

Control of male production in the swarm-founding wasp, *Polybioides tabidus*

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Abstract

In social insect colonies, male production may involve conflicts over the sex ratio, worker vs. queen reproduction, and each queen's contribution to the males when there are multiple queens. We examined male production in the swarm-founding, multiple-queened wasp, *Polybioides tabidus*, for which previous work suggested worker control of the sex ratios. We found that queens produced the males in accord with the collective worker preference. We also found that diploid males were produced, but only in association with haploid males. Simulations show they should have been produced in other colonies as well and their absence indicates that they were killed in some of these other colonies. The pattern of their removal indicates that *P. tabidus* cannot distinguish diploid from haploid males, and that haploid males would have been removed from these colonies too. This provides evidence that the workers are able to manipulate male production when collective preferences dictate.

Introduction

Social insect colonies are remarkably integrated cooperative groups, characterized by altruistic workers who sacrifice their own reproduction for the benefit of the colony. However, these altruistic individuals are genetically distinct with unique genetic interests, and their pursuit of their own interests may lead to disruptive conflicts within the colony. It is important that we understand how such conflicts are resolved for the maintenance of cooperation (Trivers & Hare, 1976; Crozier & Pamilo, 1996; Queller & Strassmann, 1998; Keller, 1999).

The production of males can lead to several kinds of conflict. The best studied is sex ratio conflict. In haplodiploid ants, bees and wasps, workers are often more related to female brood than male brood and therefore favour more female-biased investment than queens favour (Trivers & Hare, 1976). A second conflict concerns

who produces the males. In haplodiploids, males arise from unfertilized haploid eggs, so unmated workers are potentially able to produce sons. Finally, even if workers do not produce males, there may be conflict if there are multiple queens. Any queen would gain inclusive fitness if she produced sons while the other queens took care of worker production. This could lead to a tragedy of the commons in which each queen overproduces sons, to the detriment of the colony as a whole (Frank, 1995). Queens might, for example, produce males at times when the colony should be growing instead of reproducing.

Workers, as the most numerous caste, may have greater power to impose their interests in such conflicts (Trivers & Hare, 1976), provided there is a collective worker interest. In sex ratio conflict, worker control has been well documented both by female-biased investment ratios, by 'split' sex ratios in which colonies specialize in rearing males or females according to worker relatedness differences (Boomsma & Grafen, 1990, 1991; Queller & Strassmann, 1998), and by worker killing of queen-produced males (Sundstrom, 1996).

Conflict over whether queens or workers produce males generates a surprising conclusion. A worker will

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always be more highly related to her own sons than to any other males, but collective worker interests may favour queen production of males (Starr, 1984; Woyciechowski & Lomnicki, 1987; Ratnieks, 1988). Worker policing is the idea that the disparate preferences of each individual worker are controlled by the collective actions of all the other workers combined (Ratnieks, 1988). Although a worker may prefer to produce her own sons, she may be prevented from doing so if the other workers would rather rear the queen's sons than hers. Worker policing has been well documented in honey bees, where multiple mating causes workers to be more related to the queen's sons than to each others' sons (Ratnieks & Visscher, 1989). The opposite relationship holds in singly mated stingless bees and, as predicted, workers often compete for and win rights to some male production (Peters *et al.*, 1999).

The third source of conflict, overproduction of males by multiple queens, has not been studied. However, it is clear that workers would have an interest in limiting any costs to the colony.

Swarm-founding wasps provide particularly interesting contexts for examining these conflicts. The swarm-founding habit has evolved several times (Ross & Carpenter, 1991) and is associated with very high average queen numbers. However, wherever it has been examined, queen number is cyclical, with a new cohort of queens being produced only after the old cohort of queens has been reduced to one or a few (West-Eberhard, 1978; Queller *et al.*, 1993; Hastings *et al.*, 1998; Henshaw *et al.*, 2000b; Tsuchida *et al.*, 2000). Male production occurs at higher queen numbers. This cycle may be driven by worker split sex ratio interests because the worker relatedness asymmetry favouring female production is greatest at low queen numbers (Queller *et al.*, 1993). However, there has been no direct evidence that workers control the timing of male production.

The multiple-queen habit of the swarm-founding wasps also makes them interesting from the standpoint of worker policing. Like multiple mating, multiple queens tend to make workers more related to the sons of the queens than to the sons of the workers, so it provides a distinct context to look for worker policing. In agreement with theory, those species that have been examined show queen production of males (Hastings *et al.*, 1998; Henshaw *et al.*, 2000a), but again, no direct evidence of worker policing is available.

Finally, the presence of multiple queens leads to the potential for a tragedy of the commons over queen production of males. Workers could control such conflicts by eliminating excess males, but their ability to do so is not documented.

We recently examined male production in the African swarm-founding wasp *Polybioides tabidus* (Fabricius). *Polybioides tabidus* builds perennial colonies, initiated by

a swarm containing a dozen or more queens and hundreds of workers, and attaining sizes of 2000–6000 workers when mature. Thus, collective worker control should be effective at all colony stages. *Polybioides tabidus* has the usual queen-number cycle, suggesting worker control over the timing of sexual production. New queens are produced at an average effective queen number (N_e) of 1.67 (derived from a relatedness among the queens of 0.50). Conversely, males are produced when colonies are at higher queen numbers (N_e from 5.7 to 16.6; Henshaw *et al.*, 2000b).

In this study we focus on two questions. First, do queens or workers produce the males, and is this consistent with collective worker interests? Secondly, can workers adjust male production in a way that would allow them to be effective in enforcing their interests with respect to sex ratios and overproduction of males by queens?

The second question was made possible by the finding that some of our study colonies were producing diploid males. In the Hymenoptera, sex is often determined by heterozygosity at a single, highly polymorphic sex determining locus. Diploid males arise when diploid individuals, who would normally be heterozygous at the sex determining locus and develop as females, are homozygous at the sex determining locus and develop as males (Cook, 1993). Diploid males are sterile, and all parties agree that they should be eliminated to reduce the colony's wasted investment, provided they can be identified. However, we have found a striking pattern of diploid male production which suggests that workers cannot effectively differentiate between diploid and haploid males. Evidence for the selective elimination of diploid males therefore indicates that normal males would also be eliminated.

Materials and methods

Colony collection and dissections

Between January 22, 1998, and February 4, 1998, we collected 14 colonies of *P. tabidus* from forests adjacent to the Kakamega Forest Reserve, Kenya, the easternmost fragment of the African equatorial rainforest. We dissected 2971 adult wasps from all 14 colonies and determined the sex of every wasp. For every female, we counted the number of mature eggs, nearly mature eggs, and measured the largest mature egg or oocyte. Based on the dissections, we confirmed that workers, queens and males could be identified based on morphological differences (Turillazzi *et al.*, 1994) and we determined the sex and caste of an additional 5368 wasps to better estimate the proportions of queens, workers and males in each nest. All morphologically identified queens were later dissected to evaluate their ovarian condition and insemination status.

Genetic analyses

We genotyped a total of 250 mated queens, 336 workers and 247 males from the 14 study colonies at eight microsatellite loci: Ptab22AAT, Ptab31AAT, Ptab65TAG, Ptab91GAC, Ptab131AAT, Ptab157CAG, Ptab188CAT and Ptab238CAT (Henshaw *et al.*, 2001). We followed protocols which were slightly modified from published protocols (Strassmann *et al.*, 1996). Genotypes were scored independently by two people and their scores compared. We made two or three attempts to amplify a sample before scoring it as missing.

Relatedness calculations

We calculated regression relatedness from the microsatellite data according to the method of Queller & Goodnight (1989), using the program Relatedness 5.0 (Goodnight & Queller, 1999c). We weighted colonies equally to calculate allele frequencies, and calculated standard errors by jackknifing over loci for within-colony estimates and over colonies for population estimates. *F* statistics were calculated using the program Relatedness 4.2c (Goodnight & Queller, 1999b).

Haploid male production

In colonies with at least five haploid males, and for all colonies combined, we implemented a maximum likelihood (ML) procedure which uses the allele frequencies in the queens, workers and haploid males to estimate the fraction of the haploid males produced by the queens (Q) and workers (1 - Q), respectively (Arévalo *et al.*, 1998; Hastings *et al.*, 1998; Henshaw *et al.*, 2000b). We present the ratio Lik_Q/Lik_{max} , dividing by the maximum value of Lik_Q to scale the ratios from 0 to 1.

Diploid male production

We found that six of seven haploid male producing colonies were also producing diploid males, whereas none of the colonies without haploid males were producing them (Fisher's Exact Test, $P = 0.0047$). We estimated the probability that diploid males were missing from these other colonies by chance by sorting the diploid males into groups of full siblings and estimating the frequency of diploid male-producing queens.

Polybioides tabidus queens mate only once (Henshaw *et al.*, 2000b), so each queen's offspring will belong to a single full sibling group which can be reconstructed using the computer program Kinship 1.4 (Goodnight & Queller, 1999a). Kinship 1.4 estimates the likelihood of a hypothesized relationship against an alternative hypothesis. We tested the primary hypothesis that diploid males were full siblings against the closest possible alternative hypothesis (given that queens mate singly) that they were cousins. We ran 100 000 simulations to

estimate significance levels for the likelihood ratios reported by Kinship. As long as a diploid male could not be excluded from a matriline based on his genotype, we included him in that sib group if he was significantly more likely to be a full sibling than a cousin to any of the matriline members. When a diploid male could be assigned to one of several sib groups, we included him in the group with the highest likelihood. Some males were not likely to belong to any of the reconstructed groups, but could not be excluded from existing ones either. Because it was uncertain whether these males constituted additional sib groups, they were not assigned to any. This provides a conservative estimate of the number of diploid male-producing queens in the haploid male-producing colonies.

There should not be any difference between the frequency of diploid male-producing queens in colonies with and without haploid males. Thus, the frequency of diploid male-producing queens in the haploid male-producing colonies provides an estimate of the frequency for the entire population. We used the computer program Resampling Stats 4.0 (Bruce *et al.*, 1995) to estimate the probability that the colonies without males would lack diploid male-producing queens by chance. We created simulated groups of queens equal in size to that observed in each of the seven colonies without males, and assigned them to a diploid male-producing class with a probability equal to one of the observed probabilities in the seven male-producing colonies. Probabilities were assigned randomly and were selected separately for different colonies and for each colony in different replicates. We ran 10 000 replicates to estimate significance. The frequency of replicates which contained no diploid male-producing queens provides an estimate of the probability that diploid male-producing queens were missing from the seven colonies without males by chance.

Results

Male-producing colonies

Seven colonies produced males (Table 1). Male-producing colonies did not differ in size from colonies which did not produce males [2096 ± 536 (mean \pm S.E.) vs. 1912 ± 725 workers; two-tailed *t*-test, $t_{13} = 0.372$, $P = \text{n.s.}$]. Male-producing colonies with more workers did produce more males however, suggesting that males are produced in proportion to colony resources (linear regression; males = $-166.403 + 0.165^* \text{ workers}$; $P = 0.0018$; $R^2 = 0.878$). In accord with previously published results for other swarm-founding wasps (Strassmann *et al.*, 1998), male-producing colonies did not differ in queen number from colonies which did not produce males (34 ± 8.2 vs. 37 ± 15.7 queens; two-tailed *t*-test, $t_{13} = 0.528$, $P = \text{n.s.}$) nor was there a relationship between queen number in male-producing colonies and

Table 1 A demographic summary of the 14 *Polybioides tabidus* colonies in this study.

Nest	Percentage of nests examined	Estimated number of workers	Estimated number of queens	Estimated number of males	Estimated number of diploid males	Estimated number of diploid male-producing queens
4	100	434	12	0	0	–
5	100	449	7	0	0	–
6	55	1048	11	5	20	4
7	55	1941	52	23	4	2
8	41	1832	27	19	32	4
9	100	240	17	0	0	–
10	13	2710	8	316	23	2
11	16	5018	25	711	19	1
12	10	1797	52	176	41	2
13	33	1192	12	0	0	–
14	30	1921	53	0	0	–
15	49	325	63	4	0	0
68	23	4947	124	0	0	–
69	18	4200	34	0	0	–

the number of males produced (linear regression; males = $313.566 - 3.954 * \text{Queens}$; $P = \text{n.s.}$; $R^2 = 0.107$).

Normal male production

Workers were significantly more highly related to the queens than to each other in each nest (average relatedness among workers = 0.20 ± 0.04 , average relatedness of workers to queens = 0.26 ± 0.03 ; paired difference = 0.065 ± 0.024 , paired t -test, $t_{13} = 2.96$, $P = 0.01$). Thus, workers would be more highly related to the sons of the queens than to the sons of other workers on average, and would be expected to prevent worker reproduction.

In accord with the relatedness-based preferences of the workers, the queens produced all of, or at least the vast majority of, the males. Queens possessed on average 7.9 mature, or nearly mature eggs in their ovaries whereas no workers possessed any mature or nearly mature eggs in their ovaries. The complete lack of worker ovarian development suggests that workers were not laying eggs. The results of the ML analysis confirm that males are produced exclusively, or nearly so, by the queens (Fig. 1). The only uncertainty arises in nest 10. Three males in this colony possessed an allele at a single locus which was not observed in the collected queens, but which was observed in the workers. Thus, it appears that they were produced by a worker or workers. However, given that only seven queens were located for this colony and that no workers possessed eggs, it is possible that a queen produced these males, but was not collected, possibly because of recent queen reductions associated with cyclical oligogyny (Henshaw *et al.*, 2000b).

Diploid male production

Six of the seven haploid male-producing colonies also produced diploid males (Table 1). Diploid males were

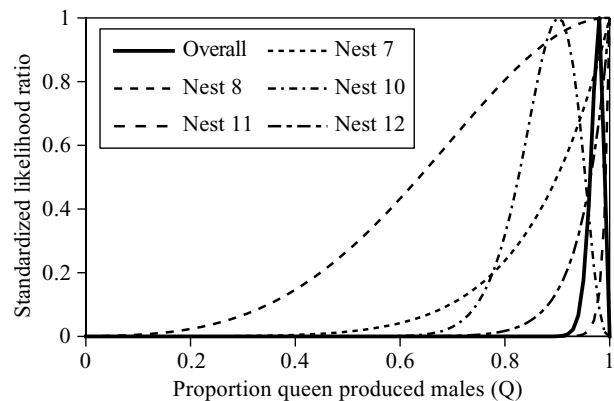


Fig. 1 Standardized likelihood ratios for all values of Q , the proportion of queen-derived males, for five of the seven male-producing colonies and all seven colonies combined.

probably produced by the queens in these colonies as no workers were mated and diploid males arise from fertilized eggs. We identified diploid males as individuals who were morphologically male but heterozygous at two or more loci (to control for the possibility of mutation or errors in genotyping, we conservatively eliminated seven males who were only heterozygous at one locus). Based on the heterozygosities of the eight microsatellite loci, we should have detected $\geq 99\%$ of diploid males in this manner.

We found no evidence of inbreeding in this population (inbreeding coefficient = 0.002 ± 0.030 ; $F \pm \text{S.E.}$), which might have explained the high frequency of diploid males. Diploid males probably occur in this population because of reduced allelic diversity at the sex determining locus. This may be because of the small size of the Kakamega Forest Reserve and the isolation of this relatively small *P. tabidus* population from larger west African populations.

Male-producing colonies each possessed at least 0–4 diploid male-producing queens (Table 1). Resampling indicates that it is significantly unlikely that all seven colonies lacking normal males would lack diploid male-producing queens. Only six in 10 000 replicates possessed no diploid male-producing queens, and more than 95% of the simulated replicates drew six or more diploid male-producing queens. Thus, it is likely that diploid male-producing queens did occur in these other colonies, but that diploid male brood were eliminated.

Discussion

Polyboides tabidus evolved the multiple-queen, swarm-founding habit independently from the neotropical epiponine wasps (Ross & Carpenter, 1991), on which most work has been carried out. We had previously shown that *P. tabidus* have the same queen number cycle, in accord with worker sex ratio interests (Henshaw *et al.*, 2000b). Here we have shown that *P. tabidus* is also convergent with respect to who produces the males. As in epiponines (Hastings *et al.*, 1998; Henshaw *et al.*, 2000a), workers are more related to the sons of queens than to the sons of workers, and queens produce the males. This result is consistent with workers policing each other, although we have no direct evidence of the workers' role. Destruction of worker-produced males seems unnecessary in this species, as workers appeared never to have developed ovaries. However, it could be that workers police each other by suppressing each others' ovarian development.

The unexpected results with diploid males did provide real evidence for collective control, probably by workers, of male production by queens. Diploid males were never reared in colonies without haploid males. However, our simulations show that there should have been diploid male-producing queens in some of these colonies, and such queens would produce some diploid males when they try to produce workers. This suggests that diploid males were killed in some or all of these colonies.

Killing diploid males makes sense because they are sterile and a drain on the colony. No triploid females were detected in 586 genotyped females, and even if triploid offspring were produced they would likely be sterile. Elimination of diploid male larvae is known from honeybees (Woyke, 1963) and stingless bee workers have been shown to kill and replace queens who produce diploid males (Kerr, 1987; this option is not open in multiple-queen species because the workers would not know which queens to kill). However, it seems unlikely that *P. tabidus* diploid males were being killed because they were diploid males, as we would then expect diploid males to be killed in all colonies. Instead, their nearly perfect co-occurrence with normal males suggests that the two could not be distinguished and they were killed because they were males. These males were probably eliminated primarily by the workers as it is unlikely that

even a few dozen queens could effectively police thousands of cells.

The selective elimination of males demonstrates a crucial ability for effective worker policing in swarm-founding wasps and extends the diversity of taxa in which such abilities have been demonstrated. The precise reasons the workers are eliminating males are not clear. Males are produced by the queens, and the workers prefer queen-derived to worker-derived males, so the diploid males were not eliminated to satisfy the worker's preferences concerning who produces the males. Rather, it appears to be the selective elimination of males to preserve the timing of male production.

There are several possible reasons why workers might eliminate males. In the swarm-founding wasps, collective worker preferences on sex ratios favour queen production at low queen numbers and male production at higher queen numbers (Queller *et al.*, 1993; Hastings *et al.*, 1998; Henshaw *et al.*, 2000b) so the workers would be expected to eliminate males produced at low queen numbers. It has been difficult to demonstrate a significant queen number difference between colonies which are producing males and others which are not producing them (Strassmann *et al.*, 1998), and the same holds for this study. The obvious reason is that there are other factors, such as the need for growth, that make male production unprofitable. In a single-queen species, queens would refrain from producing males at such times, so there would be no need for workers to evolve enforcement mechanisms. However, multiple queens face a potential tragedy of the commons, and might benefit from sneaking in some males. The removal of diploid males by workers may be a counter to what they perceive to be a 'rogue' queen who is specializing on male production at a time when her female offspring are certain to become workers and not new queens.

Most of the nonmale producing colonies in our sample appear to fit this mould. Male production may be unprofitable in new swarms, such as nest 4 which had not yet had any pupal emergences, as well as in colonies that are small for any other reason (Nests 5, 9, 15). Colonies that have just requeened may also need a period of growth, to make up for the period when emerging females become queens instead of workers. Nests 68 and 69 appear to have recently requeened. Nest 68 had an extraordinarily large number of queens while many of the morphologically distinct queens in nest 69 had yet to mate.

Thus, workers appear to be executing males that are produced at certain times, an event signalled by the absence of diploid males that should otherwise be there. A possible alternative explanation is that workers can discriminate between diploid and haploid males, but with a high error rate. A high error rate could impose a cost on the elimination of diploid males when the colony is producing haploid males, because many haploid males would then be inadvertently destroyed. This could favour

a cessation of diploid male removal when some other independent cue (e.g. relatedness) indicated that haploid males were being produced. If so, then the removal of diploid males would be a mechanism to minimize the negative effects of diploid males on the colony, not a manipulation of the timing of male production. But it would still operate by workers distinguishing males from females in situations where they did not have to make the trickier distinction between haploid and diploid males. Thus, even if this alternative hypothesis were true, *P. tabidus* workers must have all the tools necessary to identify and eliminate male offspring. Theory shows that they have the motive to kill males, and the results of this study show that they have the means, and quite likely employ them.

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