

Short Review

Testing kin selection with sex allocation data in eusocial Hymenoptera

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Sex allocation data in eusocial Hymenoptera (ants, bees and wasps) provide an excellent opportunity to assess the effectiveness of kin selection, because queens and workers differ in their relatedness to females and males. The first studies on sex allocation in eusocial Hymenoptera compared population sex investment ratios across species. Female-biased investment in monogyne (= with single-queen colonies) populations of ants suggested that workers manipulate sex allocation according to their higher relatedness to females than males (relatedness asymmetry). However, several factors may confound these comparisons across species. First, variation in relatedness asymmetry is typically associated with major changes in breeding system and life history that may also affect sex allocation. Secondly, the relative cost of females and males is difficult to estimate across sexually dimorphic taxa, such as ants. Thirdly, each species in the comparison may not

represent an independent data point, because of phylogenetic relationships among species. Recently, stronger evidence that workers control sex allocation has been provided by intraspecific studies of sex ratio variation across colonies. In several species of eusocial Hymenoptera, colonies with high relatedness asymmetry produced mostly females, in contrast to colonies with low relatedness asymmetry which produced mostly males. Additional signs of worker control were found by investigating proximate mechanisms of sex ratio manipulation in ants and wasps. However, worker control is not always effective, and further manipulative experiments will be needed to disentangle the multiple evolutionary factors and processes affecting sex allocation in eusocial Hymenoptera.

Keywords: ants, kin selection, relatedness asymmetry, sex ratio, social Hymenoptera

Introduction

Kin selection extends natural selection to include the indirect transmission of copies of genes through related individuals (Hamilton, 1964). This is a powerful concept, which may explain many facets of the co-operation and conflicts associated with social life. However, empirical tests of kin selection have proved difficult, because social behaviour has been shaped by the interaction of multiple ecological and genetic factors over evolutionary time. Studies of sex allocation in hymenopteran societies have emerged as prime tests of kin selection, because sex allocation theory provides quantitative predictions based on variation in relatedness (Bourke & Franks, 1995; Crozier & Pamilo, 1996).

Kin selection and expected sex ratios in social Hymenoptera

Kin selection theory predicts that the value of new queens and males is influenced by their relatedness to other colony members (Hamilton, 1964). The haplodiploid sex-determination system (with males arising from unfertilized eggs and females from diploid eggs) in social Hymenoptera results in

asymmetries in the relatedness of workers to females and males. Thus, full sisters are three times more related to each other than they are to their brothers. Hence, Fisherian sex ratio selection should result in a 3:1 female bias in social Hymenoptera when workers (which are always females) raise full siblings and control sex allocation (Trivers & Hare, 1976). However, because queens are equally related to their sons and daughters, they should favour a 1:1 population sex investment ratio. Thus, a potential conflict exists between the individuals that lay the eggs (the queens) and those that rear the brood (the workers). Kin selection predicts that the sex investment ratio will be determined by the relative power of different colony members in controlling sex allocation.

Kin selection combined with Fisher's sex ratio theory provides a quantitative prediction linking sex investment ratios to genetic relatedness asymmetry at both the population and colony levels (Table 1). If workers control sex allocation, female bias should be 3:1 at the population level when all colony members are the offspring of a single queen mated with a single male. Female bias is expected to decrease concomitantly with a decrease in relatedness asymmetry (Boomsma 1989). The most important factors reducing relatedness asymmetry are: (i) the presence of more than one reproductive queen (polygyny) per colony when queens are related; (ii) multiple mating by queens; and (iii) worker reproduction. A first approach to test kin selection and workers' ability to

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Table 1 Sex ratio theory for social Hymenoptera

Hypothesis	Assumptions	Predictions at the		Additional predictions
		population level	colony level	
Genetic relatedness asymmetry (1,2)	worker control sex allocation	greater female bias when higher relatedness asymmetry (RA) (1)	split sex ratio; colonies with high RA produce more females, colonies with low RA produce more males (2)	workers can distinguish the sex of the brood and assess the RA in their colony; workers may actively manipulate sex allocation
Local mate competition (3)	males competing for matings are related	greater female bias when competition among related males is intense	female bias increases with brood size; males always produced	limited dispersal of males; mates of the same queen or of nestmate queens are related
Local resource competition (4)	competing females are related	greater male bias when competition among related females is intense	male bias increases with brood size; females always produced	limited dispersal of females; nestmate queens are related
Local resource enhancement (5)	related queens cooperate, which increases per capita productivity	degree of female bias depends on optimal queen number and on brood size	female bias decreases with brood size	per capita productivity is a convex function of the number of queens
Multifaceted parental investment (6)	egg number or other specific resource limits brood size	female bias if the number of eggs is the limiting factor	female bias decreases when number of eggs increases	increasing the number of eggs or decreasing food resource should decrease female bias, and vice versa
Endoparasites	maternally transmitted endoparasites influence sex allocation	degree of female bias depends on the prevalence and efficiency of endoparasites	greater female bias if endoparasites present	antibiotic treatment should decrease female bias due to bacteria

References: 1, Trivers & Hare (1976); 2, Boomsma & Grafen (1990, 1991); 3, Hamilton (1967); 4, Clark (1978); 5, Schwarz (1988); 6, Rosenheim *et al.* (1996).

control sex allocation is thus to compare sex investment ratios among species with different levels of relatedness asymmetries.

At the colony level, workers benefit by producing the sex to which they are relatively more related, as compared to the population average (Nonacs, 1986; Boomsma & Grafen, 1990, 1991). For example, consider a population where most colonies are headed by a single-mated queen. Under worker control, the population numerical sex ratio should be close to 3:1 (assuming no sexual dimorphism). Workers in a colony headed by a double-mated queen would benefit by producing males only, because the mating success of males exceeds the relatedness asymmetry in this particular colony. The mating success of males is three times that of females, because the population sex ratio is 3:1. In contrast, workers in this colony are only twice as related to females than to males, because the queen is double-mated and females represent a mixture of full and half sisters. Hence, workers in a colony headed by a double-mated queen would transmit more copies of their genes to the next generation by investing colony resources into males rather than females. The general prediction is that workers

should rear mainly or only females in colonies with relatedness asymmetries above the population average and mainly or only males in colonies with relatedness asymmetries below the population average (Boomsma & Grafen, 1990, 1991). Under worker control, sex ratio should be split among colonies with high or low relatedness asymmetry, and the variation predicted among colonies is much greater than the variation predicted at the population level. Hence, a second and powerful approach to test kin selection and queen-worker conflict over sex allocation is to compare the sex investment ratios among colonies differing in relatedness asymmetry.

A third approach consists of investigating directly whether, and how, queens and workers manipulate colony sex allocation in their favour (Pamilo, 1991). The queen controls the primary sex ratio by regulating the proportion of haploid (male) and diploid (female) eggs she lays, but workers may subsequently adjust the sex investment ratio in their own interest by selective rearing of the brood. Hence, consistent differences between egg and adult sex ratios are expected if workers, and not queens, control sex allocation.

Sex ratio variation across species

The first cross-species comparison of population-level sex investment ratios was performed by Trivers & Hare (1976). Subsequently, this analysis has been refined and extended by many authors (Nonacs, 1986; Boomsma, 1989; Bourke, 1989; Pamilo, 1990; Bourke & Franks, 1995; Herbers & Stuart, 1998). The most striking result of this approach is that the population sex investment ratio is globally female-biased (1.7:1) in monogyne ants, slightly male-biased (1:1.25) in polygyne ants, and unbiased in slave-making ants (Bourke & Franks, 1995).

Female-biased sex investment ratio in monogyne ants, but not in polygyne and slave-making ants, is consistent with the hypothesis that workers in free-living ant species control sex allocation and achieve their preferred optimum set by relatedness asymmetry. A lower than 3:1 investment ratio in predominantly monogyne ant species is still consistent with workers being in full control and achieving their preferred sex investment ratio, as long as a significant part of the colonies have reduced relatedness asymmetries due to multiple mating by queens, occasional presence of multiple related queens, or worker reproduction (Boomsma, 1989). Lower female bias in polygyne ants bolsters the argument, because the presence of several related queens decreases the average relatedness asymmetry (Nonacs, 1986; Boomsma, 1993), and nestmate queens are generally related in ants (Crozier & Pamilo, 1996). Finally, unbiased investment ratio had been predicted in slave-making ants, because all brood care is performed by heterospecific slave workers. The slave workers are unrelated to the brood of the slave-maker, and therefore they may have no evolutionary stake in biasing sex allocation in their parasite (Trivers & Hare, 1976).

The contrast across species thus suggests that workers frequently control population sex allocation in monogyne ant species. This result has been extremely important historically, triggering further theoretical and empirical research in the field (Bourke & Franks, 1995; Crozier & Pamilo, 1996). However, a number of correlated factors may confound the pattern of sex investment ratio across species.

A first problem is that cross-species variation in relatedness asymmetry is typically associated with major changes in the breeding system, life history and mode of colony reproduction that are also expected to influence investment patterns. For example, in polygyne species, young queens often return to their parental colony after mating (Keller, 1995), and new colonies frequently arise in the proximity of old ones. Such limited dispersal of females may result in local resource competition (Table 1), which in turn may promote male-biased sex allocation (Trivers & Hare, 1976). Moreover, in polygyne ants workers frequently accompany queens founding a new colony. In this case, the production of workers represents an investment into females (Pamilo, 1991). Consequently, sex investment ratio estimated from winged males and females may underestimate the actual female bias in polygyne ants.

A second problem arises from the difficulty in estimating an essential element of cross-species comparisons, the relative cost of female and male production (Boomsma, 1989; Crozier & Pamilo, 1996). Initially, costs have been measured in terms of dry mass (Trivers & Hare, 1976). However, when females and

males differ in size or chemical composition, the mass ratio is a poor indicator of the energetic cost ratio, because metabolism and maintenance costs vary between the sexes (Boomsma, 1989). When females are larger and contain more lipids than males, the female to male mass ratio tends to be larger than the ratio of energy needed to produce a female and a male. Monogyne ant species are generally characterized by a higher queen to male dimorphism than polygyne and slave-making ant species (Trivers & Hare, 1976; Keller & Passera, 1989; Stille, 1996), and young monogyne queens tend to have more fat reserves than their polygyne counterparts (Keller & Passera, 1989). Therefore, the energetic cost of producing a female, as compared to a male, may be overestimated in monogyne ant species when it is measured as the ratio of dry mass. Moreover, investment ratios should be estimated with respect to the factor limiting the production of reproductive individuals, which is not necessarily energy (multifaceted investment model, Rosenheim *et al.*, 1996; Table 1). Egg number or any particular resource necessary for growth may be the limiting factor, so that it may become extremely difficult to assess the relative cost of females and males across species.

A third problem of cross-species comparisons is that each species may not represent an independent data point, because of phylogenetic relationships among species. Therefore, the analyses performed so far might have overestimated the number of degrees of freedom, and other conclusions might emerge when modern comparative methods based on molecular phylogenies will be applied to the same data set (e.g. Harvey & Pagel, 1991).

Further confounding factors may also play some role. For example, a recent survey detected the presence of *Wolbachia* bacteria in 25 out of 50 ant species tested (Wenseleers *et al.*, 1998), and in some circumstances these maternally transmitted endoparasites induce female-biased sex ratios in their hosts (Hurst *et al.*, 1997). Whether *Wolbachia* or other endoparasites actually affect sex allocation in social insects is unknown, and the question constitutes an exciting area for future research.

Overall, the comparison of sex investment ratios across many species might be affected by a wealth of confounding factors and potential problems. Therefore, we believe that this approach does not provide compelling evidence that workers manipulate sex allocation in response to relatedness asymmetry.

Sex ratio variation across colonies

Studies of variation in sex investment ratios across colonies have emerged as powerful ways to assess the role that kin selection, relatedness asymmetry and worker control play in sex allocation. Theory predicts that, under worker control, colonies with high or low relatedness asymmetry should specialize in producing females or males, respectively (Boomsma & Grafen, 1990, 1991). This prediction can be tested within populations where colonies differ by a single factor altering their genetic structure, but are similar in other characteristics. Such intraspecific tests are more powerful than cross-species comparisons, because they do not rely on precise estimates of the cost of males and females, and avoid confounding

variation in breeding system (Boomsma, 1993). Moreover, the prediction can be tested independently in many species, and experimental manipulations can be performed.

Overall, relatedness-induced split sex ratio has been documented in 17 species of social Hymenoptera (Queller & Strassmann, 1998). As expected under worker control, colonies with higher relatedness asymmetry produced more females than colonies with lower relatedness asymmetry. Such sex ratio specialization occurred when relatedness asymmetry varied because of differences in the number of matings by queens in monogyne ants (Sundström, 1994; Sundström *et al.*, 1996), variation in the number of queens in ants and wasps (Herbers, 1984; Queller *et al.*, 1993; Chan & Bourke, 1994; Deslippe & Savolainen, 1995; Evans, 1995) or replacement of the mother queen by one of her daughters in halictid bees (Boomsma, 1991; Mueller, 1991; Packer & Owen, 1994). In contrast, only two studies did not detect the expected association between relatedness asymmetry and colony sex ratio specialization. Higher relatedness among nestmate workers (which should usually reflect higher relatedness asymmetry) was not associated with specialization into female production in the ants *Formica sanguinea* (Pamilo & Seppä, 1994) and *Pheidole pallidula* (Aron *et al.*, 1999a). Workers may fail to control sex allocation in these particular cases. For example, heterospecific slave workers perform part of the brood care in *Formica sanguinea*, which may prevent sex ratio manipulation. Alternatively, relatedness among workers may not reflect accurately relatedness asymmetry, if queen number and relatedness among queens covary. For example, colonies headed by unrelated queens may have low worker relatedness but high relatedness asymmetry (Boomsma, 1993).

Experimental manipulation of relatedness asymmetry provides even more compelling evidence of worker control. In the eusocial bee *Augochlorella striata*, the foundress queen is replaced by one of her daughters when removed experimentally (Mueller, 1991). Such queen turnover decreases relatedness asymmetry from 3:1 to 1:1 (a female being equally related to nieces and nephews, as well as to sons and daughters). As predicted by kin selection and sex ratio theories, the sex investment ratio was significantly more female-biased in queenright colonies than in colonies where the foundress had been removed (Mueller, 1991). Further experimental manipulations of colony relatedness asymmetry would be useful to assess the effectiveness and frequency of worker control across the many species of social Hymenoptera.

Mechanisms of sex ratio manipulation

Hymenopteran queens can control the primary sex ratio within nests by adjusting the proportion of unfertilized (male-destined) and fertilized (female-destined) eggs they lay. Subsequently, workers may alter colony sex allocation by eliminating one sex, by influencing caste determination (e.g. by forcing female larvae to develop into workers), or by regulating the amount of resources invested in each sex.

The comparison of numerical sex ratios between the egg and adult stages thus provides a direct way to examine if the conflict between queen and workers is manifest. So far, such

comparisons have been conducted in five species of ants. The sex ratio was more female biased in adults than in eggs for the four species where a sex ratio conflict between queen and workers was expected (Aron *et al.*, 1994; Aron *et al.*, 1995; Keller *et al.*, 1996; Sundström *et al.*, 1996). By contrast, there was no significant change in sex ratio between the egg and adult stages in *Plagiolepis xene*, a social parasite that has lost the worker caste and completely relies on the host workers to raise its brood (Aron *et al.*, 1999b). The reduction in the proportion of males in the four ant species with a queen-worker conflict but not in *P. xene* supports the view that changes in colony sex ratio stem from workers actively manipulating sex ratio, rather than from differential mortality of males (Aron *et al.*, 1999b). Moreover, facultative sex ratio manipulation by workers has been documented in the monogyne population of the ant *Formica exsecta*. As predicted by split sex ratio theory, males were eliminated in colonies headed by a single-mated queen, but not in colonies headed by a double-mated queen (Sundström *et al.*, 1996).

This conditional elimination of males indicates that workers possess accurate discrimination ability at two stages. First, they are able to assess the relative level of relatedness asymmetry in their colony. Experimental data suggest that workers of the ant *Myrmica tahoensis* use larval cues to assess the mean genetic diversity in their colony, which indirectly reflects the level of relatedness asymmetry (Evans, 1995). When the genetic diversity was increased experimentally by transferring larvae among unrelated colonies, a lower proportion of colonies produced females. Secondly, workers must identify the sex of the brood before it becomes too costly to eliminate males. The developmental stage reached by males at the time they are eliminated seems to vary. In the Argentine ant *Linepithema humile*, about half of the male brood was eliminated just after hatching and the rest shortly before pupation (Passera & Aron, 1996). In *Formica exsecta*, many males were eliminated at a late developmental stage (Chapuisat *et al.*, 1997). This late elimination of males suggests that workers reduce brood size to cope with resource limitation, and that early sex ratio biasing is prevented either by the cost of errors in determining the sex of young brood or by limitations in the number of female eggs. In contrast to the previous studies, workers did not discriminate supernumerary males in the queenless ant *Diacamma* sp. (Nakata, 1998). More manipulative experiments are needed to determine when workers are able to distinguish the sex of the brood, and under which ecological conditions they actually raise females and males differently. It would be particularly interesting to manipulate the sex ratio among eggs, the number of eggs, and the level of colony resources.

To date, very little is known about the mechanisms of caste determination. Adult workers may control caste determination by regulating the food supplied to larvae. However, larvae may also evolve counter-manipulative mechanisms, for example by changing the threshold for the switch between queen and worker developmental trajectories, or by varying their own feeding rate. Thus, the question arises whether the brood itself might play some role in sex allocation. Furthermore, Haig (1992, 1998) has suggested that there might be an intragenomic

conflict over colony sex allocation. Because paternally inherited genes are not present in brothers, they should favour exclusive investment in females. In contrast, maternally inherited genes are equally related to brothers and sisters, and their optimum is an equal investment in males and females. Hence, an intragenomic conflict over colony sex ratio manipulation might occur within workers (Haig, 1992, 1998). Interestingly, a similar conflict might also occur within female larvae, but this time over caste determination. Maternally inherited genes have a greater incentive to let the female larva develop into a worker, resulting in a higher relative investment in males in the current cohort. In contrast, paternally inherited genes should favour brood development into queens. Whether this sort of intragenomic conflict occurs in social insects is unknown. However, recent empirical evidence that sex might be determined by a mechanism of genomic imprinting in *Nasonia vitripennis* (Dobson & Tanouye, 1998) should trigger further empirical and theoretical work on imprinting and intragenomic conflict in Hymenoptera.

Sexual discrimination may also occur after pupation. An unusual way to funnel colony resources into females has been observed in the social wasp *Polistes dominulus*, where workers forcibly 'stuff' young males head-first into empty nest-cells when foragers return to the colony (Starks & Poe, 1997). 'Stuffed' males are temporarily unable to feed, which seems to ensure that food is preferentially distributed to larvae.

Conclusion

A large body of data is consistent with kin selection having a substantial impact on sex allocation in the social Hymenoptera. Comparisons across species first suggested that workers manipulate sex allocation to match their relatedness asymmetry to males and females. Recently, much stronger evidence that workers manipulate colony sex ratio in their favour arose from studies showing relatedness-induced sex ratio specialization among colonies within single populations, and revealing the proximate mechanisms used in sex ratio biasing.

Lately, it has been suggested that the control of sex allocation by workers might generate surprising and counter-intuitive selection pressures on the mating behaviour of social insects. Queens might be selected to mate with multiple males in order to decrease the relatedness asymmetry in their colonies and induce workers to bias colony sex allocation towards males (Queller, 1993; Ratnieks & Boomsma, 1995). Indeed, a recent study has demonstrated that facultative sex allocation by workers results in higher fitness for double-mated than single-mated queens in field colonies of the ant *Formica truncorum* (Sundström & Ratnieks, 1998). In contrast, the fitness of the mates of the queens is zero when colonies produce only males (because of haplo-diploidy, males transmit genes through daughters only). This might result in an unusual tug of war between the sexes, with females eager to mate and males showing restraint (Boomsma, 1996). For example, males may avoid mating with queens that have already mated. Alternatively the second male may transfer a small amount of sperm to ensure relatively high relatedness asymmetry and female-biased sex allocation in the future colony (Boomsma,

1996). These new and fascinating theoretical developments now await empirical testing.

Relatedness-induced worker control has been observed frequently in social Hymenoptera, but is not universal. For example, bumblebees are monogynous, but exhibit male-biased population sex investment ratios (Trivers & Hare, 1976; Bourke, 1997). Whether or not worker control is realized depends on multiple parameters, such as the balance of power between queens and workers, the ability of workers to distinguish male and female brood, the level of resources, and the timing of production of reproductive individuals. A particular response may occur in part of this multifactorial space (e.g. when resources are limited), and not in others (e.g. when resources are plentiful). Moreover, the important factors explaining sex ratio variation among colonies within a population may differ from those determining sex ratio variation at the population or species levels. Indeed, long-term studies of population sex investment ratios in ants have revealed variations between years that seem independent of relatedness asymmetry (e.g. Herbers, 1990; Evans, 1996).

Finally, we would like to stress that the large number of factors that can potentially affect sex allocation, and the wealth of alternative hypotheses on sex ratio evolution (Table 1), weaken the conclusions of correlational studies. Basically, any pattern can be explained by one of the numerous alternative hypotheses, or by the effect of various uncontrolled factors. Hence, further manipulative experiments are needed. Controlling for factors such as relatedness asymmetry or resource levels may be difficult and time-consuming, but such experiments are necessary to reveal how multiple factors interact in determining the evolution of sex allocation.

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