	0	2	0	0	0.04
9	10	270	235	14	14	100	2	0	3	0	0	0.11
10	7	580	312	9	20	80	2	0	2	0	1	0.24
11	20	130	131	35	18	86	3	0	1	2	1	0.35
12	12	160	252	17	38	100	7	0	6	0	0	0.24
13	5	65	63	5	10	100	2	0	3	0	0	0.32
14	7	225	274	79	50	86	10	0	8	0	2	0.10
15	6	142	109	28	16	100	3	1	3	1	0	0.19
16	8	360	222	64	42	67	7	3	1	3	7	0.11
17	12	600	196	11	0	—	0	0	0	0	0	0.26
18	20	670	383	19	42	56	7	1	1	0	7	0.27
19	10	105	79	9	2	100	1	0	0	1	0	−0.01
20	5	80	65	5	12	100	1	2	3	0	0	0.17
21	7	240	134	32	68	67	13	0	5	2	9	0.31
22	15	415	189	13	36	76	9	0	4	0	4	0.18
23	8	85	59	25	2	100	0	0	1	0	0	0.72
24	3	60	118	3	0	—	0	0	0	0	0	0.30
25	13	430	211	15	0	—	0	0	0	0	0	0.31
26	6	280	254	28	30	91	5	0	4	1	1	0.28
27	12	270	171	44	114	81	20	1	12	2	8	0.32
28	12	160	96	28	36	85	7	0	4	0	2	0.40
29	17	430	262	13	56	75	8	5	5	0	5	0.14
30	17	290	266	29	38	73	8	0	3	0	4	0.24
31	3	110	134	98	32	50	6	0	0	0	6	0.05
32	5	205	467	92	24	55	2	2	1	0	5	0.07
33	20	350	536	88	78	78	17	2	8	2	8	0.15
34	21	55	111	77	18	60	0	2	1	0	2	0.07

Males were aggressors in only three of the sampled aggressive interactions.

Age and Ovarian Measures

To assess age and ovarian development, we dissected all 779 collected individuals (aggressors, victims and random samples). We classified individuals into four relative age categories using the degree of sclerotization of the last sternite (Gastreich et al. 1993). We determined whether a female was mated by the presence of sperm in her spermatheca. We considered ovaries to be developed if they contained any detectable oocytes or eggs; we classified any female lacking such ovarian development as a worker and any mated female with such ovarian development as a queen. Twenty-three per cent of the dissected females had some ovarian development, but only 7% of all dissected females had mature or nearly mature eggs. We measured the length of the longest oocyte or egg and recorded the number of mature and nearly mature eggs in each female's ovaries. We determined the sex of all collected individuals. Our random sample did not have enough mated females to make comparisons with victim and aggressor females. To determine whether mated victims and aggressors had lower levels of ovarian development than the average mated female, we made comparisons with mated females from previous collections of *P. colobopterus*.

Genotyping

We stored the adults at −80 °C until DNA extraction. We extracted genomic DNA from adults either from the thorax or from the abdomen (Strassmann et al. 1996a). To estimate average genetic relatedness in each colony, we amplified five microsatellite loci using 10-μl PCR-reactions and ^{35}S d-ATP to label products (Strassmann et al. 1996a). We attempted to genotype all collected victims and aggressors, and either 12 or five adults from the random sample, for a total of 479 wasps, at five highly variable microsatellite loci: PACO3155, PACO3304, PACO3417, PACO3457 and PACO3107 (Strassmann et al. 1996b). We genotyped five random adults on colonies from which we collected no victims or aggressors or from which we collected mostly male victims. We also genotyped five random individuals for three other colonies with few adults. We ran these PCR products out on 6% polyacrylamide gels and visualized them by exposing the dried gel to a Kodak BioMax MR film. We then assessed the size of the products by comparing bands with an M13 sequencing reaction. Genotypes were scored independently by two people and a genotype was scored as missing only after multiple attempts to amplify the locus. Strassmann et al. (1996a) provides more detail on all protocols used.

Relatedness Estimation

We estimated average relatedness among colonymates based on trinucleotide microsatellite genotypes from the five loci listed above, using Relatedness 5.08 (available at http://gsoft.smu.edu/GSoft.html). For relatedness estimations we weighted colonies equally. We used 479 individuals (victims, aggressors and the random samples) from all 34 colonies to estimate population allele frequencies for relatedness estimates. To obtain standard errors and statistical tests, we jackknifed over the five loci, under the assumption that the pseudovalues are *t* distributed with four degrees of freedom (Queller & Goodnight 1989). We used relatedness among the randomly sampled adults in each colony to estimate overall average colony relatedness.

Statistical Methods

To determine whether victims, aggressors and randomly sampled wasps differed in ovarian development, we modelled the distribution of females with ovarian development among these groups with a Poisson regression. This analysis allowed us to evaluate both colony and behavioural class effects on ovarian development. All dissected females were included in the analysis. We scored females with any detectable oocytes, developing eggs or layable eggs as having ovarian development. The model uses the following log-link function to specify a log-linear relationship between the number of females expected with ovarian development and two treatments, the colony (n) and the behavioural class (c), adjusted by the logarithm of the number of females observed (N):

$$m_{ij} = N_{ij} \exp(\mu + n_i + c_j).$$

Here m_{ij} is the expected count of females with ovarian development for the ith colony and jth behavioural class, where $i =$ (colonies 1, 2, 3, …, 34) and $j =$ (victims, randoms or aggressors), μ is the overall mean of the logarithm of the proportion m_{ij}/N_{ij}, n_i is the ith treatment level for the nest treatment, and c_j is the jth treatment level for the behavioural class treatment level (Agresti 1990). We evaluated the differences between estimated treatment means using contrasts. We used the same modelling technique to evaluate whether males or mated females showed a higher probability of being victims or aggressors than all females (mated and unmated) or unmated females, respectively.

To determine whether victims and aggressors differed in age, we used an ordinal logistic regression, modelling the probabilities of victims, aggressors and randomly sampled females belonging to each age class. This model takes into account the colony effects. Because the age classes are ordinal, we used the cumulative logit model described below:

$$p1_{ij} + p2_{ij} + p3_{ij} + p4_{ij} = 1$$

$$\text{logit}\, p1_{ij} = \log(p1_{ij}/(1 - p1_{ij})) = \mu_1 + n_i + c_j$$

$$\begin{aligned}\text{logit}(p1_{ij} + p2_{ij}) &= \log((p1_{ij} + p2_{ij})/(1 - p1_{ij} - p2_{ij})) \\ &= \mu_2 + n_i + c_j\end{aligned}$$

$$\begin{aligned}\text{logit}(p1_{ij} + p2_{ij} + p3_{ij}) = \log((p1_{ij} + p2_{ij} + p3_{ij})/(1 - p1_{ij} \\ - p2_{ij} - p3_{ij})) = \mu_3 + n_i + c_j\end{aligned}$$

Here pX_{ij} is the probability of the Xth response for the ith colony and the jth behavioural class, μX is the overall mean of the logit of the Xth response, n_i is the estimated treatment effect of the ith colony, and c_j is the estimated treatment effect of the jth behavioural class with $i = (1-34)$ and $j =$ (victims, randoms and aggressors) and $X =$ (age classes 1–4), where 1 is the oldest and 4 is the youngest age class (Agresti 1990).

RESULTS

Age and Ovarian Development

On average, across all colonies, a greater proportion of victims and aggressors had ovarian development than did randomly sampled females (Fig. 1, Table 2). Victims were 2.35 times more likely to have ovarian development than were randomly sampled females (95% CI: 1.54–3.60). Similarly, aggressors were 1.99 times more likely to have ovarian development than were randomly sampled females (95% CI: 1.33–2.99). We found no significant differences between the aggressors and the victims with respect to ovarian development (Table 2).

Victims were significantly younger than randomly females and aggressors were significantly older than random females (Table 3). Victims were 1.68 times more likely than randomly sampled females to belong to the two youngest age classes (95% Wald CI: 1.10–2.54). In contrast, aggressors were 0.57 times less likely to belong to the two youngest age classes than were randomly sampled females (95% Wald CI: 0.40–0.81).

The Presence of Males

Colonies from which we collected males had higher proportions of empty comb cells (one-tailed Mann–Whitney U test: $U = 41.5$, $N_1 = N_2 = 17$, $P < 0.001$) and lower proportions of comb cells containing eggs ($U = 67$, $N_1 = N_2 = 17$, $P < 0.01$), pupae ($U = 83$, $N_1 = N_2 = 17$, $P < 0.05$) and larvae ($U = 94$, $N_1 = N_2 = 17$, $P < 0.05$) than colonies without males. For each of these analyses, one-tailed tests were appropriate because we predicted that the presence of males should cause productivity costs.

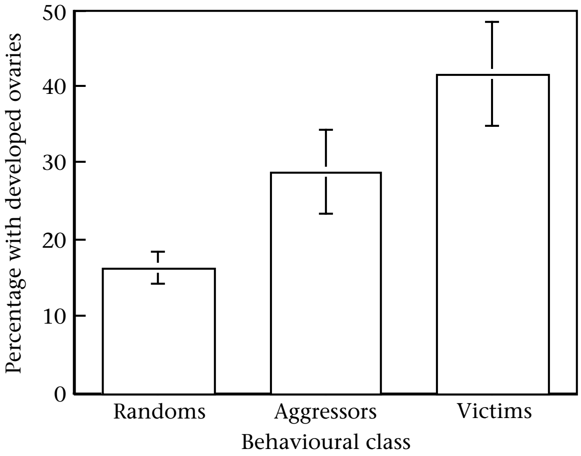

Figure 1. The percentage of victims, aggressors and randomly sampled females with ovarian development ($\overline{X} \pm$ SE, $N = 34$ colonies). We scored any female with any detectable oocytes or eggs as having ovarian development. The percentage with ovarian development for each behavioural class was calculated by averaging across all colonies.

Aggression levels involving only females were higher in colonies with males (Mann–Whitney U test: $U = 54$, $N_1 = N_2 = 17$, $P < 0.01$). Males were more likely to be observed as victims than as either randomly sampled individuals or aggressors (Table 2) and 7.28 times more likely to be victims than to be randomly sampled individuals (95% CI: 4.23–12.55). However, males were not more likely to be aggressors than to be randomly sampled individuals (Table 2). Males were observed as aggressors in only three of 184 aggressive interactions, and in each case, the interaction involved male food solicitation that escalated into outright aggression.

Mated Females and Aggression

Mated females comprised only 8.8% of all victims and aggressors collected. Of these females, 67% of victims and 53% of aggressors did not have any mature eggs in their ovaries, indicating that they were not active queens. The same was true for only 14% of the mated females from a previous collection of 22 completely collected colonies with 58 mated females (Henshaw et al. 2000 and other earlier collections). Because the victims and aggressors had so few mature eggs, for comparisons we included both mature and nearly mature eggs. Victims and aggressors had fewer mature plus nearly mature eggs than did the mated queens from the earlier study (victims: $\overline{X} \pm \mathrm{SD} = 3.0 \pm 2.3$, $N = 15$, range 0–9; aggressors: $\overline{X} \pm \mathrm{SD} = 1.4 \pm 1.5$, $N = 19$, range 0–3; earlier study: $\overline{X} \pm \mathrm{SD} = 11.0 \pm 8.0$, $N = 58$, range 1–20; Kruskal–Wallis test: H_2 corrected for ties $= 45.77$, $P < 0.0001$; Fig. 2). We also compared victims and aggressors to queens we knew to be reproductively active for number of mature plus nearly mature eggs. Reproductively active queens were determined behaviourally in a previous study of two colonies (Strassmann et al. 2002). Victims and aggressors had fewer mature plus nearly mature eggs than did the behavioural queens from the earlier study (behavioural queens from the earlier study: $\overline{X} \pm \mathrm{SD} = 40.5 \pm 10.3$, $N = 17$, range 1–20; Kruskal–Wallis test: H_2 corrected for ties $= 35.0$, $P < 0.0001$). The lack of egg-laying capability of the mated victims and aggressors is consistent with their being either young developing queens, or queens with regressed ovaries, but not with mature, reproductively active queens.

Mated females were 9.13 times more likely to be victims (95% CI: 3.46–24.12) and 6.59 times more likely to be aggressors (95% CI: 2.54–17.14) than to be randomly sampled females. However, mated females were not more likely to be observed as aggressors than as victims (Table 2).

Relatedness and Aggression

Higher proportions of randomly sampled young females had ovarian development in colonies with higher

Table 2. Poisson regression for colony and behavioural class effects on distribution of males, mated females and ovarian development

Group	Contrast	Contrast estimates ± SE	χ^2_1	*P*
Ovarian development	Victims—Randoms	0.86 ± 0.22	15.62	<0.0001
	Aggressors—Randoms	0.69 ± 0.21	11.09	<0.001
	Victims—Aggressors	0.17 ± 0.21	0.63	0.4291
Mated females	Victims—Randoms	2.21 ± 0.50	19.91	<0.0001
	Aggressors—Randoms	1.89 ± 0.49	14.97	<0.001
	Victims—Aggressors	0.32 ± 0.35	0.85	0.3576
Males	Victims—Randoms	1.98 ± 0.28	51.14	<0.0001
	Aggressors—Randoms	0.24 ± 0.38	0.42	0.5173
	Victims—Aggressors	1.74 ± 0.30	33.44	<0.0001

Analyses based on all wasps collected that belonged to the corresponding group.

relatedness compared with those in colonies with lower average colony relatedness (one-tailed Spearman rank correlation: $r_S = 0.35$, $N = 32$, $P < 0.05$). For this analysis we excluded two colonies for which we randomly sampled only one female of the younger age classes. We found no significant correlation between average colony relatedness and the frequency of per capita aggression involving only females ($r_S = 0.04$, $N = 34$, $P = 0.4144$). One-tailed tests were appropriate for these analyses because we predicted that lower relatedness should lead proportionally more females to develop their ovaries, leading to higher levels of aggression from other females.

DISCUSSION

The main results of this study support the hypothesis that genetic conflicts of interest among colony members motivate aggression by workers towards young females attempting to reproduce. Workers may attack females to suppress them from reproducing when there are already queens in the colony (Strassmann et al. 2002).

Aggression Involving Males

In colonies where males are present, males are often the victims of aggression. The most likely explanation for this aggression is that it serves to drive males from the colony, as has been reported for many other social wasps (e.g. Evans & West-Eberhard 1970, page 150). This would limit further investment in males and reduce further productivity costs for the colony.

Conflict Involving Mated Females

Overall there was much more aggression involving unmated females than mated females. However, we found that mated females were disproportionately both the targets and the instigators of aggressive interactions. Mated females with levels of ovarian development consistent with active queens were not involved in aggression, either as actors or as recipients, a finding in accord with that of Herman et al. (2000). Rather, the mated females involved in aggressive interactions had poorly developed ovaries. The number of queens on *P. colobopterus* nests cycles temporally, with the production of new queens occurring only when there are few or no queens remaining (West-Eberhard 1978; Strassmann et al. 1991, 2002; Queller et al. 1993). At this time newly emerged females mate as a distinct age cohort. The poorly developed ovaries of the mated aggressors and victims indicate that some females from this cohort do not actually become reproductively active queens at all, or are quickly forced out of that role. Our findings along with those of Herman et al. (2000) and Strassmann et al. (2002) suggest that aggression is the mechanism suppressing these mated females. Since workers are unable to discriminate matrilines within the colony (Strassmann et al. 1997), it may be that workers suppress these mated females from being active queens because they are less fertile and thus are less likely to be their mother (Forsyth 1978; Noll & Zucchi 2000). The role of mated females as aggressors suggests that these females may use aggression to suppress one another in

Table 3. Ordinal logistic regression for colony and behavioural class effects on age

Behavioural class	Number of colonies	Number of wasps	Maximum likelihood estimate ± SE (randoms as baseline)	χ^2_1	*P*
Victims	27	189	0.53 ± 0.13	15.52	<0.0001
Aggressors	29	184	−0.55 ± 0.12	20.95	<0.0001

Analysis based on all collected aggressors and victims.

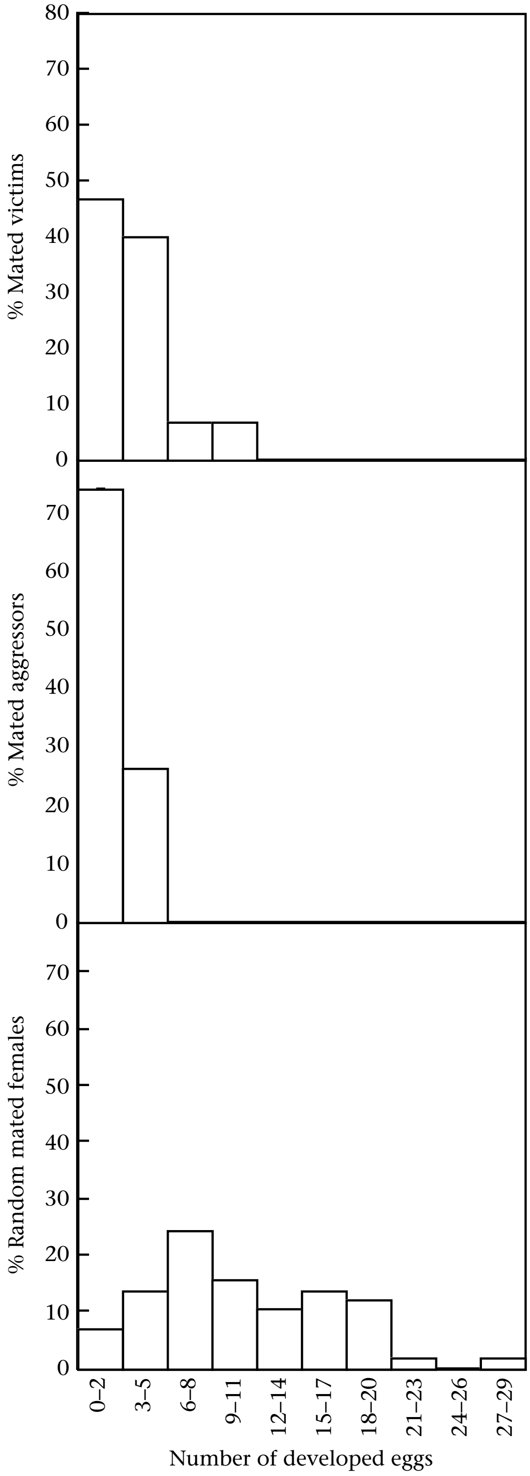

Figure 2. The percentage of mated victims, mated aggressors and randomly sampled mated females having 0—29 mature and nearly mature eggs in their ovaries across 10 three-egg intervals.

competition over reproductive rights (West-Eberhard 1978, 1981). Alternatively, once mated females have been repressed from being active queens, they themselves may become active in suppressing other females from developing their ovaries.

Conflict Involving Unmated Females

Females with ovarian development suffered a disproportionately large share of aggression, particularly when they were young. If these unmated females were not repressed, they could produce male eggs, or they could mate and produce females. It is generally in the other workers' interests to prevent young females from producing either males or females as long as there are viable queens in the colony (Strassmann et al. 2002). The results of this study and the finding that the experimental removal of all queens leads to decreased aggression and more ovarian development and mating in young females suggest that aggression is linked to the conflict over who becomes a queen (Strassmann et al. 2002). Females involved in aggressive interactions (both victims and aggressors) had more ovarian development than their randomly sampled female colonymates, but not nearly as much as queens had. Victims were younger and aggressors were older than their randomly sampled female colonymates. Aggressive interactions involved older females attacking younger females, with both parties usually having some ovarian development.

We did not observe a relationship between relatedness and the per capita frequency of aggression involving females, indicating that there may be constant suppression of newly emerging workers at many stages of the queen number cycle. However, we may not have detected such a relationship between aggression and relatedness because only one of our 34 colonies had very high relatedness, indicating low queen number, during the study period. We did find, however, that a higher proportion of young females had ovarian development when average colony relatedness was higher and thus queen number was lower, a result also reported in Strassmann et al. (2002). This increase in proportion of females with ovarian development could be related to the imminent production of queens.

In *P. colobopterus* there is a substantial payoff motivating emerging females to become queens, but allowing this is in the genetic interests of existing workers only when queen number is low, or zero, based on both the benefits of split sex ratios (Queller et al. 1993) and the relatedness ratios associated with replacing old queens with new queens (Strassmann et al. 2002). Thus, without a mechanism regulating queen production, a tragedy of the commons of too many queens could occur, resulting in decreased colony function (Frank 1995; Bourke & Ratnieks 1999; Ratnieks 2001; Strassmann et al. 2002; Wenseleers et al. 2003). The aggressive suppression of emerging females could serve as a mechanism by which older workers prevent others from becoming queens, thereby preventing untimely production of queens (West-Eberhard 1978; Herman et al. 2000; this study). Aggression decreased following the experimental removal of queens from the colony, indicating that when there are no queens on the nest, workers reduce suppression of ovarian development in emerging females, thereby allowing them to become queens (Strassmann et al. 2002). This process results in the cyclical oligogyny pattern that typifies these wasps (West-Eberhard 1978; Queller et al. 1993).

Other Studies of Aggression in Social Insects

Although our data support the hypothesis that genetic conflicts of interest motivate aggression, this is not necessarily the only motive for aggression. Several studies of other epiponine wasps indicate that aggressive interactions have a role in stimulating and organizing polyethism (Jeanne 1991; O'Donnell 2001a). In *Polybia occidentalis*, biting interactions on the exterior nest surface have been shown to stimulate victims to begin foraging and are not related to the ovarian development of either the aggressor or the victim (O'Donnell & Jeanne 1995; O'Donnell 2001a). This finding does not, however, exclude the possibility that young, developing females are the targets of aggression inside the nest envelope (O'Donnell 2001b), as we found in this study of aggressive interactions occurring inside the colony. Young females may be attacked within the nest, causing ovarian regression and driving them to the nest exterior where further aggression stimulates them to forage.

Ovarian development appears to be a general trigger for aggression as workers police other workers attempting to lay eggs. Studies in ants, bees and wasps have indicated that workers with ovarian development are more often the target of attacks from other workers than are workers without ovarian development (bees: Velthuis 1976; Visscher & Dukas 1995; ants: Cole 1981; Franks & Scovell 1983; Crosland 1990; Gobin et al. 1999; wasps: Pardi 1948; Barth et al. 1975). Moreover, other studies have demonstrated cases where larvae developing into gynes receive aggression from workers in polygynous fire ants, *Solenopsis invicta* (Fletcher 1986) and Argentine ants, *Iridomyrmex humilis* (Vargo & Passera 1991). Such attacks suggest a form of policing that suppresses females from developing their ovaries and reproducing.

It is interesting that control of worker reproduction is so often behavioural, and not entirely fixed during development. This may indicate that emerging females will be allowed to reproduce often enough that complete ovarian suppression should not be fixed. If queens die or become unproductive, the colony can salvage some reproduction only if some of its workers are able to reproduce. Reproductive plasticity generally seems to be limited to young workers, as was the case in our study, although this is not always the case. For example, in *Polistes exclamans*, older workers have more ovarian development and take over if the queen dies (Strassmann & Meyer 1983).

Worker Collective Control

The results of this study suggest that workers use aggression to regulate the timing of queen production in accord with their interests. Several other forms of reproductive conflict in *P. colobopterus* have previously been investigated. Worker collective interests are also satisfied in conflict over the timing of male production (Queller et al. 1993) and conflict over who produces males (Henshaw et al. 2000). Such decentralized colony control has probably been important in the evolution of social insect societies by facilitating colony-level adaptations and thereby the emergence of complex societies (Jeanne, in press).

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