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DIRECT, MATERNAL, AND SIBSOCIAL GENETIC EFFECTS ON INDIVIDUAL AND COLONY TRAITS IN AN ANT

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Abstract.—When social interactions occur, the phenotype of an individual is influenced directly by its own genes (direct genetic effects) but also indirectly by genes expressed in social partners (indirect genetic effects). Social insect colonies are characterized by extensive behavioral interactions among workers, brood, and queens so that indirect genetic effects are particularly relevant. I used a series of experimental manipulations to disentangle the contribution of direct effects, maternal (queen) effects, and sibsocial (worker) effects to variation for worker, gyne, and male mass; caste ratio; and sex ratio in the ant *Temnothorax curvispinosus*. The results indicate genetic variance for direct, maternal, and sibsocial effects for all traits, except for male mass there was no significant maternal variance, and for sex ratio the variance for direct effects was not separable from maternal variance for the primary sex ratio. Estimates of genetic correlations between direct, maternal, and sibsocial effects were generally negative, indicating that these effects may not evolve independently. These results have broad implications for social insect evolution. For example, the genetic architecture underlying social insect traits may constrain the realization of evolutionary conflicts between social partners.

Key words.—Caste ratio, indirect genetic effects, kin selection, queen-worker conflict, sex ratio, social evolution.

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The study of social evolution has been dominated by two main concepts for the past 40 years. First, inclusive fitness or kin selection theory argues that individuals may gain fitness not only by reproducing, but also by helping relatives to reproduce (Hamilton 1964a,b). Second, parent-offspring conflict theory predicts a conflict of interests between parents and offspring because parents must balance investment among current and future offspring, whereas individual offspring often benefit by monopolizing parental resources (Trivers 1972). Social insects emerged as a model system to further develop and study these concepts (Hamilton 1964a,b, 1972; Alexander 1974; Michener and Brothers 1974; Trivers and Hare 1976). Unfortunately, our understanding of the evolution of specific social insect phenotypes that are the focus of kin selection and parent-offspring conflict, such as offspring size, caste ratio, and sex ratio, is greatly mitigated because little is known of the underlying genetic architecture. This is a serious limitation because evolutionary responses to selection are shaped by genetic architecture (Falconer and Mackay 1996; Roff 1997).

When social interactions occur, an individual's phenotype is affected not only by its genotype, but also by the social environment it experiences (Cheverud and Moore 1994). Social insect colonies are notable for extended interactions among queen, worker, and larval nestmates, so that the social environment plays a particularly important role. This social complexity and its importance present a special difficulty for the study of the genetic architecture underlying social insect phenotypes, particularly in disentangling the effects of genotype and the social environment (Linksvayer and Wade 2005). One approach has been to search for phenotypic variation among genotypic classes within colonies, holding the social environment constant (e.g., Stuart and Page 1991; Bargum et al. 2004; Schwander et al. 2005). Studies that have considered variation in the social environment have shown that genetic effects are mediated by the social environment (Keller and Ross 1995; Goodisman et al. 1999; Rüppell et al. 2001), or that the social environment itself has a genetic component (Pankiw et al. 2002; Ross and Keller 2002).

Indirect genetic effect models provide a means to formally study the complex genetic basis of social insect phenotypes because genetic components to the social environment are explicitly considered. In an indirect genetic effect model, the phenotype of an individual is influenced both directly by its own genes (direct genetic effects) and indirectly by genes expressed in social partners (indirect genetic effects), so that phenotypes are the property of genotypes of multiple interacting individuals (Wilham 1963; Cheverud 1984; Lynch 1987; Cheverud and Moore 1994; Moore et al. 1997). Typically, indirect effects are measured as the social partner's performance, the composite effect of the social partner's phenotype on the focal individual's phenotype (Cheverud and Moore 1994). In social insects, genes expressed in queens affect offspring traits indirectly by affecting maternal performance, as do genes expressed in care-giving workers through sibsocial performance (Linksvayer and Wade 2005). An indirect genetic effect approach has been used to study the genetic architecture underlying phenotypes in a variety of organisms with parental care (e.g., Cheverud 1984; Kölliker et al. 2000; Agrawal et al. 2001; Hunt and Simmons 2002; Rauter and Moore 2002). Despite the potential for insight into the evolution of social insect phenotypes, indirect genetic effect models have only rarely been applied to social insects (Bienefeld and Pirchner 1990; Linksvayer and Wade 2005).

Here, I use an indirect genetic effects approach to study the genetic architecture underlying worker, gyne, and male mass; caste ratio; and sex ratio in the ant *Temnothorax curvispinosus*. Specifically, I estimated the heritability of direct, maternal, and sibsocial effects as well as the genetic correlations between these effects as measures of the evolutionary importance of direct, maternal, and sibsocial effects. I chose

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to study mass, caste ratio, and sex ratio because they are likely to be closely related to fitness and the focus of kin selection and parent-offspring conflict.

Materials and Methods

Natural History of Study Species

Temnothorax (= *Leptothorax* [*Myrafant*]) *curvispinosus* is an acorn ant that nests in nuts and other preformed cavities and is widespread across the eastern United States (Mackay 2000). This species, along with other closely related species, has been well studied and is readily maintained in the laboratory (e.g., Alloway et al. 1982; Herbers 1983). Colonies of *T. curvispinosus* vary in queen number, from one to several (Alloway et al. 1982). Single mating has been found in close relatives (Herbers and Grieco 1994; Foitzik et al. 1997), and in this study, I assume that queens were singly mated. Colony size ranges from a few workers to a few hundred (for this study, mean = 41, SD = 24, $N = 470$). *Temnothorax curvispinosus* and several other acorn ant species have seasonal polydomy, in which colonies overwinter in one nest and then spread out to multiple nests in the summer (Alloway et al. 1982). Eggs laid in the late summer and early fall overwinter as larvae, and in the spring these larvae develop into workers or gynes (virgin queens) or males. Diploid eggs laid in the spring and early summer develop exclusively as workers in the summer (A. Buschinger, pers. comm.).

Collection of Study Colonies

Acorn and hickory nut nests were collected from late winter to early spring (i.e., 18 March to 20 April 2004). Colonies were collected as early as possible to minimize the amount of larval development prior to collection. In a previous study, colony mean larva mass, measured at the time of collection, did not predict colony mean adult gyne mass ($P > 0.05$, $N = 55$; T. A. Linksvayer, unpubl. data). Overwintered larvae are very small (mean colony mean larva mass = 0.065 mg, SD = 0.038, $N = 130$; T. A. Linksvayer, unpubl. data), less than one-twelfth their final adult mass (mean colony mean gyne mass = 0.822 mg, SD = 0.147, $N = 55$; T. A. Linksvayer, unpubl. data). Thus, the vast majority of larval growth occurred during the course of the experiment. Furthermore, caste determination has been experimentally shown to occur after overwintering in *T. curvispinosus* (Wesson 1940).

Nests were collected 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. The number of occupied nests within 1 m of each nest was recorded as a measure of local nest density, but nest density was not correlated with any of the studied phenotypes and is not discussed further. Each nest was treated as an individual colony (Herbers 1990) and was censused and moved into an artificial nest in the laboratory (nest design after Alloway 1979). All occupied colonies were collected, but only single-queen colonies (369 of 470 colonies) and colonies with at least 10 workers (360 of 369 colonies) were included in the current analysis.

Study Traits

The phenotypes of individuals derived from overwintered larvae were the focus of this study. The mass of reared workers, gynes, and males; caste ratio; and sex ratio were the study traits. The mass of worker pupae and adult gynes and males (see below) was measured to the nearest 0.001 mg with a Sartorius MC-5 microbalance (Sartorius, Edgewood, NY). Preliminary results indicated that wet mass was strongly correlated with dry mass for workers ($r = 0.865$, $P < 0.001$, $N = 210$), gynes ($r = 0.921$, $P < 0.001$, $N = 580$), and males ($r = 0.931$, $P < 0.001$, $N = 202$), and wet mass was used in all analyses. The caste ratio of a colony is defined as the proportion of new adult females that are gynes. The sex ratio of a colony is defined as the proportion of new adult sexuals that are male. Note that both caste ratio and sex ratio are influenced by the number of gynes produced and thus are expected to be correlated.

Experimental Design

A powerful experimental approach that has been used to separate direct and maternal genetic effects in several subsocial species is cross-fostering (e.g., Cheverud 1984; Agrawal et al. 2001). I used a similar approach to disentangle the contribution of direct, maternal, and sibsocial genetic variance and covariance to total phenotypic variance for worker, gyne, and male mass; caste ratio; and sex ratio. Colonies were randomly assigned to one of seven treatments that involved none, one, or a combination of the three following experimental manipulations: (1) removal of the colony queen; (2) replacement of workers with an equal number of individuals from a mixture of workers from at least 15 colonies; and (3) replacement of larvae with an equal number of individuals from a mixture of larvae from at least 15 colonies (Fig. 1). These treatments experimentally control which factors contribute to among-colony variance for traits expressed by developing larvae (Table 1). Specifically, the removal of the colony queen eliminates the possibility of variance in (postmanipulation) maternal effects contributing to among-colony variance, and mixing workers or larvae among colonies minimizes variance among colonies due to sibsocial or direct effects, respectively. 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, larvae; Q, queen; and W, workers). The intact, unmanipulated members of the colony contribute to among-colony variance, whereas the removed or mixed members do not (Fig. 1, Table 1).

The worker/larvae mixing procedure involved taking 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 (after Ross and Keller 2002). Colonies in treatments without the worker or larvae mixing manipulation were similarly treated. Workers in newly assembled colonies generally behaved as workers in unmanipulated colonies and showed little aggression toward nestmates.

Colonies were maintained in incubators under spring conditions of 13:11 h light:dark photoperiod and 20:10°C day:night temperature cycle until 21 May 2004, and then summer

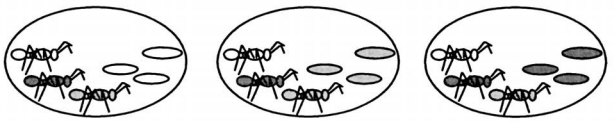
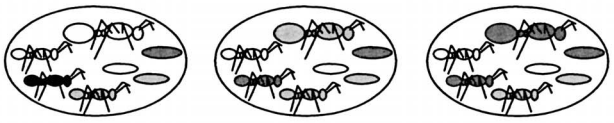
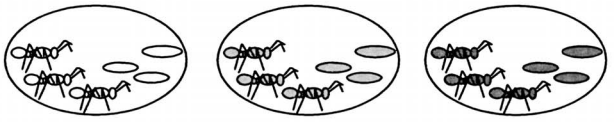
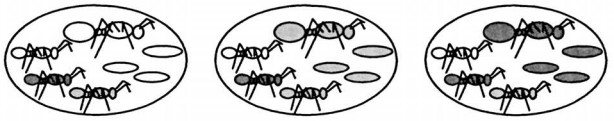
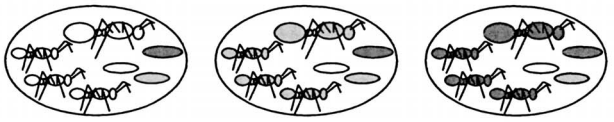
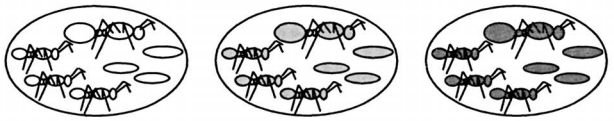
Treatment	Remove queen	Mix workers	Mix larvae	Diagram of three colonies per treatment
L	X	X	–	
Q	–	X	X	
W	X	–	X	
WL	X	–	–	
QL	–	X	–	
QW	–	–	X	
QWL	–	–	–	

FIG. 1. Combination of the three experimental manipulations used for each of the seven treatments. X indicates that the manipulation was used. Diagrams of three colonies for each treatment are shown, with shading indicating the colony of origin of queens, workers, and larvae. Each of the three colonies in the last row (treatment QWL) is unmanipulated and contains a queen, workers, and larvae of a single shade. The three colonies in treatment L only vary for the shade of larvae, indicating that among-colony variance for treatment L is due to variation among groups of full-sib larvae. Similarly, colony diagrams for treatment Q and treatment W only vary for queen shade and worker shade, indicating that among-colony variance for these treatments is due to variation among queens and groups of workers, respectively.

TABLE 1. Coefficients of causal (co)variance components that contribute to among-colony variance for each treatment for haplodiploid females in colonies with a single, singly-mated queen. The first three columns are for direct, maternal, and sibsocial genetic variance, and the second three are for direct-maternal, direct-sibsocial, and maternal-sibsocial genetic covariance.

Treatment	Direct	Maternal	Sibsocial	Direct-maternal	Direct-sibsocial	Maternal-sibsocial
L	3/4	0	0	0	0	0
Q	0	1	0	0	0	0
W	0	0	3/4	0	0	0
WL	3/4	0	3/4	0	3/2	0
QL	3/4	1	0	1	0	0
QW	0	1	3/4	0	0	1
QWL	3/4	1	3/4	1	3/2	1

conditions of 14:10 h light:dark and 22:18°C day:night until 16 August 2004, when the last gynes were removed (after Buschinger 1973). Water, freshly frozen adult fruit flies (*Drosophila melanogaster*), and 10% sucrose solution were provided ad libitum and refreshed as needed (sucrose feeding setup after Evans and Pierce 1995).

Colonies were checked biweekly for new pupae. Worker pupae were identified by morphology and removed as they appeared, and the first 15 from each colony were frozen and weighed. In treatments including a queen (QW, QL, QWL), queen eggs laid after the start of the experiment could potentially develop into worker pupae before the end of the experiment. To minimize inclusion of these worker pupae, only workers that eclosed within 60 days of the start of the experiment, and not more than three weeks after the first group of workers in a colony had pupated, were included in the study. Males were removed after they eclosed as adults and the first 15 from each colony were frozen and weighed. Gynes were removed and the first 15 from each colony frozen and weighed two weeks after eclosion, because in at least some ant species, gynes gain most of their weight in the first few weeks of their adult life (e.g., *Solenopsis invicta*, Tschinkel 1993).

Statistical Analyses

Based on standard full-sib analysis, ANOVA was used to partition residual total phenotypic variance (after removing site and colony demographic effects, see below) for all five traits into among- and within-colony variance components for each of the seven treatments separately. Restricted maximum likelihood using the Statistica 6.1 variance components module (StatSoft 2002) gave nearly identical results (results not shown). To calculate the heritability of direct, maternal, and sibsocial effects, I first computed the intraclass correlation, $t = \mathrm{Var}_{\mathrm{among}}/\mathrm{Var}_{\mathrm{total}}$ for each treatment. In standard full-sib analysis, heritability, $h^2 = (1/2\theta)t$, where 2θ is the relatedness (twice the ''coefficient of coancestry'' Lynch and Walsh 1998) among full-sibs. Analogously, the intraclass correlations for treatments L, Q, and W were used to estimate the heritability of direct effects, h^2_{direct} (see caveat below for direct effects for sex ratio), the heritability of maternal effects, h^2_{maternal}, and the heritability of sibsocial effects, $h^2_{\mathrm{sibsocial}}$, respectively, for each trait, controlling for all other direct/indirect effects. For each heritability estimate, a different coefficient based on the relatedness between social partners must be used (see Table 1, Appendix). To estimate genetic correlations among direct and indirect effects, I calculated the appropriate covariances by subtraction (Riska et al. 1985; Lynch and Walsh 1998; Wolf 2003) and divided the covariance by the square root of the product of the appropriate variances (see Table 1, Appendix). Finally, I estimated total heritability, h^2_{total}, which takes into account all direct and indirect genetic variance and covariance terms (Dickerson 1947; Wilham 1963; see Appendix). I used 10,000 bootstrap samples across families for each treatment to estimate parameters and 95% confidence intervals (Lynch and Walsh 1998). For further details and assumptions of the indirect genetic effect model used, see the Appendix.

An important caveat is that the estimate of variance for direct effects for sex ratio is confounded with variance due to premanipulation maternal effects. One limitation of cross-fostering studies is that estimates of genetic effects are always confounded with prefoster effects (Lynch and Walsh 1998). For mass and caste ratio, premanipulation effects can be reasonably assumed to be negligible because premanipulation larval mass does not predict adult mass (see above) and caste determination is known to occur after the time the manipulations occurred (Wesson 1940; T. A. Linksvayer, unpubl. data). However, for sex ratio, variation among queens in the primary sex ratio of eggs laid has been shown to be an important determinant of adult sex ratio in some ant species (Passera et al. 2001; Rosset and Chapuisat 2006). Estimates of (postfoster) maternal and sibsocial effects for sex ratio are not similarly affected because related larval groups were mixed.

Queen removal is expected to result in changes in mean caste ratio and sex ratio (Franks et al. 1990). Queen removal could also affect phenotypic variance in addition to the expected effect on maternal effect (co)variance components if queen removal is associated with increased environmental variance or variance due to an interaction between worker/larvae genotype (i.e., if genotypes respond differently to queen removal). To evaluate whether there were any such queen removal effects on phenotypic variance, I compared the 95% confidence intervals for among-colony variance for all traits in colonies with and without queen removal.

Mass.—I grouped worker pupae into four age classes based on the degree of eye and body pigmentation, because pupae are expected to lose mass as they develop. Masses of worker pupae in these four pigmentation classes were standardized to a mean of zero to remove age effects. Males and gynes were collected as fully pigmented adults. Collection site, initial number of workers, initial number of larvae, number of larvae per worker, and total colony production of workers plus gynes plus males were considered as potential covariates of mean colony worker, gyne, and male mass. Differences among colonies associated with collection site, colony size, and colony productivity variables are likely mainly due to uncontrolled environmental differences among colonies prior to collection. Statistically removing these effects minimizes the contribution of environmental effects to the observed among-colony variance (e.g., Rauter and Moore 2002). The significance of these potential covariates was tested using the Statistica 6.1 general regression models in the general linear models module (StatSoft 2002). Because the focus of the study is the comparison of components of variance for residual mass among treatments, predictors that were significant for any treatment were used to build a model for all treatments, and this model was used to compute residual mean colony mass for each treatment. Residual mass for individuals was then computed and was used for subsequent quantitative genetic analyses.

Caste ratio and sex ratio.—Caste and sex are binary traits, meaning that standard quantitative genetic analyses are not immediately applicable. However, if the unobserved, continuous, and normally distributed character liability is assumed to underlie a binary trait, such that individuals with liability below a threshold value express one phenotype and individuals with liability above a threshold value express the alter-

TABLE 2. Estimates of the heritability of direct effects, maternal effects, and sibsocial effects for each trait, with 95% confidence intervals in parentheses. The estimate of h^2_{direct} for sex ratio is bracketed to indicate that it should be interpreted with caution because it is confounded with premanipulation maternal variation for primary sex ratio. *n*, total number of individuals used for each trait; *N*, total number of colonies.

Trait	h^2_{direct}	$h^2_{maternal}$	$h^2_{sibsocial}$	*n*	*N*
Worker mass	0.37 (0.23, 0.43)*	0.19 (0.12, 0.27)*	0.12 (0.03, 0.24)*	3828	341
Gyne mass	0.74 (0.51, 0.89)*	0.47 (0.34, 0.58)*	0.43 (0.30, 0.57)*	2101	232
Male mass	0.99 (0.64, 1.19)*	0.11 (−0.12, 0.30)	0.37 (0.14, 0.56)*	639	135
Caste ratio	0.65 (0.52, 0.76)*	0.52 (0.45, 0.58)*	0.49 (0.39, 0.59)*	11,464	355
Sex ratio	[1.02 (0.80, 0.57)]*	0.35 (0.28, 0.42)*	0.48 (0.38, 0.57)*	4400	267

* 95% CIs do not include zero.

nate phenotype, then standard quantitative genetic analyses can be used for liability (Falconer and Mackay 1996; Lynch and Walsh 1998). Mean liabilities of groups can be calculated, given the proportional representation of each morph in the groups (Falconer and Mackay 1996; Lynch and Walsh 1998). If the groups are relatives of known relatedness, then the variance of group mean liabilities can be used to estimate genetic variance (Moorad 2005).

I transformed the proportion of females that were gynes (caste ratio) and the proportion of sexuals that were male (sex ratio) for each colony to colony mean liability for caste and sex (Falconer and Mackay 1996; Lynch and Walsh 1998). Because liability is undefined when a colony's caste or sex ratio is zero or one, colony caste or sex ratios that were equal to zero or one were transformed from $p = 0$ to $p' = 0 + 1/(2N_{total})$ or from $p = 1$ to $p' = 1 - [1/(2N_{total})]$, respectively, where N_{total} is the total number of females or sexuals (Moorad 2005). A general regression model (as for mass) was then used to assess the significance of the effects of collection site, colony size, and total colony productivity. After effects that were significant for any treatment were removed, residuals were used to estimate causal (co)variance components. Queen dry mass was also considered as a potential covariate for treatments with queen removal (L, W, and WL), but it did not have a significant effect after the other covariates were considered and is not discussed further.

RESULTS

A total of 360 monogynous colonies with 10 or more workers were collected. These colonies produced a total of 7928 workers, 3536 gynes, and 864 males, of which 3828 workers, 2101 gynes, and 639 males were weighed. Workers in all treatments behaved normally (e.g., collected fruit flies and cared for larvae) and reared most larvae to adulthood. The treatment effects on the colony means will be analyzed in a separate paper. It is worth noting here that, as expected, queen removal had a large effect on mean caste ratio and sex ratio (results not shown). However, queen removal was not associated with increased phenotypic variance for any traits (see Table S1 in Supplementary Material available online only at http://dx.doi.org/10.1554/06-011.1.s1), indicating that queen removal did not cause increased environmental variance or variance due to genotype-by-environment interaction. The treatment effects on intraclass correlations are shown in Table S2 of the Supplementary Material available online. Heritabilities are summarized in Table 2, and genetic correlations and total heritabilities are summarized in Table 3.

As expected, degree of pigmentation, the indicator of developmental stage, had a significant negative effect on worker pupal mass ($F_{4,3823} = 65.787$, $P < 0.001$). After this age effect was removed, site and colony covariates accounted for an average of 3.4% of total phenotypic variance for residual worker mass across all treatments. Site and colony covariates accounted for an average of 17.7%, 49.7%, 13.1%, and 5.7% of total phenotypic variance for gyne mass, male mass, liability for caste ratio, and liability for sex ratio, respectively (see Table S2 in Supplementary Material available online).

Worker, Gyne, and Male Mass

Estimates of direct, maternal, and sibsocial heritabilities for residual worker and gyne mass were significant (i.e., the 95% CI did not include zero, Table 2). Maternal and sibsocial heritabilities of residual worker and gyne mass were lower (36–62%) than the direct heritability for residual worker and gyne mass. The genetic correlations for residual worker mass ranged from −0.04 to −0.47, and none were significant (Table 3). For residual gyne mass, the genetic correlations ranged

TABLE 3. Estimates of the genetic correlations between direct and maternal effects, direct and sibsocial effects, and maternal and sibsocial effects, as well as estimates of total heritability, incorporating all direct and indirect variance and covariance components. The 95% confidence intervals are in parentheses. Estimates for sex ratio involving (co)variance due to direct effects are bracketed to indicate that they should be interpreted with caution because they also include (co)variance components due to premanipulation maternal effects on primary sex ratio.

Trait	$r_{direct\ maternal}$	$r_{direct\ sibsocial}$	$r_{maternal\ sibsocial}$	h^2_{total}
Worker mass	−0.47 (−0.94, 0.23)	−0.41 (−0.85, 0.22)	−0.04 (−0.89, 1.52)	0.28 (−0.08, 0.64)
Gyne mass	−1.15 (−1.62,−0.47)*	−0.25 (−0.57, 0.28)	−1.25 (−1.83,−0.59)*	−0.46 (−1.09, 0.18)
Caste ratio	−1.06 (−1.29,−0.73)*	−0.47 (−0.68,−0.19)*	−1.45 (−1.65,−1.19)*	−1.00 (−1.58,−0.43)*
Sex ratio	[−0.99 (−1.23,−0.69)]*	[−0.74 (−1.01,−0.27)]*	−0.50 (−0.93, 0.08)	[−0.13 (−0.44, 0.21)]

* 95% CIs do not include zero.

from −0.25 to −1.25, and all estimates were significantly less than zero (Table 3). Note that correlation estimates with an absolute value of greater than 1.0 are possible because the correlations were not product-moment correlations, but were calculated using the appropriate covariance and variance components. Total heritability estimates for residual worker and gyne mass were indistinguishable from zero, despite significant heritability for direct, maternal, and sibsocial effects considered separately (Table 3). For residual male mass, direct and sibsocial heritabilities were significant (Table 2). The estimate of sibsocial heritability of male mass was approximately 40% that of direct heritability of male mass. Because there were only five and six male-producing colonies in treatments QWL and QL, respectively, I did not calculate any correlation or total heritability estimates for residual male mass.

Liability for Caste and Sex

Although estimates of direct, maternal, and sibsocial heritability for residual liability for caste and sex were all significant, the direct heritability estimate of sex ratio is confounded with premanipulation maternal variation for primary sex ratio (Table 2). Estimates for (postmanipulation) maternal and sibsocial heritability of residual sex liability were of similar magnitude. The estimate of sibsocial heritability for residual caste liability was lower (50–60%) than the corresponding estimates of maternal heritability and direct heritability. The correlation estimates for residual caste and sex liability were all significantly negative except for the maternal-sibsocial covariance for residual sex liability (Table 3). The estimate of total heritability for residual liability for sex was negative but not significantly so, whereas the estimate of total heritability for residual caste liability was significantly less than zero. As for direct heritability for residual sex liability, genetic correlations involving direct effects and total heritability should be interpreted with caution because they are confounded with premanipulation maternal effects (Table 3).

Discussion

Heritability of Direct, Maternal, and Sibsocial Effects

I used an indirect genetic effect approach to study the genetic architecture underlying worker, gyne, and male mass; caste ratio; and sex ratio in the ant *T. curvispinosus*. The main result of this study is that there were generally high levels of among-colony variance for all traits attributable to variation in direct effects, maternal (queen) effects, and sibsocial (worker) effects. Only the estimate of variance due to maternal effects for male mass was not significant. Because most environmental factors contributing to differences among colonies were experimentally or statistically controlled, these results indicate genetic variance for direct, maternal, and sibsocial effects on worker mass, gyne mass, and caste ratio and for direct and sibsocial effects on male mass. Because the estimate of variance due to direct effects on sex ratio includes maternal variance for primary sex ratio, for sex ratio, the results indicate genetic variance for (postmanipulation) maternal and sibsocial effects as well as for direct effects plus premanipulation maternal effects.

A genetic component to body size has been demonstrated in many organisms, with both direct and indirect genetic effects contributing to size variation in a variety of taxa (e.g., Roff 1997). Previous studies of the genetic basis of size variation in social insects have implicated the social environment (e.g., Goodisman et al. 1999; Rüppell et al. 2001). The estimates of heritability for maternal and sibsocial effects for mass in the current study confirm that the social environment can strongly affect mass, and further that the social environment has a genetic basis.

In contrast to body size, genetic influences on caste and sex in social insects are usually not considered (Crozier and Pamilo 1996). Caste determination is assumed to occur conditionally, in response to environmental factors (e.g., nutritional quality and quantity). In haplodiploids, sex is determined primarily by whether an egg is fertilized, and social insect sex ratio models typically focus on social environmental factors such as colony size and relatedness among nestmates (Crozier and Pamilo 1996; but see Pamilo 1982; Roisin and Aron 2003; Helms et al. 2005). However, a direct genetic component to caste (Kerr 1950; Winter and Buschinger 1986; Julian et al. 2002; Volny and Gordon 2002), queen polymorphism (Buschinger 2005), and worker polymorphism (Fraser et al. 2000; Hughes et al. 2003; Rheindt et al. 2005) has been demonstrated in a variety of social insect taxa. Furthermore, subfamily membership in honeybee colonies was found to affect the likelihood that individual workers rear queens versus workers (Page et al. 1989; Robinson et al. 1994), suggesting sibsocial genetic variance for caste ratio. Other studies have demonstrated maternal genetic variance for primary sex ratio in ants (Passera et al. 2001; Rosset and Chapuisat 2006), and the proportion of male comb produced in honeybee hives, a trait under worker control, has been shown to be heritable (Page et al. 1993), suggesting sibsocial genetic variance for sex ratio. These studies, along with the results of the current study, suggest that both direct and indirect genetic influences on caste ratio and sex ratio may be more common than previously thought (Linksvayer et al. 2006).

The estimates of heritability of direct effects were generally higher than the heritability of maternal and sibsocial effects for all traits, indicating that direct genetic effects contribute a larger proportion of total phenotypic variance. In general, these results are in agreement with those from subsocial animals in which variance in maternal genetic effects usually contributes less, but can contribute as much as variance in direct genetic effects to total phenotypic variance for offspring traits (Cheverud 1984; Hunt and Simmons 2002; McAdam et al. 2002; Rauter and Moore 2002).

Genetic Correlation between Direct and Indirect Effects

In addition to consistently detecting genetic variation for direct, maternal, and sibsocial effects, the other main result of this study is that the estimates of genetic correlations between direct and indirect effects were consistently negative for all traits (Table 3). Negative direct-maternal genetic correlations have also been found in a variety of domestic mam-

mals (Wilson and Reale 2006) as well as natural animal populations (e.g., Agrawal et al. 2001; reviewed in Roff 1997). In addition, Bienefeld and Pirchner (1990) found strong negative genetic correlations between queen and worker effects on colony traits such as honey production and aggressiveness in the honeybee *Apis mellifera carnica.*

Genetic covariances and correlations are important evolutionary parameters that imply genetic constraints, because genetically correlated traits cannot independently respond to selection (Lande 1979; Arnold 1992). Despite the generally high estimates of heritable variation for direct, maternal, and sibsocial effects on mass, caste ratio, and sex ratio, estimates of total heritability for these traits, incorporating all genetic variance and covariance components, were negative or not different than zero (Table 3). These results suggest that the direct and indirect genetic components cancel each other out to some degree, leading to a genetic constraint on the evolutionary response to selection. Even though the direct, maternal, and sibsocial components of the phenotype all have a genetic basis, selection on any one component may cause opposing correlated responses to selection for the other components (Cheverud and Moore 1994).

Genetic correlations are caused by pleiotropy or linkage disequilibrium (Falconer and Mackay 1996). Theory suggests that stabilizing selection on a trait influenced by direct and indirect genetic effects can build up a negative correlation due to linkage disequilibrium or antagonistic pleiotropy because certain gene combinations (e.g., a positive maternal effect and a negative direct effect) produce intermediate phenotypes that are favored (Wolf and Brodie 1998). This process can be considered to be the coadaptation of parent and offspring genes (Wolf and Brodie 1998; Agrawal et al. 2001). Directional selection may also result in the buildup of negative correlations because alleles with antagonistic pleiotropy are maintained longer than alleles with other patterns of pleiotropic effects (Falconer and Mackay 1996).

Implications for Kin Selection, Parent-Offspring Conflict, and Queen-Worker Conflict

The genetic basis of offspring size, caste ratio, and sex ratio has traditionally been modeled in terms of kin selection and parent-offspring conflict theory. For example, the evolutionary origin of the sterile worker caste is often attributed to the spread of ''kin-selected-altruism'' or ''offspring-control'' genes expressed in offspring (Hamilton 1964a,b; Michod 1982) or ''parental-manipulation'' genes expressed in social partners (Alexander 1974; Michener and Brothers 1974; see Linksvayer and Wade 2005). For the studied population of *T. curvispinosus*, there was genetic variance for direct, maternal, and sibsocial effects for most traits (Table 2), demonstrating that genes expressed in both developing larvae and in their social partners have the potential to influence the evolutionary dynamics of these traits.

As explained above, negative correlations between direct, maternal, and sibsocial effects may constrain or complicate evolutionary responses to selection. For example, with a negative direct-maternal genetic covariance for caste, positive selection on direct effect genes that increase the probability a larva develops into a gyne would cause a correlated response to selection in the opposite direction on the maternal effect, decreasing gyne-biased development. A possible mechanistic explanation is that genotypes that produce larval phenotypes with an increased propensity to beg for food (Kaptein et al. 2005) may also produce queen and worker phenotypes that respond less to larval begging stimuli (Agrawal et al. 2001; Kölliker 2005; Kölliker et al. 2005). Similarly, selection for queens to suppress the gyne development of their female offspring through a negative maternal effect would result in a corresponding increased bias toward gyne development through the correlated direct genetic effect.

Selection on, for instance, larvae and the queen or the queen and workers, may be in opposing directions so that there is a predicted evolutionary conflict between social partners, but this conflict may never actually be realized in terms of an evolutionary response to selection (Cheverud 1984; Lynch 1987; Cheverud and Moore 1994; Wolf et al. 1998). Just as costs and limitations on information and power can constrain the evolution of conflicts among queen, worker, and larval nestmates (Heinze 2004; Korb and Heinze 2004), so can the genetic architecture underlying social insect phenotypes (Boomsma et al. 2003). These results suggest that consideration of the genetic architecture underlying traits that are likely the focus of parent-offspring and queen-worker conflict is necessary to fully understand the evolutionary dynamics of these traits.

More generally, this study demonstrates some of the complexity of the genetic architecture underlying social insect phenotypes. The evolutionary dynamics of social insect phenotypes involve the coevolution of genes expressed in interacting brood, workers, and queens. Theoretical and empirical approaches that consider this complexity should help to elucidate all aspects of the study of social evolution.

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Appendix

I used the following linear model to partition total phenotypic variance into causal components and to calculate the coefficients of each component (after Cheverud 1984; Lynch 1987; Cheverud and Moore 1994):

$$z = a_d + e_d + M + S, \tag{A1}$$

where the phenotype (z) of a focal individual is determined by the following genetic and environmental effects: direct additive genetic effects (a_d), direct environmental effects (e_d), the effect of the social environment provided by the queen (maternal performance M), and the effect of the social environment provided by adult sib workers (sibsocial performance S). Maternal and sibsocial performance are also determined by genetic and environmental effects:

$$M = a'_m + e'_m \quad \text{and} \tag{A2}$$

$$S = a'_s + e'_s, \tag{A3}$$

where a'_m and a'_s are maternal and sibsocial additive genetic effects and e'_m and e'_s are maternal and sibsocial environmental effects. The primes indicate that the social performance effects are due to genetic and environmental effects experienced by social partners. Substituting for M and S,

$$z = a_d + e_d + a'_m + e'_m + a'_s + e'_s. \tag{A4}$$

Under this model, all genetic effects are assumed to be additive, that is, dominance and epistasis are ignored. The variable z in social partners is assumed to have no effect on z in the focal individual (i.e., nonreciprocal effects; Moore et al. 1997). There are assumed to be no interactions between any genetic and environmental effects (i.e, no genotype-by-environment interactions) or between any two genetic effects (i.e., no genotype-by-genotype interactions), and queen removal is assumed to not be associated with increased environmental variance, as described above. Furthermore, except for premanipulation maternal effects on primary sex ratio, premanipulation effects are assumed to be negligible. If these assumptions are not true, the genetic estimates will be biased.

With these assumptions, and assuming no covariance due to environmental effects, the covariance between individuals 1 and 2 for trait z is (after Wilham 1963):

$$\begin{aligned}\mathrm{Cov}(z_1, z_2) = {} & 2\theta_{dd}G_{dd} + 2\theta_{mm}G_{mm} + 2\theta_{ss}G_{ss} \\ & + (2\theta_{dm} + 2\theta_{md})G_{dm} + (2\theta_{ds} + 2\theta_{sd})G_{ds} \\ & + (2\theta_{ms} + 2\theta_{sm})G_{ms},\end{aligned} \tag{A5}$$

where G_{dd}, G_{mm}, and G_{ss}, are genetic variance for direct, maternal, and sibsocial effects, respectively, and G_{dm}, G_{ds}, and G_{ms} are direct-maternal, direct-sibsocial, and maternal-sibsocial genetic covariance, respectively. $2\theta_{xy}$ is the relatedness between social partners x and y within colonies, with d indicating larvae, m indicating the queen, and s indicating the workers. For female full-sibs in a colony with their mother queen and full-sib workers (i.e., unmanipulated colonies in treatment QWL), this covariance is:

$$\begin{aligned}&\mathrm{Cov}(z_1, z_2)_{\text{haplodiploid female full sibs}} \\ &\quad = 3/4G_{dd} + G_{mm} + 3/4G_{ss} + G_{dm} + 3/2G_{ds} + G_{ms}.\end{aligned} \tag{A6}$$

The coefficients of causal variance components were similarly calculated for the other treatments and are shown for haplodiploid full-sib females in Table 1. Coefficients for males are different (e.g., $2\theta_{dd} = ½$ for full-sib males). Because of the different coefficients of males and females, the coefficients for sex ratio are complex. I used the mean colony sex ratio across all treatments to calculate the mean relatedness between individuals and their social partners. For example, $2\theta_{dd} = p_{\text{male}}(0.50) + (1 - p_{\text{male}})[(1 - p_{\text{male}})(0.75) + p_{\text{male}}(0.25)]$, because brother-sib relatedness is 0.50, sister-sister relatedness is 0.75, and sister-brother relatedness is 0.25.

Among-colony variance for treatments L, Q, and W include only one causal variance component each and are directly proportional to G_{dd}, G_{mm}, and G_{ss}, respectively (Table 1). Among-colony variance for the remaining treatments (WL, QL, QW, QWL) include more than one causal variance component as well as at least one causal covariance component (Table 1). Causal covariance components then must be indirectly estimated by subtraction of the observed among-colony variance components of certain treatments (cf. Riska et al. 1985; Wolf 2003):

$$G_{dm} = \mathrm{Var}_{QL} - \mathrm{Var}_{Q} - \mathrm{Var}_{L}, \quad \text{(A7a)}$$

$$G_{ds} = \mathrm{Var}_{WL} - \mathrm{Var}_{W} - \mathrm{Var}_{L}, \quad \text{and} \quad \text{(A7b)}$$

$$G_{ms} = \mathrm{Var}_{QW} - \mathrm{Var}_{Q} - \mathrm{Var}_{W}. \quad \text{(A7c)}$$

In social insect species with larger colony size and multiple related queens (or the potential to create related queens with controlled breeding; e.g., *Solenopsis invicta* and *Apis mellifera*,), it is theoretically possible to estimate these covariance components directly by estimating the covariance of direct, maternal, and sibsocial effects of replicate related larval groups, queens, and worker groups (cf. Agrawal et al. 2001).

I calculated genetic correlations with the appropriate covariance and variance components, for example the direct-maternal genetic correlation, $r_{dm} = G_{dm}/(G_{dd}G_{mm})^{1/2}$. I also calculated the heritability of total genetic effects or total heritability, h^2_{total}, which takes into account all direct and indirect genetic variance and covariance terms (Dickerson 1947; Wilham 1963). The total heritability is the covariance of breeding value (a) and phenotype (z), divided by total phenotypic variance (P) (after Wilham 1963):

$$\begin{aligned} h^2_{\text{total}} &= \mathrm{Cov}(a, z)/P \\ &= \mathrm{Cov}(a_d + a_m + a_s, a_d + e_d + a'_m + e'_m + a'_s + e'_s)/P, \\ &= [G_{dd} + 2\theta_{dm}G_{mm} + 2\theta_{ds}G_{ss} + (1 + 2\theta_{ds})G_{ds} \\ &\quad + (1 + 2\theta_{dm})G_{dm} + (2\theta_{dm} + 2\theta_{ds})G_{ms}]/P. \end{aligned} \quad \text{(A8)}$$

For example, with full-sib haplodiploid females, in a colony with full-sib workers and their mother queen (i.e., unmanipulated colonies in treatment QWL), the total heritability, h^2_{total}, equals (G_{dd} + 1/2G_{mm} + 3/4G_{ss} + 7/4G_{ds} + 3/2G_{dm} + 3/2G_{ms})/P. Note that in the absence of indirect effects (i.e., $G_{mm} = G_{ss} = G_{ds} = G_{dm} = G_{ms} = 0$), this is simply $h^2_{\text{direct}} = G_{dd}P$, as expected. Colonies in treatment QWL were not manipulated and I used the total phenotypic variance for these colonies as an estimate of total phenotypic variance, P, for total heritability.