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Queen–worker–brood coadaptation rather than conflict may drive colony resource allocation in the ant *Temnothorax curvispinosus*

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Abstract Conflicts of interest among genetically heterogeneous nestmates in social insect colonies have been emphasized as driving colony resource allocation. However, potential intracolonial conflicts may not actually be realized so that resource allocation could be shaped primarily by among-colony selection that maximizes colony productivity. To elucidate the causal basis of patterns of resource allocation, I experimentally manipulated three fundamental aspects of colony social structure (relatedness among workers, relatedness among larvae, and queen presence) in the ant *Temnothorax curvispinosus* and measured effects on colony resource allocation to new workers, gynes, and males. The experimental manipulations had widespread effects on patterns of colony resource allocation, but there was little evidence for realized conflicts over the sex ratio and caste ratio. Decreasing nestmate relatedness caused decreased colony productivity, suggesting that more closely related nestmates have more favorably interacting phenotypes. Together, these results suggest that resource allocation in *T. curvispinosus* may be shaped more by among-colony selection than intracolonial conflict, leading to queen–worker–brood coadaptation.

Keywords Caste ratio · Intracolonial conflict · Levels of selection · Social evolution · Sex ratio

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Introduction

Traditionally, social insect colonies were considered cohesive units, sometimes referred to as “superorganisms” (Wheeler 1911; Wilson 1971). In this view, interactions among queen, worker, and brood nestmates are shaped by among-colony selection that maximizes the total colony output of reproductive males and females. However, because colonies are genetically heterogeneous (i.e., nestmates are not clones), potential conflicts of interest between colony members arise and may lead to the evolution of selfish phenotypes (Hamilton 1964a,b). The primary conflict predicted to occur is between haplodiploid queens and workers over the proportional investment of resources in males versus females (i.e., the colony sex allocation ratio, Trivers and Hare 1976). Haplodiploidy causes an asymmetry in the relatedness among nestmates such that workers are more closely related to their sisters than brothers, and the extent of this relatedness asymmetry depends on colony social structure (e.g., queen mating frequency, and the number of and relatedness among queens). In contrast, queens are equally related to their daughters and sons. As a result, queens and workers are predicted to disagree over colony sex ratios, depending on colony social structure (Trivers and Hare 1976; Boomsma and Grafen 1991). Additional related conflicts are predicted to occur among nestmates over the caste fate of female brood, investment in colony growth versus reproduction, and the production of males (reviewed by Ratnieks et al. 2006).

Although these conflicts are predicted by theory and have been a major focus of social insect research, it remains unclear to what degree they are actually realized and are important driving forces in social insect evolution (Ratnieks and Reeve 1992; Heinze 2004; Korb and Heinze 2004; Ratnieks et al. 2006). Colony-level costs and limits on information or power available to potentially selfish nestmates may constrain selection favoring selfish phenotypes (Ratnieks and Reeve 1992; Beekman and Ratnieks 2003; Reuter et al. 2004), and a lack of genetic variation or genetic correlations between traits may constrain evolutionary responses to selection for these phenotypes (Boomsma et al. 2003; Linksvayer 2006). As a result, predicted conflicts may often be resolved or greatly mitigated, so that the primary force influencing the evolution of colony resource allocation is selection among colonies for total reproductive output, favoring colonies with integrated queen, worker, and brood phenotypes (Ratnieks and Reeve 1992; Ratnieks et al. 2006).

The first studies of queen–worker conflict over the sex ratio assumed a priori that conflicts occur and drive patterns of investment and determined whether population-level sex allocation ratios were most consistent with queen or worker control (reviewed by Chapuisat and Keller 1999). Further studies investigated factors that might explain variation in sex ratio among colonies within populations (Boomsma and Grafen 1990, 1991), such as factors that theoretically influence optimal worker strategies (i.e., relatedness asymmetry, Sundström et al. 1996) or factors that theoretically influence the relative power of queens and workers to impose their optimal strategies (Herbers 1984, 1990; Bourke and Chan 1999). Many of these studies have supported predicted patterns, while others have not (reviewed in Heinze 2004; Ratnieks et al. 2006).

Experimental study using direct manipulations of interacting nestmates is necessary to elucidate the causal basis of observed patterns, but only a handful of studies have used an experimental approach to study the predictions of queen–worker conflict theory. Helms et al. (2000) and Passera et al. (2001) used experimental manipulations to verify that basic assumptions of queen–worker conflict theory hold, namely that both ant queens and workers can affect the sex ratio. Mueller (1991) manipulated the identity of the dominant reproductive in colonies of the primitively eusocial bee *Augochlorella striata* to produce colonies with or without relatedness asymmetry. As predicted, colonies with higher relatedness asymmetry produced a more female-biased sex ratio. Evans (1995) manipulated relatedness among larvae in the ant *Myrmica tahoensis* by transferring larvae among unrelated colonies, and Foitzik et al. (2003) similarly manipulated relatedness among workers in the ant *Temnothorax nylanderi* by transferring worker pupae among related colonies. Both of these studies found that colonies containing unrelated nestmates produced a more male-biased sex ratio. This result was predicted in the first study because some *M. tahoensis* colonies have multiple related queens so that, in this species, reduced relatedness is usually coupled with reduced relatedness asymmetry (Evans 1995). In contrast, the result was not predicted in the second study because *T. nylanderi* is monogynous and monandrous and processes that reduce relatedness in this species do not also cause reduced relatedness asymmetry (Foitzik et al. 2003). Colonies with unrelated workers produced fewer gynes (virgin queens), and hence a male-biased sex ratio, than colonies with full-sib workers. As an alternative to explanations based on queen–worker conflict, Foitzik et al. (2003) proposed that male-biased sex ratios and reduced total sexual production were non-adaptive epiphenomena caused by incompatibility between genetically heterogeneous nestmates. Additional studies have manipulated queen number to test other factors besides intracolonial conflicts that may influence resource allocation, such as local mate competition (Cremer and Heinze 2002; Kümmerli et al. 2005), and many other studies have experimentally examined effects of resource availability on colony resource allocation (e.g., Backus and Herbers 1992; Deslippe and Savolainen 1995; Bono and Herbers 2003).

In this study, I report results of an experimental study of colony resource allocation in the ant *Temnothorax curvispinosus* (Mayr 1866). I manipulated three fundamental aspects of colony social organization (relatedness among workers, relatedness among larvae, and queen presence), and I studied how these factors separately and in combination affected components of colony resource allocation. The main hypothesis I considered was the “conflict hypothesis,” which posits that resource allocation is driven primarily by selection among different groups of individuals within colonies, i.e., conflicts among queen, worker, and brood nestmates. The predictions, based on queen–worker conflict/split sex ratio theory (Trivers and Hare 1976; Boomsma and Grafen 1990, 1991) and caste conflict theory (Bourke and Ratnieks 1999; Wenseleers et al. 2003), are: First, if workers can detect large changes in nestmate relatedness and also can influence the colony sex ratio and caste ratio, reducing the relatedness among larvae or workers should cause more male-biased sex allocation ratios and less gyne-biased caste allocation ratios because reduced relatedness is expected to be associated with decreased relatedness asymmetry in *T. curvispinosus* (this species has facultative polygyny with related queens, Stuart et al. 1993). Second, if larvae can detect changes in nestmate relatedness and can influence their own caste development, reducing relatedness among larvae or workers should result in increased gyne-biased development because the relatedness of larvae to their nestmates' potential offspring will be reduced. Finally, if queens suppress worker and larval manipulation of caste and sex ratios (e.g., Herbers 1984, 1990; Bourke and Chan 1999), queen removal should cause more gyne-biased caste and sex ratios as well as increased responses of caste and sex ratios to changes in nestmate relatedness.

Materials and methods

Study species and study sites

T. curvispinosus nests in nuts and other preformed cavities and is widespread across the eastern USA (Mackay 2000). This species and close relatives are generally well studied and, in particular, have frequently been used to study the predicted conflicts among queens, workers, and brood over resource allocation (e.g., Herbers 1990; Foitzik et al. 2003). Each *T. curvispinosus* nest contains zero to several queens (Alloway et al. 1982) and from a few to several hundred workers (in this study, mean=41, SD=24, N=470). In this and closely related North American species, colonies are initiated by a single queen, but subsequently, in some colonies, additional daughter queens are readopted, leading to multiple related queens within a colony (Alloway et al. 1982; Stuart et al. 1993; Herbers and Grieco 1994; Herbers and Stuart 1996a). In contrast, most European *Temnothorax* (e.g., *T. nylanderi*) are monogynous (Foitzik et al. 2003). Single mating has been found in all *Temnothorax* studied (Herbers and Grieco 1994; Herbers and Stuart 1996a; Foitzik et al. 1997), and in this study, I assume that queens were singly mated. Colonies of *T. curvispinosus* and close relatives overwinter in one nest and then spread out to multiple nests in the summer (Alloway et al. 1982). Eggs laid in the late summer overwinter as larvae and, in the spring, develop into workers, gynes, or males. Colony investment in males, gynes, and workers derived from overwintered larvae was the focus of this study.

I collected 467 total nests, beginning as soon as winter snow had melted, between 18 March and 20 April 2004, from five sites, all within approximately 1 km of each other, at the Indiana University Research and Teaching Preserve at Griffy Woods and the Griffy Lake Nature Preserve, Bloomington, Indiana. Nests were collected as early as possible to minimize the amount of larval development after the onset of spring warming and to minimize the number of nests that were colony fragments due to seasonal polydomy. Overwintered larvae are very small, on average less than 1/12 their final adult mass (Linksvayer 2006), so that the vast majority of larval growth (and caste determination; Wesson 1940) occurred after overwintering and during the course of the experiment. Each nest was treated as an individual colony (Herbers 1990). Colonies were removed from their nests, censused, assigned at random to an experimental treatment (see below), and moved into artificial nests in the laboratory (Linksvayer 2006).

Study traits

I measured several colony traits that reflect colony resource allocation: mean colony worker mass, gyne mass, and male mass; colony worker production, gyne production, and male production; and caste ratio (=number of gynes/number of gynes plus workers) and sex ratio (=number of males/number of males plus gynes). Whereas most studies present sex allocation ratios (the proportion of resources invested in males) because they are the focus of sex ratio selection, I chose to study all components of the allocation ratios listed above. I was interested in the *relative responses* of each component to the experimental manipulations, not how close observed allocation ratios were to certain predictions (e.g., a sex allocation ratio of 0.5 or 0.25, the theoretical optima for queens and workers, respectively; Trivers and Hare 1976). Furthermore, I wanted to determine the mechanistic basis for how different ratios were achieved (Foitzik et al. 2003), e.g., whether male-biased sex ratios were achieved by the increased production of males or decreased production of gynes.

Experimental design

Colonies with one queen (N=366) were randomly assigned to one of seven treatments with none, one, or a combination of the three following experimental manipulations: replacement of all full-sibling workers from a colony with an equal number of mixed, unrelated workers; replacement of all full-sibling larvae with an equal number of mixed, unrelated larvae; removal of the colony queen. These treatments enable estimation of the main and interaction effects of experimental reduction of relatedness among worker or larval nestmates, and queen removal, respectively. Replacing full-sib workers or larvae with a mixture of unrelated workers or larvae simulates an extreme form of natural processes that reduce nestmate relatedness such as polygyny (Evans 1995; Foitzik et al. 2003). Queen removal is directly analogous to natural queen loss. The names of the seven treatments used hereafter (L, Q, W, WL, QL, QW, and QWL) refer to the members of the initial colony that were kept intact and *not manipulated*; “L” stands for larvae, “Q” queen, and “W” workers. By this scheme, QWL refers to unmanipulated control colonies. I created unrelated worker/larvae mixtures by taking all workers/larvae from at least 15 colonies, combining them in a 10-cm Petri plate, blowing on them and vortexing the plate, and cooling the mixture in a refrigerator (to disrupt nestmate recognition cues and facilitate cross-fostering; after Ross and Keller 2002). As a control for the manipulations, workers and larvae in treatments that did not involve worker or larvae mixing were similarly treated and returned to their natal nest. Workers in experimental colonies constructed with the worker and/or larvae mixtures generally behaved as did workers in unmanipulated colonies and showed little aggression toward nestmates. Colonies that initially had no queen (N=72) were randomly assigned to treatments that included queen removal (i.e., L, W, WL), but results from colonies varying in initial queen number were analyzed separately.

Colonies were maintained in incubators simulating seasonal conditions (Buschinger 1973; for details see Linksvayer 2006). Water, freshly frozen adult fruit flies (*Drosophila melanogaster*), and 10% sucrose solution were provided ad libitum and refreshed as needed. Colonies were checked biweekly. Worker pupae were identified by morphology, removed as they appeared, and up to 15 per colony were frozen and weighed to the nearest 0.001 mg with a Sartorius MC-5 micro balance. To minimize the possibility that worker pupae were derived from queen eggs laid after the start of the experiment, in treatments with a queen, only workers that pupated within 60 days of the start of the experiment or within 3 weeks of the first workers to pupate in a colony were included in the study. Male and gyne larvae must overwinter before completing development (Buschinger 1973), so there was no possibility that males or gynes were derived from eggs laid after the start of the study. Males were removed after they eclosed as adults, and up to 15 per colony were frozen and weighed to the nearest 0.001 mg. Gynes were not removed and weighed until 2 weeks after eclosion because, in at least some ant species, gynes gain much of their weight in the first few weeks of their adult life (Tschinkel 1993). Wet mass was strongly correlated with dry mass (Linksvayer 2006), and wet mass was used in all analyses.

Statistical analyses

First, I estimated the mean values and confidence intervals for the studied traits for each treatment separately. Because only some of the traits were normally distributed, I used bootstrapping to estimate parameters for each trait and treatment (Lynch and Walsh 1998). Specifically, for each trait and treatment, 10,000 bootstrap samples were created, and the median value and 2.5 and 97.5% values were taken as the parameter estimate and 95% confidence intervals, respectively. Next, to further disentangle the main and interaction effects of the three experimental manipulations as well as the effects of potential categorical and continuous covariates associated with initial colony condition and colony productivity, I used a general regression model or generalized linear model. The full potential model considered for a colony trait, z, was:

$$\begin{aligned} z = {} & \text{intercept} + \text{queen removal} + \text{worker mixing} + \text{larvae mixing} \\ & + \text{queen removal} * \text{worker mixing} + \text{queen removal} * \text{larvae mixing} \\ & + \text{worker mixing} * \text{larvae mixing} + \text{collection site} + \text{larvae number} \\ & + \text{worker number} + \text{number of larvae per worker} \\ & + \text{total number of individuals produced} + \text{total number of sexuals} \\ & \quad \text{produced} + \text{residual}. \end{aligned}$$

The total numbers of individuals or sexuals produced were not considered as potential covariates for productivity variables because they were correlated by definition. Similarly, the total number of sexuals produced was not considered as a covariate for caste ratio. The full model was first considered, and then, a backward removal process was used in which the least significant covariates were sequentially removed until only significant covariates remained in the model (Boomsma and Nachman 2002). Worker, gyne, and male mass data were analyzed using the Statistica 6.1 (StatSoft, Tulsa, OK, USA) general regression models: general linear models. The remaining traits are counts or proportions composed of count data and were analyzed using the generalized linear models module (Wilson and Hardy 2002). For traits involving counts, I used a generalized linear model with Poisson distributed residuals and a log link function, i.e., a log-linear model (Wilson and Hardy 2002). For sex ratio and caste ratio, I followed the protocol of Boomsma and Nachman (2002) except that I used a probit link function instead of a logit link function for the generalized linear model with binomially distributed residuals (Sorenson and Gianola 2002).

Results

Of the 467 total colonies collected, 72 had no queen, 366 had a single queen, and 29 had multiple queens. Colonies in all treatments appeared to behave normally and successfully reared workers, gynes, and males. A total of 9,083 worker pupae, 4,799 adult gynes, and 1,477 adult males were produced, of which 4,455 workers, 2,906 gynes, and 1,032 males were weighed. Results of the numbers of individuals produced, the resulting colony caste and sex ratios, and the mean mass of individuals produced are summarized by treatment in Tables 1 and 2 for colonies with zero and one initial queen. Data from colonies with multiple queens were not analyzed because there were only three to six multiple queen colonies per treatment. In a separate paper, I use a quantitative genetic approach to partition total phenotypic variance for the traits measured into (co)variance components for direct and indirect genetic effects (Linksvayer 2006).

Covariates

Categorical and continuous covariates that significantly predicted variables are summarized in Table 3. The strong positive correlation of initial larvae number with the number of individuals produced was expected. More interesting are the other significant covariates. For example, the number of larvae per worker in a colony was negatively correlated with the mean mass of new workers, the number of gynes produced, and the

Table 1 Resource allocation and productivity for colonies in which queen presence and relatedness among workers and larvae were experimentally manipulated

Treatment	Queen number	Workers produced	Gynes produced	Males produced	Total production	Caste ratio	Sex ratio	*N*
QWL	1	37.0 (30.5–43.6)	5.0 (2.3–8.1)	1.9 (0.0–5.0)	44.0 (37.2–51.1)	0.11 (0.05–0.17)	0.24 (0.10–0.43)	49
QW	1	23.6 (18.6–28.9)	2.1 (1.2–3.3)	1.9 (1.2–2.6)	27.6 (22.0–33.5)	0.08 (0.04–0.12)	0.54 (0.41–0.67)	50
QL	1	25.2 (20.6–30.2)	7.1 (4.4–10.3)	1.5 (0.2–3.2)	33.8 (28.7–39.1)	0.20 (0.13–0.28)	0.10 (0.03–0.21)	55
Q	1	20.0 (15.6–24.7)	5.4 (3.2–8.0)	2.3 (1.5–3.1)	27.6 (22.8–32.9)	0.23 (0.15–0.32)	0.41 (0.30–0.52)	48
WL	1	15.7 (12.3–19.3)	19.7 (15.2–24.5)	1.8 (0.8–3.4)	37.2 (31.3–43.9)	0.51 (0.42–0.59)	0.07 (0.03–0.13)	59
W	1	8.7 (6.3–11.5)	15.0 (11.4–18.8)	2.4 (1.7–3.2)	26.2 (21.7–30.9)	0.60 (0.51–0.69)	0.20 (0.13–0.28)	49
L	1	23.7 (18.9–28.8)	11.3 (8.0–15.1)	4.3 (1.6–8.7)	39.4 (32.0–47.9)	0.32 (0.24–0.40)	0.20 (0.11–0.31)	56
WL	0	7.0 (3.8–11.3)	16.0 (11.5–21.2)	7.6 (3.5–12.9)	30.6 (23.0–40.5)	0.74 (0.63–0.83)	0.25 (0.14–0.38)	38
W	0	5.1 (2.5–9.2)	14.4 (10.1–19.3)	1.8 (1.0–2.6)	21.4 (14.9–29.1)	0.76 (0.65–0.85)	0.10 (0.06–0.14)	22
L	0	11.3 (5.1–18.1)	2.9 (1.2–4.9)	10.9 (3.3–20.5)	25.3 (18.5–32.6)	0.32 (0.10–0.59)	0.54 (0.28–0.79)	12

Means are shown with 95% confidence intervals in parentheses.
The names of the seven treatments refer to the members of the initial colony that were kept intact and *not manipulated.*
L stands for larvae, *Q* queen, and *W* workers. By this scheme, *QWL* refers to unmanipulated control colonies. *Queen number* indicates whether the experimental colonies were derived from field-collected colonies with a single or no queen. *N* number of colonies.

caste ratio. Colonies that reared more individuals produced a more gyne-biased sex ratio, but colonies that produced more sexuals produced a more male-biased sex ratio.

Main and interaction effects of worker mixing, larvae mixing, and queen removal

Queen removal had the most widespread effects. Colonies with the queen removed produced more and heavier gynes, more males, fewer workers, and a more gyne-biased caste ratio and sex ratio (Table 3). However, most of these effects of queen removal depended on whether colonies contained unmixed, full-sib workers or mixed, unrelated workers, as evidenced by the significant interactions of queen removal and worker mixing (Table 3, see Fig. 1). There was a main effect of worker mixing such that colonies with mixed, unrelated workers produced smaller workers than colonies with unmixed, full-sib workers (Table 3). There was a main effect of larvae mixing such that colonies with mixed, unrelated larvae produced fewer workers, fewer total individuals, fewer gynes, and a more male-biased sex ratio relative to colonies with unmixed, full-sibling larvae (Table 3). For sex ratio, there was a significant interaction of larval mixing and queen removal (Table 3, see Fig. 2e). Finally, there was a significant interaction between worker mixing and larvae mixing for the total number of individuals produced and the total numbers of workers and gynes produced (Table 3, see Fig. 3).

Initial variation in queen number

To study the longer-term effects of queen absence, I compared experimental colonies in queen removal treatments (i.e., treatments WL, L, and W) derived from colonies

Table 2 Effects of the treatments on mass of workers, gynes, and males

Treatment	Queen number	Worker mass (mg)	Gyne mass (mg)	Male mass (mg)	N
QWL	1	0.618 (0.592–0.642)	0.960 (0.844–1.075)	0.425 (0.368–0.481)	49
QW	1	0.648 (0.626–0.671)	0.877 (0.796–0.958)	0.472 (0.441–0.503)	50
QL	1	0.585 (0.563–0.606)	1.131 (1.071–1.182)	0.418 (0.381–0.462)	55
Q	1	0.596 (0.572–0.623)	0.989 (0.913–1.063)	0.440 (0.413–0.469)	48
WL	1	0.615 (0.592–0.638)	1.094 (1.042–1.141)	0.462 (0.421–0.505)	59
W	1	0.633 (0.614–0.654)	1.095 (1.046–1.141)	0.453 (0.422–0.485)	49
L	1	0.584 (0.564–0.604)	1.079 (1.023–1.131)	0.431 (0.393–0.470)	56
WL	0	0.607 (0.571–0.643)	1.134 (1.073–1.190)	0.481 (0.427–0.537)	38
W	0	0.646 (0.608–0.692)	1.137 (1.080–1.183)	0.451 (0.397–0.531)	22
L	0	0.573 (0.525–0.640)	1.157 (1.077–1.240)	0.425 (0.366–0.485)	12

Means are shown with 95% confidence intervals in parentheses.
Treatment names, Queen number, and N as in Table 1.

Table 3 Effects of manipulation of queen presence and relatedness among workers and larvae on colony resource allocation, as shown with a general(ized) linear model

	Workers produced	Gynes produced	Males produced	Total production	Caste ratio	Sex ratio	Worker mass	Gyne mass	Male mass
Queen removal	–, ****	+, ****	+, **		+, ****	–,****		+, *	
Worker mixing							–, **		
Larvae mixing	–, ****	–, *		–, ****		+, ****			
Queen removal× worker mixing	***	**			****	****		**	
Queen removal× larvae mixing		*			*	****			
Worker mixing× larvae mixing	*	*		****					
Collection site		****	**	**	***			****	**
Worker number			–, *						
Larvae number	+, ****	+, ****	+, ****	+, ****	+, *				
Larvae per worker	+, *	–, ***	–, *		–, ****		–, **		
Total individuals produced	NA	NA	NA	NA		–,****			
Total sexuals produced	NA	NA	NA	NA	NA	+, ****		+, **	
Error *df*	357	353	352	354	348	256	332	220	124

NA indicates that the covariate was not considered for that trait.

Significant main and interaction effects of manipulations as well as significant categorical and continuous covariates are shown for each trait. For main effects and continuous covariates, the direction of the effect on the trait is indicated by a plus or minus sign. Only experimental colonies derived from field-collected colonies with a single queen were included.

*$p<0.05$

**$p<0.01$

***$p<0.001$

****$p<0.0001$

that originally had a single queen and colonies that originally did not have a queen. Because queenless colonies were smaller than colonies with a queen (Linksvayer 2006), I used a generalized linear model including initial queen number, larvae number, worker number, and the number of larvae per worker. Experimental colonies in treatments with queen removal but without larvae mixing (i.e., WL and L) that were derived from queenless colonies produced more males than experimental colonies derived from single queen colonies ($p<0.0001$). Experimental colonies in treatments with queen removal but without worker mixing (i.e., WL and W) that were derived from queenless colonies produced a more gyne-biased caste ratio than experimental colonies derived from monogynous colonies ($p<0.0001$).

Discussion

Sex ratio and caste ratio patterns are inconsistent with the conflict hypothesis

If workers can detect changes in nestmate relatedness and can manipulate the colony sex ratio, by killing or underfeeding male brood (Sundström et al. 1996) or controlling larval caste determination (Hammond et al. 2002), they should respond to decreases in nestmate relatedness by producing more male-biased sex ratios and less gyne-biased caste ratios. In contrast, if larvae can detect changes in nestmate relatedness and can influence their own caste development, e.g., by begging for more food (Kaptein et al. 2005), they should respond to reduced nestmate relatedness by biasing their own development toward becoming a reproductive gyne. I found no evidence for such worker and larval responses to decreased nestmate relatedness. Larvae mixing resulted in more male-biased sex ratios; however, this was not because fewer males were produced in colonies with full-sib larvae, but because colonies with mixed, unrelated larvae produced fewer gynes (Table 3). Finally, if queens suppress worker and larval manipulation of caste and sex ratios, queen removal should be associated with increased gyne-biased caste and sex ratios (see next paragraph) and increased responses of caste and sex ratios to changes in nestmate relatedness. The effects of worker and larvae mixing depended on queen presence (i.e., there were interactions between queen removal and both worker mixing and larvae mixing, Table 3, Figs. 1 and 2), but not in the predicted patterns. Colonies without a queen did not produce more males or fewer gynes when relatedness among larvae or workers decreased, as would be expected if workers could better respond to changes in nestmate relatedness when the queen was gone (Figs. 1 and 2). Colonies without a queen and with mixed, unrelated larvae produced a more gyne-biased caste ratio as expected if queens suppressed larval influences on caste determination, but this was because these colonies produced fewer workers, not more gynes (Fig. 2).

Queen removal resulted in the production of more gynes and slightly more males, fewer workers, and more gyne-biased caste and sex ratios (Table 3). Experimental colonies with workers derived from originally queenless colonies produced an even more gyne-biased caste ratio, and experimental colonies with larvae from queenless colonies produced more males, presumably derived from worker-laid eggs (Table 1). Increased gyne production with queen absence has often been interpreted as evidence of queen–worker conflict over the sex ratio (e.g., Herbers 1984, 1990; Backus 1995; Bourke and Chan 1999). My results are more consistent with the interpretation that queen removal causes fundamental shifts in colony resource allocation from growth and maintenance to reproduction associated with colony orphanage (Franks et al. 1990; Bourke and Franks 1995). That is, the effects of queen presence do not indicate queen suppression of nestmates, but a queen signal to nestmates so that the colony can respond to the current social structure and invest appropriately in growth and reproduction, as favored by among-colony selection (Keller and Nonacs 1993; Dietemann et al. 2005; Iwanishi and Ohkawara 2005).

Why was their no evidence of predicted queen–worker–brood conflict?

Orphanage is an inescapable life history stage for colonies so that selection on worker and brood responses to queen loss is likely strong. In contrast, processes that reduce relatedness among nestmates (and relatedness asymmetry) are rarer. In *T. curvispinosus*, the primary factor affecting relatedness and relatedness asymmetry is the adoption of

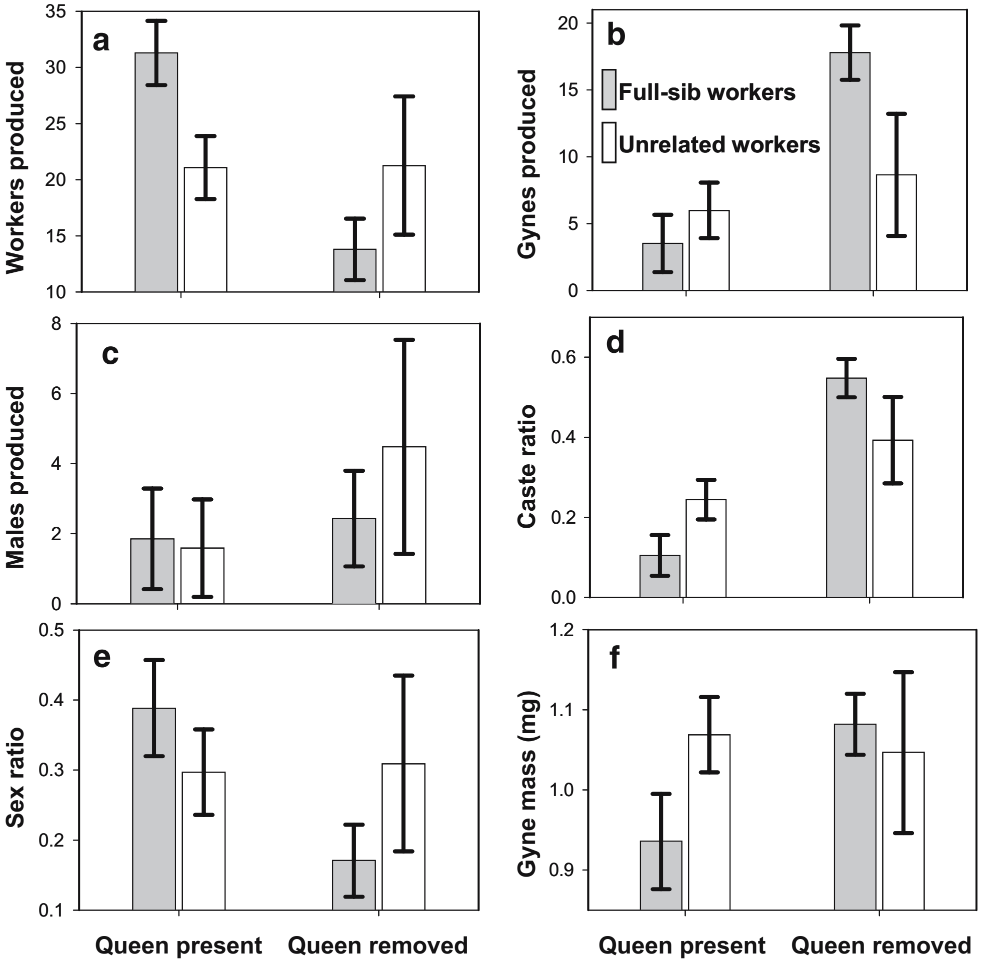

Fig. 1 Interaction of queen removal and worker mixing for worker, gyne, and male production; caste and sex ratio; and gyne mass. Colonies with full-sibling workers responded to queen removal and produced fewer workers (**a**) and more (**b**) but on average smaller gynes (**f**), a gyne-biased caste ratio (**d**), and a gyne-biased sex ratio (**e**); whereas colonies with an unrelated mixture of workers produced a similar number of workers, and number and mass of gynes regardless of whether the queen was present or removed. Estimated marginal means with 95% confidence intervals are shown

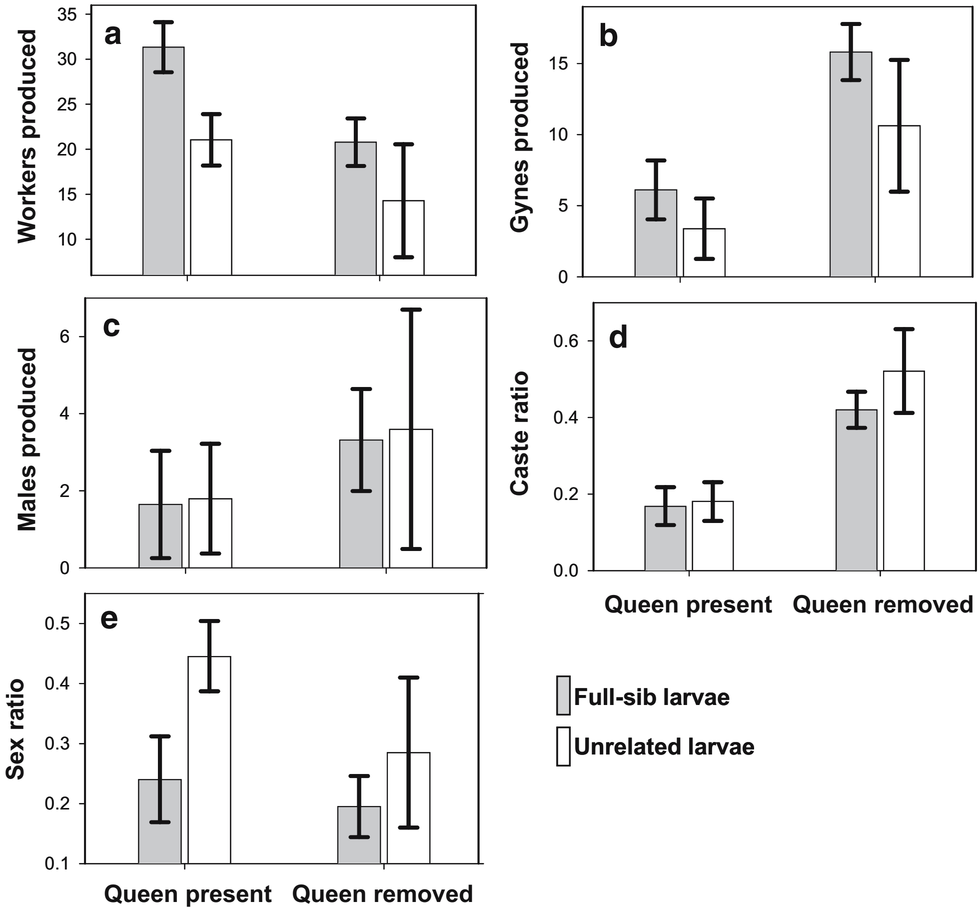

Fig. 2 Interaction of larval mixing and queen removal for worker, gyne, and male production; and caste and sex ratio. Colonies with a queen and mixed, unrelated larvae produced relatively fewer gynes (**b**) and hence a more male-biased sex ratio (**e**) relative to other colony types. Estimated marginal means with 95% confidence intervals are shown

additional related queens, but in the study population, only 6.2% (29 out of 467) of all colonies collected were polygynous (in other populations up to 17% of colonies are polygynous, Herbers and Stuart 1996b). As a result, worker and larval phenotypes associated with the ability to respond to or even detect variation in relatedness may be only weakly favored by selection (Boomsma and Grafen 1991). Alternatively, if workers do in fact have the ability to detect small differences in relatedness and distinguish between changes in relatedness and changes in relatedness asymmetry, then the predictions for the conflict hypothesis described above would not be valid, because workers are equally unrelated to male and female brood from unrelated colonies. Although there is experimental evidence that, in some species, workers may be able to generally assess relatedness asymmetry by detecting a threshold level of nestmate relatedness (e.g., Evans 1995), there is little conclusive evidence that workers in any species have the ability to detect fine differences in relatedness or relatedness asymmetry (Holzer et al. 2006, but see Hannonen and Sundström 2003 for a possible example). In the only study of its kind, Boomsma et al. (2003) found that heritable variation in recognition cues that would allow fine discrimination and the expression of nepotism was low, such that workers are constrained by lack of informative cues (see also Dani et al. 2004), in accordance with theoretical predictions (Ratnieks et al. 2007). More generally, other limits on information or power available to colony members may prevent the evolution of manipulative phenotypes (Nonacs and Carlin 1990; Beekman and Ratnieks 2003), and furthermore, such selfish phenotypes likely incur colony-level costs that may constrain their evolution (Ratnieks and Reeve 1992; Korb and Heinze 2004; Reuter et al. 2004). In addition, the genetic architecture underlying selfish phenotypes may constrain the response to selection for these phenotypes (Boomsma et al. 2003; Linksvayer 2006). One final issue that may have limited the expression of potential conflicts is that, in this study, nutritional resources were unlimited from the time the experiment began, and conflicts that may have occurred in the field, under resource-limited conditions, may have been mitigated.

Decreased relatedness causes decreased productivity: evidence for queen–worker–brood coadaptation?

Experimental reduction of nestmate relatedness did not result in the changes in sex and caste allocation predicted by the conflict hypothesis, but did affect colony productiv-

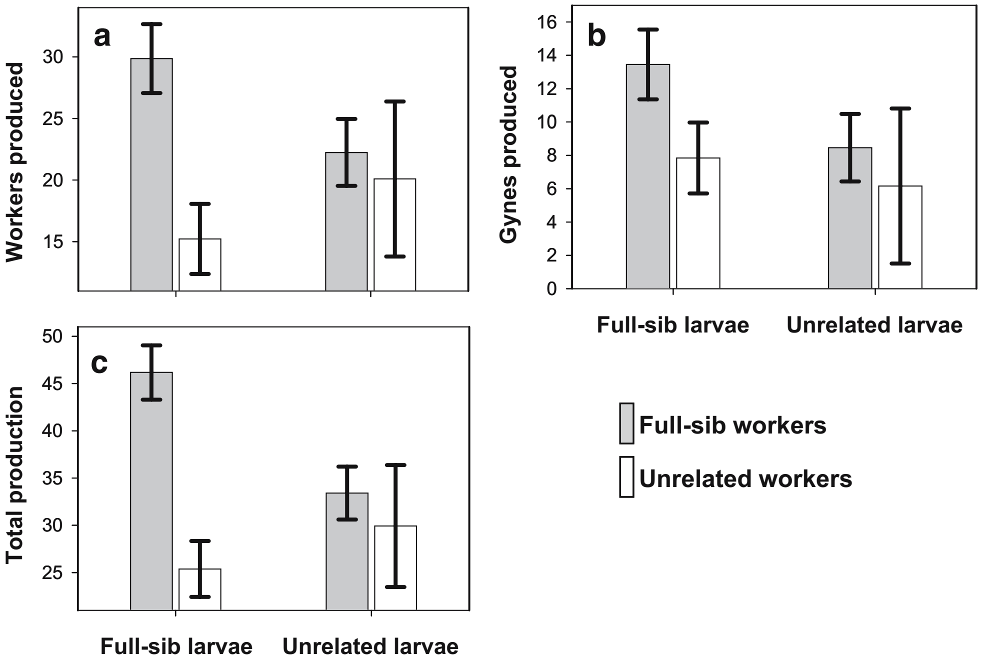

Fig. 3 Interaction between larvae mixing and worker mixing for worker, gyne, and total production. Colonies with unmixed, full-sib workers responded more to larvae mixing for worker production (**a**) and total production (**c**) than colonies with mixed, unrelated workers. Estimated marginal means with 95% confidence intervals are shown

ity. Unmanipulated colonies produced more workers than colonies in treatments with unrelated larvae or workers (Table 1). Larvae mixing had a strong and consistent effect on productivity: Colonies with mixed, unrelated larvae produced fewer workers, gynes, and total individuals than colonies with unmixed, full-sibling larvae (Tables 1, 3). Colonies with unrelated workers produced smaller workers (Tables 2, 3), but the effect of worker mixing on colony productivity was weaker and depended on the other two manipulations. In two other *Temnothorax* species, experimentally decreasing worker relatedness caused decreased colony productivity (Trampus 2001; Foitzik et al. 2003).

The interdependence of effects of queen removal, worker mixing, and larvae mixing, as evidenced by the significant interaction effects (Table 3), further indicates the influence of relatedness among nestmates on colony responses to changes in social structure. For example, colonies with unmixed, full-sib workers responded more to queen removal than colonies with mixed, unrelated workers and produced fewer workers (Fig. 1a) and more and larger gynes (Fig. 1b,f). If this response to queen loss is an adaptive shift in colony investment that is favored by among-colony selection, then reducing relatedness among workers may prevent an adaptive colony response. Similarly, Sanada et al. (1998) found that groups of *Camponotus yamaokai* workers derived from a single colony increased the frequency of trophallaxis in response to starvation conditions, whereas mixed worker groups derived from multiple colonies did not.

One explanation for the observed effects of larvae and worker mixing on colony productivity is simple nestmate discrimination. For example, if some non-nestmate larvae were recognized as such and not cared for, colonies with mixed larvae would have had decreased productivity. However, nestmate recognition in *T. curvispinosus* seems to be based mainly on transient environmental cues (Stuart 1987), that presumably were disrupted during the worker/larval mixing procedure (see “Materials and methods”) and subsequent laboratory rearing (Stuart 1987). Indeed, all larvae were initially accepted, worker aggression was generally nonexistent, and all treatments successfully reared workers, gynes, and males, suggesting that nestmate discrimination per se was relatively unimportant.

An alternative hypothesis is that nestmates did not directly assess and respond to cues of relatedness, but relatedness still affected colony performance because more closely related nestmates had coadapted and more compatible phenotypes (Foitzik et al. 2003). According to this hypothesis, introducing unrelated nestmates disrupts favorably interacting nestmate phenotypes, resulting in decreased colony productivity (Foitzik et al. 2003). Most social insect colonies have a long period of colony growth before sexuals are produced (Wilson 1971), and colonies with queen, worker, and larval phenotypes that are not well integrated are likely selected against. The result of this evolutionary process would be the buildup of linkage disequilibrium for favorably interacting queen, worker, and brood genetic effects (Wolf and Brodie 1998; Agrawal et al. 2001; Linksvayer 2006). This process can also be considered in terms of conflict resolution (Ratnieks and Reeve 1992; Ratnieks et al. 2006); that is, well-integrated colonies with complementary queen, worker, and brood phenotypes are more likely to survive and reproduce than conflict-ridden colonies with nestmates that express selfish phenotypes. Across social insect species, potential intracolonial conflicts may often be unrealized and the coadaptation of nestmate phenotypes, resulting from among-colony selection, may more strongly affect the evolutionary dynamics of social insect phenotypes.

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