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# Caste totipotency and conflict in a large-colony social insect

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In most social insects with large, complex colonies workers and queens are morphologically quite distinct. This means that caste determination must occur prior to adulthood. However workers and queens in the swarm-founding epiponine wasps are often morphologically indistinguishable, or nearly so, suggesting that caste determination in these wasps could be quite different. To determine the extent of caste lability in the epiponine, *Parachartergus colobopterus*, we removed all the queens from one colony and all but one from another colony. Worker aggression diminished after queen removal. A week later the colony with no queens had a new, young cohort of mated queens. These must have been either adults or pupae at the time of queen removal, and so could not have been fed any differently from workers. Relatedness patterns confirmed that these new queens would normally have been workers and not queens. A model of inclusive fitness interests shows that workers ought to suppress new queen production, except at low queen numbers, a prediction supported by our empirical results. The patterns of social conflict over queenship resulting from swarm founding in a many-queen society may help to explain the unusually weak caste differentiation in the epiponines.

**Keywords:** social insects; worker policing; caste; relatedness; conflict; *Parachartergus*

## 1. INTRODUCTION

Social insects are defined by a division into reproductive castes (Wilson 1971). Within the social insects, the principal functional subdivision concerns whether or not workers and queens are morphologically differentiated. Primitively eusocial insects, such as *Polistes* wasps, have no morphological castes and females are typically totipotent, with the colony social environment determining whether they become workers or queens (e.g. Solís & Strassmann 1990). In advanced social insects like honeybees, workers and queens differ morphologically, with their caste being determined before adulthood, generally by differential feeding. Advanced social insects also tend to have larger colony sizes, a sophisticated division of labour, more complex communication systems and elaborate nest structures (Bourke 1999).

The swarm-founding polistine wasps, particularly their largest and best-studied group, the Epiponini, are especially interesting for understanding the evolution of morphological castes. This large, ecologically successful group shows appreciable caste variability among species, with a transition from no caste to morphological castes occurring within the group. Differences between workers and queens range from, apparently, zero as in *Parachartergus colobopterus* (Strassmann *et al.* 1991) and *Metabolybia aztecoides* (West-Eberhard 1978) to marked size and shape differences, as in *Agelaia areata* (Jeanne & Fagen 1974). In a recent summary by Bourke (1999) of complexity in social insects, all large-colony taxa except the swarm-founding wasps were classified as having 'high' caste differentiation. The swarm-founding wasps received a 'low' classification, below the 'medium' classification received by some small-colony taxa. Thus this group contradicts the generalization that large, highly eusocial species have large caste differences. In most genera of this tribe caste differences are at best slight, involving small changes in proportions, occasionally in sizes, and seldom in fundamental structures (Richards 1978; Jeanne 1980; Jeanne *et al.* 1995; Hunt *et al.* 1996; O'Donnell 1998).

This weak caste differentiation seems anomalous because in most other respects the epiponines are like advanced eusocial insects. Colony sizes range from hundreds to hundreds of thousands (Jeanne 1980; Zucchi *et al.* 1995). The swarm-founding wasps also have other hallmarks of advanced sociality, including a sophisticated division of labour, task partitioning, alarm pheromones, trail pheromones, and long-term persistence of colonies (reviewed in Jeanne 1991). The comparative lack of differentiation is particularly puzzling given their swarm-founding behaviour. Because queens are never without workers, they should be free to evolve specialized queen phenotypes, and this has indeed happened in other swarm-founding taxa such as honeybees, stingless bees, and army ants.

The exceptional status of the epiponines is dependent upon how small their caste differences are. The claim of no caste differences in some species (Strassmann *et al.* 1991) has met with some scepticism, particularly when no abdominal characteristics were measured (O'Donnell 1998). However some of those abdominal changes could be due to expansion of the intertegmental membranes to accommodate developed ovaries, a result, not a cause, of queen caste (e.g. measure MH in Mateus *et al.* (1997)). In addition, a lack of any differences would be expected to be associated with a totipotency of adults, or perhaps pupae. There is some evidence of totipotency (West-Eberhard 1978) but this has never been demonstrated experimentally. In this study, we provide such a demonstration via queen removal experiments.

A second goal of this study is to explore a novel

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approach to understanding caste differentiation. Much of the thinking about caste evolution has focused on colony function. Advantages of caste differentiation include the optimization of different forms for different tasks. Costs include a decrease in individual flexibility, which comes into play particularly during queen replacement, because it may take longer to replace a queen in species where strong differentiation is the result of early developmental switches.

However, advantages and disadvantages to individuals within colonies should also be considered. Within-colony conflict may arise over queen determination because different parties will weigh the advantages and disadvantages differently (Strassmann 1989). Morphological differentiation of individuals into reproductive castes in social insects is environmentally, not genetically determined, as predicted by kin selection theory (Queller & Strassmann 1998). While determining the details of this differentiation is an active area of study (Corona *et al.* 1999; Evans & Wheeler 1999; Cnaani *et al.* 2000), it has long been known that the relevant environment governing differentiation is largely the social one within the colony, and that differential feeding is the mechanism by which castes are often controlled. For example, Alexander (1974) noted that workers might be manipulated into their role if their mother (or older workers) provided too little food for optimal reproductive development.

More recently, there has been increased awareness that resistance to such manipulation may be important (Bourke & Ratnieks 1999; Ratnieks 2001; Wenseleers *et al.* 2001). For example, the large surfeit of young queens in the stingless bee genus, *Melipona*, seems neatly explained by the fact that this is the only genus of stingless bees in which queens receive the same amount of food as workers. The resulting immunity to feeding manipulation allows many individuals to take a shot at the big fitness prize of becoming a functioning queen (Bourke & Ratnieks 1999; Ratnieks 2001; Wenseleers *et al.* 2001).

Individual conflict over caste seems likely to be relevant to epiponines. The lack of extreme caste differences in many epiponine wasps is probably attributable to their unusual queen situation, and the resolution of caste conflict. Most large-colony social insects including termites, hornets, fungus-growing ants, honeybees and stingless bees have a single queen (Wilson 1971; Michener 1974). By contrast, colonies of epiponine wasps have many queens at some times and very few at others. Queen number cycles from very high to low because new queens are produced and added to the colony only when it has lost all, or nearly all, of its queens (West-Eberhard 1978; Strassmann *et al.* 1991; Queller *et al.* 1993), which is consistent with worker sex ratio interests (Queller *et al.* 1993). When a batch of new queens is produced, there may be more females mating and functioning as queens than is optimum for the colony. These queens typically stay on the nest to reproduce, and do not found independently or leave with swarms before reproducing in their natal colony (Strassmann *et al.* 1998). Other workers may aggressively suppress some of these queens, forcing them to begin functioning as workers (West-Eberhard 1978; Herman *et al.* 2000; T. G. Platt *et al.*, unpublished data).

The modest caste differences in epiponines are consistent with the view that the large queen numbers may result from excess adults deciding, at a late stage, to become queens. Specifically, changes in proportion, rather than size or structure, are the probable outcome of caste determination that is delayed until late in development. After larval feeding has ceased, resources can be reallocated to different tissues, but not increased. After eclosion as an adult, of course, no structural exoskeleton changes are possible.

In this study we extend the knowledge of epiponine caste conflict in two ways. First, in our queen removal experiment, we determined whether a decrease in worker aggression precedes the appearance of new queens. Second, we modelled the interests of workers and queens regarding production of new queens.

## 2. MATERIAL AND METHODS

We observed naturally occurring colonies of *P. colobopterus* on vacant capybara pens at the Universidad Central de Venezuela, Facultad de Agronomía, Maracay, Venezuela (10° 16′ N, 67° 36′ W, 450 m elevation). We selected two colonies of average size and numbers of workers and removed the envelope enclosing the combs. This can be done without disturbing the combs because the envelope is attached directly to the substrate, and not to the combs. Because queens are morphologically indistinguishable from workers, it was necessary to observe egg laying to identify queens (Strassmann *et al.* 1991). We stimulated egg laying by increasing the numbers of empty cells and removing four to five eggs from cells on the edge of several combs. Any female inserting her abdomen into a cell to lay an egg was captured with forceps, placed in a small plastic bag, and preserved for dissection and genotyping. This caused minimal disturbance to the other wasps on the nest. The true queens from this sample were determined by evaluating whether or not their spermathecae contained sperm.

Each colony was observed for 336 min on 31 July 1997, for 440 min on 1 August 1997, and for 418 min on 2 August 1997 for a total of *ca.* 20 h per colony. Observation sessions started at 10.30 or later each morning and ended by 18.40 with short breaks for lunch or rain.

During these observations, the colony was scanned every 10 min and all aggressive acts occurring at that moment were counted. An aggressive act involves one or more wasps aggressively chewing or pulling on a victim. The victim often responds by curling over on its side (Strassmann *et al.* 1997; Herman *et al.* 2000). Following 3 days of observation we replaced the envelope and left the colonies alone for 7 days. We then collected the entire colonies on 9 August, at dusk, and returned for the next two days to pick up any straggling foragers.

Ovarian condition, insemination status, and relative age of all collected adults were assessed. The longest oocyte was measured and the total number of mature and nearly mature eggs in the ovaries were counted. Age was assessed according to the degree of sclerotization of the second to last sternite, assigning five categories, but combining them into two, old and young, for most analyses (Gastreich *et al.* 1993). For comparison to normal ovarian development levels among young workers, nine non-manipulated colonies collected from the same location at the same time of year were used (26 July–6 August 1995; Henshaw *et al.* (2000)).

We used five polymorphic DNA microsatellite loci, PACO3457AAT, PACO3304AAT, PACO3417AAT, PACO3155AAT, and PACO3107AAT, to estimate genetic relatedness among samples of workers, original queens, and final queens (Strassmann *et al.* 1996*a*). Polymerase chain reaction products on denaturing polyacrylamide gels were visualized using standard procedures (Strassmann *et al.* 1996*b*). We used Relatedness 5.07 to estimate relatedness. Relatedness estimates require estimates of population allele frequencies, which were obtained by adding to the genetic dataset of our two colonies an additional 474 wasps from 33 colonies collected and genotyped from the same site in 1999. Standard errors and tests were based on jackknifing over loci under the assumption that the pseudovalues are $t$-distributed with 4 d.f. (Queller & Goodnight 1989).

To test whether individual relationships are full sister or aunt–niece, we used Kinship 1.4 to estimate the log-likelihood ratio for the two hypotheses. The full-sister relationship was rejected if the estimated value fell in the lower 5% of 10 000 values for simulated full sisters. The aunt–niece relationship was rejected if the estimated value fell in the lower 5% of 10 000 values for simulated aunts and nieces. Simulated pairs were also obtained with Kinship 1.4, drawing from the estimated population allele frequencies.

Both Relatedness 5.07 and Kinship 1.4 are available for free download from http://gsoft.smu.edu/gsoft.html.

## 3. RESULTS

Colony A had 359 associated females and colony B had 307 females. Neither colony had any males, as expected, since males are produced primarily in very large colonies (Strassmann *et al.* 1991).

Dissections of all females in the colonies revealed only one mated female in colony A and 51 mated females in colony B. To prove that queens collected at the end of the experiment were newly produced, we needed to exclude the possibility that we failed to remove all the old queens. The number of individuals inserting their abdomens into empy cells over the 3 day collection period generally decreased suggesting that most of the existing queens had been removed. In the two colonies, respectively, 9 and 15 females with their abdomens in cells were removed on the first day, 12 and 3 females on the second day, and 6 and 4 females on the third day. Later dissections showed that some of these females were not mated queens, and disregarding these females further strengthens the observed trend. In colony A, seven mated females were removed on the first day, one the next day, and none on the last day. In colony B, all nine mated queens were removed on the first day and none on the following two days. The remaining females we caught putting their abdomens into cells were not queens. They were all uninseminated, and many had no mature eggs in their ovaries (13 out of 20 in colony A; 11 out of 14 in colony B).

Stronger evidence comes from relatedness patterns. The removed queens will be referred to as 'old queens' and the queens collected and the end of the experiment as 'final queens'. Queens and workers typically show different relatedness patterns in epiponines. In particular, because new queens are normally produced only at very low queen numbers, they are more highly related to each other than are the workers (Strassmann *et al.* 1991; Hughes *et al.* 1993; Queller *et al.* 1993). Queens are also more related to workers than workers are to each other. Therefore, if final queens match the high-relatedness patterns of old queens but not the low-relatedness patterns of workers, then they are likely to be old queens that we failed to remove. If, on the other hand, final queens match the relatedness patterns of workers but not old queens, we can conclude that these are members of the worker cohort who became new queens. Figure 1 shows the relevant relatedness comparisons. For colony B (shown first), the relatednesses of the 20 genotyped final queens (light grey bars) to themselves, to workers, and to old queens, were all indistinguishable from the corresponding relatednesses of workers (open bars; $p > 0.40$ for all three comparisons). However, the final-queen relatednesses were significantly different from all three old-queen relatednesses (dark grey bars). Final queens were less related among themselves than old queens were ($r = 0.165 \pm 0.057$ versus $r = 0.673 \pm 0.077$, $p < 0.001$; one-tailed paired test); final queens were less related to workers than old queens were to workers ($r = 0.181$ versus $r = 0.345$, $p < 0.05$); and final queens were less related to old queens than old queens were related among themselves ($r = 0.268$ versus $r = 0.673$, $p < 0.01$). This conclusion is confirmed by inspection of the nine old-queen genotypes, which are consistent with a single full-sister group (at every locus, they share one allele—from their single haploid father—and at most two other alleles—from their diploid mother). None of the 20 genotyped final queens is consistent with that full-sister group.

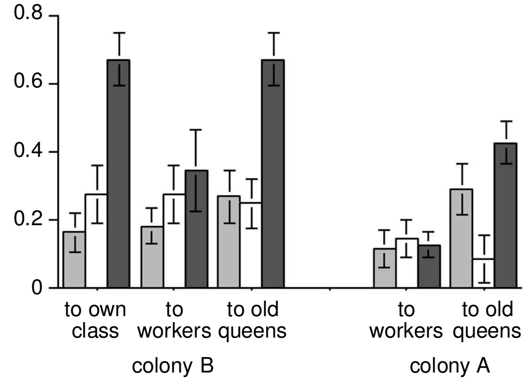

Figure 1. Observed within-nest relatedness (and standard errors) of various parties to themselves and to each other. Final queens are indicated by light grey bars, workers by open bars, and old queens by dark grey bars. Note that colony A is shown second. Because there was only one final queen collected in colony A, relatedness to own type is not shown for this colony. If final queens are derived from existing workers, then light grey bars should be similar to open bars. If final queens are old queens that we failed to collect, then light grey bars should be similar to dark grey bars.

In colony A, there was only one final queen, therefore figure 1 excludes the within-category comparisons. This queen is harder to classify. Consistent with being an old queen, her relatedness to workers and to old queens is the same as old-queen relatedness to these two categories ($p > 0.08$ and $p > 0.20$, respectively). However, consistent with being a new queen, her relatedness to workers and to old queens is the same as worker relatedness to these two categories ($p > 0.67$ and $p > 0.84$, respectively). This final queen might be ambiguous because she is a member of a rare matriline. Because her average relatedness is inconclusive, not aligning her clearly with original queens or with young workers, we decided to test whether she could be a full sister to anyone. If she is a full sister to an old queen, then she must be an old queen. Conversely, if she is a full sister to a worker, then she must be a worker. This queen was excluded as a full sister to all genotyped individuals except four, one old queen and three workers. Her estimated relatedness was higher to the old queen (0.69) than to the workers (0.58, 0.52, 0.42), suggesting that the old queen was a more probable full sister. The test based on log-likelihoods (full sister/aunt–niece) rejected each worker as a full sister to the new queen ($p < 0.05$) but did not reject the old queen ($p = 0.20$). None of the four relationships could be rejected as an aunt–niece, but the old queen came close to being rejected ($p = 0.08$). A full-sister relationship to the workers is unlikely for another reason. The three workers all have significant full-sister likelihoods to each other and form a consistent full-sister group. Although the final queen could be a full sister to each worker individually (though not significant with any), she cannot be a member of their full-sister group as a whole. We conclude that the single final queen on this colony was an old queen that we failed to remove.

This conclusion is also supported by the ovarian dissections. This queen had 25 mature and nearly mature eggs in her ovaries, far above the range of new queens in the other colony ($x = 3.6 \pm$ s.d. 1.6, range 0–6), but not different from old queens removed from her colony ($x = 36.6 \pm$ s.d. 14.1, range 4–50).

In an effort to determine what caused workers to become queens after the old queens were removed, we first looked at ovarian development in all young females. There was much more ovarian development in females in the two colonies where queens had been removed than there was in a control set of nine colonies (Mann–Whitney $U$, $p < 0.0001$). In the non-manipulated set there was more ovarian development in those with very low queen numbers (and resultant high relatednesses among workers) that were close to requeening, and in a colony that had just requeened, than in the colonies with a large group of active queens (figure 2).

Requeening generally appears to be accomplished by younger females. In the nine control colonies only 48 out of the 2650 (1.81%) old females had any development at all (27 of these females were from the same colony). In the experimental colonies only 56 out of 431 (13.0%) old unmated females had some development. In both experimental and control colonies a minority of old females with ovarian development actually had mature eggs. It was not possible to determine whether or not the old females with some development achieved it as old females, or retained development after they aged. Among young females in control colonies, 93 out of 1024 (8.3%) had some ovarian development. In the experimental colonies 158 out of 182 (86.8%) had some ovarian development. Overall, these differences are highly significant ($G$-squared tests $p < 0.0001$). When compared within colonies with at least five females with developed ovaries (five colonies excluded), five out of six colonies showed that young females were significantly more likely than old females to have ovaries with some development. The colony with no difference (V21-9) had just requeened naturally. In general, our results support the view that beyond a certain age, females have little chance of becoming queens.

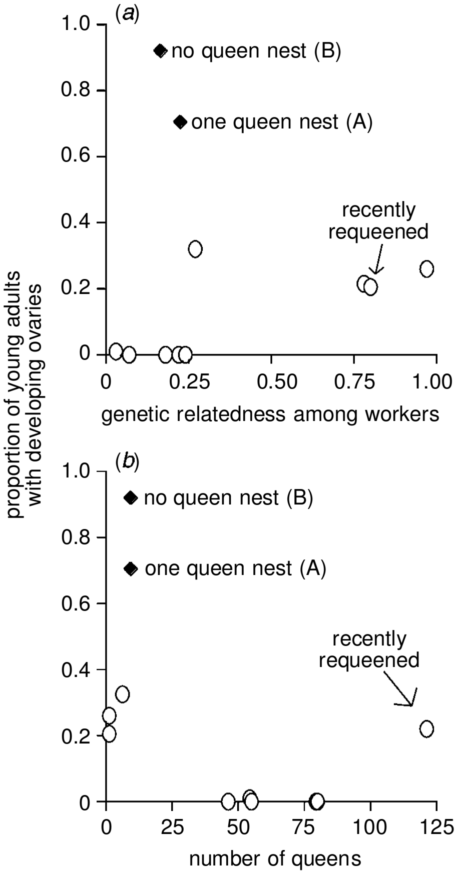

Figure 2. Ovarian development as a function of relatedness (*a*) and queen number (*b*) among nine control (open circle) and two experimental (filled diamond) colonies. Colonies where requeening is imminent (experimental colony A) or has just occurred (experimental colony B and one of the control colonies) have workers with developing ovaries. Colonies with many queens and low relatedness have very few or no workers with any ovarian development at all.

This experiment indicates that emerging females who are expected to become workers can become queens. What prevents them from always doing so? One possibility is that worker aggression towards emerging females normally suppresses ovarian development. Our results are consistent with this hypothesis. We found that aggression at the 10 min scans was higher before all the queens were removed, averaging 2.6 (s.e. = 0.653, $n = 10$ scans) and 4.3 (s.e. = 0.56, $n = 10$ scans) aggressive acts at each scan for colonies A and B respectively (figure 3). This was significantly greater than the aggression after queen removal in colony B where we removed all queens (1.62 acts per scan, s.e. = 0.138, $n = 81$ scans, $p < 0.0001$). There was, however, no significant decrease in aggression after the detected queens were removed from colony A where all but one queen was removed (1.67 acts per scan, s.e. = 0.214, $n = 81$ scans, $p < 0.16$).

Workers can be demonstrated to have preferences in

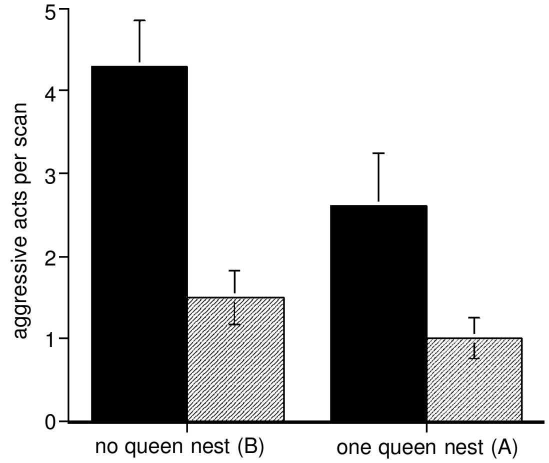

Figure 3. Average (standard errors) aggression declined significantly in the experimental colony where all queens were removed but not in the experimental colony where all but one queen was removed (see § 3 for statistical tests). Filled bars denote before queen removals and hatched bars denote after queen removals.

queen replacement based on their relatedness to original and new queens. In principle, new queens could be produced at any time in the colony cycle, however, changes in kin structure during the cycle will affect the inclusive fitness payoffs differently for different classes. Let the reproductive benefit accruing to each new queen be $b_{\text{new}}$. Because new queens remain in the parental colony, we assume that each imposes some cost, $c_{\text{old}}$, on the reproduction of any remaining old queens. In contrast to other models (Ratnieks 2001; Wenseleers *et al.* 2001), no assumptions were made about the relationship between benefits and costs. These might change with queen number, but our main intent was to compare the parties at any fixed queen number. For any party related to the progeny of the old queens by $r_{\text{old}}$ and to the progeny of the new queens by $r_{\text{new}}$, production of a new queen will be favoured if

$$br_{\text{new}} > cr_{\text{old}} \text{ or } \frac{b_{\text{new}}}{c_{\text{old}}} > \frac{r_{\text{old}}}{r_{\text{new}}}. \qquad (3.1)$$

For a colony with $q$ queens related by $r_q$, table 1 gives the relatednesses for three parties: the old queens, workers and potential new queens. Substitution of these values gives the following three conditions:

old queens,

$$\frac{b_{\text{new}}}{c_{\text{old}}} > 2; \qquad (3.2)$$

workers,

$$\frac{b_{\text{new}}}{c_{\text{old}}} > \frac{4 + 2(q-1)r_q}{3 + (q-1)r_q}; \qquad (3.3)$$

potential new queens,

$$\frac{b_{\text{new}}}{c_{\text{old}}} > \frac{2 + (q-1)r_q}{2q}. \qquad (3.4)$$

Table 1. The relatedness of various parties to the progeny of old queens, $r_{\text{old}}$, and to the progeny of the new queens, $r_{\text{new}}$. The values assume a colony headed by $q$ old queens, related by $r_q$. The colony is assumed to invest according to the population sex-investment ratio, and that queens (not workers) produce the males (supported by Henshaw *et al.* 2000) so that life-for-life relatedness to females and males can be averaged. For the $r_{\text{new}}$ of the potential new queen, only her relatedness to her own progeny is used, because it is assumed that her choice is whether she herself becomes a queen, not whether others become new queens.

| | $r_{\text{old}}$ | $r_{\text{new}}$ |
|---|---|---|
| old queen | $\frac{1}{q}\times\frac{1}{2}+\frac{q-1}{q}\times\frac{r_q}{2}$ | $\frac{1}{q}\times\frac{1}{4}+\frac{q-1}{q}\times\frac{r_q}{4}$ |
| worker | $\frac{1}{q}\times\frac{1}{2}+\frac{q-1}{q}\times\frac{r_q}{4}$ | $\frac{1}{q}\times\frac{3}{8}+\frac{q-1}{q}\times\frac{r_q}{8}$ |
| potential new queen | $\frac{1}{q}\times\frac{1}{2}+\frac{q-1}{q}\times\frac{r_q}{4}$ | $\frac{1}{2}$ |

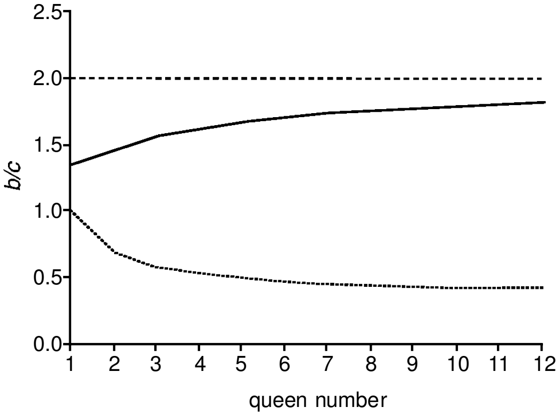

Figure 4. Ratio of benefit to new queens over cost to old queens required for production of new queens to be favoured. A dashed line denotes old queen, continuous line, worker and dotted line, new queen. Potential new queens favour becoming replacement queens at consistently lower benefit-to-cost ratios. The interests of the old queen are the hardest to meet. Workers' interests are intermediate, and most favourable to new queen production at low queen numbers.

Note that equations (3.2) and (3.3) apply to whether old queens or workers should suppress the class of new queens, whereas equation (3.4) pertains to the question of whether a potential new queen should decide to become a new queen instead of a worker.

The three parties' interests with respect to queen replacement are illustrated in figure 4. Old queens require a high $b/c$ ratio; they are always twice as related to the progeny of old queens as to the progeny of new queens. Potential new queens have the lowest threshold. A new queen is more related to her own progeny than to anyone else's, so the $b/c$ threshold for becoming a new queen is less than 1. But the new queen's threshold is highest at low queen number; she is less prone to becoming a new queen at that time because she could rear full sisters. The *b*/*c* thresholds for worker suppression of new queens are intermediate. They are lowest at low queen number because the progeny of the new queens would be replacing full siblings. Therefore, only the worker condition matches the observation that new queens are produced at low queen numbers, supporting worker control of queen replacement.

## 4. DISCUSSION

Our results support the view of West-Eberhard (1978, 1981) that, in many epiponines, caste is determined late, even in the adult stage, and that it is mediated by social conflict with workers. *Parachartergus colobopterus* has the ability to requeen rapidly. Because we waited only 7 days between removing the last queen and collecting the colonies, the new queens in colony B must have come either from the class of young workers already present in the colony, or from females that were pupae when the queens were removed. They could not have been larvae because pupal development takes longer than 7 days in this species (personal observation) and in other epiponine wasps (in *Polybia occidentalis* pupal development takes about 13 days; K. Howard, personal communication). Thus, caste determination in this species does not result from feeding differences or other manipulations during the larval stage, a conclusion that corresponds with the lack of any size differences between queens and workers (Strassmann *et al.* 1991).

Furthermore, new queens were generally not in the youngest age category of adults, suggesting that they may already have been adults, or older pupae, at the time queens were removed. Three were in the oldest category possible for non-foraging queens (corresponding to an age of at least 5 days), 13 were in a younger category (corresponding to an age of 3–5 days), and one was in the youngest category (corresponding to an age of 0–2 days).

West-Eberhard (1978, 1981) has shown that new queens arise from amongst existing workers or pupae in two other epiponines, *M. aztecoides* and *Synoeca surinama*. However, as both these colonies were at the single-queen stage, it remains theoretically possible that workers sensed that a queen replacement event was imminent, and that the new queens had been treated differently during the larval stage. This study removes that possibility, because workers could not have anticipated our queen removals. The fact that the relatedness coefficients of new queens in colony B were similar to that of a normal worker cohort, rather than like a normal queen cohort, shows that these individuals would have developed as workers in the absence of the manipulation.

An important consequence of this mode of caste determination is that power relationships between competing parties are different from those seen in other advanced social insects. Instead of workers controlling relatively helpless larvae, caste determination becomes an issue of conflict among relatively equally matched adults. Our model shows the interests of the various parties. The key finding, supporting previous thinking (West-Eberhard 1981; Bourke & Ratnieks 1999) is that each female has a greater interest in becoming a new queen than the other colony members have in letting her become one ('potential new queen' curve lowest in figure 4). Therefore, if the decision to become a queen or not is left to the differentiating individual, the result may be a tragedy of the commons, with too many queens for optimal colony function (Wenseleers *et al.* 2001). Some independent evidence exists for this in one epiponine, *Polybia occidentalis*. Forsyth (1978, 1980) showed that the productivity of young colonies was strongly related to worker number, but not queen number; most colonies could have been just as successful with far fewer queens.

However, for the differentiating individual, the relatedness gains of becoming a new queen are lowest at low numbers of old queens, precisely when new queens are actually produced. These two facts are not necessarily inconsistent, as the benefits of producing new queens could well be highest at this point, but it does raise the question of whether the timing could be due to some other caste exercising its interests. The workers' relatedness interest in raising new queens is highest at this point (figure 4), suggesting that workers might control the process, suppressing the development of new queens most of the time, but allowing their development when the number of old queens falls. The required benefit-to-cost ratios are still greater than one for workers, indicating that the old queen(s) will always be preferred if they can fulfil the colony's need for eggs. This may mean that new queens are never favoured until there are no queens left. Our experiment seems to support this view, because removing all but one queen was insufficient to generate a new cohort of queens. However, unmated females in this colony had greater levels of ovarian development than in a control sample of colonies, so the road to new queen production may have been initiated. Similarly, most of the control colonies with the many ovary-developed workers were those with few queens. We cannot determine whether these workers would actually become queens or if they would have been suppressed.

We explained, previously, the delaying of queen production to the period when only one queen remains as the result of split sex ratios (Queller *et al.* 1993). The logic is that workers ought to prefer to raise queens in colonies with the greatest asymmetry in female/male relatedness, which occurs when there is only one queen (Boomsma & Grafen 1991; Pamilo 1991). Workers in colonies with multiple queens cannot favour sisters, and so are left to specialize on males (Henshaw *et al.* 2000). Our new model shows that, in addition to this male–female trade-off, workers also prefer this timing because of the trade-off between old queens and new queens. Whether sex ratio or queen replacement interests are paramount is hard to assess since both factors favour the same outcome. If colonies produce new queens only after losing all the original queens, this could be support for the queen replacement theory. Our data support this view but are not extensive enough to rule out the alternative of queen production at low queen numbers.

Whatever the cause, our data are consistent with the view (West-Eberhard 1978, 1981) that females are totipotent at emergence as adults, but that they are quickly suppressed by existing workers when one or more queens are present. When we removed all queens from colony B, aggression declined and new queens developed. A smaller, non-significant decline in colony A, which had one old queen remaining, might indicate that this colony was also preparing to requeen. It is unlikely that the old queens are suppressing emerging females because *P. colobopterus* queens engage in very little activity at all apart from laying eggs and soliciting workers for food (Herman *et al.* 2000). Worker suppression of young females that keeps them from becoming queens is a form of worker policing, the behavioural control of an aspect of reproduction in favour of the worker's collective interests. It is conceptually similar to another form of policing, that of worker consumption of worker-produced male eggs (Ratnieks 1988).

It is not known how widely these issues of caste choice and conflict extend in the epiponines. Morphological caste differences have been reported for many species, suggesting pre-imaginal caste determination (positive cases reviewed by O'Donnell (1998)) but some of these may be questionable and others may be still be consistent with caste conflict between adults. The most questionable are those reporting minor differences in size from very few colonies, sometimes only one. Queens in epiponines are usually a discrete age cohort that could be affected by a common temporal environmental factor, such as a temporary abundance or scarcity of food. Queens and workers in a single colony might therefore differ because of chance environmental differences.

Even if size or shape differences are consistent across multiple colonies, it does not prove that caste determination occurs in the larval stage, or that conflict among adults is not important. Shape differences could develop after the larval stage (Jeanne *et al.* 1995; Jeanne 1996). In an early pupa, the available tissue might be slightly reorganized to favour queen over worker morphological features, as appears to be the case in the stingless bees of the genus *Melipona* (Bourke & Ratnieks 1999).

There are, however, some genera of epiponine wasps with distinctly larger queens that would be difficult to explain in this way (Jeanne 1980, 1991). However, it is clear that a queen cycle occurs in all of them that is similar to that of epiponines lacking caste differences. As in *P. colobopterus*, new queens are only produced by one, or a few, final queens even in genera with huge colonies like *Brachygastra* and *Agelaia* (West-Eberhard 1990; Hastings *et al.* 1998). In the species where queens are significantly larger than workers, they have presumably been fed more as larvae. But it is important to remember that in a typical collected colony, old queen cohort is being compared with a much younger cohort of workers, while the cohort of workers produced at around the same time as the queens has died off. It could be that around the time of queen production, everyone gets more food, and workers are also larger. This could be by design, with workers opting to feed the brood more as the time of requeening approaches. Alternatively, it could be for purely mechanistic reasons if total egg output decreases when only one or a few queens are laying eggs. A smaller number of mouths to feed may mean more food per mouth, resulting in larger individuals around the time of new queen production. Either way, social competition and conflict over queenship among adults might still occur. Either scenario would predict that caste differences are sometimes at their lowest, or are nonexistent, in young colonies (Hastings *et al.* 1998; Noll & Zucchi 2000).

These issues of conflict may provide some insight into why most epiponines have failed to evolve the highly distinct castes that normally accompany the evolution of larger and more complex societies. One of our arguments is based on unpredictability and the other on social competition. In epiponines, workers prefer to rear new queens at times that are relatively unpredictable, that is, when the old queens happen to die. It may therefore be difficult to plan queen rearing far enough in advance to manipulate larvae. Of course, our theoretical result that workers should prefer their old queens to new queens is not unique to epipoinines; it should be true of most social insects. However, in most social insects, new dispersing queens can be produced at comparatively little cost to the old queen—the opportunity cost of not having used those resources on workers. In species where new queens stay in the old colony, or leave with a swarm, each new queen exacts a far higher price on the reproduction of the old queen(s) because each effectively commandeers a portion of the future workforce of the colony. Therefore, workers in swarming species should be more prone to waiting for the death of the old queens. Against this argument, other swarming groups, such as the honeybees and the non-*Melipona* stingless bees, have managed to evolve distinct castes, although they may have done so before swarming evolved.

The epiponine path of choosing queens by social competition among adults may also mitigate against morphological caste evolution. Imagine how a morphological queen caste would invade an epiponine species lacking castes. When a queen vacancy occurs, workers could begin feeding larvae better, to create morphologically better-adapted queens. But these larvae would have to do more than be better egg layers. By the time they emerged as adults, a cohort of undifferentiated adults who opted to become queens immediately would already be established. They would therefore have to at least hold their own against competitors who had a significant head start. This barrier to caste evolution is not necessarily insuperable, but it is one that other species do not have to surmount, and it would apply at every step in the process of pushing the age of caste determination earlier.

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