SOCIAL EVOLUTION IN ANTS: DIRECT AND INDIRECT GENETIC EFFECTS

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Submitted to the faculty of the University Graduate School

in partial fulfillment of the requirements

for the degree

Doctor of Philosophy

in the Department of Biology

Indiana University

October 2005

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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7 October, 2005

ACKNOWLEDGEMENTS

First I would like to thank the members of my committee: Mike Wade, Butch Brodie, Loren Rieseberg, and Curt Lively. Mike has had a tremendous impact on how I think about and do science. His unique perspective and his insights into social evolution, as well as his excitement for science in general have made him an ideal graduate advisor for me. Butch has been something like a co-advisor for me in terms of his impact on the development of my studies of indirect genetic effects. Indeed, Mike and Butch together have been a major factor making Indiana University such a good environment for my studies. Although I interacted much less with Loren and Curt, they provided thoughtful criticism and discussion at my committee meetings, and during the review of my dissertation.

Besides my committee members, my experience at Indiana University was largely shaped through interactions with my fellow graduate students. A number of them have been extremely helpful by discussing my variance component designs and especially by discussing how to analyze the results. Jake Moorad and Erik Osnas have been particularly helpful. I would also like to thank Aneil Agrawal, Jeremiah Busch, Ben Ridenhour, Liz Lehman, and Desiree Allen. In addition, Liz Lehman, Erik Osnas, and Jeremiah Busch helped with nest collection and Liz Lehman and Maurine Neiman provided comments on several drafts of my dissertation chapters.

My dissertation research would not have been possible without the help of several undergraduate assistants. In particular, Gabe Harp, Stephanie McAfee, and Patrick McChesney each weighed thousands of ants and helped to maintain hundreds of colonies.

I would also like to thank Alfred Buschinger for providing advice on how to rear the colonies and for many insights into formicoxenine biology. Joan Herbers provided useful comments on several chapters. My dissertation research was funded mainly by grants from the Indiana Academy of Sciences Graduate Research Grant program and Indiana University Teaching and Research Preserve Grant Program.

Finally, I would like to acknowledge earlier influences on my development as a scientist. Given the topic of my dissertation, I cannot forget parental and sib effects, and I believe that my interest in science (and ants) sprang from a large fraction of my childhood spent playing in the wilds of my backyard. Later, during college, Mark McKone encouraged my scientific interest in ants and it was his tropical rainforest ecology class to Costa Rica that convinced me that ants were what I wanted to study.

ABSTRACT

When social interactions occur, the phenotype of an individual can be influenced both directly by its own genes and indirectly by genes expressed in social partners. Eusocial insect colonies are notable for extensive behavioral interactions among larval, worker, and queen nestmates. In particular, developing larvae are reliant on care provided by workers and queens. Social insect phenotypes are thus affected by zygotic genes expressed during development (direct genetic effects), genes expressed in care-giving adult workers (sibsocial genetic effects), and genes expressed in queens (maternal genetic effects). The purpose of this dissertation was to incorporate this complexity into models of social insect evolution and to empirically study the evolutionary importance of direct and indirect genetic effects on ant phenotypes.

The first chapter reviews existing models for the evolutionary origin and maintenance of eusociality in social insects and proposes a new model incorporating both direct and indirect genetic effects. The second chapter uses a quantitative genetic approach to estimate variation for direct and indirect genetic effects on worker, gyne, and male mass, caste ratio, and sex ratio within a population of the ant *Temnothorax curvispinosus*. There was genetic variation for direct, maternal, and sibsocial effects for all traits, suggesting that larval, queen, and worker influences on mass, caste ratio, and sex ratio can respond to selection. However, there was also evidence for negative genetic correlations between each effect, demonstrating a potential constraint to the independent evolution of these effects. The third chapter uses the same ant colonies to study the effects of experimental queen removal and manipulation of relatedness among workers

and larvae on mean colony worker, gyne, and male mass, caste ratio, and sex ratio. The fourth chapter examines the contribution of direct and sibsocial genetic effects to phenotypic differences between three *Temnothorax* species. The results demonstrate that among-species differences are influenced by the interaction of genes expressed in developing larvae and care-giving workers. Overall, this dissertation demonstrates the evolutionary importance of both direct and indirect genetic effects to social insect phenotypes.

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CHAPTER 1

THE EVOLUTIONARY ORIGIN AND ELABORATION OF SOCIALITY IN THE ACULEATE HYMENOPTERA: MATERNAL EFFECTS, SIB-SOCIAL EFFECTS, AND HETEROCHRONY

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(The Quarterly Review of Biology 80: 317-336)

ABSTRACT

We discuss the evolutionary origin and elaboration of sociality using an indirect genetic effects perspective. Indirect genetic effects models simultaneously consider zygotic genes, genes expressed in social partners (especially mothers and siblings), and the interactions between them. Incorporation of these diverse genetic effects should lead to more realistic models of social evolution. We first review haplodiploidy as a factor that promotes the evolution of eusociality. Social insect biologists have doubted the importance of relatedness asymmetry caused by haplodiploidy and focused on other predisposing factors such as maternal care. However, indirect effects theory shows that maternal care evolves more readily in haplodiploids, especially with inbreeding and despite multiple mating. Because extended maternal care is believed to be a precondition for the evolution of eusociality, the evolutionary bias towards maternal care in haplodiploids may result in a further bias towards eusociality in these groups. Next, we compare kin selection and parental manipulation and then briefly review additional hypotheses for the evolutionary origin of eusociality. We present a verbal model for the evolutionary origin and elaboration of sib-social care from maternal care based on the modification of the timing of expression of maternal care behaviors. Specifically, heterochrony genes cause maternal care behaviors to be expressed prereproductively towards siblings instead of postreproductively towards offspring. Our review demonstrates that both maternal effect genes, expressed in a parental manipulation manner, and direct effect zygotic genes, expressed in an offspring control, kin selection manner are likely involved in the evolution of eusociality. We conclude by describing theoretical and empirical advances with indirect genetic effects and sociogenomics, and

provide specific quantitative genetic and genomic predictions from our heterochrony model for the evolutionary origin and elaboration of eusociality.

The nature and amount of parental investment varies widely across taxa. In some species, gamete size appears to be reduced to a minimum, indicating minimal investment, while in others, parents expend many resources on large gametes and/or postnatal parental care (Trivers 1972; Clutton-Brock 1991). Brood defense and provisioning are forms of parental investment found in a wide variety of animals, such as some crustaceans, spiders, mites, scorpions, millipedes, insects, and vertebrates (Wilson 1971). Social systems characterized by these types of parental care are described as being "subsocial" (sensu Wheeler 1923; Wilson 1971; Alexander et al. 1991). Although behaviorally similar to parental care, alloparental or sib-social care has a more restricted taxonomic distribution. By "sib-social" care, we mean those instances in which young adults remain for some time in their natal nest to help rear siblings, as is found in some birds, mammals, and insects (Wilson 1975). Finally, in some taxa described as being "eusocial," sib-social helpers remain at their natal nest more or less permanently, and there is a reproductive division of labor, overlapping of adult generations, and cooperative brood care (Michener 1969; Wilson 1971). Traditionally, only the ants and some bees and wasps (all found in the insect order Hymenoptera) and the termites were considered to be eusocial. More recently, eusociality has been discovered in naked mole rats (Jarvis 1981), aphids (Aoki 1982; Itô 1989), an ambrosia beetle (Kent and Simpson 1992), thrips (Crespi 1992), and snapping shrimp (Duffy 1996). There has been discussion about broadening the definition of eusociality to also include taxa with helpers that provide sib-social care only temporarily (e.g., Gadagkar 1994; Crespi and Yanega 1995; Sherman et al. 1995).

The evolution of parental care is often understood as maximizing the direct fitness of the parents (i.e., individual-level selection) (e.g., Alexander 1974; Clutton-Brock 1991). However, indirect genetic effect models reveal that assigning components of offspring fitness, such as early survival to parents, can lead to incorrect evolutionary inferences (Wolf and Wade 2001). The evolution of sib-social care is more complex because the beneficiaries of care are not offspring of the caregivers but rather kin to them with varying degrees of relatedness. Darwin (1859) suggested that selection at the family level could result in the evolution of sterile helpers, as found in eusocial insects. Hamilton (1963, 1964a,b, 1972) formalized these ideas in his theory of inclusive fitness and showed that altruistic behaviors evolve when the genetic relatedness (r) between social partners is greater than the ratio of fitness costs (c) to the performer over fitness benefits (b) to the recipient: $r > c/b$. This relationship, known as Hamilton's Rule, is the foundation of inclusive fitness or kin selection theory. Wade (1979, 1980, 1982b, 1985) and others have shown that kin selection is a combination of two levels of selection, namely selection among individuals within kin groups and selection among kin groups. In this theoretical context, Hamilton's Rule specifies the conditions under which selection among kin groups is stronger than opposing selection within kin groups.

In this paper, we discuss the evolutionary origin and elaboration of social behaviors in the aculeate Hymenoptera and offer maternal effects and indirect genetic effects theory as a complement to existing models of social evolution. Individual and colony phenotypes of social insects are influenced by genes expressed zygotically as well as by genes expressed in social partners (i.e., mother, sibling brood, sibling workers) (Figure 1). These direct and indirect genetic effects and interactions among them are

likely to strongly influence the evolutionary dynamics of social insect traits (e.g., Cheverud 1984, 2003; Wolf et al. 1998), and we believe that incorporating these various effects will lead to more comprehensive and realistic models of social evolution.

Several authors have discriminated between the evolutionary origin, maintenance, and elaboration of eusociality, emphasizing that the selection pressures involved in the evolutionary origin may be very different than those involved in the maintenance or elaboration of eusociality (e.g., Crespi 1996; Michener 2000). We focus largely on the evolutionary origin of eusociality, specifically the genetic and developmental basis of sib-social care. We also discuss the evolutionary elaboration of eusociality (e.g., queen-worker phenotypic divergence), but stress that the genetic and developmental machinery underlying the origin of eusociality is also likely to be involved in the elaboration of eusociality.

First we review the role of haplodiploidy in the evolution of maternal and sib-social care, because this genetic system facilitates the evolution of maternal effect genes just as it sometimes does altruism genes through kin selection. Second, we introduce parental manipulation, a traditionally recognized alternative to kin selection for the evolution of eusociality, and review the differences between kin selection genes and parental manipulation genes. We then review more proximate, mechanistic hypotheses and present a scenario for the evolutionary origin and elaboration of eusociality based on heterochrony genes that modify the timing of expression of maternal care. Finally, we discuss how quantitative genetic and sociogenomic approaches can be used to study the evolution of eusociality. Although we focus primarily on the social aculeate

Hymenoptera, we believe that our remarks are applicable to the evolution of sociality in all taxa in which sib-social care is derived from maternal care.

HAPLODIPLOIDY AND THE EVOLUTIONARY ORIGIN OF EUSOCIALITY

The "Haplodiploid Hypothesis"

For much of the past century, social insect biologists believed that eusociality arose more than ten times in the order Hymenoptera (wasps, bees, and ants) but only once in nonhymenopteran insects (termites) (e.g., Wheeler 1923; Wilson 1971). As a corollary to kin selection theory, termed the "haplodiploid hypothesis" by West-Eberhard (1975), Hamilton (1964b) suggested that a sterile female caste had more frequently evolved in the haplodiploid Hymenoptera because of the asymmetry of genetic relatedness that exists between haplodiploid females and their relatives. Full sib haplodiploid females are more closely related to one another ($r = 0.75$) than they are to their brothers ($r = 0.25$) or to their own sons and daughters ($r = 0.5$). In contrast, diplodiploid male and female siblings are equally related to one another and to their offspring ($r = 0.5$). Thus, alleles causing haplodiploid females to care for their sisters instead of their own offspring spread more easily than such alleles in diplodiploids.

Although the haplodiploid hypothesis was initially embraced (e.g., Wilson 1971, 1975; Trivers and Hare 1976), it is now doubted by most social insect biologists (reviewed by Andersson 1984; Bourke and Franks 1995; Crozier and Pamilo 1996; Queller and Strassmann 1998). Because haplodiploid sisters are less closely related to their brothers than diplodiploids, female haplodiploid helpers must invest more resources

in rearing sisters than brothers to capitalize on the high relatedness (Trivers and Hare 1976; Wade 1979). Furthermore, colonies with helpers must bias resource investment towards females to a greater degree than the rest of the population so that female-biased investment and helping behavior become associated (e.g., Charlesworth 1978; Charnov 1978b; Iwasa 1981; Grafen 1986). While several authors have proposed models by which this may occur (e.g., Seger 1983; Grafen 1986; Godfray and Grafen 1988; Frank and Crespi 1989), the conditions that favor the evolution of eusociality in haplodiploids are not as easily met as initially proposed by Hamilton (1964b).

In addition, multiple queens (polygyny) and multiple mating (polyandry) are common in the eusocial Hymenoptera (reviewed by Bourke and Franks 1995; Crozier and Pamilo 1996), and both reduce the relatedness among female siblings (Hamilton 1972; Wade 1982a). These phenomena often reduce relatedness to values lower than that expected between parents and offspring or between diplodiploid siblings so that the theoretical benefit of haplodiploidy disappears or is greatly mitigated (e.g., Gadagkar 1991). This has been taken as evidence refuting the haplodiploid hypothesis, although conditions found in highly eusocial populations likely do not reflect conditions in populations at the evolutionary origin of eusociality (Crespi 1996).

The phylogenetic association between haplodiploidy and eusociality also seems to be weaker than it was once perceived. Although all Hymenoptera are haplodiploid, only four aculeate hymenopteran clades contain eusocial species (Hunt 1999). Several other large taxonomic groups are haplodiploid but do not have eusocial members (e.g., some mites, scale insects, whiteflies, and beetles) (Normark 2003), and eusociality occurs in

several diplodiploid groups, including termites, naked mole rats, aphids, snapping shrimp, and an ambrosia beetle (Gadagkar 2001).

Other predisposing traits for the evolution of eusociality

Other characteristics besides the relatedness asymmetry caused by haplodiploidy may explain the prevalence of eusociality in the Hymenoptera. For example, Hamilton (1972:206) stated, "Male haploidy is certainly not the only prerequisite for evolving a sterile caste. Perhaps the preadaptations of solitary nesting Hymenoptera as porters and builders are equally important." Similarly, a variety of other traits may be predispositions, for example, maternal care, nest building, mandibulate mouthparts, the female sting, above average chromosome numbers, short lifespan of adults relative to juvenile development time, and protogyny enabled by haplodiploidy (Wilson 1971; Sherman 1979; Andersson 1984; Starr 1985; Queller 1989; Alexander et al. 1991; Crespi 1996; Hunt 1999). Some authors stress the uniqueness of each lineage in which eusociality has arisen and the complexity of factors influencing the evolutionary origin of eusociality, such that searching for a small number of common factors underlying the evolutionary origin of eusociality is not likely to be successful, and may even be misleading (Crespi 1996; Hunt 1999). Nevertheless, Alexander et al. (1991) argued that parental care is a universal and necessary precondition for the evolution of eusociality and noted that maternal care is found more commonly in the Hymenoptera than in any other arthropod group.

Maternal care, protected invasion theory, and maternal effects theory

Despite the difficulties described above for the haplodiploid hypothesis to explain the distribution of eusociality, "some crusaders still search among the rubble of haplodiploidy" (Hunt 1991:426-427). With the discovery of eusociality in some species of haplodiploid thrips (Crespi 1992), Reeve (1993) reassessed the importance of haplodiploidy in the evolution of eusociality. "Protected invasion theory" indicates that dominant maternal care and female alloparental care alleles are less likely to be lost by drift when rare, and are more likely to fix by selection relative to paternal and male alloparental care alleles in haplodiploids or parental or alloparental care alleles in diplodiploids (Reeve 1993; Reeve and Shellman-Reeve 1997).

Using a maternal effects model, Wade (2001) similarly showed that maternal effect alleles (including alleles for maternal care) fix more readily in haplodiploids relative to diplodiploids. In addition, multiple mating does not restrict the evolution of maternal effect genes as it does kin selection genes (Wade 2001). Furthermore, Wade (2001) found that, unlike under kin selection, inbreeding differentially facilitates the evolution of maternal care in haplodiploids relative to diplodiploids. Inbreeding theoretically has strong effects on the probability of complex sociality evolving (Wade and Breden 1981; Breden and Wade 1991) and may be involved in the evolution of sociality in a number of groups such as naked mole rats, spiders, termites, and ambrosia beetles (reviewed in Choe and Crespi 1997). Thus, genetic system and mating system affect the probability of sociality evolving, and both protected invasion theory and maternal effects theory predict that maternal care should evolve more readily in haplodiploid relative to diplodiploid populations.

The association of haplodiploidy with subsociality and eusociality

An association between subsociality and haplodiploidy has long been noted (Borgia 1980; Andersson 1984; Alexander et al. 1991). "Indeed, in arthropods, haplodiploidy seems more closely correlated with subsociality than with eusociality" (Alexander et al. 1991:18). For example, subsociality in mites and ticks (subclass Acari) is only found in haplodiploid species (Saito 1997). Subsociality and haplodiploidy also co-occur in some thrips (Thysanoptera, Crespi and Mound 1997), bees and wasps (aculeate Hymenoptera), and ambrosia beetles (Coleoptera: Xyleborini, Kirkendall 1993).

It is commonly accepted that subsociality is a precondition for the evolution of eusociality (e.g., West-Eberhard 1987; Alexander et al. 1991; Hunt 1994). If subsociality is more likely to evolve in haplodiploid populations (Reeve 1993; Wade 2001), and eusociality is derived from subsociality, then eusociality is also more likely to be found in haplodiploid populations. Despite the difficulties discussed above for the haplodiploid hypothesis, there are still strong theoretical reasons to expect an association between haplodiploidy and eusociality. Below we review models for the evolutionary origin of eusociality and then describe a new model for the evolutionary origin of eusociality from subsociality.

PARENTAL MANIPULATION AS AN ALTERNATIVE TO KIN SELECTION

Two hypotheses, mutualism and parental manipulation, are traditionally presented as alternatives to kin selection for the evolutionary origin of eusociality. The mutualism hypothesis suggests that eusociality evolves through mutualistic group living (e.g.,

Michener 1958; Lin and Michener 1972; West-Eberhard 1978; Itô 1993), wherein individuals live together and reciprocally assist one another in procuring food and defending a common nesting site. Many authors (e.g., Hamilton 1972; Andersson 1984; Bourke and Franks 1995; Crozier and Pamilo 1996) have argued that mutualism alone cannot lead to eusociality, however, and thus we do not discuss the mutualism hypothesis further. As a second alternative to kin selection, Alexander (1974) and Michener and Brothers (1974) suggested that eusociality evolves through parental manipulation, wherein mothers restrict the reproductive options of some offspring so that they assist in the rearing of additional fully-fertile offspring. Reviews have emphasized that such parental manipulation is not a mutually exclusive alternative to kin selection (Michod 1982; Andersson 1984; Bourke and Franks 1995; Crozier and Pamilo 1996), and each may operate sequentially or in concert (Craig 1979). Furthermore, parental manipulation requires interactions between kin (parents and offspring), and need not be considered distinct from kin selection theory (Michod 1982; Andersson 1984; Bourke and Franks 1995; Crozier and Pamilo 1996). Indeed, the spread of alleles causing parental manipulation can be understood in terms of among-family selection (i.e., among kin group selection, or "kin selection"), just like kin selected altruism alleles, although the former is often incorrectly considered to be only individual-level selection (cf. Wolf and Wade 2001).

In theory, however, there are expected differences between the evolutionary dynamics of alleles that cause parental manipulation and kin selection alleles that cause worker altruism (e.g., Charnov 1978a; Craig 1979; Crespi and Ragsdale 2000; Wade 2001). The primary difference lies in the nature of the genetic underpinnings of the

evolving behaviors. In most kin selection models, the altruistic genes are located in and expressed in the genomes of the caregiving relatives. This can be considered "offspring control" in the case of the evolution of eusociality, because whether an offspring helps raise its sibs directly depends on its own genotype (Michod 1982). In parental manipulation models, the genes in question are located in and expressed in the maternal genome, although there may also be genes in the zygotic genome that direct offspring response to parental manipulation (Craig 1979).

Because of these differences in the location of genes underlying the behaviors, the benefit to cost ratio necessary for alleles to spread by parental manipulation is often less than kin selection (Charnov 1978a; Craig 1979; Wade 1998, 2001; Crespi and Ragsdale 2000). For example, in many models the benefit to cost ratio necessary for parental manipulation alleles is half that of kin selection alleles so parental manipulation alleles spread more easily (Michod 1982). In addition, a new parental manipulation mutation will have an initial advantage relative to a kin selection mutation expressed zygotically (Alexander 1974; Seger 1991). When a new parental manipulation mutation is expressed in a mother, it causes some portion of her offspring to express sib-social care (assuming the offspring already possess the capability to express sib-social care, see Charlesworth 1978; Craig 1979; Crespi and Ragsdale 2000), benefiting the remaining fully-fertile offspring, half of which carry the new mutation. Alternatively, when a new kin selection mutation is expressed, the individual bearing the mutation expresses sib-social care and bears a cost, but the sibling beneficiaries likely do not carry the mutation (just as with mutations causing aposematism, see Brodie and Agrawal 2001).

Throughout this paper, we consider "offspring control alleles" to be zygotic alleles that, when expressed, cause an individual to behave "altruistically" and provide care to fully-fertile siblings at the expense of its own reproduction (alleles with a negative direct effect and a positive sib-social effect). "Parental manipulation alleles," in contrast, are maternal effect alleles that cause a female to manipulate some of her offspring to help rear additional, fully-fertile siblings (alleles with a negative maternal effect and a positive sib-social effect).

Additional Models for the Evolutionary Origin of Eusociality

Because kin selection theory provides no insight into the developmental, physiological, or ecological basis of eusociality (Alexander 1974; Michener and Brothers 1974; West-Eberhard 1987; Hunt 1999), several authors have searched for more proximate, mechanistic explanations for the evolution of eusociality. In this sense, parental manipulation theory is appealing because it provides a specific underlying behavioral mechanism for sib-social care, namely, "mom made me do it" (Alexander 1974; Michener and Brothers 1974). Scenarios for the evolutionary origin of eusociality, such as the subfertility hypothesis (West-Eberhard 1975; Craig 1983) and especially the nutritional scenario (Hunt 1991, 1994, 1999), provide further explicit ecological and behavioral mechanistic details. West-Eberhard's (1996) "ovarian groundplan" scenario has a developmental focus and describes how queen and worker phenotypes diverge based on an ancestral developmental program (see also Gadagkar 1997, 2001).

Other authors have attempted to identify particular selection pressures that might favor offspring that stay at their natal nest and help rear sibs (reviewed by Queller 1996), such as defense of nests against parasites and predators (Lin and Michener 1972), the potential to inherit proven nest sites (Andersson 1984; Alexander et al. 1991), and various demographic factors **(**Queller 1989, 1996; Gadagkar 2001). Another approach, reproductive skew modeling, uses phenotypic optimization to build upon Hamilton's Rule (Hamilton 1963, 1964a,b, 1972), identifying the expected reproductive decisions and the distribution of reproduction based on relatedness among social group members, constraints to solitary breeding, and benefits to group living (e.g., Keller and Reeve 1994; Emlen 1995; Jeon and Choe 2003).

It has been difficult to empirically study the various hypotheses and factors described above for the origin of eusociality. For example, studies of sex allocation are described as providing strong support for kin selection theory (e.g., Queller and Strassmann 1998; Chapuisat and Keller 1999), but do not provide evidence for the existence of kin-selected altruism alleles causing sib-social care (Alonso and Schuck-Paim 2002). One difficulty in studying the origin of eusociality is finding the right study system: most highly eusocial lineages with sterile workers and large societies (e.g., many ants, honey bees, stingless bees) are highly derived and have been eusocial for millions of years (Danforth 2002). The selection pressures and traits of these taxa are likely to be very different than those of taxa at or closer to the origin of eusociality. Other lineages such as xylocopine bees (Michener 1985, 2003), halictid bees (Danforth 2002), and vespid wasps (Hunt 1999), contain genera, species, and sometimes populations within species that range from subsocial to eusocial and have traits and selection pressures more

relevant to understanding the origin of eusociality (Danforth 2002). Phylogenetic study of these lineages suggests that eusociality is frequently lost and study of taxa, once eusocial or trapped at the threshold, may shed light on the necessary traits and selection pressures for the maintenance of eusociality (Wcislo and Danforth 1997). For example, Michener and Brothers (1974) attempted to distinguish between worker altruism and parental manipulation using behavioral observations of a halictid bee. Recently, Langer et al. (2004) tested assumptions of skew theory in a halictid, and several authors used phylogenetic approaches to study factors important in the evolution of eusociality (e.g., in vespids, Carpenter 1991; Hunt 1999, in halictids, Danforth 2002, in xylocopines, Schwarz et al. 2003; Cronin 2004).

It is important to note that the hypotheses, scenarios, or factors discussed above for the origin of eusociality are not mutually exclusive. Each explains different aspects of the origin of eusociality. For example, among-family selection provides an evolutionary mechanism for the evolution of sib-social care and sterile castes, while the specific benefits to group living provide the possible underlying causes of among-family selection. Scenarios for particular lineages, such as the nutritional scenario for social vespids, provide more mechanistic, physiological, developmental, and life historical details for how a specific case(s) of sociality evolved. A unique feature of our heterochrony model (below) is that it provides details of the genetic and developmental basis of sib-social care behaviors (cf. ovarian groundplan scenario, West-Eberhard 1996).

THE MODEL: THE EVOLUTION OF SIB-SOCIAL CARE FROM MATERNAL CARE WITH HETEROCHRONY

Our model for the evolutionary origin of eusociality from subsociality is based on the premise that sib-social care behaviors are developmentally homologous with and evolutionarily derived from maternal care behaviors (see Dawkins 1979; West-Eberhard 1987, 1996; Alexander et al. 1991; Hunt 1994; Bourke and Franks 1995). More specifically, we propose that prereproductive sib-social care is caused by the early expression of genes for maternal care. In the ancestral condition, maternal care behaviors are expressed as one of the final steps in a coordinated series of physiological and behavioral changes that occur through reproductive development (West-Eberhard 1996). Thus, ancestrally, maternal care genes are expressed only after mating and other steps of reproductive development. In the derived condition, aspects of the reproductive developmental program are co-opted so that maternal care behaviors are expressed prereproductively towards siblings instead of offspring (see West-Eberhard 1996). That is, genes for maternal care are expressed prereproductively in female helpers in the derived condition. This is a case of behavioral heterochrony, that is, modifications of the expression of genes regulating behavioral development that cause a change in the timing of expression of behaviors. Behavioral heterochrony is thought to be important in a wide variety of animal groups (West-Eberhard 2003), and has been explicitly invoked in the evolution of eusociality in termites (Nalepa and Bandi 2000; West-Eberhard 2003) and the evolution of helping behavior in birds (Jamieson 1989). In our model, genetic variation underlies variation in the timing of the expression of maternal care behaviors (for discussion of whether genetic variation must underlie the evolution of eusociality, see West-Eberhard 1987, 1992b; Crozier 1992; Bourke and Franks 1995). In this view,

sib-social care is an evolutionarily derived trait and the evolution of the capacity for females to provide care prereproductively towards their sibs is a first step in the evolutionary origin of eusociality from subsociality.

The key point of our model is that sib-social care is based on the altered expression of maternal care genes and that the origin and elaboration of eusociality involves the evolution and regulation of this altered expression so that sib-social care is expressed when it is adaptive. We stress heterochrony and sib-social care as the prereproductive expression of maternal care genes above, however, the exact nature of altered expression of maternal care genes is dependent on life history, when, for example, in most extant aculeate Hymenoptera, mating and dispersal occur at about the same time, followed by nest foundation and the expression of brood care behaviors (Hölldobler and Wilson 1990; Ross and Matthews 1991; O'Neill 2001; Michener 2000). In this case, opportunities for sib-rearing only exist before mating and dispersal so that sib-social care must be expressed prereproductively. In other cases, mating and dispersal are not so closely linked, and for example, mated offspring overwinter in the natal nest (e.g., Michener 2000). In this case, opportunities for sib-rearing exist after mating. Sib-social care then could be expressed postreproductively, similar to the ancestral condition. Finally, in some cases, mated offspring females may lay eggs in their natal nest. Then care behaviors are expressed toward a combination of their own offspring and their siblings (or nieces and nephews as in semisocial models, e.g., Michener 1958; West-Eberhard 1978; Itô 1993). In all of these cases, sib-social care behaviors are based on the expression of maternal care genes and the evolutionary elaboration of sib-social care involves the regulation of expression of these genes.

The developmental basis of sib-social care

The expression of sib-social care must be conditional such that both helper and normal reproductive phenotypes can be produced by a single genotype. Discrete alternative phenotypes, or polyphenisms (e.g., alternative horn morphs in male dung beetles, alternative castes in female social insects), are typically induced by environmental stimuli such as photoperiod, crowding, and nutrition level (reviewed by Nijhout 2003). Caste in the eusocial Hymenoptera is determined primarily by nutritional signals received during larval development (reviewed by Wheeler 1986; Hartfelder and Engels 1998; O'Donnell 1998). However, in some taxa, caste determination seems to occur primarily in the adult stage and dominant, egg-laying females suppress the ovarian development of underdominants through behavioral interactions or pheromones (Wheeler 1986; O'Donnell 1998; Gadagkar 2001). Nutrition and behavioral dominance are environmental cues mediated by endocrine mechanisms, such as hormone titer with a threshold, which initiate alternative developmental pathways into reproductive females or workers (Nijhout and Wheeler 1982; Wheeler 1986; West-Eberhard 1996; Robinson and Vargo 1997; Hartfelder and Engels 1998; Nijhout 2003). Polyphenisms are thought to be derived from phenotypically plastic traits, using preexisting physiological and endocrine developmental mechanisms (Nijhout 2003). Thus, the evolution of discrete castes in the eusocial Hymenoptera involves the elaboration and regulation of preexisting endocrine and developmental mechanisms sensitive to environmental conditions (Wheeler 1986; West-Eberhard 1987, 1996; Nijhout 2003).

In our model, the behavioral switch between normal and precocious maternal care via the heterochronic expression of maternal care genes is also largely determined by the social environment mediated by the endocrine system. The sensitivity of the behavioral switch to environmental signals and the endocrine response is affected by genes expressed zygotically during development (West-Eberhard 1987, 1996). As stressed by indirect genetic effects theory (e.g., Moore et al. 1997; Cheverud 2003), the social environment is affected by genes expressed in social partners such as the mother, other sibling brood, and sibling adults. Thus, both zygotic genes (direct genetic effects) and genes expressed in the mother and other social partners (indirect genetic effects) affect the expression of sib-social care (Figure 1, 2B). The evolution of sib-social care may proceed as a series of sequential maternal and zygotic evolutionary events or the simultaneous coevolution of integrated maternal-zygotic behaviors (see Craig 1979).

The evolutionary origin of sib-social care in our model is based on preexisting behavioral traits, with the regulation of the expression of sib-social care based on preexisting physiological and endocrine machinery (Dawkins 1979; West-Eberhard 1992a, 1996). We suggest that there may often be a small number of genes underlying this behavioral heterochrony, permitting rapid social evolution once the appropriate mutations arise (see Michener 1985). These may be rare or, alternatively, they may be common in subsocial populations, but only rarely adaptive (see Michener 1985).

The expression of sib-social care in socially polymorphic species

It is possible that the first step in the evolution of eusociality, the expression of sib-social care, could occur long before the evolution of a permanent sterile caste and in a

wide range of subsocial taxa where opportunities for sib-rearing exist. This appears to be the case in some bee lineages, such as Apidae: Xylocopinae. Michener (1985:303; see also Tierney et al. 2002) states, "The existence of a minority of nests containing colonies, some of them with castes (i.e., semisocial and eusocial), in various species of *Ceratina*, in most allodapines, and perhaps also in *Xylocopa*, suggests that this polymorphism or at least a potential for it arose in a remote common ancestor of the modern species and has persisted, without ever proceeding to fixation." Alternatively, alleles enabling sib-social care (i.e., heterochrony alleles in our model) may be fixed, but the sib-social behaviors are expressed only under certain conditions (indeed, alleles causing sib-social care must be conditionally expressed, but in some populations the necessary environmental conditions may never occur so that sib-social care is never expressed) (Soucy and Danforth 2002). That is, the social polymorphism may be environmentally determined. Parental manipulation requires that the manipulated offspring already possess the ability to provide sib-social care (Charlesworth 1978; Craig 1979; Crespi and Ragsdale 2000). Experimental manipulation of colonies can confirm the capacity of prereproductive adults to provide sib-social care under certain ecological conditions, and induction of eusociality has been shown for some normally subsocial bees in the genus *Ceratina* (Sakagami and Maeta 1982; Michener 1985).

Environmental conditions related to altitude, latitude, and day length are known to be important in the expression of sociality in some socially polymorphic halictid bees (Wcislo 1997; Yanega 1997). Plateaux-Quénu et al. (2000) transplanted foundress females from eusocial and noneusocial populations of the halictid *Lasioglossum* [*Evylaeus*] *albipes* into conditions simulating those normally experienced by the alternate

social form (see also Cronin 2001). In general, changing rearing conditions did not change the expression of worker behavior, which suggests that it may have a genetic basis (Plateuax-Quénu et al. 2000). Soucy and Danforth (2002) studied the phylogeography of a socially polymorphic halictid, *Halictus rubicundus*, and found that populations expressing eusociality belonged to different genetic lineages than noneusocial populations, a finding also consistent with a genetic basis to sociality (see also Zayed and Packer 2002). Transplant or common garden experiments are a useful first step in distinguishing genetic from environmental causes of sociality and complement correlational findings from phylogenetic studies. As described in the next section, a quantitative genetics approach provides techniques to further study the relative environmental and genetic contributions to the expression of sociality.

THE QUANTITATIVE GENETIC BASIS OF SIB-SOCIAL CARE

Standard social evolution models use an optimality approach to predict evolutionary outcomes based only on assumed selective pressures. Selection does not equal evolution (Fisher 1958; Arnold and Wade 1984; Moore and Kukuk 2002), however. Something of the genetic architecture underlying traits must be understood before the response to selection can be reliably predicted. A quantitative genetic approach compares phenotypes among individuals of known relatedness to estimate components of the genetic architecture underlying traits, such as the additive genetic variance for a trait (usually described as a proportion of the total phenotypic variance, or heritability) and the genetic covariance between traits (usually presented as a genetic

correlation) (Falconer and MacKay 1996; Roff 1997, for discussion of application of a basic quantitative genetic approach to social insects, see Moritz 1986; Owen and Harder 1995; Moore and Kukuk 2002). Even if there is genetic variance for a trait, it may not respond to selection as expected if the trait genetically covaries with other traits. Genetic covariance between traits can act as an evolutionary constraint because correlated traits do not evolve independently (Falconer and MacKay 1996; Roff 1997).

When the social environment affects the expression of a trait, genes expressed in social partners (indirect genetic effects) as well as zygotic genes (direct genetic effects) affect a phenotype (Figure 1, 2). In social insects, the social environment is clearly important in the expression of individual and colony phenotypes (West-Eberhard 1996; Wcislo 1997) so that indirect genetic effects likely play a major role in determining the evolutionary dynamics of these traits. However, traditional social evolution models do not include both direct and indirect genetic effects (e.g., Cheverud 1984, 2003). Additionally, if genes have pleiotropic effects on traits expressed by different life stages (e.g., larval traits and maternal traits), then the traits will be genetically correlated (Figure 2), potentially causing evolutionary constraints analogous to constraints caused by genetic correlations among traits expressed within a single individual. Optimality approaches ignore these evolutionary constraints even though they potentially have important impacts on evolutionary dynamics (Cheverud 1984; Lynch 1987; Cheverud and Moore 1994; Wolf et al. 1998; Cheverud 2003; Wolf 2003). For example, when genetic correlations between direct and indirect effects are considered, Hamilton's Rule must be altered to include a genetic correlation term, which may often dominate the inequality (Cheverud 1984, 2003; Cheverud and Moore 1994; Wolf et al. 1998). We

have highlighted the potential importance of both direct and indirect effects in our heterochrony model. It is also clear under our model that there will be a genetic correlation between maternal and sib-social care (Figure 2, see below).

It is possible to experimentally dissect the relative contributions of direct, maternal, and sib-social effects, and the interactions among them. Cross-fostering offspring between unrelated foster mothers is a powerful approach to estimate direct and maternal effects on a phenotype (Cheverud and Moore 1994; Roff 1997; Lynch and Walsh 1998). If a full factorial design is used, such that mothers rear some of their own offspring and some unrelated offspring, the phenotypic variance due to direct effects, maternal effects, and the direct-maternal interaction can be estimated. This approach has recently been used to study the evolution of maternal care in several natural systems, including burying beetles (Rauter and Moore 2002), dung beetles (Hunt and Simmons 2002), burrower bugs (Agrawal et al. 2001a), and a passerine bird (Kölliker et al. 2000). Alternatively, in social insects, a series of manipulations (e.g., mixing larvae among colonies, mixing workers among colonies, and removing queens) can be used to estimate the relative magnitude of among colony variance due to direct effects, maternal effects, sib-social effects, queen by larval interaction, worker by larval interaction, and queen by worker interaction.

The experimental designs described so far estimate the relative importance of direct and indirect effects to the evolutionary dynamics of traits within populations. Analogous cross-fostering manipulations between populations or species would estimate the relative importance of direct and indirect effects in the fixed differences between populations or species. Cross-fostering between a subsocial population or species and a

eusocial population or species would be especially insightful in understanding the fixed genetic differences between them underlying the expression of sociality. For example, if subsocial offspring reared by eusocial mothers express sib-social care and eusocial offspring reared by subsocial mothers do not express sib-social care, then maternal effect, "parental manipulation" genes are important. If subsocial offspring reared by eusocial mothers do not express sib-social care, and eusocial offspring reared by subsocial mothers do express sib-social care, then direct effect, "offspring control" genes are important. Ideally, a full factorial design could be used in which mothers from eusocial and subsocial populations rear offspring from their own population as well as all other populations. This design enables the estimation of variance due to direct effects, maternal effects, and direct-maternal interaction. If maternal and direct effect genes evolve in concert, then only certain combinations of maternal and zygotic genotypes may result in offspring expressing sib-social care, which suggests that the coadaptation of maternal and zygotic traits are important (Wade 1998; Wolf and Brodie 1998; Wolf 2000a,b).

Genetic Constraints to the Evolutionary Divergence of Maternal and Sib-social Care

Under our model, maternal care and sib-social care are both the pleiotropic expression of the same set of maternal care genes, so as sib-social care spreads through a population, there will be a strong positive genetic correlation between sib-social care and maternal care (r_{ms} in Figure 2B). Allelic variation affecting maternal care will similarly

affect sib-social care. Genotypes that produce good mothers will also produce good sib helpers. This positive genetic correlation between maternal and sib-social care means that selection on one trait will cause a correlated response to selection on the second trait. At first glance, this seems unimportant, because, initially, maternal and sib-social care can be thought of as a single trait. However, when alleles affecting brood care behaviors are expressed in helpers, as well as in mothers, the total fitness effects of these alleles are changed**.** For example, if there is a synergistic effect between maternal and sib-social care, such that colonies that add on helpers produce more fully-fertile adults than colonies without helpers (e.g., West-Eberhard 1975; Oster and Wilson 1978; Queller 1989, 1996), then alleles causing increased levels of brood care may be favored.

Subsequent to the initial spread of sib-social care, mutations that affect one trait (e.g., sib-social care) positively and the other trait (e.g., maternal care) negatively may arise and will spread if the sum of the two effects on fitness is positive. The genetic correlation between maternal and sib-social care may then diminish and permit maternal and sib-social care to diverge to some extent and specialize as separate traits. In this way, some alleles could produce good "queens" and other alleles could produce good "workers." In addition, gene duplication can further enable the divergence of queen and worker phenotypes, given that each caste has a separate set of genes, or more simply, the evolution of caste-specific gene expression (West-Eberhard 1996; Gadagkar 1997, 2001). However, genes that have pleiotropic expression in both queens and workers might not be able to be optimized for both queen and worker phenotypes, and the divergence of queen and worker phenotypes may be constrained to some degree.

The evolutionary elaboration of queen-worker dimorphism may be constrained in many eusocial species because each queen founds a colony independently and must provide care for the first brood, just as workers provide care for subsequent broods. Thus, though queens and workers in these species are divergent in many morphological, physiological, and behavioral traits, there are times in the colony cycle that each must perform similar brood care behaviors. Alleles affecting brood care are then likely to have pleiotropic effects on both maternal care and sib-social care, and sustain a genetic correlation between maternal and sib-social care.

Only antagonistically pleiotropic alleles affecting one trait positively and another trait negatively are likely to remain segregating in the population because alleles that affect both traits positively or negatively will quickly fix or be lost (Falconer and MacKay 1996; Roff 1997). Segregating antagonistically pleiotropic alleles may cause a negative genetic correlation between maternal and sib-social care. This could result in a genetic constraint to the further divergence of maternal and sib-social care. Similarly, negative genetic correlations between male and female fitness have been found in fruit flies, showing strong evidence for antagonistic pleiotropy ("intralocus conflict") between the sexes (Chippindale et al. 2001). These negative genetic correlations are thought to be important constraints to the divergence of male and female traits and the evolution of sexual dimorphism (Rice and Chippindale 2001; Badyaev 2002), just as we suggest that negative genetic correlations between maternal and sib-social care may be a constraint to the divergence of maternal and sib-social care and the evolution of queen-worker dimorphism.

Obligate swarm-founding bees, wasps, and ants, as well as socially parasitic species, do not found colonies independently (reviewed by Peeters and Itô 2001). Queens are never without workers and they never have to perform brood care behaviors (Jeanne 1991; Peeters and Itô 2001). Pleiotropy for genes affecting brood care may be less of a constraint to queen-worker divergence for some phenotypes in these species. Of all social insects, obligate swarm-founding species, such as honey bees (*Apis* spp.), stingless bees (e.g., *Melipona* spp.), swarm-founding Polistinae, army ants (e.g., *Eciton* spp.), and driver ants (e.g., *Dorylus* spp.) have some of the most divergent queen and worker morphology, highest queen egg-laying-rates, and largest colonies (Hölldobler and Wilson 1990; Jeanne 1991; Bourke and Franks 1995; Bourke 1999; Michener 2000).

SOCIOGENOMICS AND THE MOLECULAR BASIS OF EUSOCIALITY

A quantitative genetic approach will provide insight into the genetic architecture of social traits (i.e., sib-social care), and this should lead to increasingly realistic models of social evolution. But a sociogenomic approach (sensu Robinson 1999) will identify specific genes underlying the social traits and will elucidate the molecular basis of these traits (Krieger and Ross 2002). Our heterochrony model for the evolution of eusociality makes explicit predictions regarding patterns of gene expression for the expression of sib-social care. In our model, sib-social care first appears as the heterochronic expression of maternal care genes. We predict that many of the same genes will be expressed in adults performing sib-social care behaviors as in adults performing maternal care behaviors. This will be especially true in populations of incipiently eusocial species but less so in

those with an advanced degree of eusociality, where more genes are expected to have caste-limited expression due to selection for the elaboration of queen-worker divergence (see West-Eberhard 1996; Gadagkar 1997; 2001). However, because the evolutionary elaboration of sib-social care behaviors and queen-worker phenotypic differences is likely based upon simple modification of preexisting physiological, behavioral, and genetic machinery, queen and worker traits, even in highly eusocial species, are expected to have a common molecular basis. We further suggest that some of the key genes involved in the evolution of sib-social care are genes that regulate the timing of expression of maternal care behavior genes.

Social insect biologists have been successful in identifying regions of the genome involved in social traits in two model systems, the honey bee, *Apis mellifera*, and the imported red fire ant, *Solenopsis invicta*, both of which have a highly derived degree of eusociality (Robinson 1999). Ross and Keller and collaborators have demonstrated that the locus *Gp-9* affects male and female size, colony queen number, and overall social organization of *S. invicta* (Keller and Ross 1995; Ross and Keller 1998; Keller and Parker 2002; Krieger and Ross 2002). A sociogenomic approach has been used to study the molecular basis of caste differentiation in *A. mellifera*. Screening for differential gene expression in queen- and worker-destined honeybee larvae reveals that many genes are indeed expressed differentially (Corona et al. 1999; Evans and Wheeler 1999, 2001; Hepperle and Hartfelder 2001).

Robinson and colleagues have also been successful in elucidating the molecular basis of worker behavioral development in *A. mellifera*. In honeybee colonies, division of labor from hive to foraging is associated with an age-related transition of workers that

involves changes in brain chemistry, brain structure, endocrine activity, and gene expression (reviewed by Robinson 2002). Specific genes involved in this age-related behavioral transition have been identified (Toma et al. 2000; Ben-Shahar et al. 2002), and these genes can be considered behavioral heterochrony genes. In addition, genes underlying this transition have pleiotropic effects on genes involved in the sequential expression of phases in the reproductive cycle (Amdam et al. 2004). Thus, worker behavioral development and maternal reproductive development have a common genetic basis, and genes regulating worker behavioral development seem to be derived from genes that regulate maternal reproductive development (Amdam et al. 2004). These results are consistent with the predictions of our model above, as well as West-Eberhard's (1996) ovarian groundplan scenario. Furthermore, Robinson and Ben-Shahar (2002) used a comparative genomics approach to ask whether social evolution, in a general sense, in model organisms (including *A. mellifera*) usually involves gene diversification or changes in gene regulation that influences spatial or temporal patterns of gene expression. As predicted under our model, it seems that new gene regulation is often involved in the evolution of social behaviors (Robinson and Ben-Shahar 2002).

The honeybee genome sequencing project should help to further elucidate the molecular bases of social behavior and caste determination (Robinson and Ben-Shahar 2002). Once genes involved in caste differentiation and social behaviors have been identified in honeybees, probes can be developed to search for homologues in nonmodel social Hymenoptera. It would be particularly interesting to determine if these genes are conserved across social taxa.

Although most sociogenomic study has concentrated on genes expressed during zygotic development (i.e. direct effect genes) (but see Ross and Keller 2002), we emphasize that indirect effect genes expressed in social partners are also likely to be involved in the expression of social traits such as sib-social care. Identification of the specific ways that individual genes influence the expression of social traits will enable biologists to explicitly study the roles and interplay between direct effect (e.g., offspring control) and indirect effect (e.g., parental manipulation) genes.

SUMMARY AND CONCLUSIONS

We make five main points: (1) The role for haplodiploidy in the evolution of eusociality has been downplayed, and other predisposing factors, such as maternal care, have been highlighted. However, maternal care genes evolve more readily in haplodiploids relative to diplodiploids, as shown by protected invasion theory (Reeve 1993) and maternal effects theory (Wade 2001). It is widely accepted that eusociality is evolutionarily derived from subsociality, so the prevalence of subsociality in haplodiploids (such as the aculeate Hymenoptera) may make the evolution of eusociality in these groups more likely. (2) Although both offspring control and parental manipulation genes evolve through among-family selection, there are expected evolutionary differences in the dynamics of these genes due to their different genomic locations (i.e., zygotic versus maternal). (3) We propose that the evolutionary origin of sib-social care involves heterochrony, by the condition-dependent, prereproductive expression of maternal care behaviors in females towards their siblings instead of their

offspring. The expression and regulation of sib-social care behaviors are likely to be based on the modification of existing developmental, physiological, and endocrine mechanisms. Genes expressed in both the zygotic genome and the genomes of social partners (e.g., maternal genome) are likely to affect the expression of sib-social care. (4) Quantitative genetic approaches, especially using indirect genetic effect models, enable the separation of direct and indirect environmental and genetic effects on social traits, and may be particularly insightful when applied to socially polymorphic species. Because sib-social care arises from maternal care in our model, there will be a genetic correlation due to pleiotropy between maternal care and sib-social care, which may constrain the divergence of these two traits. We predict that this genetic correlation will be strongly positive in incipiently eusocial populations and it will be more negative in more advanced eusocial species. (5) The emerging field of sociogenomics will yield insight into the molecular basis of sib-social care behaviors, and we predict that many of the genes involved in the expression of sib-social care behaviors are also involved in the expression of maternal care behaviors. Recent study of the genetic basis of worker behavioral development in the honeybee *Apis mellifera* supports these predictions (Amdam et al. 2004). Identification of the specific genes involved in the expression of sib-social care may further elucidate the roles of direct effect, zygotic genes and indirect effect genes expressed in social partners in the evolutionary origin and elaboration of eusociality.

Throughout this paper, we used the evolutionary perspective of indirect genetic effects. Indirect genetic effects theory makes unique predictions about social evolution relative to traditional optimality model approaches (see Roff 1994) because it explicitly considers both direct and indirect genetic effects, and interactions between the two

(Cheverud 1984, 2003; Cheverud and Moore 1994; Wolf et al. 1998; Agrawal et al. 2001b). We believe that modeling social evolution using an indirect genetic effects framework will allow social insect biologists to develop more realistic models. Importantly, quantitative genetics provides techniques to empirically study the assumptions of optimality models and the predictions of indirect genetic effects models (see Kölliker et al. 2000; Agrawal et al. 2001a; Kölliker and Richner 2001; Rauter and Moore 2002; Wolf 2003). Ideally, this top-down phenotypic, quantitative genetics approach can be used in concert with a bottom-up molecular, sociogenomics approach. We suggest that quantitative genetic and sociogenomics approaches should be used to build upon existing social evolution models, such as kin selection, parental manipulation, and skew models (see Roff 1994).

Acknowledgments

We would like to thank Aneil Agrawal, Butch Brodie, and Ben Ridenhour for discussion of ideas and Aneil Agrawal, Butch Brodie, Jeremiah Busch, Joan Herbers, Liz Lehman, Maurine Neiman, and two anonymous reviewers for helpful comments on the manuscript. We acknowledge support from NIH (GM65414) and NSF (DEB 0206628) to MJW.

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Figure captions:

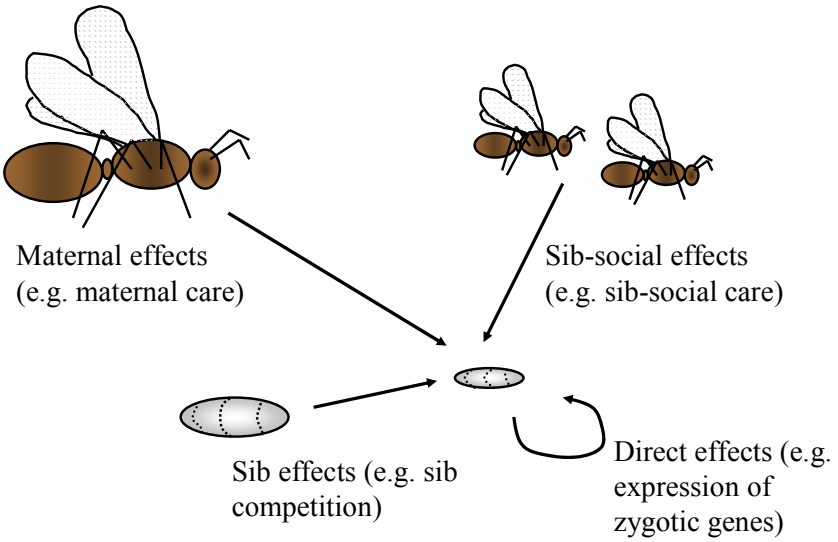
FIGURE 1. DIRECT AND INDIRECT EFFECTS ON A LARVAL PHENOTYPE

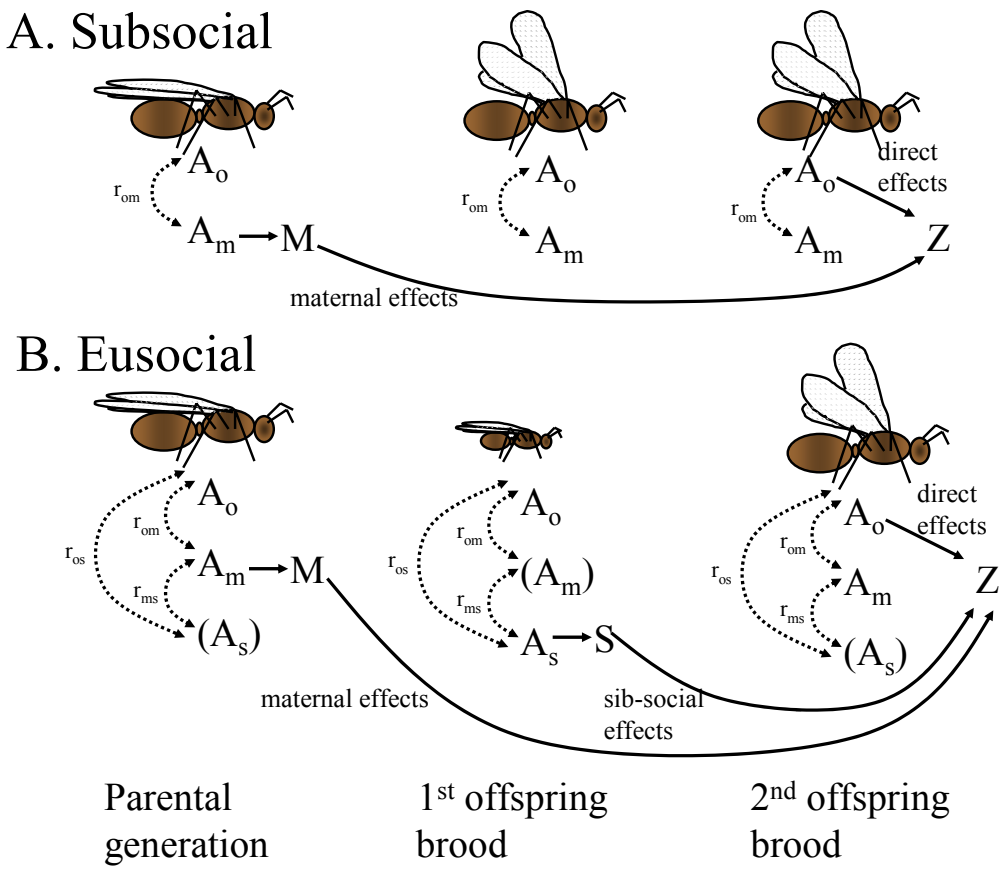
Social insect phenotypes are influenced by a variety of direct and indirect effects. These different influences on a focal larva's phenotype are shown by the arrows. Siblings expressing sib-social care are shown to be smaller than the mother to indicate that only individuals in poor condition express sib-social care.

FIGURE 2. PATH DIAGRAM FOR INFLUENCES OF DIRECT AND INDIRECT ADDITIVE GENETIC EFFECTS ON A PHENOTYPE OF A SECOND BROOD OFFSPRING

A mother with folded wings is shown on the left and her first and second brood female offspring are in the center and right, respectively. Panel A. shows a subsocial colony in which offspring of both broods reproduce, disperse (hence the extended wings), and express maternal care genes toward their own offspring (not shown). Panel B. shows a eusocial colony in which first brood offspring remain at the natal nest (hence folded wings) and express sib-social genes toward their sibs (second brood dispersing offspring with extended wings) instead of reproducing, dispersing, and expressing maternal care genes toward their own offspring. The first brood offspring is smaller than the other individuals to indicate that it is in poor condition and cannot successfully reproduce on its own. The symbol, A_o, is for direct additive genetic effects that influence the phenotype of the individual in which they are expressed, while A_m represents maternal additive genetic effects that influence offspring phenotypes through their effect on maternal performance, M. The A_s represents sib-social additive genetic effects that influences sibling phenotypes through their effect on sib-social performance, S. Genetic effects in parentheses are not

expressed (i.e., the eusocial mother and her second brood offspring express maternal care genes but do not express sib-social care genes, while smaller first brood offspring express sib-social care genes but do not express maternal care genes). Solid arrows represent influences on a phenotype Z of the second brood offspring. Double-headed dotted arrows represent genetic correlations between additive genetic effects due to pleiotropy: (1) r_{om} is the direct maternal additive genetic correlation; (2) r_{os} is the direct sib-social additive genetic correlation; and, (3) r_{ms} is the maternal sib-social additive genetic correlation. In panel A., phenotype Z of the second generation offspring is influenced by maternal and direct effects. In panel B., phenotype Z of the second brood offspring is influenced by maternal and direct effects, as well as by sib-social effects from the first brood offspring. Note that sib-social care initially is merely the pleiotropic expression of maternal care genes, so that at least initially, $r_{os} = r_{om}$ and $r_{ms} = 1$. For clarity, no environmental effects, paternal effects, or effects due to competition among sibs, are shown. Paths for the transmission of additive genetic effects from mother to offspring weighted by maternal-offspring relatedness (1/2) are also not shown nor is the genetic relatedness between sibs (3/4 in haplodiploids with a singly-mated mother) shown, although relatedness among social partners is accounted for in indirect genetic effect models.


Maternal effects
(e.g. maternal care)
Sib-social effects
(e.g. sib-social care)
Sib effects (e.g. sib
competition)
Direct effects (e.g.
expression of
zygotic genes)


A. Subsocial
A_o
r_{om}
A_m → M
A_o
r_{om}
A_m
A_o
direct effects
r_{om}
A_m
Z
maternal effects
B. Eusocial
A_o
r_{om}
r_{os}
A_m → M
r_{ms}
(A_s)
A_o
r_{om}
r_{os}
(A_m)
r_{ms}
A_s → S
A_o
direct effects
r_{om}
r_{os}
A_m
Z
r_{ms}
(A_s)
maternal effects
sib-social effects
Parental generation
1st offspring brood
2nd offspring brood

CHAPTER 2

THE EVOLUTIONARY IMPORTANCE OF DIRECT, MATERNAL, AND SIBSOCIAL GENETIC EFFECTS ON MASS, CASTE RATIO, AND SEX RATIO IN AN ACORN ANT

Timothy A. Linksvayer

ABSTRACT

Social insects are model systems for the study of social evolution, particularly for concepts such as kin selection and parent-offspring conflict. However, little is known about the genetic architecture underlying social insect traits. Knowledge of genetic architecture is necessary to predict the evolutionary dynamics of traits because patterns of genetic variation and covariation can alter expected evolutionary trajectories. Both genes expressed within an individual ("direct genetic effects") and genes expressed in social partners ("indirect genetic effects") affect the phenotypes of individuals. Indirect genetic effects are of particular relevance to social insects, where brood are reliant on care provided by the queen and workers. This complexity has not previously been incorporated in genetic models of social insect trait evolution. I used a novel quantitative genetic approach to study the relative contributions of genetic variance and covariance due to direct effects, maternal effects, and sibsocial effects to total phenotypic variance for mass, colony caste ratio, and colony sex ratio. The results suggest genetic variance (i.e. heritability) for direct effects, maternal effects, and sibsocial effects for all traits, with direct effects being relatively more important. In addition, estimates of direct-maternal, direct-sibsocial, and maternal-sibsocial genetic covariance were large and negative, suggesting that these effects may not evolve independently. Total heritability, incorporating all genetic (co)variance components, for all traits was not different than zero. Negative genetic covariance components may prevent an evolutionary response to selection, despite heritable variation for all direct and indirect effects. These results are relevant to social evolution theory. Evolutionary conflicts between social partners that are

predicted to occur based on opposing selection pressures may not actually be realized as opposing evolutionary responses to selection.

The study of social evolution has been dominated by two main concepts for the past forty years (Lynch and Walsh 1998). The first, inclusive fitness or kin selection, suggests that some individuals should be selected to give up individual reproductive options in order to provide aid to social partners, depending on the specific fitness costs and benefits and the degree of relatedness between social partners (Hamilton 1963, 1964a,b). The second, parent-offspring conflict, suggests that there is a conflict of interests between parents and offspring because parents must balance investment in present offspring against loss of future reproduction while offspring are interested in immediate gain (Trivers 1972).

Social insects quickly emerged as a model system to further develop and study these concepts: Hamilton (1964a,b, 1972) proposed that a sterile female worker caste frequently arose in haplodiploid insects through kin selection, because haplodiploid females are more closely related to their sisters than their own offspring. As an alternative to kin selection, Alexander (1974) and Michener and Brothers (1974) proposed that eusociality evolved through parental manipulation, in which mothers coerce some of their offspring to forgo personal reproductive options in order to aid their siblings. Trivers and Hare (1976) extended parent-offspring conflict theory to include queen-worker conflict over colony sex ratio, because queens and workers are expected to favor different sex ratios. More recent models suggest that queens and workers should also favor different strategies of colony growth versus reproduction (Pamilo 1991; Herbers et al. 2001).

These major concepts of social evolution have mainly been modeled with a phenotypic optimality approach (reviewed by Crozier and Pamilo 1996). This approach is

useful for predicting expected evolutionary outcomes given certain selective pressures, but the evolutionary dynamics of traits are determined by both patterns of selection and the underlying genetic architecture (Roff 1994). Genetic constraints, in the form of limited genetic variance for traits or genetic covariance between traits, shape the evolutionary response to selection and may make some evolutionary outcomes unavailable to a population, at least in the short term (Lande 1979; Arnold 1992; Falconer and Mackay 1996). Genetic constraints may be especially likely when social interactions are involved. In this situation, genetic constraints can occur both between genes within individuals and between genes expressed in different individuals. For example, the same genes expressed in individuals at different life stages, e.g., parents and offspring, may commonly affect a variety of parent and offspring traits, so that these traits cannot evolve independently (Cheverud 1984; Lynch 1987; Cheverud and Moore 1994; Wolf et al. 1998). Unfortunately, little is known about the genetic architecture underlying social insect traits that are likely the focus of kin selection and parent-offspring conflict, e.g., offspring size, caste and sex ratio (Page et al. 1993; Robinson et al. 1994; Rüppell et al. 2001).

This lack of attention is not surprising given that social insect phenotypes are expected to be strongly influenced by the social environment, and it is difficult to empirically disentangle the effects of genes from those of social environment (Calderone and Page 1991; Keller and Ross 1995; Rüppell et al. 2001; Linksvayer and Wade 2005). Indirect genetic effect models provide a means to partition the effects of genes expressed in individuals (direct genetic effects) and their social partners (indirect genetic effects)

from environmental effects (e.g., Wilham 1963; Cheverud 1984; Lynch 1987; Kirkpatrick and Lande 1989; Cheverud and Moore 1994; Wolf et al. 1998).

In the present study, I developed a novel quantiatitve genetic approach to study the genetic architecture underlying body size, female caste ratio, and sex ratio in the ant *Temnothorax curvispinosus*. I was specifically interested in the relative contribution of direct, maternal, and sibsocial genetic variance and covariance to total phenotypic variance for these traits. I chose these traits because they are likely to be both closely related to fitness and the focus of kin selection and parent-offspring conflict. Furthermore, these traits are likely influenced by both direct and indirect genetic effects.

METHODS

Study traits

Body size is a typical continuous and often normally distributed quantitative trait. A genetic component to body size has been established in many organisms, including social insects (e.g., Rüppell et al. 2001; Bargum et al. 2004). In addition, both direct and indirect genetic effects are known to affect body size in a variety of taxa (e.g., Cheverud 1984; Hunt and Simmons 2000; Rauter and Moore 2002; Wolf 2003). In this study, mass was used as a measure of body size for workers, gynes (reproductive females), and males.

In contrast to body size, caste and sex are binary traits. A female adult is either a worker or a gyne and a sexual adult is either a male or female. Genetic influences on caste and sex are usually not considered (Crozier and Pamilo 1996). Caste determination is assumed to occur conditionally, in response to environmental factors (e.g., nutrition quality and quantity). In haplodiploids, sex is determined primarily by whether an egg is

fertilized, such that fertilized diploid eggs develop into females and unfertilized haploid eggs develop into males. Social insect sex ratio models focus on (social) environmental factors such as colony size, queen age, queen number and mating frequency, and relatedness among nestmates (Crozier and Pamilo 1996). However, empirical evidence for a genetic influence on caste has been found in a variety of social insect groups (Kerr 1950; Buschinger 1975; Winter and Buschinger 1986; Julian et al. 2002; Volny and Gordon 2002; Buschinger 2005). Similarly, genetic variation for sex ratio has been detected in a variety of animals, including haplodiploid parasitoid wasps (Orzack and Gladstone 1994; Kobayashi et al. 2003) and the honeybee *Apis mellifera* (Page et al. 1993). In this study, the caste ratio of a colony is defined as the proportion of new adult females that are gynes, and the sex ratio of a colony is the proportion of new adult sexuals that are male.

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 USA (Mackay 2000). This species, along with other closely-related species, has been well studied and is readily maintained in the lab (e.g., Alloway 1979; 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 (Hebers 1986; 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). Several 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; Herbers and Tucker 1986). Colonies produce two types of brood: Eggs laid in the late summer and early fall overwinter as larvae, and in the spring, these larvae develop into workers or gynes, or males. In addition, diploid eggs laid in the spring and early summer develop exclusively as workers in the summer (A. Buschinger, pers. comm.). The phenotypes of individuals derived from overwintered larvae were the focus of this study.

Collection of study colonies

Acorn and hickory nut nests were collected in late winter / early spring, from 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 gyne mass ($p>.05$, N=55, Linksvayer, unpublished data). This is probably because overwintered larvae are very small (mean colony mean larva mass 0.065 mg, 0.038 SD, N=130, Linksvayer, unpublished data) less than one twelfth their final adult mass (mean colony mean gyne mass 0.822 mg, 0.147 SD, N=55, Linksvayer, unpublished data) (Wesson 1940). Thus, the vast majority of larval growth occurred during the course of the experiment. Caste determination has also been experimentally shown to occur after overwintering in *T. curvispinosus* (Wesson 1940). Nests were also collected early in the season to minimize the number of nests that were colony fragments due to seasonal polydomy.

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. 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 out of 470 colonies) were included in the current study. I used only single-queen colonies in order to keep colony queen number, an important social trait, constant, and to maximize the probability that colony larvae and workers were full sibs. In addition, only colonies with at least 10 workers were used (360 out of 369 colonies).

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; Rauter and Moore 2002; Wolf et al. 2002). In this approach, offspring are reared by unrelated dams in order to separate direct and maternal influences on offspring traits. I used a similar approach to disentangle the contribution of direct, maternal, and sibsocial variance and covariance to total phenotypic variance.

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 from a mixture of workers from at least fifteen colonies, and (3) replacement of larvae with an equal number from a mixture of larvae from at least fifteen colonies (see Table 1). These treatments experimentally minimize certain factors contributing to among-colony variance. Specifically, the removal of the colony queen eliminates the possibility of

variance in (post manipulation) maternal effects contributing to among-colony variance. Mixing workers among colonies creates a common social environment due to sibsocial effects so that variance among colonies due to sibsocial effects is minimized post manipulation. Similarly, mixing larvae among colonies minimizes variance among colonies due to direct effects so that the remaining among-colony variance is due to variance in the social environment.

The names of the seven treatments used hereafter (L, Q, W, QL, WL, QW, and QWL) refer to the members of the initial colony that were kept intact; "L" stands for larvae, "Q" queen, and "W" workers. The intact, unmanipulated members of the colony contribute to among-colony variance while the removed or mixed members do not. For example, in treatment L, the larvae were kept intact but the queen was removed and the workers were replaced with a mixed group of workers. In this treatment, among-colony variance is expected to be due to inherent differences among groups of full-sib larvae (i.e. variance in direct effects), because the social environment provided by workers is experimentally made constant across colonies. Similarly, only variation among worker groups (i.e. sibsocial effects) contribute to among-colony variance for treatment W, and variation among queens (i.e. maternal effects) contribute to among-colony variance for treatment Q (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 (see Ross and Keller 2002). This procedure presumably diminished or mixed up chemical cues used in nestmate recognition. At least 15 colonies were used to create worker and larvae mixtures to

minimize the contribution of any one colony's workers / larvae to the mixture, and to minimize variance among these mixtures. Workers in newly assembled colonies generally behaved as workers in unmanipulated colonies and showed little aggression towards nestmates. One exception was that a subset of workers from treatments that included worker mixing but no queen removal (QL and Q) frequently behaved aggressively towards the queen. These colonies were monitored for the first 24 hours of the study. When any workers displayed aggression towards the queen, the colony was blown on and cooled in a refrigerator, to facilitate queen adoption by the mixed workers (see Ross and Keller 2002).

Colonies were maintained in incubators under "spring" conditions of 13:11 hours light: dark photoperiod and 20:10 C day: night temperature cycle, until 21 May 2004, and then "summer" conditions of 14:10 hours light: dark and 22:18 C day: night, until 16 August 2004, when the last gynes were removed (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, removed as they appeared, and then frozen and weighed to the nearest 0.001 mg. 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 were included in the study. In addition, workers usually pupate at approximately the same time in a colony, and worker pupae that appeared more than three weeks after the first group of workers in a colony had

pupated were not included in the study. Males were removed, frozen, and weighed to the nearest 0.001 mg after they eclosed as adults. Gynes were not removed and weighed until 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). Preliminary results indicated that wet mass was strongly correlated with dry mass for workers ($r=0.865$, $p<0.000$, $N=210$), gynes ($r=0.921$, $p<0.000$, $N=580$), and males ($r=0.931$, $p<0.000$, $N=202$), and wet mass was used in all analyses.

Quantitative genetic 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 gave nearly identical results. I estimated the relative importance of (co)variance components involving direct effects, maternal effects, and sibsocial effects in two ways:

(1) I computed the intraclass correlation, $t=Var_{among}/Var_{total}$ for each treatment. In standard full-sib analysis, the heritability of direct genetic effects, $h^2_{direct} = (1/2\theta)t$, where 2θ is the relatedness (twice the "coefficient of coancestry", Lynch and Walsh 1998) among full sibs. For diploids with a full sib relatedness of 1/2, this leads to the traditional $h^2_{direct} = 2t$ (Falconer and Mackay 1996). Because the manipulations directly affected which aspect of the social environment was kept intact, the intraclass correlations for certain treatments can be used to estimate heritability of direct and indirect effects, e.g., h^2_{direct}, $h^2_{maternal}$, $h^2_{sibsocial}$, controlling for all other direct/indirect effects. For each

heritability estimate, a different coefficient based on the relatedness between social partners must be used. To calculate the coefficients, and to partition total phenotypic variance into causal components, I used the linear model:

$z = o + m' + s' + e,$

where the phenotype (z) of a focal individual is assumed to be determined by direct genetic effects (o), maternal genetic effects expressed in the queen (m', the superscript prime indicates that the effect is indirect and expressed in a social partner), sibsocial genetic effects expressed in workers (s'), and environmental effects (e). Under this model, indirect environmental effects are ignored, and all genetic effects are assumed to be additive, i.e., dominance and epistasis are ignored. If there is variance due to indirect environmental effects, dominance, or epistasis, the estimates of additive genetic variance will be inflated. With these assumptions, the covariance between traits z_1 and z_2 in two individuals is (after Wilham 1963):

$$Cov(z_1,z_2)=2\theta_{oo}G_{oo}+2\theta_{mm}G_{mm}+2\theta_{ss}G_{ss}+(2\theta_{om}+2\theta_{mo})G_{om}+(2\theta_{os}+2\theta_{so})G_{os}+(2\theta_{ms}+2\theta_{sm})\ G_{ms},$$

Where G_{oo}, G_{mm}, and G_{ss}, are genetic variance for direct, maternal, and sibsocial effects, respectively, and G_{om}, G_{os}, 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 o 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:

$$Cov(z_1, z_2)_{\text{haplodiploid female full sibs}} = 3/4G_{oo} +G_{mm}+3/4G_{ss}+G_{om}+3/2G_{os}+G_{ms}.$$

The coefficients of causal variance components were similarly calculated for the other treatments and are shown for haplodiploid females in Table 1. Coefficients for males are different (e.g., $2\theta_{oo} = ½$ 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, $2\theta_{oo}=p_{male}(0.5)+(1-p_{male})((1-p_{male})(0.75)+p_{male}(0.25))$, because brother-sib relatedness is 0.5, sister-sister relatedness is 0.75, and sister-brother relatedness is 0.25.

(2) I used the observed among-colony variance components to directly solve for the causal (co)variance components, G_{oo}, G_{mm}, G_{ss}, G_{om}, G_{os}, and G_{ms}, with the coefficients determined above (Table 2). Among-colony variance for treatments L, Q, and W include only one causal variance component each and are directly proportional to G_{oo}, G_{mm}, $G_{ss,}$ respectively (Table 2). The causal variance components can be estimated using the among-colony variance estimate multiplied by the reciprocal of the correct coefficient. For example, for female full-sib haplodiploids, $Var_L=3/4G_{oo}$, and $G_{oo}=4/3Var_L$, where Var_L is among-colony variance for treatment L. Among-colony variance for the remaining treatments (WL, QL, QW, QWL) include more than one causal variance components as well as at least one causal covariance component. Causal covariance components then must be estimated by subtraction of the observed among-colony variance components of certain treatments: $G_{om}=Var_{QL} – Var_Q – Var_L$, $G_{os}=Var_{WL} –Var_W – Var_L$, and $G_{ms}=Var_{QW} – Var_Q – Var_W$ (after Riska et al. 1985; Wolf 2003).

I calculated genetic correlations with the appropriate covariance and variance components, e.g., the direct-maternal genetic correlation, $r_{om}= G_{om}/\sqrt{(G_{oo}G_{mm})}$. I also

calculated the "total heritability," which takes into account all direct and indirect genetic variance and covariance terms (Wilham 1963; Cheverud and Moore 1994). The total heritability is the covariance of breeding value (a) and phenotype (z), divided by total phenotypic variance (P) (after Cheverud and Moore 1994):

$h^2_{Total} = cov(a,z)/P$

$= cov(o+m+s, o+m'+s')/P$

$= (G_{oo}+G_{mm}+2\theta_{ss}G_{ss}+(1+2\theta_{os})G_{os}+(1+2\theta_{om})G_{om}+(1+2\theta_{ms})G_{ms})/P$

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 $(G_{oo}+G_{mm}+3/4G_{ss}+7/4G_{os}+3/2G_{om}+3/2G_{ms})/P$.

Note that in the absence of indirect effects (i.e. $G_{mm}=G_{ss}=G_{os}=G_{om}=G_{ms}=0$), this is simply G_{oo}/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.

I used bootstrapping across families to estimate parameters and upper and lower confidence intervals. 10,000 bootstrap samples across families were produced for all treatments, observed within- and among-colony variance components were estimated with ANOVA, and the intraclass correlations, causal (co)variance components, correlations, and total heritabilities were computed. I used the median values as the parameter estimates and I used the upper (97.5%) and lower (2.5%) bootstrap values to construct 95% confidence intervals (Lynch and Walsh 1998).

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. In addition, collection site, nest density at the collection site, initial worker number, 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 mass. These collection site, colony size, and colony productivity variables are caused mainly by uncontrolled environmental factors prior to collection. Statistically removing these effects minimizes the contribution of environmental effects to the observed among-colony variance. Finally, queen dry mass for treatments involving queen removal (L, W, and WL) was also included as a potential covariate because queen mass may be a predictor of queen reproductive status. The significance of these potential covariates was tested using the STATISTICA 6.1 general regression models: general linear models module (Statsoft 2002). Site number was used a categorical predictor and the remaining variables were used as continuous predictors. Forward stepwise model building was used, with entry and removal levels set at P=0.05 and P=0.10, respectively (Dohm et al. 1996). 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 as mass minus mean colony mass minus residual mean colony mass, and individual residual mass was used for subsequent quantitative genetic analyses.

Males and gynes were collected as fully pigmented adults so that degree of pigmentation was not used as an initial covariate. As for worker mass, significant effects of collection site, colony size, and total colony production were removed and residual male and gyne mass were used for further analyses. In a previous study, I found that gyne size was bimodally distributed, with large individuals (macrogynes) capable of successfully rearing a first brood of workers, and small individuals (microgynes) incapable of successfully rearing a first brood of workers (Linksvayer, unpublished results). Gyne size has been found to be bimodally distributed in other close relatives of *T. curvispinosus*, as well (e.g., Herbers 1986; Hamaguchi and Kinomura 1996; Rüppell et al. 1998). One way to deal with a bimodally distributed trait is to treat it as a binary threshold trait (as caste and sex, see below) and focus exclusively on variation between size morphs (Roff 1997; Roff 2001; Moorad 2005). This approach was used to study the heritability of gyne morph in *Temnothorax* [*Leptothorax*] *rugatulus* (Rüppell et al. 2001). Alternatively, standard ANOVA techniques can be used to estimate variance components regardless of the underlying distribution, but standard significance tests are no longer valid (Lynch and Walsh 1998). Because I was interested in both the within- and between-morph variance, and because the bimodality was not detectable after controlling for site and colony demographic effects, I chose to use standard ANOVA techniques, as above. As with all other traits, I used bootstrapping to construct confidence intervals.

Caste and sex

Caste and sex are binary traits and standard quantitative genetic analyses are not immediately applicable. If an 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 alternate phenotype, then standard quantitative genetic analyses can be used for liability (Falconer 1965; Falconer and Mackay 1996; Lynch and Walsh 1998). Heritability can be estimated on the observed scale (i.e. one morph is coded as "0" and the alternate morph as "1") and then transformed to heritability on the liability scale (Dempster and Lerner 1950; Van Vleck 1972; Roff 1997). Alternatively, the mean liabilities of groups can be calculated, given the proportional representation of each morph in the groups (Falconer 1965; Falconer and Mackay 1996; Lynch and Walsh 1998). If the groups are relatives of known relatedness (e.g., full-sibs), then the variance of group mean liabilities can be used to estimate genetic variance (Moorad 2005). Because of known biases associated with the first approach (Van Vleck 1972; Lynch and Walsh 1998), I used the second approach (see Moorad 2005 for a comparison of the two approaches).

The threshold approach is a type of probit analysis. It is similar to and gives similar results to those of logistic regression, except that probit analysis uses the cumulative normal instead of the logistic to fit binomial data (Searle et al. 1992; Sorenson and Gianola 2002). Logistic regression has been used in the analysis of sex ratio data (Orzack and Gladstone 1994; Boomsma and Nachman 2002; Wilson and Hardy 2002). In many biological systems, it may be more intuitive to use probit analysis and assume an underlying normal distribution of effects (e.g., phenotypes controlled by many genetic or environmental factors of small effect) (Sorenson and Gianola 2002). As with logistic regression, qualitative and quantitative covariates can be controlled for. I

transformed the proportion of gynes ("caste ratio" = N_{gynes} / (N_{gynes} + $N_{workers}$)) and proportion of male sexuals ("sex ratio" = N_{males}/ (N_{males} + N_{gynes}) for each colony to mean colony liability for caste and sex, respectively. Because liability is undefined when the family incidence is 0 or 1, colonies with caste/sex ratios equal to zero were transformed from p=0 to p'=0+1/(2N_{total}), where N_{total} is the total number of females or sexuals. Colonies with caste/sex ratios equal to 1 were transformed from p=1 to p'=1-1/(2N_{total}). A general regression model (as for mass) was then used to assess the significance of the effects of collection site, nest density, colony size, and total colony productivity. After effects that were significant for any treatment were removed, residuals were used in analyses of intraclass correlations and causal (co)variance components. Queen dry mass was also considered as a potential covariate for treatments L, W, and WL. Analyses weighted by total brood number may be preferable because the caste/sex ratios and mean liabilities of larger families are estimated more accurately (Boomsma and Nachman 2002; Wilson and Hardy 2002). However, weighting is potentially unfavorable in quantitative genetic analyses because it may bias the analysis towards families (and genotypes) with large colony sizes, whereas the purpose of the analysis is to obtain an unbiased estimate of genetic variance for the whole population (J Moorad, pers. comm.). Weighted and unweighted analyses gave similar results, but below I present only the results of the unweighted analyses.

RESULTS

A total of 360 monogynous colonies with 10 or more workers were collected. These colonies produced a total of 7,928 workers, 3,536 gynes, and 864 males, of which 3,828 workers, 2,101 gynes, and 639 males were weighed. The treatment effects on the colony means (i.e. mean worker, gyne, and male mass, caste ratio, and sex ratio) will be analyzed in a separate paper. The treatment effects on variance components are summarized in tables 3 and 4.

Worker and gyne mass

As expected, degree of pigmentation, the indicator of developmental stage, had a significant effect on worker pupal mass ($F_{4,3823}$=65.787, p<0.000). After this age effect was removed, the number of workers predicted mean colony residual worker mass for treatment WL ($F_{1,50}$=5.938, p=0.018). Thus, worker number was used as a covariate for residual worker mass for all treatments, and accounted for 0-7% of the total phenotypic variance for residual worker mass (Table 2). Collection site had a significant effect on mean colony gyne mass for treatments L ($F_{4,36}$=4.257, p=0.006) and QL ($F_{4,23}$=2.952, p=0.042), and the total number of individuals produced predicted mean colony gyne mass for treatments W ($F_{1,42}$=4.108, p=0.049) and QW ($F_{1,23}$=12.400, p=0.002). Site and total number of individuals produced were used as predictors for gyne mass and these variables accounted for 6-38% of the total phenotypic variance (Table 2).

The intraclass correlations ranged from 0.09 to 0.36 for residual worker mass, and 0.32 to 0.89 for residual gyne mass. Unmanipulated colonies (treatment QWL) had the highest intraclass correlations for worker and gyne mass. The 95% confidence intervals

for the estimates of the intraclass correlations for all treatments for worker and gyne mass did not overlap zero, showing significant among-colony variance (Table 2).

Of particular note are the intraclass correlation estimates for treatments L, Q, and W, because among-colony variance for these treatments is due to only one causal variance component. Intraclass correlations for treatments Q and W were lower than the intraclass correlation for treatment L for gyne and worker mass, but only treatment W for residual worker mass was significantly lower than treatment L. These trends were the same for heritabilities of direct effects, maternal effects, and sibsocial effects that were computed from the intraclass correlations for treatment L, Q, and W, respectively. Heritabilities of maternal and sibsocial effects were lower (36-62%) than the heritability of direct effects for worker and gyne mass. These results indicate that variance in direct, maternal, and sibsocial effects all contribute to total phenotypic variance for gyne and worker mass, although direct effects are relatively more important.

As the within-colony variance components for all treatments and traits were generally of similar magnitude, the estimates of causal variance components for direct effects, maternal effects, and sibsocial effects mirrored the results for the corresponding intraclass correlation estimates (Table 3). All covariance estimates for worker and gyne mass were negative, with direct-maternal and maternal-sibsocial covariances for residual gyne mass being significantly lower than zero. The corresponding correlations ranged from -0.04 to -0.47 for residual worker mass and -0.25 to -1.25 for residual gyne mass. Total heritability for residual worker and gyne mass were indistinguishable from zero, despite significant heritability for direct, maternal, and sibsocial effects considered separately (Table 3). Total heritability incorporates all causal covariance as well as causal

variance components, and the negative covariance components cancelled out the positive variance components.

Male mass

Collection site had a significant effect on male mass for treatment W ($F_{4,29}$=4.689, p=0.005), and total number of individuals produced predicted male mass for treatment WL ($F_{1,14}$=9.297, p=0.009). These two variables accounted for 24-87% of total phenotypic variance for male mass, although two treatments (QL and QWL) only had 5 or 6 male-producing colonies (Table 2). The intraclass correlations for these treatments were not bounded away from zero, but because of small sample size, these estimates are not very meaningful. The intraclass correlation estimate for treatment Q for male mass also was not bounded away from zero, despite a much larger sample size (27 colonies), suggesting that maternal effects are relatively less important for male mass. The estimate of heritability for sibsocial effects was approximately 40% that for direct effects, suggesting that as for worker and gyne mass, variance in direct effects account for a relatively larger proportion of total phenotypic variance. Because there were only 5-6 male-producing colonies in treatments QL and QWL, I did not calculate any covariance or correlation estimates for male mass.

Liability for caste and sex

Site had a significant effect on liability for caste for treatment W ($F_{4,42}$=7.279, p=0.046). In addition, the number of workers predicted liability for caste for treatments W ($F_{1,42}$=4.666, p=0.037) and QL ($F_{1,52}$=24.0157, p<0.001), the number of larvae per

worker predicted liability for caste for treatments L ($F_{1,53}$=4.977, p=0.030), W ($F_{1,42}$=5.255, p=0.027), and QWL ($F_{1,45}$=4.685, p=0.036), and the total production of individuals predicted liability for caste for treatment QW ($F_{1,47}$=9.177, p=0.004). These variables accounted for 6-25% of total phenotypic variance for liability for caste (Table 2). The initial number of workers predicted liability for sex for treatment L ($F_{1,43}$=4.419, p=0.041) and QL ($F_{1,26}$=5.636, p=0.025), the number of larvae predicted liability for sex for treatment WL ($F_{1,48}$=4.072, p=0.049), and the total production of individuals predicted liability for sex for treatments W ($F_{1,46}$=4.225, p=0.045), Q ($F_{1,34}$=6.067, p=0.019), and QL ($F_{1,26}$=7.974, p=0.009). These variables accounted for 0.6-13% of the total phenotypic variance for liability for sex (Table 2). For treatment W, queen mass was correlated with liability for caste (r=0.312, p=0.041, N=43), but it was also correlated with worker number (r=0.565, p<0.000, N=43), larvae number (r=0.692, p<0.000, N=43), and total production of individuals (r=0.371, p=0.014, N=43), and when all potential variables were included in a regression model, queen mass did not have a significant effect on liability for caste.

The intraclass correlations for all treatments for residual liability for caste and sex were significantly greater than zero (Table 2). The relative magnitudes of intraclass correlations for residual liability for sex followed a similar pattern as for mass, with the intraclass correlation for unmanipulated colonies (treatment QWL) being the highest, and the intraclass correlation for treatment L being higher than those for treatments Q and W. The estimate for heritability of direct effects for residual sex liability was twice as large as the estimates for heritability for maternal and sibsocial effects (Table 2). The intraclass correlations for residual caste liability were different, with the intraclass correlation for

treatment Q being the largest, which, together with intraclass correlations for treatments L, QL, WL, and QWL were of similar magnitude and statistically indistinguishable. Intraclass correlations for treatments W and QW were relatively lower in magnitude (Table 2). The estimate of heritability for sibsocial effects on residual caste liability was lower (50-60%) than the corresponding estimates of heritability for maternal effects and direct effects. These results indicate that there are significant levels of among-colony variance due to direct effects, maternal effects, and sibsocial effects for residual caste and sex liability, although variance due to direct effects is relatively more important for sex, and variance due to sibsocial effects is relatively less important for caste.

As for mass, the covariance estimates for residual caste and sex liability were all significantly negative except for the maternal-sibsocial covariance for residual sex liability. The corresponding correlations ranged from -1.5 to -0.5 for residual liability for caste, and -1.0 to -0.5 for residual liability for sex (Table 3). The total heritability for liability for sex was negative but not significantly less than zero, while the estimate of total heritability for residual caste liability was significantly less than zero. As for mass, these results indicate that the negative covariance components can greatly reduce the estimated total heritability.

DISCUSSION

A number of studies of social insects provide evidence for a genetic component to individual and colony traits (e.g., Calderone and Page 1988; Stuart and Page 1991; Ross and Keller 1998; Krieger and Ross 2002; Boomsma et al. 2003). However, despite the

known importance of the social environment in mediating these genetic effects (e.g., (Calderone and Page 1992; Robinson et al. 1994; Keller and Ross 1995; Rüppell et al. 2001; Ross and Keller 2002), these studies typically focus exclusively on direct genetic effects or potentially confound direct effects with the effects of genes expressed in the social environment (i.e. indirect genetic effects). I used an indirect genetic effect model that explicitly includes direct, maternal, and sibsocial influences on phenotypes.

The main result of this study is that there were high levels of among-colony variance due to direct, maternal, and sib-social effects for mass, caste ratio, and sex ratio in the ant *Temnothorax curvispinosus*. These among-family "origin effects" are often interpreted as providing evidence for genetic variance, i.e. heritability for direct and indirect effects (e.g., Owen and McCorquodale 1994; Kölliker et al. 2000). Before further discussing the quantitative genetic estimates and their implications, I review controlled and uncontrolled factors that may have influenced the results.

Controlled and uncontrolled environmental effects

Because the colonies used in this study were field collected, environmental factors contributed to variation among-colonies for traits such as the size and condition of the queen, workers, and overwintered larvae, and the number of workers and larvae in the colony. I controlled for these pre-foster environmental effects by using residuals to estimate quantitative genetic parameters after effects due to collection site, colony size, and total colony production were removed. For some traits and treatments, these variables accounted for a large proportion of the total phenotypic variance (Table 2). Access to unlimited resources post-foster may have minimized pre-foster environmental effects on

the measured phenotypes because all colonies, regardless of the starting condition were not constrained due to lack of resources. In studies of the related *Temnothorax longispinosus*, colony demographic traits affected total production, but had a much smaller effect on allocation (i.e. mass, caste ratio, and sex ratio, Herbers 1990; Backus 1995).

Queen mass is potentially related to reproductive status or queen condition and may be expected to predict colony patterns of investment. Queen dry mass was included as a potential covariate for all traits in treatments with queen removal (i.e. L, W, and WL), but it did not have a significant effect on any trait for these three treatments. Additional factors associated with queen condition (e.g., queen age and reproductive status), may have influenced the measured traits. However, these factors may be correlated with queen mass, colony size, and colony productivity, which were controlled for.

I did not control for among-colony variance due to mean worker size or mean larvae size. However, mean colony larvae mass did not predict mean colony gyne mass in a previous study of the same population (Linksvayer, unpublished data), just as egg mass did not predict gyne mass in *Temnothorax rugatulus* (Rüppell et al. 2001). Thus, though uncontrolled, the contribution of pre-foster size variation in workers and larvae to among-colony variation in the studied phenotypes may be minimal.

I experimentally minimized post-foster environmental variation by maintaining all colonies under the same temperature, climate, and feeding conditions. Ideally, in quantitative genetic sib analyses, replicated groups of full-sibs can be reared so that post-foster common environment effects do not contribute to among-family variance (Lynch

and Walsh 1998). In the case of social insects, colonies can be split into replicate colony fragments. This is difficult when using colonies with small average colony size, as *Temnothorax curvispinosus*, but would be possible with larger colony species. In addition, the use of colony fragments of the same size would experimentally remove the potential for initial colony size parameters to contribute to among-fragment variance. Such a replicated design could not be used in the present study because I was also interested in maternal effects, and single queens cannot be replicated.

The uncontrolled environmental effects potentially contributed to among-colony variance for the studied traits, and affected the estimates of underlying genetic (co)variance components. In addition, non-additive genetic variance due to dominance and epistasis could inflate the estimates of additive genetic variance. Thus, unlike a model system, it was impossible to control for all environmental factors that potentially contributed to among-colony variance. This means that the quantitative genetic parameter estimates reported here should be interpreted with some caution.

One final complication is that all colonies may not have been composed of full-sib larvae and workers, e.g., because of instances of multiple mating by the queen, intraspecific brood raiding, or the fusion of two colonies (Herbers and Grieco 1994; Foitzik and Heinze 2001). These phenomena would result in increased levels of within-colony variance and corresponding decreased levels of among-colony variance, so that the magnitude of (co)variance components would be underestimated. Thus, considering all workers and larvae as full-sibs should result in conservative quantitative genetic estimates. In the following sections, I discuss the quantitative genetic estimates and their implications.

Variance for direct, maternal, and sibsocial effects: mass

The results of this study are suggestive of genetic variance for direct effects, maternal effects, and sibsocial effects on mass. The heritability of direct effects on mass was higher than the heritability of maternal and sibsocial effects on mass, 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 to total phenotypic variance as variance in direct genetic effects (e.g., Cheverud 1984; Hunt and Simmons 2002; Rauter and Moore 2002).

Heritable variation for direct effects on mass indicates variation for zygotic genes expressed during development that influence individual mass. Heritable variation for sibsocial effects on mass indicates variation for genes expressed in workers that influence the social environment of developing larvae, perhaps through the amount of care provided (Linksvayer and Wade 2005). Evidence for a genetic component to colony division of labor has been found in a variety of social insect taxa, including a congener of *T. curvispinosus*, *T. rudis* (Calderone and Page 1988; Page et al. 1989; Stuart and Page 1991; Snyder 1992; O'Donnell 1996). These genetic differences among worker groups are interpreted as a genetic influence on the probability a worker will perform a certain task. Presumably, these genetic differences have a measurable (indirect) effect on the phenotype of brood reared. These findings are thus suggestive of the importance of sibsocial genetic variance. Heritable variation for maternal effects on mass indicates variation for genes expressed in queens that affect the social environment of developing

larvae. One example of these maternal effect genes might be genes that influence chemical signals the queen produces that influence worker behavior (Vargo and Passera 1991; Keller and Nonacs 1993).

It is interesting to note that unmanipulated colonies (treatment QWL) had the highest intraclass correlations for worker and gyne mass. This was expected, because all genetic (co)variance components contribute to among-colony variance in unmanipulated colonies. Rüppell et al. (2001) estimated the heritability of gyne size in *Temnothorax rugatulus*, an ant with dimorphic gynes. Similar to the results of the present study, they found that heritability estimates using unmanipulated colonies reared in the lab were very different (much higher in colonies with a single queen morph, but not different than 0 in colonies with both queen morphs) than heritability estimates using larvae reared by foster worker groups. The authors attributed these differences in heritability estimates to maternal effects (Rüppell et al. 2001). Their results along with mine further demonstrate how aspects of the social environment contribute to among-colony variance and can confound estimates of heritability of direct effects if they are not controlled for.

It is also interesting to note that the intraclass correlations for residual worker mass were generally lower across all treatments relative to the intraclass correlations for residual gyne and male mass. Bargum et al. (2004) used a maternal half sib analysis to estimate heritability for gyne and worker size in the ant *Formica truncorum*, and found significant narrow-sense heritability for queen size in one of the two study years and low and non-significant heritability for worker size. They suggested that low heritability for worker size may be favored by colony-level selection because a variably-sized workforce may be advantageous (Bargum et al. 2004).

Variance for direct and indirect effects: caste and sex

Traditionally, among-colony variance for sex and caste ratio is attributed to environmental factors. For example, among-colony variance for caste ratios may be explained by variation in colony size, age, or the amount of available larval nourishment (Hölldobler and Wilson 1990; Herbers et al. 2001). Variation among colonies for sex ratio is also usually attributed to such factors, along with factors affecting within-colony relatedness such as queen number and mating frequency (Crozier and Pamilo 1996). Split sex ratio theory predicts large among-colony variance, with some colonies producing mainly gynes and some colonies producing mainly males, depending on within-colony relatedness (Grafen 1986; Ratnieks and Boomsma 1997). I attempted to experimentally or statistically control many of these potentially important environmental variables (see above). I explicitly manipulated social environmental factors (queen presence and the relatedness between social partners) and estimated residual variation among colonies with the same experimentally imposed social system. I suggest that the remaining among-colony variance for caste and sex ratio is at least partly due to an additional factor not usually considered, direct and indirect genetic (co)variance (Pamilo 1982; Crozier and Pamilo 1996). Empirical studies of sex and caste ratio in social insects usually find a large amount of unexplained among-colony variance after all variables relating to colony relatedness, colony size, site effects, etc. are removed (e.g., Herbers 1990; Backus 1995). Variation among colonies for direct and indirect genetic effects may well contribute to this residual phenotypic variance (see Pamilo 1982; Crozier and Pamilo 1996).

The results of the current study suggest a heritable basis to direct effects, maternal effects, and sibsocial effects on caste ratio and sex ratio. As for mass, variation in direct effects contributed relatively more than variation in maternal and sibsocial effects to total phenotypic variance for caste and sex ratio. Note though that the estimate of genetic variance for direct effects for sex ratio may be largely due to pre-foster among-colony differences in the ratio of haploid to diploid larvae. Maternal and sibsocial variance estimates should not be similarly affected, because all colonies in treatments used to estimate these parameters (e.g., treatments Q and W) should have received similar initial proportions of haploid to diploid larvae.

Traits that may affect caste ratio include larval traits underlying the developmental switch of caste determination (Wheeler 1986) or the propensity to beg for food (Kaptein et al. 2005). Heritability of direct effects on caste ratio may be indicative of genetic variation for these traits. Heritability of maternal and sibsocial effects for caste ratio suggests genetic variation for worker and queen traits that affect the nutritional and pheromonal environment of developing larvae. Page et al. (1989; Robinson et al. 1994) showed that subfamily membership in honeybee colonies predicted the likelihood of individual workers taking part in worker- versus queen-rearing. These results are suggestive of sibsocial genetic variance for caste.

All of the factors affecting caste ratio are also likely have an influence on sex ratio because colonies which rear a larger proportion of gynes from female larvae will have a more female biased sex ratio than colonies that raise primarily workers (Crozier and Pamilo 1996). In addition, genetic variation for direct effects on sex ratio may affect the relative viability of male and female sexuals. Genetic variation for maternal effects

genes may directly influence the ratio of haploid to diploid eggs laid (Crozier and Pamilo 1996). Genetic variation for sibsocial effects may influence relative worker investment in males and gynes.

Pamilo (1982) developed a deterministic simulation model of sex ratio evolution with genes controlling sex ratio expressed only in queens (i.e. maternal effect genes), only in workers (i.e. sibsocial effect genes), or both. The results suggest that at equilibrium more variance may be maintained for maternal effect genes relative to sibsocial effect genes (Pamilo 1982; Pamilo and Crozier 1996). In contrast, I found indistinguishable levels of variance due to maternal and sibsocial effects. Additional theoretical models incorporating the full complexity of social insect colonies are needed to make predictions about expected levels of variation in direct, maternal, and sibsocial effects on sex ratio, and additional empirical studies are needed to test these predictions.

Heritability for threshold traits is often higher than continuous traits (Falconer and Mackay 1996; Roff 1998). This is thought to be because individual-level selection on threshold traits is inefficient, and depends not only on the strength of selection, but also the incidence of each morph. Only genotypes with mean liability above the threshold express the phenotypic trait that is seen by selection. As the selected morph becomes more common, it becomes harder to select for that morph further. However, the response of threshold traits to family-level selection is expected to be much more efficient (Falconer and Mackay 1996). This is because incidence at the family level reflects mean family liability, so that at the family-level, selection can effectively act on liability directly. This is interesting given that traits affecting individual and colony fitness such as propensity to develop into a reproductive female are expected to be acted on by

individual- and colony-level selection in opposite directions (Wade 1979, 1980; Bourke and Ratnieks 1999). Consideration of caste and sex as threshold traits may lead to insights about the evolutionary dynamics of these traits.

Covariance for direct and indirect genetic effects

Another main result of this study is that negative genetic correlations between direct and indirect effects were consistently detected for all traits (Table 3). However, these covariance estimates should be interpreted with some caution. They were calculated by subtracting among-colony variance estimates of two treatments from that of one treatment (e.g., $G_{om} = Var_{QL} - Var_{Q} - Var_{L}$). If pre- or post-manipulation environmental factors consistently inflated the estimates of among-colony variance for all treatments (see above), then the covariance estimates would be consistently biased downwards.

However, inspection of the intraclass correlations for the treatments also provides evidence consistent with negative covariances (Table 2). Although intraclass correlations cannot be summed because they are made up of both among- and within-group variance components, the magnitude of intraclass correlations of treatments that include multiple causal variance components (i.e., QL, WL, QW, QWL) are not as high as expected given the magnitude of intraclass correlations of treatments that include single causal variance components (i.e., Q, W, and L). A possible explanation is that the treatments that include multiple casual variance components also contain one or more negative causal covariance components.

Negative direct-maternal genetic covariances have been found in a variety of domestic mammals as well as natural animal populations (e.g., Cheverud and Moore

1994; Agrawal et al. 2001). Genetic covariances are expected to be negative in a variety of situations. If a trait is influenced by both direct and maternal effects and there is stabilizing selection for intermediate trait values, then negative genetic correlations between maternal and direct effects will build up due to linkage disequilibrium (Wolf and Brodie 1998). This is because certain combinations of genetic effects (e.g., a positive maternal effect and a negative direct effect) are favorable. Stabilizing selection may also favor negative genetic correlations due to pleiotropy (Wolf and Brodie 1998). Directional selection for increased or decreased trait values can also lead to negative genetic correlations due to pleiotropy, because alleles that are unconditionally positive or negative (e.g., through both direct and maternal effects) will fix or be lost, respectively. Only alleles with antagonistic pleiotropy (e.g., a positive direct effect and a negative maternal effect) will remain segregating (Falconer and Mackay 1996). In addition, because most mutations have negative fitness effects, it is likely that mutations with an overall favorable fitness effect will act positively through only one effect (e.g., direct effect), and negatively through other effects (e.g., maternal effect) (Roff 1996). Mutations that spread may sequentially have a positive maternal effect and a negative direct effect, then a positive direct effect and maternal effect, etc. (cf. Rice and Chippindale 2001).

Genetic covariances and correlations are important evolutionary parameters because they imply genetic constraints, in that genetically correlated traits cannot independently respond to selection (Lande 1979; Arnold 1992). Despite the high estimates of heritable variance for direct, maternal, and sibsocial effects, estimates of total heritability for all traits, incorporating all these effects and the corresponding covariances, were negative or not different than zero (Table 3). Even though direct

effects, maternal effects, and sibsocial effects all have a genetic component and can respond to selection, selection on any one of these effects may cause a correlated response to selection in the opposite direction for the other effects. Consequently, the combination of the effects causes no change in the phenotype of the focal individual.

Implications for kin selection, parent-offspring conflict, and queen-worker conflict

Genes underlying caste determination and sex ratio have often been imagined in terms of kin selection and parent-offspring conflict theory. For example, the evolutionary origin of worker behavior is often attributed to the spread of "kin selected altruism"/ "offspring control" genes (Hamilton 1964a,b; Michod 1982) or "parental manipulation" genes (Alexander 1974; Michener and Brothers 1974). The discovery of among-colony variance for direct effects on caste is suggestive of variation for zygotic genes involved in caste determination. These genes could be interpreted as offspring control or kin selected altruism genes (Linksvayer and Wade 2005). Among-colony variance for indirect (maternal or sibsocial) effects suggests variation for genes expressed in the colony queen(s) or workers, and these genes could be interpreted as parental manipulation genes (Linksvayer and Wade 2005). The magnitude of genetic variance for direct and indirect effects on mass, caste, and sex are a measure of the potential of these effects (and thus the traits) to respond to selection. For *T. curvispinosus*, it appears that all of these direct and indirect effects can respond to selection, suggesting that both "offspring control" and "parental manipulation" genes influence the evolutionary dynamics of mass, caste ratio, and sex ratio. It would be very interesting to study the relative importance of direct and indirect genetic effects on mass, caste ratio, and sex ratio in additional social insect taxa,

especially those that may possess phenotypes associated with the origin of eusociality (e.g., halictid bees, vespid wasps, Linksvayer and Wade 2005).

While the results of this study suggest that direct, maternal, and sibsocial effects on mass, caste, and sex can respond to selection, as explained above, the independent response of these effects to selection may be complicated by genetic correlations between these effects. For example, with a negative direct-maternal genetic covariance, selection on larvae to have a higher propensity to develop into gynes would cause a corresponding decrease in gyne-biased development due to the negatively correlated maternal genetic effect. Similarly, selection for queens to suppress the gyne development of their female offspring through a negative maternal effect on the offspring could result in a corresponding increased bias towards gyne development through the direct genetic effect. Thus, selection on larvae and the queen may be in opposing directions so that there is predicted evolutionary conflict, but this conflict may never be realized in terms of an evolutionary response to selection (Cheverud and Moore 1994). In addition, despite potential evolutionary change in direct and indirect effects, the composite of these effects on the focal individual's phenotype may not change, so that the trait of interest cannot respond to selection, as suggested by estimates of total heritability that are not different than zero (Table 3). These results suggest that consideration of the genetic architecture underlying traits that are likely the focus of parent-offspring and queen-worker conflict may be necessary to fully understand the evolutionary dynamics of these traits.

Conclusions

The results of this study suggest that direct, maternal, and sibsocial effects all have a heritable basis and can affect the evolutionary dynamics of mass, caste ratio, and sex ratio. Thus, this study demonstrates the evolutionary importance of genes expressed in the social environment, as well as genes expressed within individuals. While caste ratio and sex ratio are usually considered to be affected only by environmental factors, this study and others suggests that genetic factors may also often influence these traits. In general there was no evidence of evolutionary constraints due to lack of genetic variance for direct, maternal, and sibsocial effects. However, the results of this study suggest that genetic correlations between direct, maternal, and sibsocial effects may be an important evolutionary constraint for mass, caste ratio, and sex ratio. Using indirect genetic effect models and empirical approaches, as described here, together with optimality models and other more traditional approaches should lead to a more thorough understanding of social evolution.

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TABLE 1. Experimental manipulations used for each of the seven treatments and the coefficients of causal (co)variance components that contribute to among-colony variance for each treatment. G_{oo}, G_{mm}, and G_{ss} are direct, maternal, and sibsocial genetic variance, and G_{os}, G_{om}, and G_{ms} are direct-sibsocial, direct-maternal, and maternal-sibsocial genetic covariance. The coefficients shown are for haplodiploid females.

Treatment	Manipulation			Coefficients of causal (co)variance components contributing to among-colony variance					
	Remove queen	*Mix workers*	*Mix larvae*	*Direct (G_{oo})*	*Maternal (G_{mm})*	*Sibsocial (G_{ss})*	*Direct-maternal (G_{om})*	*Direct-sibsocial (G_{os})*	*Maternal-sibsocial (G_{ms})*
L	X	X		3/4					
Q		X	X		1				
W	X		X			3/4			
WL	X			3/4		3/4		2/3	
QL		X		3/4	1		1		
QW			X		1	3/4			1
QWL				3/4	1	3/4	1	2/3	1

TABLE 2. The proportion of total phenotypic variance explained by collection site, colony size, and colony productivity for each treatment and trait, intraclass correlation estimates and 95% confidence intervals, and heritability estimates and 95% confidence intervals for treatments L, Q, and W, corresponding to heritability for direct effects, maternal effects, and sibsocial effects, respectively. t, intraclass correlation, the proportion of total phenotypic variance contributed by among-colony variance; N, number of colonies; n, total number of individuals, for each treatment.

Trait	*Treatment*	*Proportion of variation explained by site and colony size / productivity*	*t*	*Lower CI for t*	*Upper CI for t*	*heritability*	*n*	*N*
Worker mass								
	L	0.0305	0.245	0.174	0.319	0.326	620	54
	Q	0.0266	0.189	0.115	0.269	0.189	511	46
	W	0.0307	0.0931	0.0228	0.170	0.1241	313	44
	QL	-0.00963	0.347	0.264	0.424		639	50
	WL	0.0542	0.225	0.123	0.321		537	52
	QW	0.0331	0.245	0.160	0.337		580	49
	QWL	0.0701	0.358	0.268	0.443		628	46
Gyne mass								
	L	0.216	0.552	0.381	0.666	0.736	390	41
	Q	0.0646	0.465	0.336	0.579	0.465	198	30
	W	0.0701	0.322	0.222	0.427	0.430	438	44
	QL	0.256	0.546	0.237	0.752		256	28
	WL	0.122	0.613	0.496	0.710		576	49
	QW	0.379	0.371	0.721	0.563		94	25
	QWL	0.134	0.892	0.797	0.939		149	15
Male mass								
	L	0.237	0.496	0.322	0.595	0.993	133	20
	Q	0.308	0.111	-0.121	0.302	0.111	103	27
	W	0.445	0.276	0.105	0.421	0.368	125	34
	QL	0.871	0.0190	-0.195	0.0812		57	6
	WL	0.521	0.284	0.0416	0.485		87	16
	QW	0.314	0.288	0.107	0.437		93	27
	QWL	0.781	0.188	-0.368	0.434		41	5
Caste ratio								
	L	0.139	0.490	0.390	0.570	0.653	1963	55
	Q	0.0637	0.524	0.453	0.578	0.524	1202	49
	W	0.248	0.367	0.290	0.443	0.330	1166	46
	QL	0.233	0.448	0.362	0.527		1779	54
	WL	0.0905	0.459	0.372	0.532		2043	55
	QW	0.0670	0.258	0.173	0.353		1278	49
	QWL	0.0734	0.411	0.304	0.491	0.548	2033	47
Sex ratio								
	L	0.0638	0.514	0.402	0.598	1.0247	883	45
	Q	0.0681	0.353	0.284	0.415	0.415	368	36
	W	0.0389	0.302	0.241	0.359	0.479	856	48
	QL	0.1337	0.329	0.226	0.426		477	29
	WL	0.0378	0.352	0.183	0.516		1267	50
	QW	0.00581	0.413	0.327	0.485		201	38
	QWL	0.0515	0.644	0.460	0.739		348	21

TABLE 3. Causal (co)variance, correlation, and total heritability estimates and 95% confidence intervals for each trait.

Trait	*Causal variance component*	*(co)variance estimate*	*Lower CI*	*Upper CI*	*Correlation*	*Lower CI*	*Upper CI*
Gyne mass							
	Direct	0.0238	0.0119	0.0340			
	Maternal	0.0264	0.0170	0.0367			
	Sibsocial	0.0175	0.0104	0.0263			
	Direct-maternal	-0.0283	-0.0463	-0.00947	-1.151	-1.624	-0.471
	Direct-sibsocial	-0.00498	-0.0143	0.00411	-0.249	-0.565	0.280
	Maternal-sibsocial	-0.0267	-0.0430	-0.0105	-1.253	-1.825	-0.591
	Total heritability	-0.458	-1.09	0.179			
Worker mass							
	Direct	0.00443	0.00304	0.00610			
	Maternal	0.00303	0.00173	0.00463			
	Sibsocial	0.00168	0.000432	0.00295			
	Direct-maternal	-0.00169	-0.00418	0.000679	-0.468	-0.941	0.229
	Direct-sibsocial	-0.00107	-0.00255	0.000481	-0.407	-0.849	0.218
	Maternal-sibsocial	-0.0000885	-0.00249	0.00267	-0.0389	-0.892	1.522
	Total heritability	0.284	-0.0750	0.642			
Caste							
	Direct	1.279	0.853	1.770			
	Maternal	1.099	0.828	1.371			
	Sibsocial	0.774	0.544	1.062			
	Direct-maternal	-1.247	-1.762	-0.727	-1.061	-1.294	-0.727
	Direct-sibsocial	-0.466	-0.799	-0.155	-0.472	-0.680	-0.190
	Maternal-sibsocial	-1.328	-1.715	-0.957	-1.449	-1.651	-1.192
	Total heritability	-1.002	-1.582	-0.430			
Sex							
	Direct	1.813	1.151	2.548			
	Maternal	0.546	0.397	0.708			
	Sibsocial	0.576	0.423	0.747			
	Direct-maternal	-0.976	-1.423	-0.563	-0.992	-1.228	-0.686
	Direct-sibsocial	-0.739	-1.180	-0.240	-0.736	-1.014	-0.273
	Maternal-sibsocial	-0.275	-0.576	0.0382	-0.495	-0.930	0.0768
	Total heritability	-0.132	-0.435	0.212			

CHAPTER 3

EXPERIMENTAL STUDY OF COLONY RESOURCE ALLOCATION: EFFECTS OF QUEEN REMOVAL AND RELATEDNESS BETWEEN WORKERS AND LARVAE IN THE ANT *TEMNOTHORAX CURVISPINOSUS*

Timothy A. Linksvayer

ABSTRACT

Social insect colonies have come to be understood as foci of both cooperation and conflict. Among-colony selection favors the integration of nestmate behaviors that maximize colony productivity, while within-colony genetic heterogeneity is predicted to cause conflicts of interest among nestmates over allocation of colony resources to males versus females and to colony growth and maintenance versus reproduction. However, constraints, due to limited information or power, costs of manipulation, or the genetic architecture underlying manipulative behaviors, may prevent these conflicts from being realized. Correlations among colony attributes such as sex ratio and queen number or nestmate relatedness are commonly used to infer the interactions among nestmates that cause those patterns, such as queen-worker conflict. Experimental manipulations of colony social structure are rare but necessary to elucidate the mechanistic and causal bases of observed correlations. I manipulated three fundamental aspects of colony social structure in the ant, *Temnothorax curvispinosus*: queen presence, relatedness among workers, and relatedness among larvae. I measured the effects of these manipulations on colony resource allocation to workers, gynes, and males. The results did not support the predictions of queen-worker conflict theory. I suggest that many aspects of colony resource allocation are shaped primarily by among-colony selection, leading to queen-worker-larva coadaptation, rather than queen-worker-larva conflict.

Traditionally, social insect colonies have been understood as cohesive units, sometimes referred to as "super organisms" (Wheeler 1911; Wilson 1971). In this view, interactions among queen, worker, and brood nestmates are shaped by among-colony selection acting on the colony output of reproductive males and females. Hamilton's gene-centered theory of inclusive fitness formally explained how genes for "altruism" that benefit related nestmates could spread, but it also emphasized the potential for conflicts of interest among genetically heterogeneous nestmates (Hamilton 1964a,b, 1970, 1972). Thus, social insect colonies have come to be understood as being foci of both cooperation and conflict (Bourke and Franks 1995).

The primary conflict to emerge from the conceptual framework of inclusive fitness was that between haplodiploid queens and workers over the colony sex ratio (Trivers and Hare 1976). Specifically, in colonies with a single, singly mated queen, the queen is equally related to her male and female offspring, but worker females are more closely related to their female than male sibs. As a result, in theory, queens "favor" equal investment in the sexes, while workers favor female-biased investment (Trivers and Hare 1976). Additional related conflicts may also occur among nestmates, for example over investment in colony growth versus reproduction (Pamilo 1991; Herbers et al. 2001), in the production of males (Crozier and Pamilo 1996), and in the caste fate of female brood (Bourke and Ratnieks 1999).

These predicted conflicts may be prevented or mitigated by various constraints (Heinze 2004). For example, limits on the information or power available to colony members may prevent them from manipulating colony resource allocation (e.g., Nonacs and Carlin 1990; Beekman and Ratnieks 2003; Boomsma et al. 2003). Manipulative

behaviors are also likely to incur costs to total colony productivity that may constrain the evolution of these behaviors (Ratnieks and Reeve 1992; Korb and Heinze 2004). Finally, the genetic architecture underlying manipulative traits may constrain their evolution (Boomsma et al. 2003; Linksvayer, Chapter 2). The degree to which these constraints and conflicts exist, and their mechanistic bases remain largely unresolved.

Most studies of queen-worker conflict have been observational or correlational (reviewed by Queller and Strassmann 1998; Chapuisat and Keller 1999). For example, many observational field studies have identified sex investment ratios at the population level in order to determine who "wins" queen-worker conflict, with female-biased sex ratios taken as evidence of workers controlling colony investment decisions, and equal sex ratios indicating queen control. Further studies investigate the correlation between colony sex investment ratios and factors that theoretically directly influence optimal worker strategies (e.g., average relatedness between workers and brood; Evans 1995; Sundstrom et al. 1996) or factors that theoretically influence the relative power of queens and workers to impose their optimal strategies (e.g., relative numbers of queens and workers, Herbers 1984, 1990; Bourke and Chan 1999). These studies use observed sex ratios or the correlation of colony variables with colony sex ratios to infer specific sorts of interactions among nestmates, e.g., the manipulation of brood sex ratios by workers associated with queen-worker conflict.

Experimental study using direct manipulations of interacting nestmates are necessary to elucidate the causal basis for the observed patterns. Only a handful of studies have used experimental manipulation to study colony resource allocation. These studies have manipulated available nesting and nutritional resources (e.g., Backus and

Herbers 1992; Herbers and Banschbach 1998; Bono and Herbers 2003; Foitzik et al. 2004), queen presence (Mueller 1991) or number (Kümmerli et al. 2005), and relatedness among nestmate larvae (Evans 1995). Additional studies exchanged queens (Passera et al. 2001) or brood (Helms et al. 2000) between colonies which previously produced only a single sex.

In colonies of the ant, *Temnothorax curvispinosus*, collected in nature and transferred to the laboratory, I manipulated queen presence, relatedness among workers, and relatedness among larvae and examined treatment effects on components of colony resource allocation (worker mass, gyne mass, male mass, worker production, gyne production, male production, caste ratio and sex ratio). By manipulating these three fundamental aspects of social structure and studying changes in the components of colony allocation, I can study the proximate social causes and mechanistic basis of changes in resource allocation. In general, the results reported below are not consistent with the predictions of queen-worker conflict theory. I suggest that the results are most consistent with patterns of colony resource allocation being shaped by among-colony selection that has led to queen-worker-larvae coadaptation, not queen-worker-larva conflict.

METHODS

Natural history of study species

Temnothorax [=*Leptothorax* (*Myrafant*)] *curvispinosus* nests in nuts and other preformed cavities and is widespread across the eastern USA (Mackay 2000). This

species, along with other closely-related species, has been well studied and is readily maintained in the lab (e.g., Alloway 1979; 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 (Hebers 1986; 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 to a few hundred workers (mean 41, s.d. 24, N=470). Several 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; Herbers and Tucker 1986). Colonies produce two types of brood: (1) eggs laid in the late summer and early fall overwinter as larvae, and in the spring, these larvae develop into workers or gynes, or males; and (2) diploid eggs laid in the spring and early summer develop exclusively as workers in the summer (A. Buschinger, pers. comm.). Colony investment in individuals derived from the first type of brood, i.e. those developing from overwintered larvae, was the focus of this study.

Collection of study colonies

Acorn and hickory nut nests were collected in late winter / early spring, from 18 March to 20 April, 2004, 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. In a previous study, colony mean larva mass, measured at this time of collection, did not predict colony mean gyne mass or colony caste ratio ($p>.05$, N=55, Linksvayer, unpublished data). Overwintered larvae are very small (mean colony mean larva mass 0.065 mg, 0.038 s.d., N=130, Linksvayer, unpublished data), less than one

twelfth their final adult mass (mean colony mean gyne mass 0.822 mg, 0.147 s.d., N=55, Linksvayer, unpublished data) (see also Wesson 1940). Thus, the vast majority of larval growth occurred during the course of the experiment. Caste determination has also 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. Each nest was treated as an individual colony (Herbers 1990). Colonies were removed from their acorn nest, censused, and immediately assigned at random to an experimental treatment (see below). After experimental colonies were constructed, they were moved into artificial nests in the laboratory (nest design after Alloway 1979). In this way, the members of colonies in all treatments experienced the same disruptions during the setup of the experiment.

Experimental design

Colonies with at least one queen 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 from a mixture of workers from at least fifteen colonies, and (3) replacement of larvae with an equal number from a mixture of larvae from at least fifteen colonies (see Table 1). Colonies with no queen were randomly assigned to treatments that included queen removal (i.e. these queenless colonies could not be used in the treatments without queen removal). Results from colonies varying in initial queen number were analyzed separately.

The names of the seven treatments used hereafter (L, Q, W, QL, WL, QW, and QWL) refer to the members of the initial colony that were kept intact and were not experimentally manipulated; "L" stands for larvae, "Q" for queen, and "W" for workers (Table 1). For example, in treatment L, the larvae were kept intact but the queen was removed and the workers were replaced with a mixed group of workers. In this treatment, the larvae were not mixed and are thus full-sibs, while the workers were mixed and are assumed to be unrelated. For colonies in treatment Q, the queen was not removed, but both the workers and larvae were mixed and thus there were unrelated groups of both workers and larvae. For colonies in treatment W, the workers were the original unmixed, full-sibs, but the queen was removed and the larvae were mixed. Colonies in treatment QL had the original queen and full-sib larvae but mixed, unrelated workers. Colonies in treatment WL had no queen, full-sib larvae, and full-sib workers. Colonies in treatment QW had the original queen and full-sib workers, but mixed, unrelated larvae. Finally, colonies in treatment QWL were unmanipulated.

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 (see Ross and Keller 2002). Workers and larvae in treatments that did not involve worker or larvae mixing were similarly treated. This procedure was meant to facilitate cross-fostering by diminishing or mixing up chemical cues used in nestmate recognition. At least 15 colonies were used to create worker and larvae mixtures to minimize the contribution of any one colony's workers / larvae to the mixture, and to minimize variance among these mixtures. Workers in newly assembled colonies generally behaved as did workers in unmanipulated colonies and

showed little aggression towards nestmates. One exception was that a subset of workers from treatments that included worker mixing but no queen removal (QL and Q) frequently behaved aggressively towards the queen. These colonies were monitored for the first 24 hours of the study. When any workers displayed aggression towards the queen, the colony was blown on and cooled in a refrigerator, in order to facilitate queen adoption by the mixed workers (see Ross and Keller 2002).

Colonies were maintained in incubators under "spring" conditions of 13:11 hours light: dark photoperiod and 20:10 C day: night temperature cycle, until 21 May 2004. "Summer" conditions of 14:10 hours light: dark and 22:18 C day: night were then used through 16 August 2004, when the last gynes were removed (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, removed as they appeared, and then frozen and weighed to the nearest 0.001 mg. In treatments with 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 were included in the study. In addition, because workers tend to pupate at approximately the same time in a colony, worker pupae that appeared more than three weeks after the first group of workers had pupated were not included in the study. Males were removed, frozen, and weighed to the nearest 0.001 mg after they eclosed as adults. Gynes were not removed and weighed until 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). Preliminary results indicated that wet mass was strongly correlated with dry mass for workers ($r=0.865$, $p<0.000$, $N=210$), gynes ($r=0.921$, $p<0.000$, $N=580$), and males ($r=0.931$, $p<0.000$, $N=202$), and wet mass was used in all analyses.

Statistical analyses

First, I estimated the average 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. Specifically, for each trait and treatment, 10,000 bootstrap samples were created and the median value, 2.5%, and 97.5% values were taken as the parameter estimate and 95% confidence intervals.

Next, in order to further disentangle the main and interaction effects of the three experimental manipulations (queen removal, worker mixing, and larvae mixing) as well as the effects of potential categorical covariates (i.e. collection site), and continuous covariates (e.g., larvae number, worker number, total number of individuals produced), I used a general regression model or generalized linear model. This enabled me to simultaneously consider the effects of the experimental manipulation as well as effects of covariates such as colony size and colony productivity. The full potential model considered for a colony trait, z, was:

$$z = \text{intercept} + \text{queen removal} + \text{worker mixing} + \text{larvae mixing} + \text{queen removal} * \text{worker mixing} + \text{queen removal} * \text{larvae mixing} + \text{worker mixing} * \text{larvae mixing} +$$

collection site + larvae number + worker number + number of larvae per worker + total number of individuals produced + total number of sexuals produced + residual.

Variables for the total number of individuals or sexuals produced were only considered as potential covariates for body size traits (i.e. mean colony worker, gyne, and male mass), and sex ratio. The full model described above was first considered, and then a backwards removal process was used in which the least significant factors were sequentially removed until only significant factors remained in the model (Boomsma and Nachman 2002). Worker, gyne, and male mass data were analyzed using the STATISTICA 6.1 general regression models: general linear models (Statsoft 2002). The remaining traits are counts or proportions composed of count data and were analyzed using the generalized linear models module (Wilson and Hardy 2002)(Statsoft 2002). For traits involving counts (i.e. total production of workers, gynes, and workers, and the total number of individuals produced), 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 data, Wilson and Hardy (2002) and Boomsma and Nachmann (2002) recommend using a generalized linear model with binomially-distributed residuals and a logit link function (i.e. logistic regression). In particular, Boomsma and Nachmann (2002) provide a detailed protocol for analyzing ant sex ratios data with this approach. I followed this protocol, except that I used a probit link function instead of a logit link function. These different link functions are very similar and give nearly identical results. However, when many quantitative factors with normally distributed effects are assumed to underlie the trait of interest, a probit link function ("probits" are normal equivalent

deviates) is most appropriate (Searle et al. 1992; Sorenson and Gianola 2002). Sex ratios and caste ratios are likely influenced by a variety of environmental and genetic factors, together which can be modeled as having an underlying normal distribution of effects (Falconer and Mackay 1996; Linksvayer, Chapter 2), so I used a probit model.

RESULTS

I collected a total of 467 colonies; 72 with no queen, 366 with a single queen, and 29 with multiple queens. These colonies produced a total of 9,083 worker pupae, 4,799 adult gynes, and 1,477 adult males, of which 4,455 workers, 2,906 gynes, and 1,032 males were weighed. Queenless colonies were significantly smaller (mean worker number 34.49, s.d. 19.51; mean larvae number 35.89, s.d. 22.20; N=72) than single queen colonies (mean worker number 41.92, s.d. 24.26; mean larvae number 50.74, s.d. 30.47; N=366) (both worker number and larvae number $p<0.05$). Results of the mean mass of individuals produced, the numbers of individuals produced, and the resulting colony caste and sex ratios are summarized by treatment in Tables 2 and 3 for colonies with zero and one queen. Data from colonies with multiple queens were not analyzed because there were only 3-6 multiple queen colonies per treatment.

For single queen colonies, the treatments had effects on all colony traits except for the number of males produced and mean male mass (Tables 2, 3). For example, experimental colonies in treatments without a queen and full-sib workers (treatments W and WL) produced more gynes than those in treatments with a queen (treatments QWL, QW, QL, and Q). Comparing colonies in treatments with mixed larvae (QW, W, and Q)

is insightful because these colonies all had larvae from the same mixture, but differed only in queen presence and worker relatedness. All three of these treatments produced the same total number of individuals, but treatment W produced more gynes and fewer workers, further demonstrating the strong effect of queen removal. There were also differences among the treatments for sex ratio. For example, colonies in treatments QW and Q had a more male-biased sex ratio than colonies in treatments QL, WL, and W. Because there were no differences among treatments in male production, these differences in sex ratio are mainly due to differences among treatments in gyne production. The effects of the three experimental manipulations (queen removal, worker mixing, and larvae mixing) on all colony traits are examined more fully below using general regression models or generalized linear models.

Mean colony worker mass

Factors that affected mean colony worker mass included the number of larvae per worker, the total number of individuals produced, and larvae mixing (Table 4). The number of larvae per worker was negatively correlated with worker mass and the total number of individuals produced by a colony was positively correlated with worker mass. Larvae mixing was the only experimental manipulation that affected worker mass. Colonies composed of mixed, unrelated larvae produced larger workers than colonies with unmixed, full-sib larvae.

Mean colony gyne mass

Factors affecting mean colony gyne mass included the total number of sexuals produced, collection site, queen removal, worker mixing, and the interaction between queen removal and worker mixing (Table 4). The total number of sexuals produced by a colony was positively correlated with mean colony gyne mass. Colonies that had the queen removed produced larger gynes than colonies with a queen. Colonies that contained a mixture of unrelated workers also produced larger gynes than colonies that contained unmixed, full-sib workers. However, the effects of queen removal and worker mixing were interdependent. Colonies with a queen and unmixed, full-sib workers produced smaller gynes than colonies with a queen and unrelated workers or colonies without a queen and full-sib/unrelated workers (Figure 1).

Mean colony male mass

The only factor affecting mean male mass was collection site (Table 4). None of the experimental manipulations had a detectable effect on male mass.

Total worker production

The number of larvae, the number of larvae per worker, queen removal, larvae mixing, the interaction of worker mixing * queen removal, and the interaction of worker mixing * larvae mixing affected the number of workers a colony produced (Table 4). Initial larvae number and the number of larvae per worker were positively correlated with worker production. Colonies with mixed, unrelated larvae produced fewer workers than colonies with full-sib larvae. However, this effect was only detectable in colonies with full-sib workers; colonies with mixed, unrelated workers produced similar numbers of

workers regardless of whether larvae were full-sibs or unrelated (Figure 3). Overall, queen removal resulted in lower worker production. This effect occurred in colonies with full-sib workers, but did not occur in colonies with mixed, unrelated workers (Figure 1).

Total gyne production

Factors affecting total gyne production included larvae number, the number of larvae per worker, collection site, queen removal, larvae mixing, the interaction of queen removal * worker mixing, the interaction of queen removal * larvae mixing, and the interaction of worker mixing * larvae mixing (Table 4). Worker number was positively correlated with total gyne production while the number of larvae per worker was negatively correlated with total gyne production. As discussed above, queenless colonies produced more gynes than colonies with a queen. This effect depended on whether the workers were full-sibs or unrelated: The increase in gyne production in colonies with unrelated workers was insignificant, while there was a large detectable increase in the production of gynes in colonies with full-sib workers (Figure 1). There was also a marginally significant effect of queen removal * larvae mixing, such that the effect of queen removal was greater in colonies with mixed larvae. Overall there was a negative main effect of larvae mixing such that colonies with full-sib larvae produced more gynes than colonies with unrelated larvae. This effect also depended on worker mixing, such that colonies with full-sib workers and full-sib larvae produced the most gynes and colonies with unrelated workers and larvae produced the fewest (Figure 3).

Total male production

Total male production was affected by the number of workers, the number of larvae, the number of larvae per worker, the collection site, and queen removal (Table 4). The initial number of larvae was positively correlated with male production. Both worker number and the number of larvae per worker had a marginally significant negative effect on male production. Queen removal had a small positive effect on male production, such that queenless colonies produced more males than colonies with a queen.

Total colony productivity

The total number of individuals produced by a colony was affected by larvae number, collection site, larvae mixing, and the interaction between worker mixing and larvae mixing (Table 4). As expected, the number of initial larvae in a colony was positively correlated with the total number of individuals produced. Colonies with mixed, unrelated larvae produced fewer total individuals than colonies with full-sib larvae. However, this effect depended on worker mixing. Colonies composed of full-sib workers produced fewer total individuals when the larvae were unrelated than when the larvae were full-sib. There was no significant effect of larvae mixing when workers were also mixed (Figure 3).

Caste ratio

Caste ratio, the proportion of total females that were gynes, was affected by collection site, the number of larvae, the number of larvae per worker, queen removal, the interaction of queen removal * worker mixing, and the interaction of queen removal * larvae mixing (Table 4). The number of larvae was negatively correlated with caste ratio

and the number of larvae per worker was positively correlated with caste ratio. There was a main effect of queen removal such that colonies without a queen had more gyne-biased caste ratios. However, this effect depended on both worker and larvae mixing. Colonies with full-sib workers responded to queen removal by producing more gynes and fewer workers, and hence gyne-biased caste ratios, but colonies with mixed, unrelated workers produced the same caste ratio whether or not the queen was removed (Figure 1). Furthermore, colonies with both full-sib larvae and unrelated larvae responded to queen removal by producing a more gyne-biased caste ratio, although colonies with mixed, unrelated larvae produced an even more gyne-biased caste ratio by producing fewer workers (Figure 2).

Sex ratio

Sex ratio, the proportion of colony sexual production that was males, was affected by the total number of individuals produced, the total number of sexuals produced, queen removal, larvae mixing, the interaction between queen removal * worker mixing, and the interaction between queen removal * larvae mixing (Table 4). The total number of individuals produced was positively correlated with sex ratio and the total number of sexuals produced with negatively correlated with sex ratio. Colonies that had the queen removed had female-biased sex ratios compared to colonies with a queen. Colonies with unrelated larvae produced a male-biased sex ratio relative to colonies with full-sib larvae. However, there was an interaction with queen removal and larvae mixing such that only colonies with a queen and unrelated larvae produced fewer gynes, and hence a male-biased sex ratio relative to other colony types (Figure 2). There was also an interaction

between queen removal and worker mixing such that there was an effect of queen removal in colonies with full-sib workers, but not in colonies with unrelated workers. Specifically, colonies with full-sib workers and a queen produced fewer gynes, and hence a more male-biased sex ratio than colonies with full-sib workers and no queen (Figure 1).

Initial variation in queen number

While comparison among the experimental colonies in treatments with and without queen removal demonstrates that colonies rapidly respond to queen removal, it is also possible to exploit natural variation in original colony queen number to study the longer term effects of queen absence. Specifically, I compared experimental colonies in queen removal treatments (i.e. treatments WL, L, and W) that were derived from colonies 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, 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 ($p<0.0001$). In many species, workers in queenless colonies lay haploid eggs (Bourke and Franks 1995). Thus, the extra males in experimental colonies containing workers derived from queenless colonies were likely produced by workers. 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 ($p<0.0001$). Thus, longer-term

queen absence had similar, but more extreme effects on caste ratio compared to the queen removal effects demonstrated in experimental colonies derived from single queen colonies.

DISCUSSION

This study demonstrates that changes in the colony social structure of *Temnothorax curvispinosus* have complex effects on how colonies invest resources in rearing overwintered female and male larvae. Each of the three experimental manipulations, queen removal, worker mixing, and larvae mixing, had widespread effects on patterns of colony resource allocation, and in many cases, these effects were not independent. Patterns of colony allocation are usually considered to be driven by queen-worker conflict over investment in males versus females and growth versus reproduction (Queller and Strassmann 1998; Chapuisat and Keller 1999; Mehdiabadi et al. 2003). In the sections below, I discuss the results in relation to the predictions of queen-worker conflict theory. I suggest that the results are more consistent with patterns of colony allocation being driven not by queen-worker-larva conflict, but by queen-worker-larva coadaptation.

Effects of queen removal

Queen removal had widespread effects on colony traits including gyne mass, the number of workers, gynes, and males and hence the caste ratio and sex ratio. In general, queenless colonies produced more and larger gynes and fewer workers than colonies with

a queen. For several traits, the effect of queen removal depended on worker and larvae mixing. For example, queen removal only had a significant effect on gyne production, and hence sex ratio and caste ratio, in colonies with full-sib workers. Queen presence is known to affect colony traits in a wide variety of ant species (Bourke and Franks 1995). Typically, colonies or colony fragments with queens produce mainly workers and males and colonies without queens rear more gyne-biased caste and sex ratios.

The effect of queen presence is often interpreted as evidence for queen control of worker behavior and larval development. Alternatively, queens may be viewed as signaling their presence 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). Recent experimental studies explicitly designed to distinguish between queen signaling and queen suppression are consistent with a queen signaling interpretation (Dietemann et al. 2005; Iwanishi and Ohkawara 2005). Queenless colonies may be expected to allocate resources differently than colonies with a queen because the production of diploid offspring ceases after the queen dies; in most ant species, workers can only produce haploid, male-destined eggs (Bourke and Franks 1995). After queen loss, colonies may enter into an "orphanage period" during which the remaining workers rear as many sexuals as possible, first by rearing queen-derived diploid eggs as gynes and subsequently by rearing worker-derived haploid eggs as males, for as long as the workers live (Franks et al. 1990; Bourke and Franks 1995). Consistent with these predictions, colonies without a queen reared more gynes and males, and fewer workers. Thus, queen removal was associated with a shift from investment in maintenance and growth to reproduction.

Comparison of experimental colonies derived from queenless colonies with experimental colonies with a queen further suggests that queen loss causes a shift in colony allocation from growth to reproduction. Colonies with workers derived from queenless colonies produced a similar, but more extreme gyne-biased caste ratio than colonies with workers derived from colonies with a queen. Furthermore, experimental colonies with unmixed larvae derived from queenless colonies produced more males relative to experimental colonies with unmixed larvae derived from colonies without a queen. These males were presumably derived from worker-laid eggs. Thus, naturally occurring orphan colonies invested resources mainly in the production of sexual females and males just as experimentally orphaned colonies.

Effects of mixing larvae and workers

The effects of mixing workers and larvae were not as widespread as those of removing the queen, although for many traits, the effect of queen removal depended on the effects of worker or larvae mixing. Generally, larva and worker mixing resulted in decreased colony productivity. Colonies with unrelated larvae produced larger but fewer workers, fewer gynes, fewer total individuals, and a more male-biased sex ratio (as a result of producing fewer gynes) than did colonies with full-sib larvae. In addition, there was a larger negative effect of larvae mixing on the number of workers, the number of gynes, and the total number of individuals produced in colonies composed of full-sib workers relative to colonies composed of unrelated workers. Besides these effects on productivity, worker mixing also had an effect on gyne mass. Colonies with a queen and full-sib workers produced smaller gynes relative to colonies with a queen and unrelated

workers or colonies without a queen. This difference in gyne mass may be another aspect of decreased investment in gynes (and increased investment in colony growth and maintenance through worker production) associated with queen presence.

Mixing workers or larvae replaces groups of full-sib workers or larvae with groups of unrelated workers or larvae. If related nestmates interact differently than unrelated individuals, for example due to shared environmental or genetic effects, then mixing nestmates may disrupt interactions among workers and larvae, resulting in the production of fewer individuals. According to split sex ratio theory, if workers have the capability to assess the relatedness among nestmates in their own colony relative to the population average, workers in colonies with higher than average relatedness should bias investment towards females, and workers in colonies with lower than average relatedness should bias investment towards males (Boomsma and Grafen 1990; Boomsma and Grafen 1991). Workers could respond to cues of within-colony genetic diversity produced by both nestmate workers and larvae (Ratnieks 1990; Evans 1995). However, larval cues would be more indicative of current changes in the status of reproductive individuals, while worker cues are indicative of past patterns of reproduction. Interestingly, larvae mixing had more widespread effects than worker mixing, although there is little indication that workers responded to larval cues by manipulating sexual allocation ratios (cf. Evans 1995).

Queen-worker conflict

Two main mechanisms have been proposed by which workers could manipulate the initial brood sex ratio produced by the queen (Queller and Strassmann 1998). First,

workers could directly manipulate the number of males a colony produces by killing male brood (e.g., Sundstrom et al. 1996). Second, workers could manipulate the sex ratio a colony produces by rearing more diploid larvae as gynes and fewer diploid larvae as workers, i.e. by manipulating the caste ratio (Hammond et al. 2002). In addition, in species with polydomy (i.e. multiple nest sites), workers may be better able to manipulate colony sex ratios (e.g., with male killing or increased gyne production) if they physically escape the influence of the queen by moving to a separate nest site (Snyder and Herbers 1991).

I found no evidence that workers manipulated colony sex ratios by killing males. There were no differences among treatments in the numbers of males produced. According to split sex ratio theory, both worker and larvae mixing should result in male-biased sex ratios (Boomsma and Grafen 1990). Worker mixing had no effect on sex ratio. Larvae mixing did result in more male-biased sex ratios, because colonies with mixed, unrelated larvae produced fewer workers and gynes, but the same number of males (see Hammond et al. 2002). Queen removal had an effect on male production such that queenless experimental colonies produced significantly more males. This effect was not large, but is contrary to the predictions of queen-worker conflict theory. Workers in queenless colonies are predicted to bias sex allocation ratios towards gynes. This effect is however consistent with queenless colonies being in an orphanage state and rearing as many sexuals as possible (Franks et al. 1990).

I also found little indication that workers manipulated sex ratios by conditionally manipulating caste ratios depending upon nestmate relatedness (cf. Hammond et al. 2002). Neither worker mixing or larvae mixing had an effect on caste ratio. The particular

caste ratio a colony produces may have more to do with investment in reproduction versus growth and maintenance than investment in males versus females.

Finally, I found no support for the hypothesis that workers manipulate colony resource allocation more when the queen is absent. There were interactions between queen removal and worker mixing / larvae mixing for caste and sex ratio but the patterns did not fit predictions of queen-worker conflict theory (Figures 1 and 2). Colonies without a queen did not produce more males or fewer gynes when relatedness among larvae or workers decreased.

Many studies of colony resource allocation have used ant species with seasonal polydomy, in which colonies fractionate in the summer and coalesce in the winter (e.g., Herbers 1984, 1990; Backus 1995; Banschbach and Herbers 1996; Bourke and Chan 1999). In these studies, more gyne-biased colony allocation in queenless nests is taken as evidence of queen-worker conflict, because colony fractionation is viewed as a strategy by which workers physically escape the controlling influence of queens (e.g., Snyder and Herbers 1991). As described above, the results of the current study are most consistent with the interpretation that increased gyne production with queen absence is not due to worker strategies associated with queen-worker conflict, but rather due to fundamental shifts in colony resource allocation from growth and maintenance to reproduction associated with queen loss (Franks et al. 1990; Bourke and Franks 1995).

Further, in many studies, the relative numbers of workers and queens in a colony are envisioned as being indicative of the relative power of these two parties over colony decisions (e.g., Herbers 1984, 1990; Backus 1995; Banschbach and Herbers 1996; Bourke and Chan 1999). Correlations of worker number and gyne-biased sex ratios and

queen number and male-biased sex ratios are taken as support for queen-worker conflict over colony sex ratio. However, just as colony life history strategies, shaped by among-colony selection, may change dramatically as colony queen number changes from one to zero (Franks et al. 1990), changes in worker number associated with colony ontogeny (Oster and Wilson 1976; Bourke and Franks 1995; Bourke 1999), or queen number associated with various ecological, demographic, or colony ontogenetic factors (Hölldobler and Wilson 1990; Herbers 1993; Bourke and Franks 1995), may also dramatically change colony life history strategies. Thus, correlations of queen number and worker number with sex ratios do not necessarily indicate queen-worker conflict.

The coadaptation of queens, workers, and larvae

The interdependence of effects of queen removal, worker mixing, and larvae mixing, as evidenced by the number of significant interaction effects, suggests the influence of relatedness between nestmates on colony responses to changes in social structure. If the production of more gynes and fewer workers by a colony in response to queen loss is favored by among-colony selection, as described above, and only experimental colonies with full-sib workers responded to queen removal, then mixing workers may prevent an adaptive colony response. Mixing workers or larvae may disrupt colony processes in a variety of ways. For example, related social partners share genes and these related sets of genes may more favorably interact. Selection among colonies should disfavor genes expressed in different social partners (i.e. queens, workers, and larvae) that do not interact favorably (cf. Wolf and Brodie 1998). While conflicts among individuals within colonies may have an influence on the evolution of social insect

colonies, the coadaptation of traits expressed in queens, workers, and larvae may well be more important (Korb and Heinze 2004). This is likely particularly true in morphologically eusocial taxa and less so in behaviorally eusocial taxa (sensu Kukuk 1994), where aggressive interactions among nestmates over reproduction are much more apparent (e.g., Michener and Brothers 1974; Platt et al. 2004).

Future work

Further models incorporating the complexity of life history strategies associated with colony growth are needed to explore the potential for queen-worker conflict (Herbers et al. 2001). Models of queen-worker conflict should also consider potential costs to total colony productivity as well as informational, power, and genetic constraints on the evolution of manipulative behaviors. Experimental studies further examining the effects of changes in the relatedness structure of workers and larvae would help to evaluate the predictions of queen-worker conflict theory. Methods such as artificial insemination of queens or cross-fostering individuals between colonies derived from full-sib queens could more finely control relatedness between nestmates in experimental colonies. The current study demonstrates that caste ratio and sex ratio can be influenced by changes in worker number, gyne number, and male number. That is, colonies can arrive at the same caste and sex ratios in a variety of different ways. For example, decreases in sex ratio do not imply changes in the number of males (Hammond et al. 2002). Careful experimental manipulations should enable the dissection of all of the factors involved in colony allocation decisions. These experiments will inform observational and correlative studies of unmanipulated, field-collected colonies.

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TABLE 1. Experimental manipulations used for each of the seven treatments. "X" indicates that the manipulation was used. Colonies in treatment QWL were unmanipulated.

Treatment name	Manipulation		
	Queen removal	*Worker mixing*	*Larvae mixing*
L	X	X	-
Q	-	X	X
W	X	-	X
WL	X	-	-
QL	-	X	-
QW	-	-	X
QWL	-	-	-

TABLE 2. Effects of the treatments and initial queen number on the number of workers, gynes, males, and total individuals produced as well as caste and sex ratio. The parameter estimates and 95% confidence intervals were computed with bootstrapping. N is the number of colonies for each combination of treatment and initial queen number.

Treatment	*Queen number*	*Worker total*	*95% CI*	*Gyne total*	*95% CI*	*Male total*	*95% CI*	*Total*	*95% CI*	*Sex ratio*	*95% CI*	*Caste ratio*	*95% CI*	*N*
QWL	1	36.95	(30.53-43.61)	4.98	(2.33-8.14)	1.92	(0.04-5.04)	44.02	(37.18-51.08)	0.238	(0.095-0.429)	0.105	(0.053-0.169)	49
QW	1	23.56	(18.60-28.86)	2.12	(1.18-3.30)	1.86	(1.20-2.64)	27.58	(22.04-33.54)	0.538	(0.407-0.668)	0.076	(0.044-0.123)	50
QL	1	25.18	(20.60-30.16)	7.09	(4.35-10.31)	1.47	(0.24-3.18)	33.78	(28.73-39.11)	0.103	(0.027-0.208)	0.203	(0.131-0.283)	55
Q	1	19.96	(15.63-24.71)	5.35	(3.23-7.96)	2.25	(1.48-3.10)	27.58	(22.83-32.85)	0.406	(0.297-0.523)	0.230	(0.147-0.321)	48
WL	0	6.95	(3.82-11.34)	16.00	(11.47-21.21)	7.58	(3.45-12.89)	30.61	(23.03-40.47)	0.251	(0.139-0.379)	0.735	(0.626-0.831)	38
	1	15.68	(12.32-19.34)	19.66	(15.19-24.49)	1.81	(0.76-3.41)	37.24	(31.29-43.88)	0.074	(0.033-0.132)	0.507	(0.423-0.588)	59
L	0	11.33	(5.08-18.08)	2.92	(1.17-4.92)	10.92	(3.25-20.50)	25.33	(18.50-32.58)	0.535	(0.278-0.792)	0.315	(0.102-0.590)	12
	1	23.66	(18.89-28.82)	11.27	(7.95-15.09)	4.25	(1.57-8.71)	39.39	(32.04-47.86)	0.204	(0.114-0.307)	0.320	(0.243-0.403)	56
W	0	5.09	(2.45-9.23)	14.36	(10.05-19.27)	1.77	(1.00-2.64)	21.36	(14.86-29.09)	0.098	(0.062-0.136)	0.759	(0.645-0.853)	22
	1	8.71	(6.33-11.53)	14.96	(11.39-18.78)	2.43	(1.69-3.24)	26.20	(21.67-30.94)	0.199	(0.132-0.279)	0.598	(0.506-0.686)	49

TABLE 3. Effects of the treatments and initial queen number on mean mass of workers, gynes, and males. The parameter estimates and 95% confidence intervals were computed with bootstrapping. N is the number of colonies for each combination of treatment and initial queen number.

Treatment	*Queen number*	*Worker mass*	*95% CI*	*Gyne mass*	*95% CI*	*Male mass*	*95% CI*	*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	0	0.607	(0.571-0.643)	1.134	(1.073-1.190)	0.481	(0.427-0.537)	38
	1	0.615	(0.592-0.638)	1.094	(1.042-1.141)	0.462	(0.421-0.505)	59
L	0	0.573	(0.525-0.640)	1.157	(1.077-1.240)	0.425	(0.366-0.485)	12
	1	0.584	(0.564-0.604)	1.079	(1.023-1.131)	0.431	(0.393-0.470)	56
W	0	0.646	(0.608-0.692)	1.137	(1.080-1.183)	0.451	(0.397-0.531)	22
	1	0.633	(0.614-0.654)	1.095	(1.046-1.141)	0.453	(0.422-0.485)	49

TABLE 4. Results of general regression model / generalized linear model showing significant main and interaction effects of experimental manipulations, as well as significant categorical and continuous covariates for all colony study traits. For main effects and continuous covariates, the direction of the effect on the trait is indicated by a plus or minus sign. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. NA indicates that the covariate was not considered for that trait.

	Worker mass	*Gyne mass*	*Male mass*	*Worker total*	*Gyne total*	*Male total*	*Total*	*Caste ratio*	*Sex ratio*
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	350	226	135	357	353	352	354	348	256

Figure captions:

FIGURE 1. Interaction between queen removal and worker mixing on total worker production, total gyne production, gyne mass, sex ratio, and caste ratio. The symbols represent mean values and the error bars 95% confidence intervals. Queen removal is indicated on the x axis. The mean of colonies with unmixed workers is indicated with a black box and black error bars and the mean of colonies with mixed workers is indicated by a grey circle and grey error bars.

FIGURE 2. Interaction between queen removal and larvae mixing on sex ratio and caste ratio. The symbols represent mean values and the error bars 95% confidence intervals. Queen removal is indicated on the x axis. The mean of colonies with unmixed larvae is indicated with a black box and black error bars and the mean of colonies with mixed larvae is indicated by a grey circle and grey error bars.

FIGURE 3. Interaction between larvae mixing and worker mixing on total worker production, total gyne production, and total production. The symbols represent mean values and the error bars 95% confidence intervals. Larvae mixing is indicated on the x axis. The mean of colonies with unmixed workers is indicated with a black box and black error bars and the mean of colonies with mixed mixed is indicated by a grey circle and grey error bars.

Figure 1

Interaction between queen removal and worker mixing

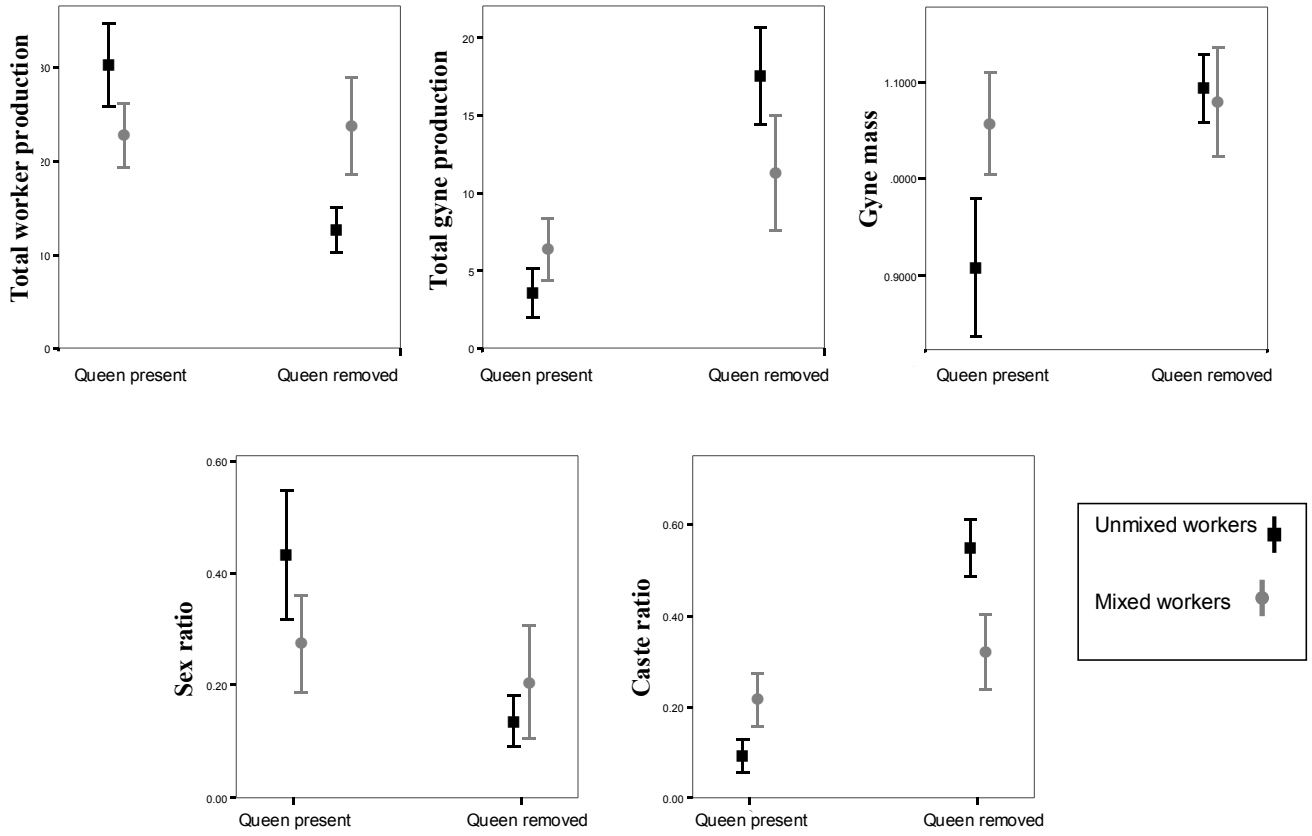


Figure 2

Interaction between queen removal and larvae mixing

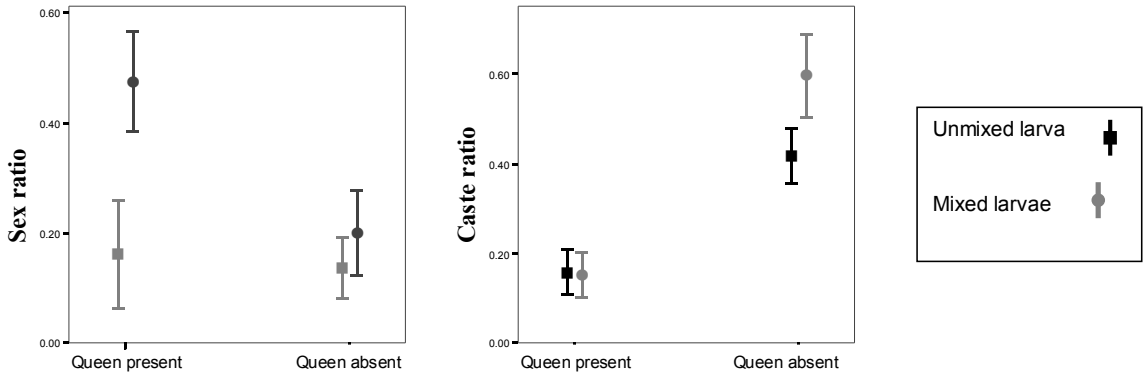
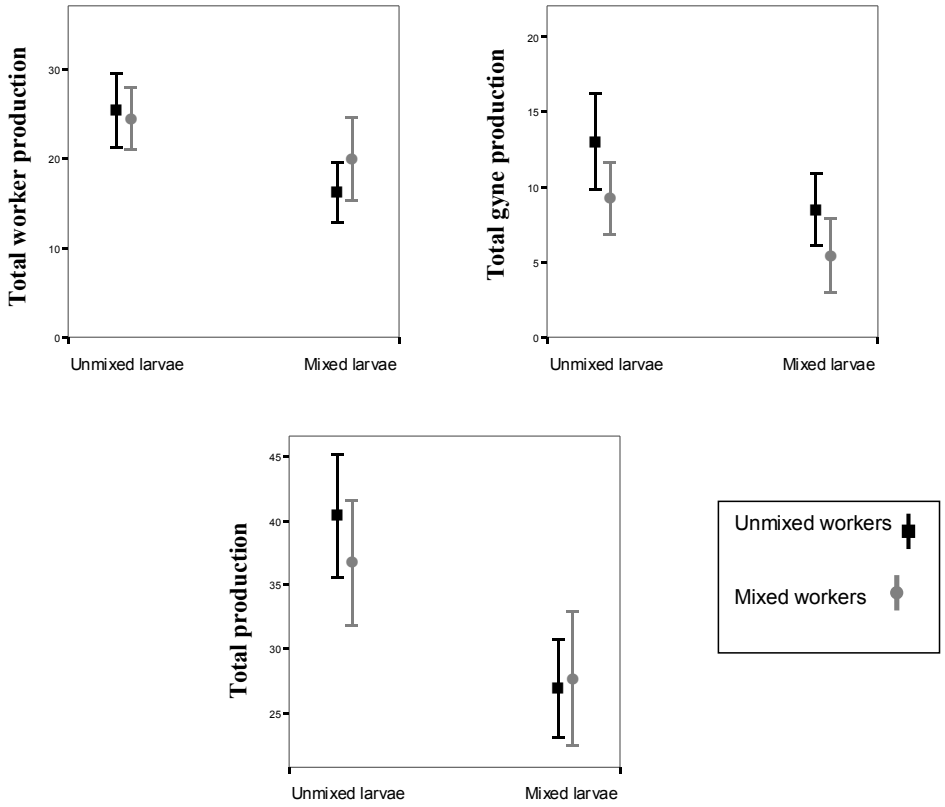


Figure 3

Interaction between larvae mixing and worker mixing



CHAPTER 4

THE DIRECT AND INDIRECT GENETIC BASIS OF PHENOTYPIC DIFFERENCES AMONG ANT SPECIES

Timothy A. Linksvayer

ABSTRACT

When social interactions occur, the phenotype of an individual can be influenced both by its own genotype and the genotypes of its social partners. Eusocial insect colonies are notable for extensive behavioral interactions among larval, worker, and queen nestmates. In particular, developing larvae are reliant on care provided by workers. Social insect phenotypes are thus strongly affected by zygotic genes expressed during development and also by genes expressed in care-giving adult worker nestmates. These two classes of genes coevolve and influence the evolutionary dynamics of traits within populations as well as the evolutionary divergence among populations. I studied the co-evolutionary divergence of direct and indirect genetic effects across taxa by cross-fostering larvae among three closely related ant species. The results suggest that phenotypic differences among species are due to fixed genetic differences in both the genomes of larvae and those of care-giving workers. This study demonstrates the importance of the interaction and coevolution of worker and larvae genomes.

Understanding the genetic basis of phenotypic differences between populations and species is a fundamental goal of evolutionary biology, linking microevolutionary processes within populations to macroevolutionary patterns across species. Phenotypic differences between individuals within populations are usually assumed to be caused by the expression of genes within those individuals ("direct effect genes") and interactions with the environment during development. In social insects, the environment of developing brood is determined by the social milieu within the colony. As a result, another class of genes expressed in social partners ("indirect effect genes") is expected to influence phenotypic differences within- and among-colonies, as well as phenotypic differences among populations and species (Wolf et al. 1998; Linksvayer and Wade 2005).

Maternal genetic effects are the best studied examples of indirect genetic effects, and they have been shown to contribute to variation for offspring phenotypes within natural populations (Hunt and Simmons 2000; Agrawal et al. 2001; Rauter and Moore 2002) and also between selected lines of mice (White et al. 1968; Wolf et al. 2002). Theory predicts that maternal effect genes and coevolved direct-maternal gene complexes may also be important in the phenotypic divergence of natural populations and species (Wade 1998; Wolf and Brodie 1998; Wolf 2000a; Wolf 2000b). Furthermore, theory predicts higher equilibrium gene diversities within populations and rapid evolution among populations for maternal effect genes owing to relaxed selection (Whitlock and Wade 1995; Wade 1998). Both of these predictions are supported by comparative molecular genetic studies across arthropod taxa (Demuth and Wade 2005, in press;

Barker et al. 2005, in press). Other types of indirect genetic effects besides maternal genetic effects may similarly contribute to among-population differences (Lynch 1987).

In social insect colonies, offspring phenotypes are not only influenced by direct effect genes expressed during development and maternal effect genes expressed by queens, but also by genes expressed in care-giving adult sibling workers (i.e. "sibsocial effect genes") (Linksvayer and Wade 2005). In a previous study, I found evidence for direct, maternal, and sibsocial genetic variance affecting individual phenotypes such as body mass in populations of the ant, *Temnothorax curvispinosus* (Linksvayer, unpublished ms). These same phenotypes differ among species in this genus (Herbers and Stuart 1996), and the goal of the present study was to investigate the degree to which phenotypic differences among species in the genus *Temnothorax* are the result of divergence for direct effect and sibsocial effect genes. In addition, I investigated whether the pattern of divergence observed among species provides evidence for the co-evolution of direct and sibsocial effects.

For direct effect genes, crosses between lineages or species are often used to elucidate the genetic basis of phenotypic differences between lineages or species (Wade et al. 1997; Lynch and Walsh 1998; Lexer et al. 2003). Detecting indirect genetic effects, however, requires cross-fostering, for example of offspring from their genetic parents to foster parents (Cheverud 1984; Cheverud and Moore 1994; Wolf et al. 2002). Cross-fostering between populations and species should elucidate the relative importance of fixed genetic differences for direct and indirect effect genes, and the importance of coevolved direct-indirect effect gene complexes. I used interspecific cross-fostering of

larvae among three species in the genus *Temnothorax* to study the relative contributions of direct and sibsocial effect genes to phenotypic differences among ant species.

METHODS

Study species and collection sites

I used three closely related ant species in the genus *Temnothorax* that are widespread across the eastern USA (Mackay 2000). All three species have small colonies, typically with less than 100 workers (Mackay 2000). Colonies were collected in August 2004 from three different sites: *T. curvispinosus* from Griffy Nature Preserve, Bloomington, IN, *T. longispinosus* from Rondeau Provincial Park, Ontario and Allegany State Park, NY. Additionally, *T. ambiguus* colonies were collected on 16 April 2005 from Cowling Arboretum, Carleton College, Northfield, MN. Although I used *T. longispinosus* colonies from two different populations, I was interested only in differences among species. Therefore, in the analyses presented below, I did not consider population of origin, only species.

Colony maintenance

Colonies were kept in incubators simulating seasonal conditions (Buschinger 1973). Colonies collected in August 2004 were overwintered from October 2004 to February 2005 using "winter" conditions (10:14 hrs. light: dark and 12:8 C day: night). Diel cycles were then slowly changed to simulate "spring" conditions. From 21 May 2005 to the end of the experiment, "summer" conditions were used (14:10 hrs. light: dark

and 22:16 C day: night). Water and freshly frozen adult fruit flies were provided ad libitum and refreshed weekly, and 1.5 ml of 10% sucrose solution was provided in May.

Experimental design

During the last two weeks of April 2005, I created experimental colonies using workers and larvae from the overwintered colonies. I used a full factorial design, in which larvae from each species were combined with conspecific workers or workers of each of the other species. This design permits estimation of the separate contributions of worker species, larvae species, and worker-larvae interaction to total phenotypic variation each of the phenotypic measures, namely, mass of adult workers, gynes (reproductive females), and males. In these three *Temnothorax* species, brood overwinter as small larvae and mature to adults in the spring. Thus, the phenotypes of interest were measured on adults derived from overwintered larvae. Most larval growth occurs during the spring, and overwintered female larvae can develop into either workers or gynes depending on the social and nutritive environments (Wesson 1940; Linksvayer, unpublished results). Thus, most larval growth occurred over the course of the experiment.

The procedure for creating experimental colonies from overwintered colonies involved the following: Queens were removed, frozen, and weighed. Workers and larvae from all colonies of a single species were removed and placed into separate Petri plates, one containing workers and one containing larvae. This process was repeated for each species. I mixed workers and larvae from all colonies of a single species to eliminate potential variation among colonies within species due to genetic and environmental factors (Linksvayer, unpublished ms). The effects measured are due to variation among

species. Each experimental colony consisted of 15 workers of species X and 25 workers of larvae Y from the respective pools of workers and larvae. As experimental colonies were created, they were grouped into experimental blocks composed of the available worker species and larvae species combinations. …Thus, full blocks consisted of the nine (3 x 3) worker species and larvae species combinations. There were 16-29 replicates of each worker-larvae species combination, so that there were 16 complete blocks with all 9 worker-larvae combinations and an additional 13 incomplete blocks.

Colonies were checked biweekly for new pupae and adults. Worker pupae and adult males were identified by morphology, removed as they appeared, and were frozen. Adult gynes were allowed one to two weeks to gain fat reserves before being collected and frozen, because in at least some ant species, gynes gain a majority of their fat reserves in the first few weeks as adults (Tschinkel 1993). All individuals were weighed to the nearest 0.001 mg. Wet mass was used as a measurement of body size. Wet and dry mass are strongly correlated in *T. curvispinosus* workers, gynes, and males (Linksvayer, unpublished data).

Statistical analysis

Phenotypic data were analyzed with the following linear model for each trait z:

$z = \mu + block + worker\ species + larvae\ species + worker\ species * larvae\ species + residual.$

Block was included as a random factor and the remaining factors were fixed (White et al. 1968; Atchley et al. 1991). The effects of biological importance are *worker species*, *larvae species*, and *worker species * larvae species*. Variation due to these effects is

caused by genetic and environmental variation among species. As described above, I did not include population in the model for *T. longispinosus* because I was interested only in between-species differences. Variation among blocks then includes between-population effects for *T. longispinosus* as well as random effects and effects due to incomplete blocks (White et al. 1968; Atchley et al. 1991). The error term includes genetic and environmental variation within species (i.e. among- and within-colonies). I also considered variables measuring total colony productivity (i.e. total number of workers, gynes, or males produced, as well as total females, and total number of individuals) as covariates for mean colony worker, gyne, and male mass, but none of these productivity variables had a significant effect and were not included in the analyses reported below.

RESULTS

Male, worker, and gyne production of experimental colonies

A total of 1,624 workers, 238 gynes, and 226 males were produced by the experimental colonies. Experimental colonies consisting of *T. ambiguus* larvae and *T. longispinosus* workers produced more workers than all other worker-larvae species combinations (Tukey HSD test, df=147, all $p<0.005$). However, the number of gynes reared did not differ among experimental colonies due to larvae species, worker species, or worker-species (all $p>0.05$). Experimental colonies with *T. longispinosus* larvae from New York produced more males and fewer females than colonies with *T. longispinosus* larvae from Ontario or larvae of the other species (Tukey HSD test, df=180, all $p<0.0001$). Presumably *T. longispinosus* colonies from the New York population

contained a higher proportion of haploid larvae. There were other small differences between *T. longispinosus* worker and larval effects from the New York and Ontario populations, but because I was interested in between-species effects, for the remainder of the analyses I only considered differences between species.

Direct and sibsocial effects on worker, gyne, and male mass

The species origin of larvae had a highly significant effect on worker, gyne, and male mass (direct effects: worker mass $F_{2,1586}$=101.17, p<0.000001; gyne mass $F_{2,188}$=15.47, p=0.000001; male mass $F_{2,189}$=29.71, p<0.000001). The mass of workers and males, but not gynes, was affected by the species identity of the rearing workers (sibsocial effects: worker mass $F_{2,1586}$=8.37, p=0.00024; male mass $F_{2,189}$=3.31, p=0.039). The mass of both workers and gynes also exhibited significant interactions between direct and sibsocial effects (interactions: worker mass $F_{4,1586}$=3.32, p=0.010; gyne mass $F_{4,188}$=2.55, p=0.041). These results demonstrate that phenotypic variations in size among the three *Temnothorax* species are caused by differences inherent in the larvae but also by differences in the rearing workers and in the larval-by-worker interactions.

Some heterospecific worker-larvae combinations produced worker phenotypes more extreme than the corresponding conspecific worker-larvae combinations. For example, *T. ambiguus* larvae reared by *T. longispinosus* workers developed into smaller workers than they did when reared by conspecific workers (Tukey HSD test, df=1586, p=0.0163). However, *T. curvispinosus* larvae reared by *T. ambiguus* workers were larger than those reared by conspecific workers (Tukey HSD test, df=1586, p=0.0380). These results are consistent with the hypothesis, described below and in the discussion, that

conspecific worker-larvae combinations produce intermediate worker phenotypes but heterospecific worker-larvae combinations produce more extreme phenotypes.

One way that conspecific worker-larvae combinations could lead to intermediate phenotypes is if larvae and worker effects on worker mass across species were negatively correlated. To estimate the correlation between larvae and worker effects, for each species within each block, I calculated the marginal mean larvae and worker effect on worker mass. Both larvae and worker marginal mean effect estimates were possible for all three species in the first 16 blocks. I then calculated Pearson correlation coefficients across the three species for these 16 blocks. There was a negative correlation between the three species' marginal mean larvae and worker effects on worker mass (chi square=4, df=1, p=0.046; median correlation coefficient with 95% bootstrap confidence intervals, -0.333, -0.621 to -0.022). *T. longispinosus* had the largest marginal mean larvae effect on worker mass (Tukey HSD test, df=1585, all $p<0.0001$) and the lowest marginal mean worker effect (Tukey HSD test, df=1585, all $p<0.0001$). Experimental colonies with *T. longispinosus* workers also produced the largest number of workers (Tukey HSD test, df=147, all $p<0.01$), suggesting that the negative correlation between worker and larvae effects on worker mass could be due to a trade-off between worker effects on worker mass and the total number of workers produced. However, the correlation across the three species for worker effect on worker mass and worker effect on total worker production was not different than zero (chi square=0.067, df=1, p=0.796).

DISCUSSION

The results of this study demonstrate that phenotypic differences in mass between *Temnothorax* ant species are determined by inherent differences within larvae of different species but also by differences in the care provided by workers of different species. These between-species differences in the effects of larvae (i.e. direct effects) and the effects of care-giving workers (i.e. sibsocial effects) are likely mainly due to genetic differences between species. These results suggest that both direct and sibsocial effect genes contribute to the fixed genetic differences underlying phenotypic differences in mass among acorn ant species.

In a separate study, I found that direct and sibsocial genetic variance contribute to within-population phenotypic variance for worker, gyne, and male mass in *T. curvispinosus* (Linksvayer, unpublished ms). That study demonstrated the potential of both direct and sibsocial effects on mass to respond to selection. The results of the current study suggest that phenotypic differences in mass between *Temnothorax* species are due to fixed genetic differences for both direct and sibsocial effects. Together, the results of these studies demonstrate that both direct and indirect genetic effects affect evolutionary processes within- and between-populations. Further study is necessary to determine the relative strength of selection on direct and sibsocial effects within populations as well as to determine whether selection on direct and sibsocial effects contributed to the observed between-species phenotypic differences.

Another main result of the current study is that interactions between worker species and larvae species contributed to total phenotypic variance for worker and gyne mass (Table 1). This demonstrates that interactions between direct and sibsocial effect genes contribute to phenotypic differences between species. Physiological interactions

between genes (i.e. within-genome epistasis) play an important role in theories of population divergence and speciation, because crosses between populations or species tend to break up coevolved gene complexes (Dobzhansky 1937; Muller 1942; Demuth and Wade 2005). Genes expressed in different genomes can also interact if the individuals are social partners (i.e. between-genome epistasis) (Wade 1998; Wolf 2000a; Wolf 2000b). In this case, the genes interact by influencing behavioral interactions between social partners. Indirect genetic effects theory suggests that coevolved direct-indirect gene complexes may also play an important role in processes of population divergence and speciation (Wade 1998; Wolf 2000a; Wolf 2000b). Crosses between populations or species may not only break up coevolved gene complexes within individuals, but also coevolved complexes of genes located in the genomes of different social partners. The results of the current study support these ideas and demonstrate the importance of interaction between direct effect genes expressed in developing larvae and sibsocial effect genes expressed in care-giving workers to worker and gyne body size.

In many organisms, body size is thought to experience stabilizing selection (Roff 1997). This is often attributed to a trade-off between offspring size and number, such that intermediate body size phenotypes are optimal (Roff 1997). Selection for intermediate phenotypes may lead to a negative genetic correlation between direct and indirect effects within populations due to linkage disequilibrium and pleiotropy (Wolf and Brodie 1998). Populations with relatively high direct genetic effects may have correspondingly lower indirect genetic effects, and vice versa, so that all populations have similar optimal intermediate phenotypes (Wolf and Brodie 1998).

Consistent with these ideas, I found a negative correlation between the direct and sibsocial effects of the three study species on worker mass. Furthermore, in some cases, heterospecific worker-larvae combinations produced workers with more extreme phenotypes than conspecific worker-larvae combinations. These heterospecific combinations may have matching high or low direct genetic and sibsocial genetic effects, resulting in direct-sibsocial gene combinations that produce phenotypes relatively larger or smaller than either conspecific worker-larvae combination. Hybridization between populations and species often leads to later generation genotypes with more extreme phenotypes than found in either parent population (Rieseberg et al. 1999). These "transgressive segregants" are thought to possess matching high or low alleles at several loci, resulting in extreme phenotypes. In contrast, genotypes of each parental population are thought to be shaped by stabilizing selection, such that each parent population possesses alternative coevolved complexes of high and low alleles, resulting in intermediate phenotypes (Rieseberg et al. 1999; Rieseberg et al. 2003). Analagously, the heterospecific worker-larvae combinations that produced relatively high or low worker phenotypes in the current study may result from the breakup of coevolved direct-sibsocial gene complexes. Stabilizing selection on worker body size in the three *Temnothorax* species may cause direct and sibsocial effects to be negatively correlated so that conspecific worker-larvae combinations produce intermediate phenotypes (Wolf and Brodie 1998). However, workers of the three species are not the same size, so that other evolutionary forces besides stabilizing selection, such as directional selection or drift, also likely played a role in the evolutionary divergence of these phenotypes.

Colony caste ratio, the proportion of female larvae that develop into workers versus gynes, is a colony trait that may be especially subject to the coevolution of direct and sibsocial genetic effects (Bourke and Ratnieks 1999; Linksvayer and Wade 2005, Chapter 1; Linksvayer et al., unpublished ms; Linksvayer, Chapter 2). While caste is thought to be determined primarily by environmental factors (e.g., nutritional quality and quantity) (Wheeler 1986), both direct and sibsocial effect genes also likely influence caste (Linksvayer, chapter 2). For example, direct effect genes expressed in developing larvae may affect the physiological basis of the developmental switch and sibsocial effect genes expressed in workers may affect the relative amount of nutrition provided to developing larvae (Linksvayer and Wade 2005). Selection within- and among-colonies may fine-tune the interactions between these genes so that the optimal proportion of gynes and workers are produced for colony maintenance and reproduction (Linksvayer et al., unpublished ms).

Presumably, crosses between populations or species could disrupt these direct-indirect effect gene interactions so that more or fewer gynes are produced. However, in the current study, all worker-larvae species combinations produced the same number of gynes. In addition, intercastes, individuals with a mixture of worker and gyne traits, were only very rarely produced, suggesting that caste determination was not disrupted by cross-fostering. One reason may be that gynes of all three *Temnothorax* species are of similar size, indicating that gyne determination for all three species occurs at a similar nutritional level. Previous ant cross-fostering studies in which larvae were reared by workers from a different genus showed changes in the proportion of gynes produced as well as a high proportion of intercastes (e.g., Plateaux 1985). Further cross-fostering

studies should elucidate the importance of the coevolution of direct and sibsocial effects on caste.

Workerless social parasite-host systems may present especially interesting study systems. Larvae of the social parasite species are cared for solely by workers of the host species. Often, microgyny, the miniaturization of reproductive gynes, occurs (Aron et al. 1999). This is thought to be a strategy to bypass worker control of larval developmental fate, so that all larvae develop into gynes (Aron et al. 1999). The evolution of socially parasitic microgynes provides an example of coevolution between direct effect genes expressed in the developing social parasite larvae and sibsocial effect genes expressed in care-giving host workers (cf. Fischer and Foitzik 2004; Brandt et al. 2005).

This study demonstrates that phenotypic variation between species is determined by genes expressed in both developing larvae and care-giving workers. Social insect phenotypes are determined by a complex web of interactions between queen, worker, and larvae nestmates. Coevolutionary dynamics of genes expressed in all of these social partners likely plays a major role in within- and between-population evolutionary processes for all social insect phenotypes. Cross-fostering at a finer scale, between populations of the same species, should elucidate the importance of these varied direct and indirect genetic effects to phenotypic differences between populations. *Temnothorax* species have been shown to display between-population variation for a variety of individual and colony traits such as body size, queen number, and sex investment ratio (e.g., Herbers and Stuart 1996). Thus, these species provide an ideal study system for studying between-population as well as between-species phenotypic differences.

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TABLE 1. Analysis of Variance for new workers, gynes, and males produced by experimental colonies formed from conspecific and heterospecific worker-larvae combinations. All factors are fixed except for block which was random. Type III SS were used.

Worker mass

Effect	*df*	*MS*	*F*	*p*
Intercept	1	173.132	9372.379	0.0000000
Worker species	2	0.0881	8.366	0.0000243
Larvae species	2	1.0650	101.165	0.0000000
Worker species * Larvae species	4	0.0349	3.315	0.010286
Block	28	0.0312	2.959	0.0000000
Error	1586	0.0105		

Gyne mass

Effect	*df*	*MS*	*F*	*p*
Intercept	1	88.74392	2258.995	0.000000
Worker species	2	0.01828	0.608	0.545532
Larvae species	2	0.46507	15.467	0.000001
Worker species * Larvae species	4	0.07670	2.551	0.040630
Block	27	0.05411	1.800	0.012719
Error	188	0.03007		

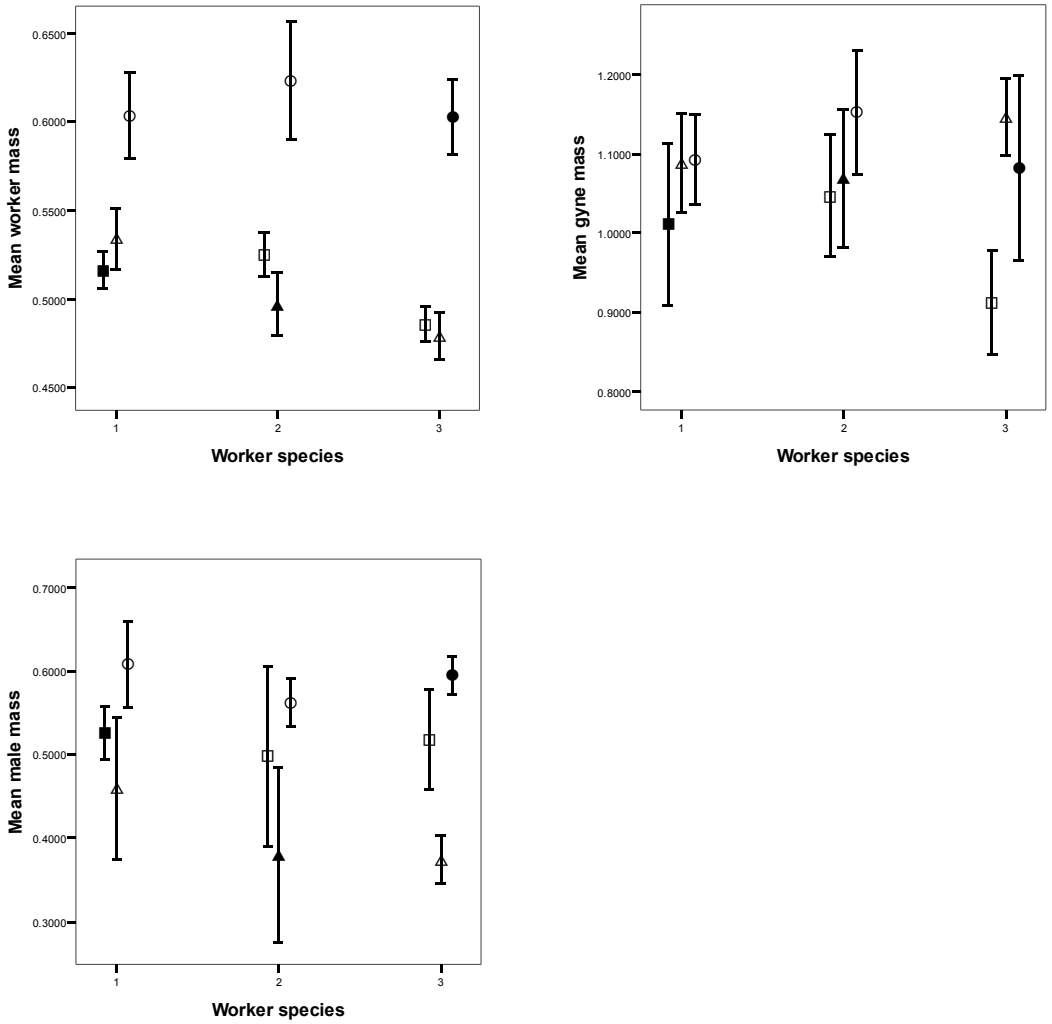
Male mass

Effect	*df*	*MS*	*F*	*p*
Intercept	1	22.739	2282.377	0.000000
Worker species	2	0.0348	3.309	0.0387
Larvae species	2	0.312	29.708	0.000000
Worker species * Larvae species	4	0.00777	0.739	0.566
Block	25	0.00930	0.884	0.627
Error	189	0.0105		

Figure captions:

FIGURE 1. Mean mass of worker pupae, adult gynes, and adult males produced by worker-larvae species combinations. The species of care-giving workers is shown on the x axis (*T. ambiguus* is species 1, *T. curvispinosus* is species 2, and *T. longispinosus* is species 3). The species of larvae is indicated by the following symbols: species 1, □, species 2, Δ, and species 3, ○. Conspecific worker-larvae combinations are indicated with a filled symbol and heterospecific worker-larvae are indicated with an open symbol.

Figure 1


Mean worker mass
0.6500
0.6000
0.5500
0.5000
0.4500
1
2
3
Worker species
Mean gyne mass
1.2000
1.1000
1.0000
0.9000
0.8000
1
2
3
Worker species
Mean male mass
0.7000
0.6000
0.5000
0.4000
0.3000
1
2
3
Worker species

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EDUCATION

Ph.D. Indiana University, Evolution, Ecology, & Behavior Program, Department of Biology. 2005.

B.A. Carleton College, Biology, Northfield, Minnesota, 1998.

RESEARCH EXPERIENCE

Dissertation Research (1999-2005) Advisor Dr. Michael Wade, Department of Biology, Indiana University. Theoretical and empirical study of social evolution, using an indirect genetic effects, quantitative genetic approach.

Undergraduate Research Intern (1996-1999)
-Advisor Dr. Marla Spivak, Department of Entomology. University of Minnesota. Bumblebee foraging ecology (1998).
-Advisor Dr. Mark McKone, Department of Biology, Carleton College. Ant community ecology, diversity, and taxonomy (1996-1999).

AWARDS AND HONORS

National Merit Scholarship, 1994-1998.
Distinction in Major, Carleton College, 1998.
Magna cum Laude, Carleton College, 1998.
National Science Foundation Graduate Research Fellowship (1999-2003).
National Science Foundation Postdoctoral Research Fellowship in Biological Informatics, with Robert Page, Arizona State University (2006-2008).

GRANTS AWARDED

Indiana University Teaching and Research Preserve Grant Program ($500): "Queen-worker conflict over colony sex ratio in the acorn ant *Leptothorax curvispinosus*" (2002).
Indiana Academy of Sciences Graduate Research Grant ($1400): "A quantitative genetic approach to study social evolution in ants: estimating direct, maternal, and

sib-social genetic variance and covariance for size in the acorn ant, *Leptothorax curvispinosus*" (2003).

Indiana University Presidential Summer Undergraduate Research Initiative ($500): "Direct and indirect genetic effects on maternal performance in the acorn ant, *curvispinosus*" (2003).

TEACHING EXPERIENCE

Mentor for undergraduate research assistants (Indiana University)
Nine students (2002-2005)

Instructor (Carleton College)
Off-campus Tropical Rainforest Ecology to Costa Rica (December 2004)

Associate Instructor (Indiana University)
L111 Evolution and Diversity (Fall 2000).

Teacher's Assistant (Carleton College)
-Carleton College Biology Seminar in Australia and New Zealand (Winter 2002)
-Biology 212, Biology Field Studies
-Biology 250, Marine Biology
-Environmental Studies 238, Physical and Cultural of New Zealand and Australia
-Environmental Studies 290, Directed Reading
-Ecology (Winter Spring 1998)
-Insects and Angiosperms Winter 1997)

PRESENTATIONS

Linksvayer TA. Maternal care, indirect genetic effects, queen dimorphism, and sympatric speciation in the acorn ant, *Leptothorax curvispinosus*.. Departmental seminar. Indiana University, Bloomington, Indiana (March 2003).

Linksvayer TA. Direct and indirect genetic effects on mass and caste dermination in an acorn ant. Internation Union for the Study of Social Insects, North American Section. Arizona State University (October 2004).

Linksvayer TA. Direct and indirect genetic effects on mass, caste, and sex ratio in the ant *Temnothorax curvispinosus*. Departmental seminar. Indiana University, Bloomington, Indiana (March 2005).

PUBLICATIONS

Linksvayer TA, AC McCall, RM Jensen, CM Marshall, JW Miner, MJ McKone. 2002. The function of hitchhiking behavior in the leaf-cutting ant *Atta cephalotes*. Biotropica 34: 93-100.

Buschinger A, **TA Linksvayer**. 2004. Novel blend of life history traits in an inquiline ant, *Temnothorax minutissimus*, with description of the male (Hymenoptera: Formicidae). Myrmecological News 6: 67-76.

Linksvayer TA, MJ Wade. 2005. The evolutionary origin and maintenance of eusociality in the aculeate Hymenoptera: maternal effects, sib-social effects, and heterochrony. The Quarterly Review of Biology 80: 317-336.

Neiman M, **TA Linksvayer**. 2006. The conversion of variance and the evolutionary potential of restricted recombination. Heredity (in press).

Linksvayer, TA, MJ Wade, DM Gordon. Genetic caste determination in harvester ants: possible origin and maintenance by cyto-nuclear epistasis. Ecology (in review).

PROFESSIONAL ACTIVITIES

Referee for

-Evolution
-Functional Ecology
-Ecological Entomology
-Biotropica
-Behavioral Ecology and Sociobiology

PROFESSIONAL AFFILIATIONS

Society for the Study of Evolution
International Union for the Study of Social Insects