

# Male parentage does not vary with colony kin structure in a multiple-queen ant

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## Abstract

Kin selection theory predicts that, in social Hymenoptera, the parentage of males should be determined by within-colony relatedness. We present a model showing that, when sex ratios are split (bimodal) as a function of colony kin structure, the predictions of kin selection theory regarding the occurrence of worker reproduction and policing (prevention of worker reproduction) require modification. To test the predictions of kin selection theory and our model, we estimated using microsatellites the frequency of worker-produced male eggs and adults in the facultatively polygynous (multiple-queen) ant *Leptothorax acervorum*. Analysis of 210 male eggs and 328 adult males from 13 monogynous (single-queen) and nine polygynous colonies demonstrated that the frequency of worker-produced males was low (2.3–4.6% of all males) and did not differ significantly between colony classes or between eggs and adults. This suggested workers' self-restraint as the cause of infrequent worker reproduction in both colony classes. Such an outcome is not predicted either by comparing relatedness values or by our model. Therefore, it appears that factors other than colony kin structure and sex ratio effects determine the pattern of male parentage in the study population. A likely factor is a colony-level cost of worker reproduction.

## Introduction

Within groups of communally living organisms there is potential conflict because the interests of group members do not always coincide (Hamilton, 1964; Trivers & Hare, 1976). Whether potential conflicts are expressed, and how they are resolved, are fundamental questions in social evolution (Frank, 1998; Keller, 1999). Kin selection theory predicts that relatedness is a critical factor underlying the occurrence of such conflicts (Hamilton, 1964). In the eusocial Hymenoptera, males are haploid and females are diploid, creating asymmetries of relatedness that lead to potential conflict over many aspects of reproduction including sex ratios and the parentage of males (Hamilton, 1964; Trivers & Hare, 1976; Ratnieks & Reeve, 1992; Bourke & Franks, 1995; Crozier & Pamilo,

1996; Queller & Strassmann, 1998; Sundström & Boomsma, 2001).

Workers of many species of eusocial Hymenoptera are capable of laying unfertilized haploid eggs that develop into males (Bourke, 1988; Choe, 1988). Successful worker reproduction may nonetheless fail to occur either because workers refrain from laying male eggs (self-restraint) or because worker-laid male eggs are destroyed by other workers (worker policing) or by queens (queen policing) (Cole, 1986; Ratnieks, 1988). In a population of colonies each headed by a single queen (monogyny) who is singly mated, potential conflict over male parentage arises because workers favour neither self-restraint nor worker policing. Instead, kin selection theory predicts that, other things equal, workers gain greater inclusive fitness from rearing sons (relatedness,  $r = 0.5$ ) or the sons of other workers (nephews,  $r = 0.375$ ) rather than the queen's male offspring (brothers,  $r = 0.25$ ), whereas the queen gains greater inclusive fitness from the rearing of sons ( $r = 0.5$ ) rather than workers' male offspring (grandsons,  $r = 0.25$ ) (Hamilton, 1964; Trivers & Hare, 1976).

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Changes in colony kin structure due to either multiple mating by queens (polyandry) or multiple reproductive queens (polygyny) can alter the expected pattern of conflict because they alter relative relatedness values. For example, under monogyny with an effective queen mating frequency greater than two, a focal reproductive worker remains more closely related to its male eggs ( $r = 0.5$ ) than to those of the queen ( $r = 0.25$ ), but the average worker becomes more closely related to queen-produced males ( $r = 0.25$ ) than to the average worker-produced male ( $0.125 < r < 0.25$ ). Under these conditions, reproductive workers are still predicted to lay male eggs (self-restraint is not favoured), but other workers are predicted to stop these eggs being reared (worker policing is favoured), for example, by eating eggs (Starr, 1984; Woyciechowski & Lomnicki, 1987; Ratnieks, 1988). Overall, assuming workers control male production, successful worker reproduction is expected when workers are more closely related, on average, to worker-produced males than to queen-produced males. By contrast, worker policing is expected when workers are more closely related, on average, to queen-produced males than to worker-produced males (Ratnieks, 1988; Pamilo, 1991).

These predictions, which are based on comparing relatedness values, could require modifying if other types of kin-selected conflict occur. A prime candidate is conflict over sex allocation involving split sex ratios, which occur when different colony classes produce systematically different sex ratios in response to variation in workers' relative relatedness to the sexes (relatedness asymmetry) (Boomsma & Grafen, 1990, 1991). Because across-species comparisons of the incidence of worker reproduction and policing (e.g. Walin *et al.*, 1998; Peters *et al.*, 1999; Foster & Ratnieks, 2001c) may be confounded by differences between species other than differences in kin structure, it is highly desirable to test the effects of colony kin structure on patterns of male parentage in populations with facultative variation in colony kin structure (e.g. Foster & Ratnieks, 2000). But such populations also typically exhibit split sex ratios (e.g. Queller & Strassmann, 1998). Walin *et al.* (1998) proposed that when split sex ratios arise from worker biasing of sex allocation by selective destruction of queen-produced males in colonies with low effective mating frequencies, the evolution of successful worker reproduction could be prevented. In a model, Foster & Ratnieks (2001b) confirmed that worker policing could evolve when selective male destruction occurs. However, this model did not involve sex ratio splitting, although it was discussed. The effect arose because the removal of males as eggs avoided the greater costs of removing males as larvae.

In this study, we derive a model for the evolution of worker reproduction when sex ratios are split. We then test the predictions of kin selection theory based on comparing relatedness values and as modified by our

model by investigating male parentage as a function of facultative variation in colony kin structure in an ant, *Leptothorax acervorum* (Fabricius). Our model is novel because it does not invoke selective destruction of males, which is absent in *L. acervorum* (Hammond *et al.*, 2002), and because it demonstrates that the fitness returns from worker reproduction are altered by the occurrence of split sex ratios. Previous studies suggested that successful worker reproduction in queenless colonies is rare in *L. acervorum* and that few adult males in two polygynous colonies were worker-produced (Heinze *et al.*, 1997). However, no previous study of this or any other ant species has estimated the frequency of worker-produced males among both eggs and adults from several monogynous and polygynous colonies using microsatellite markers. Our results show that almost all male eggs and adult males were queen-produced in both types of colony, indicating self-restraint by potentially reproductive workers regardless of the colony class. This outcome is not predicted either by a comparison of relatedness values or by our model. We therefore conclude that additional factors, such as colony-level costs of reproduction, account for patterns of male parentage in this case.

## Methods

### Model

We investigated the fitness returns from worker reproduction when sex ratios are split within populations by workers' relatedness asymmetry (relative relatedness to females) (Boomsma & Grafen, 1990, 1991). We considered a facultatively polygynous population in which monogynous colonies have one, singly mated queen and polygynous colonies have several, singly mated, related queens. Workers control sex allocation and sex ratios are split such that monogynous (M) colonies raise all or mainly females and polygynous (P) colonies raise all or mainly males. The sexes are assumed equally costly and monogynous and polygynous colonies have the same average sexual productivity. These assumptions closely match the case of *L. acervorum* in our principal study population, Santon Warren (Chan & Bourke, 1994; Chan *et al.*, 1999; Hammond *et al.*, 2001, 2002). They are also likely to be approximated by numerous other social Hymenoptera (Queller & Strassmann, 1998).

Consider the fitness gain of a rare, mutant worker replacing a queen-produced egg (queen-destined or male) with a male egg of its own, from the viewpoint of the laying worker and the nonlaying workers. It is assumed workers do not discriminate eggs by sex. This assumption is likely to be true in *L. acervorum* and may also be true in other ants where sexual brood and worker brood are reared together (Hammond *et al.*, 2002). Note that this condition does not affect the conditions for invasion of worker reproduction when sex ratios are

unsplit. This is because, in a population with monandry, monogyny and a stable, worker-controlled population sex ratio biased 3 : 1 in favour of females, females and males yield equal fitness returns to workers (because of the assumption of sex ratio equilibrium). Therefore, a worker gains the same net fitness increment from replacing a queen-produced female egg as from replacing a queen-produced male egg. In the current case, the population sex ratio, class-specific sex ratios and sex-specific reproductive values are all assumed unaffected by worker reproduction, because of the rarity of mutant workers. Hence, the model derives conditions for the initial spread of worker reproduction under split sex allocation.

All relatednesses in the model are 'life-for-life' relatednesses (incorporating the two-fold greater sex-specific reproductive value of diploid females relative to haploid males) (Grafen, 1986). Let  $q$  be the fraction of all fitness from females, that is from dispersing females (Pamilo, 1990). Let  $s$  be the population sex ratio expressed as the fraction of females; hence, if the mating success (per capita number of mates) of a female is  $1/s$ , that of a male is  $1/(1-s)$ . Let  $R = r_F/r_M$  be the ratio, average relatedness to queen-produced females/average relatedness to queen-produced males, for workers in the P colonies. The relatedness asymmetry of the M colony class (expressed as a fraction) is then  $3q/(3q+1)$  (Pamilo, 1990) and the relatedness asymmetry of the P colony class (expressed as a fraction) is  $Rq/(Rq+1)$ . In addition, let  $IF_{QF}$ ,  $IF_{QM}$  and  $IF_{WM}$  be the per capita inclusive fitness gains to workers from rearing a queen-laid female, a queen-laid male and a worker-laid male, respectively. Per capita inclusive fitness gain was calculated as the product of  $q$  (for female progeny alone), 'life-for-life' relatedness and mating success (e.g. Bourke & Franks, 1995; Crozier & Pamilo, 1996). Therefore, worker reproduction is favoured if either  $IF_{WM}/IF_{QF}$  or  $IF_{WM}/IF_{QM}$  exceeds one.

Split sex ratio theory allows for three types of stable outcome with regard to the class-specific and population sex ratios, dependent on the relative frequencies of the colony classes (Boomsma & Grafen, 1990, 1991). Adapting these for the case of facultative polygyny (cf. Boomsma, 1993) we defined three cases (varying with an increasing frequency of P colonies): Case 1: M colonies produce a mixed sex ratio (i.e. both sexes), P colonies produce all males and the population sex ratio equals the relatedness asymmetry of M colonies; Case 2: M colonies produce all females, P colonies produce all males and the population sex ratio varies between the relatedness asymmetries of M and P colonies (depending on the exact frequency of the P colonies); Case 3: M colonies produce all females, P colonies produce a mixed sex ratio and the population sex ratio equals the relatedness asymmetry of P colonies. We calculated  $IF_{QF}$ ,  $IF_{QM}$  and  $IF_{WM}$  for all three cases. In Case 2, we examined the outcome when the population sex ratio equalled the relatedness asymmetry of either the M

colonies (Case 2a) or of the P colonies (Case 2b), as these bound the range of possible values in this case.

### Field collection, sampling and genetic methods

We studied a population of *L. acervorum* in Santon Warren, Thetford Forest, Norfolk, UK. In this population, approximately 20–50% of colonies are polygynous with an average of two to five related queens per colony, of which almost all are singly mated (Chan & Bourke, 1994; Heinze *et al.*, 1995; Bourke *et al.*, 1997; Chan *et al.*, 1999; Hammond *et al.*, 2001, 2002). Sex ratios are split, with monogynous colonies producing mainly females and polygynous colonies producing mainly males (Chan & Bourke, 1994; Chan *et al.*, 1999). Whole colonies were collected on 3 and 10 June 1999 ('SD99' colonies) and 24 May 2001 ('SD01' colonies) using methods described by Chan & Bourke (1994). Colonies from the SD99 sample were frozen shortly after collection so their composition and social structure reflected that found in the field. Colonies from the SD01 sample were frozen after 8 weeks in artificial nests (Bourke, 1991) in the laboratory. From the SD99 sample, 20 colonies were selected for the investigation of male parentage (these were the same colonies whose detailed genetic structure and primary sex ratios were investigated by Hammond *et al.*, 2001, 2002). From the SD01 sample, two monogynous and male-producing colonies were selected. Thirteen of the total sample of 22 colonies (11 SD99 and two SD01 colonies) were monogynous or, in the case of one SD99 colony, recently monogynous and now queenless. The remaining nine colonies were polygynous with two to eight mated queens per colony (Hammond *et al.*, 2001).

The detailed genetic structure of the 20 SD99 colonies was determined by typing samples of adults and brood at microsatellite loci (Hammond *et al.*, 2001). These samples included mated, dealate queens (queens heading colonies and that have shed their wings, henceforth 'queens'), each queen's mate's sperm as extracted from her sperm receptacle ('sperm'), adult workers ('old workers'), worker pupae ('new workers'), adult males and male pupae ('adult males'), and eggs and first-instar larvae ('eggs'). From the two SD01 colonies, we genotyped dealate queens ( $n = 2$ ), their mates' sperm ( $n = 2$ ), old workers ( $n = 5$ ) and adult males ( $n = 92$ ). Since *L. acervorum* males take 1 year to develop from egg to adult (Buschinger, 1973), queens and old workers were potential mothers of male eggs and, from eggs laid the previous year, the adult males. Queens were the only potential mothers of old and new workers. For both haploid and diploid offspring, some individuals could have derived from queens that had died ('lost queens'), given the queen turnover in the study population (Bourke *et al.*, 1997).

We typed individuals at the microsatellite loci LXAGT1, LXAGA1, LXAGA2 (Bourke *et al.*, 1997), MYRT3 (Evans, 1993) and L18 (Foitzik *et al.*, 1997). In the Santon

Warren population, these loci have a mean expected heterozygosity of 0.88 and an average of 23 alleles per locus (Hammond *et al.*, 2001). We genotyped eggs at one to five loci and all other individuals at four to five loci using methods described by Hammond *et al.* (2001). In monogynous colonies ( $n = 13$ ) we typed a per colony average ( $\pm$ SD) of  $0.9 \pm 0.3$  queens,  $11.0 \pm 5.8$  old workers,  $9.3 \pm 7.7$  new workers,  $18.6 \pm 14.9$  adult males and  $48.2 \pm 24.9$  eggs. In polygynous colonies ( $n = 9$ ), we typed a per colony average ( $\pm$ SD) of  $3.7 \pm 1.8$  queens,  $12.8 \pm 5.1$  old workers,  $13.9 \pm 5.3$  new workers,  $9.9 \pm 6.2$  adult males and  $52.7 \pm 16.7$  eggs. Haploid, male eggs were identified as those appearing homozygous at four loci (Hammond *et al.*, 2002).

### Relatedness

We calculated regression relatedness from the genotype data with the program `RELATEDNESS` 5.07 (Goodnight Software: <http://gsoft.smu.edu/Gsoft.html>), which is based on algorithms developed by Queller & Goodnight (1989). We estimated population allele frequencies from a larger sample of dealate queens ( $n = 67$ ) collected in 1999 (Hammond *et al.*, 2001). We estimated standard errors of relatedness values by jackknifing over loci (relatednesses are quoted  $\pm 1$  standard error), and we tested differences between relatedness coefficients using two-tailed *t*-tests.

To generate predictions about the parentage of males based on comparing relatedness values, we measured relatedness among old workers and between old workers and queens. If workers are more closely related to one another than to queens, we predict successful worker male-production because workers are then more closely related to potential worker-produced male offspring than to potential queen-produced male offspring (e.g. Arévalo *et al.*, 1998). In addition, to supplement parentage analyses, we measured the relatedness of queens and old workers to male eggs and adult males that were actually present in the colonies.

### Parentage of males

We assigned male parentage to queens or old workers by comparing the multilocus genotypes of male eggs and adult males with those of queens, their mate's sperm and old workers. All SD99 queens included in this study were singly mated (Hammond *et al.*, 2001). The sperm genotypes of both SD01 queens were also compatible with single mating. We therefore identified worker-produced males as those bearing a paternal allele at one locus or more (e.g. Walin *et al.*, 1998). Since a worker-produced male has a probability of 0.5 of inheriting a maternal or a paternal allele at each locus, we calculated frequencies of worker-produced males corrected for the possibility of nondetection using eqn 2 of Foster *et al.* (2001) modified for single mating.

In monogynous colonies it was straightforward to assign males to queens or workers because the genotype of the queen was known and her mate's genotype was determined using a combination of sperm genotyping and deduction from the genotypes of diploid offspring (Hammond *et al.*, 2001). In polygynous colonies it was less straightforward to assign male parentage because there were more potential mother queens and queen turnover could have occurred (Bourke *et al.*, 1997). In some cases (three queens from three colonies) we could reconstruct the genotypes of lost queens because the genotypes of diploid offspring grouped into full sisterhoods (Hammond *et al.*, 2001). In these cases lost queens and their old, daughter workers were considered as potential mothers of adult males. In all polygynous colonies we assigned males to maternal lineages on the basis of their genotype. Maternal lineages were defined as groups of males that were genetically compatible with being the offspring of a queen (including lost queens) or her worker progeny. Males for which this procedure was successful were then classified, as in monogynous colonies, into those compatible with being queen-produced and those compatible with being worker-produced because they shared an allele with the queen's mate. (The genotypes of the mates of queens were again identified from sperm genotyping and the genotypes of diploid offspring.) Using this approach it is impossible to distinguish between males that are the offspring of daughter workers of a resident queen and males that are the offspring of a lost queen that is the daughter of the same resident queen (the daughter workers and lost queen then being full sisters). However, because it would require a daughter queen to die prior to her mother's death, this situation is likely to be rare. If it occurred, it would have led us to overestimate the frequency of worker-produced males in the polygynous colonies. In some cases (nine male eggs from five colonies and 13 adult males from six colonies) males could not be assigned to maternal lineages and were considered to be of ambiguous parentage (males whose genotypes were compatible with their being the offspring of resident queens and their worker offspring or lost queens and their worker offspring) or of unknown parentage (males whose genotypes were incompatible with their being the offspring of resident queens and their worker offspring or with reconstructed lost queens and their worker offspring).

## Results

### Model results

In monogynous colonies, there is no effect of split sex allocation on the conditions for the evolution of worker reproduction relative to when sex ratios are unsplit provided the population sex ratio equals the relatedness asymmetry of the monogynous colonies (Cases 1 and 2a in Tables 1 and 2). Laying workers and nonlaying

Case	Fitness term	Monogynous colony		Polygynous colony	
		Fitness return to laying worker	Fitness return to nonlaying worker	Fitness return to laying worker	Fitness return to nonlaying worker
1	$IF_{QF}$	$(3q + 1)/4$	$(3q + 1)/4$	NA	NA
	$IF_{QM}$	$(3q + 1)/4$	$(3q + 1)/4$	$r_M(3q + 1)$	$r_M(3q + 1)$
	$IF_{WM}$	$(3q + 1)/2$	$3(3q + 1)/8$	$(3q + 1)/2$	$r_F(3q + 1)/2$
	$IF_{WM}/IF_{QF}$	2	1.5	NA	NA
	$IF_{WM}/IF_{QM}$	2	1.5	$1/2r_M$	$r_F/2r_M$
2a	$IF_{QF}$	$(3q + 1)/4$	$(3q + 1)/4$	NA	NA
	$IF_{QM}$	NA	NA	$r_M(3q + 1)$	$r_M(3q + 1)$
	$IF_{WM}$	$(3q + 1)/2$	$3(3q + 1)/8$	$(3q + 1)/2$	$r_F(3q + 1)/2$
	$IF_{WM}/IF_{QF}$	2	1.5	NA	NA
	$IF_{WM}/IF_{QM}$	NA	NA	$1/2r_M$	$r_F/2r_M$
2b	$IF_{QF}$	$3(Rq + 1)/4R$	$3(Rq + 1)/4R$	NA	NA
	$IF_{QM}$	NA	NA	$r_F(Rq + 1)/R$	$r_F(Rq + 1)/R$
	$IF_{WM}$	$(Rq + 1)/2$	$3(Rq + 1)/8$	$(Rq + 1)/2$	$r_F(Rq + 1)/2$
	$IF_{WM}/IF_{QF}$	$2r_F/3r_M$	$r_F/2r_M$	NA	NA
	$IF_{WM}/IF_{QM}$	NA	NA	$1/2r_M$	$r_F/2r_M$
3	$IF_{QF}$	$3(Rq + 1)/4R$	$3(Rq + 1)/4R$	$r_F(Rq + 1)/R$	$r_F(Rq + 1)/R$
	$IF_{QM}$	NA	NA	$r_F(Rq + 1)/R$	$r_F(Rq + 1)/R$
	$IF_{WM}$	$(Rq + 1)/2$	$3(Rq + 1)/8$	$(Rq + 1)/2$	$r_F(Rq + 1)/2$
	$IF_{WM}/IF_{QF}$	$2r_F/3r_M$	$r_F/2r_M$	$1/2r_M$	$r_F/2r_M$
	$IF_{WM}/IF_{QM}$	NA	NA	$1/2r_M$	$r_F/2r_M$

**Table 1** Inclusive fitness returns from worker male-production when sex ratios are split.

Case 1: M colonies produce both sexes, P colonies produce all males and the population sex ratio equals the relatedness asymmetry of M colonies; Case 2: M colonies produce all females, P colonies produce all males and the population sex ratio equals the relatedness asymmetry of either the M colonies (Case 2a) or of the P colonies (Case 2b); Case 3: M colonies produce all females, P colonies produce both sexes and the population sex ratio equals the relatedness asymmetry of P colonies;  $IF_{QF}$ ,  $IF_{QM}$ ,  $IF_{WM}$  are the per capita inclusive fitness gains to workers from rearing a queen-laid female, a queen-laid male and a worker-laid male, respectively;  $q$  is the fraction of all fitness from females from dispersing females;  $r_F$  is the workers' average relatedness to queen-produced females in polygynous colonies;  $r_M$  is the workers' average relatedness to queen-produced males in polygynous colonies;  $R = r_F/r_M$ ; NA, not applicable.

workers are always selected to favour worker-produced males over queen-produced males (and females), and by doing so they increase their fitness return by a factor of 2 or 1.5, respectively (Tables 1 and 2). This is because the population sex ratio is at the monogynous colonies' equilibrium value of 3 : 1 females : males, as in the unsplit case (see Methods section).

In polygynous colonies, there is no effect of split sex allocation on the conditions for the evolution of worker reproduction (Cases 1–3 in Tables 1 and 2). Laying workers always favour worker reproduction, since  $r_M$  in polygynous colonies is always less than 1/2. Nonlaying workers favour worker reproduction if  $r_F/r_M > 2$  (Tables 1 and 2). If there are  $N$  queens related by  $G, r_F/r_M = \{[3 + (N - 1)G]/4N\}/\{[1 + (N - 1)G]/4N\}$  (Pamilo, 1990), and hence this condition becomes  $N < 1 + 1/G$ , which is the standard condition for worker reproduction under polygyny (Pamilo, 1990; Bourke & Franks, 1995). Sex ratio splitting does not affect this condition either because polygynous colonies produce all males anyhow (Cases 1 and 2a) or because the population sex ratio is at the polygynous colonies' equilibrium value (Cases 2b and 3).

However, for monogynous colonies in Cases 2b and 3, in which the population sex ratio equals the relatedness asymmetry of the polygynous colonies, conditions for worker reproduction are altered relative to the unsplit case. Laying workers and nonlaying workers favour worker reproduction when  $r_F/r_M > 1.5$  and  $r_F/r_M > 2$  respectively (Tables 1 and 2). Hence, if  $r_F/r_M < 1.5$ , workers in monogynous colonies should refrain from laying male eggs due to self-restraint. If  $1.5 < r_F/r_M < 2$ , laying workers should lay male eggs but nonlaying workers are selected to police them. These results arise because in these cases (i) workers are replacing female eggs only and (ii) males have become relatively less valuable because of the fall in the degree of female-bias of the population sex ratio to the polygynous colonies' equilibrium level. Note that the term that determines the behaviour of the workers in the monogynous colonies ( $r_F/r_M$ ) is a property of the relatedness structure of the polygynous colonies; it influences the monogynous colonies because of the effect of the polygynous colonies on the population sex ratio and the preferred sex of the monogynous colonies. For the model's predictions to be

**Table 2** Summary of predictions of model for worker reproduction when sex ratios are split, showing conditions for laying workers and nonlaying workers to favour self-restraint, worker reproduction ('lay' or 'tolerate laying') or worker policing; cases defined as in text and Table 1 legend;  $r_F/r_M$  is the average relatedness to queen-produced females/average relatedness to queen-produced males, for workers in the polygynous colonies.

Case	Colony class	Laying worker	Nonlaying worker
1	Monogynous	Always lay	Always tolerate laying
	Polygynous	Always lay	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$
2a	Monogynous	Always lay	Always tolerate laying
	Polygynous	Always lay	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$
2b	Monogynous*	Lay if $r_F/r_M > 1.5$ Self-restraint if $r_F/r_M < 1.5$	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$
	Polygynous	Always lay	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$
3	Monogynous*	Lay if $r_F/r_M > 1.5$ Self-restraint if $r_F/r_M < 1.5$	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$
	Polygynous	Always lay	Tolerate laying if $r_F/r_M > 2$ Police if $r_F/r_M < 2$

\*Situation in which predictions differ from those applying if split sex ratios were absent.

operative, monogynous workers, therefore, need to be able to assess the colony class they belong to and to have evolved to behave in response to the polygynous colonies' average level of  $r_F/r_M$  and the average relative frequency of the two colony classes in the population. Note also that, although the model assumes workers are unable to discriminate eggs by sex, worker ability to replace male eggs selectively would not alter the model's outcome in Cases 2b and 3. This is because workers have no choice in which sex of eggs they replace, since only queen-laid female eggs are present. The model, therefore, shows that split sex ratios, by selecting against the invasion of worker reproduction, can, in principle, lead to worker self-restraint and worker policing even under

monogyny and monandry, with or without workers being able to replace males selectively.

The model predicts other counter-intuitive outcomes in Cases 2b and 3. For example, when  $r_F/r_M < 1.5$ , potentially laying workers should show self-restraint in monogynous colonies but should not show self-restraint in polygynous colonies. Under these circumstances, the model predicts worker-produced male eggs in polygynous but not in monogynous colonies and therefore, if policing is inefficient, the presence of worker-produced adult males in polygynous colonies and their absence in monogynous colonies.

Finally, we briefly consider what occurs if conditions for worker reproduction (Table 2) are met and its frequency in the population rises. Then both the relative sex-specific reproductive value of males and the average relatedness of workers to males rise, leading to a fall in the class-specific relatedness asymmetries and an increase in the relative male bias of the population sex ratio under worker control (Bourke & Franks, 1995). This makes producing males relatively less valuable, so slowing down the spread of worker reproduction. However, since monogynous colonies would retain a higher relatedness asymmetry than polygynous colonies, sex ratio splitting involving female-only production by the monogynous colony class could be maintained, depending on the mechanism and accuracy with which workers assess their colony class. Therefore, the effect identified in the current model could still apply even when worker reproduction has become established in the population.

### Worker-worker and worker-queen relatedness in *Leptothorax acervorum*

In the SD99 monogynous colonies, genotypes of all diploid offspring were consistent with their being the offspring of one, singly mated queen (Hammond *et al.*, 2001), and the same was true of both SD01 colonies. Old workers were, therefore, significantly more related to one another than to queens ( $r = 0.71$  vs. 0.42;  $t_{18} = 4.87$ ,  $P < 0.001$ ; Table 3). In polygynous colonies,

**Table 3** Average regression relatedness in *Leptothorax acervorum*.

Cohort	Monogynous colonies				Polygynous colonies			
	$r$	SE	$N_C$	$N_I$	$r$	SE	$N_C$	$N_I$
Among old workers	0.71	0.03	10	135, 135	0.28	0.03	8	115, 115
Old workers to queens	0.42	0.05	10	135, 10	0.27	0.01	8	115, 29
Queens to male eggs	0.98	0.01	11	11, 142	0.37	0.07	9	32, 68
Queens to adult males	0.99	0.01	9	9, 150	0.30	0.02	8	24, 86
Old workers to male eggs	0.42	0.05	10	135, 131	0.25	0.02	8	115, 63
Old workers to adult males	0.42	0.06	9	120, 150	0.26	0.03	7	105, 80

$r$ , regression relatedness; SE, standard error;  $N_C$  number of colonies;  $N_I$  number of individuals (first category, second category, in relatedness estimation); only colonies from the SD99 collection were used in the estimation of regression relatedness.

the relatedness among old workers indicated that they were offspring of several queens (Hammond *et al.*, 2001) and this relatedness and the relatedness between old workers and queens were not significantly different ( $r = 0.28$  vs.  $0.27$ ;  $t_{14} = 0.13$ ,  $P = 0.81$ ; Table 3). Therefore, on the basis of comparing relatedness values, worker self-restraint and worker policing should have been absent in monogynous colonies and so we predicted high frequencies of worker-produced male eggs and adult males. In polygynous colonies, worker self-restraint should also have been absent and workers as a whole should have been genetically indifferent to the source of males (i.e. there should have been selection neither for nor against worker policing). Therefore, by default, we predicted intermediate frequencies of worker-produced males.

### Parentage of males in *Leptothorax acervorum*

#### Monogynous colonies

In monogynous colonies, only four of 142 male eggs and five of 242 adult males were assigned to workers (Table 4). The corrected frequencies of worker-produced males among eggs and adults were, therefore, very low and were not significantly different from one another (3.2 vs. 2.3%; Fisher's exact  $P = 0.23$ ; Table 4). Worker-produced male eggs were found in three colonies and worker-produced adult males were found in a further two colonies, i.e. we did not record worker-produced male eggs and adult males in the same colonies. Two of four worker-produced male eggs were from the recently queenless SD99 colony (see Methods). However, all remaining worker-produced males, including the five adult males, came from colonies with a queen.

Consistent with these findings, queens in monogynous colonies were significantly more closely related to both male eggs and adult males than were old workers (male eggs:  $0.98$  vs.  $0.42$ ;  $t_{19} = 9.19$ ,  $P < 0.0001$ ; adult males:

$0.99$  vs.  $0.42$ ;  $t_{16} = 9.34$ ,  $P < 0.0001$ ; Table 3). These results were as expected if queens produced almost all males, as the predicted relatednesses assuming sole queen parentage of males were 1.0 and 0.5, respectively ( $t_8 = 1.33$  and  $t_{10} = 2.00$ , respectively, both  $P > 0.07$ ).

#### Polygynous colonies

In polygynous colonies, two of 68 male eggs and three of 86 adult males were assigned to workers (Table 4). The corrected frequencies of worker-produced males among eggs and adults were also very low and were not significantly different from one another (4.2 vs. 4.6%; Fisher's exact  $P = 0.35$ ; Table 4). Furthermore, these proportions were not significantly different from those found in monogynous colonies (male eggs, 3.2 vs. 4.2%; Fisher's exact  $P = 0.53$ ; adult males, 2.3 vs. 4.6%; Fisher's exact  $P = 0.31$ ; Table 4). Worker-produced male eggs and adult males were found in two colonies each, so again there were no colonies where worker-produced male eggs and adults were found together. Because ambiguous and unknown males (Table 4) were neither more nor less likely to be worker-produced than assigned males, they were omitted from all analyses.

Relatedness calculations in the polygynous colonies also supported the conclusion of the parentage analysis that almost all males derived from queens. In the nine polygynous colonies, harmonic mean queen number (including lost queens) was 3.5 and queen–queen relatedness was 0.20. If all males derived from queens, queen–male relatedness should have equalled 0.43 and worker–male relatedness should have equalled 0.21; if all males derived from workers, queen–male relatedness should have equalled 0.21 and worker–male relatedness should have equalled 0.25 (from regression relatedness formulae in Pamilo, 1990). The predicted worker–male values are too similar to be discriminated with the current data, but the actual value for queens'

	Monogynous colonies*		Polygynous colonies	
	Male eggs	Adult males	Male eggs	Adult males
Colonies	11	11	9	8
Individuals	142	242	68	86
Mean $\pm$ SD	12.9 $\pm$ 8.1	22.0 $\pm$ 13.6	7.6 $\pm$ 3.5	10.8 $\pm$ 5.7
Assignable males ( $N_a$ )	125.53	220.25	47.3	65.9
Ambiguous parentage	0	0	6	3
Unknown parentage	0	0	3	10
Worker produced ( $N_w$ )	4	5	2	3
Percentage worker produced ( $N_w/N_a$ )	3.2	2.3	4.2	4.6

\*SD99 and SD01 colonies were included.

$N_a$  is the number of males corrected for the probability of not detecting worker-produced males (see Methods section); see text for definition of 'ambiguous' and 'unknown' males; worker-produced males were defined as those possessing a paternally derived allele at one locus or more.

**Table 4** Summary of male parentage in *Leptothorax acervorum*.

relatedness to male eggs (the value most likely to minimize effects of queen turnover; Table 3) matched far more closely the predicted value assuming sole queen parentage of males (0.37 vs. 0.43:  $t_8 = 0.86$ ,  $P = 0.42$ ) than the predicted value assuming sole worker parentage of males (0.37 vs. 0.21,  $t_8 = 2.29$ ,  $P = 0.052$ ).

## Discussion

### Male parentage in *Leptothorax acervorum*

In a facultatively polygynous population of the ant *L. acervorum* we found evidence for successful worker reproduction in the presence of the queen or queens but at very low frequencies. Queens produced almost all male eggs and adult males (95–98%) in both monogynous and polygynous colonies. However, relatedness estimates predicted a high frequency of successful worker reproduction in monogynous colonies and overall worker indifference to the source of males in polygynous colonies. Our findings, therefore, contradicted expectations based on a comparison of relatedness values, particularly in monogynous colonies.

The very low frequencies of worker-produced male eggs (3.2–4.2%) that we detected in both monogynous and polygynous colonies suggest that workers in both colony classes exercise self-restraint. Given these low frequencies, any policing of worker-produced eggs during their development to adulthood by either queens or workers would have been hard to detect. However, our egg sample conceivably contained a bias of older eggs, since DNA in older eggs could be more likely to be amplified than in young eggs containing fewer cells (16.4% of eggs failed to amplify). In other species, policed eggs tend to be eaten shortly after being laid (Ratnieks & Visscher, 1989; Monnin & Peeters, 1997; Foster & Ratnieks, 2000, 2001a). Therefore, our measured frequency of worker-produced eggs possibly underestimated the frequency of worker egg-laying and of policing. Nonetheless, it seems likely that both queen policing and worker policing are absent or rare in the study population. This view is supported by behavioural observations suggesting that *L. acervorum* queens and workers are rarely aggressive to one another and specifically not to egg-laying workers (Bourke, 1991; Heinze *et al.*, 1997), although policing of eggs may, in principle, occur independently of aggression towards adults. The likely occurrence of self-restraint in polygynous colonies is consistent with behavioural observations of low worker egg-laying rates in such colonies (Bourke, 1991). Our finding of very few worker-produced adult males in a UK *L. acervorum* population also matches the finding of Heinze *et al.* (1997) that such males were absent from a sample of 17 adult males from two colonies collected from a German population and typed at one to two allozyme loci, as well as previous genetic studies suggesting low levels of worker parentage of adult males in two monogynous *Leptothorax* species, *L. unifasciatus* and

*L. nylanderii* (Heinze *et al.*, 1997; Foitzik & Heinze, 2000, 2001).

### Effect of split sex ratios on the evolution of worker reproduction

Our model found that, in monogynous colonies, split sex ratios altered the expected fitness returns from worker egg-laying by changing the population sex ratio and the sex that workers preferred to rear. In the *L. acervorum* study population, the median value of  $r_F/r_M$  is two (Hammond *et al.*, 2002). Applying the model directly (Tables 1 and 2), this predicts that in both colony classes there should have been egg-laying by reproductive workers and indifference between policing and toleration of these eggs by nonlaying workers. (This assumes the population sex ratio is at the polygynous colonies' equilibrium level.) Despite this, our results suggested self-restraint as the cause of the low detected frequencies of worker-produced male eggs and adults in both colony classes. This is an outcome not predicted under any of the cases in our model (Table 2). We therefore suggest that some factor other than colony kin structure and sex ratio effects determines the pattern of male parentage in the study population. A likely factor is a cost to colony productivity of worker reproduction, which, as previous studies have demonstrated, can independently select for worker self-restraint and policing (Cole, 1986; Ratnieks, 1988; Nonacs & Carlin, 1990; Ratnieks & Reeve, 1992; Kikuta & Tsuji, 1999). Such costs are hard to measure but almost certainly present, because phenomena associated with worker reproduction, such as dominance behaviour, recognition errors in egg-eating when egg-eating is discriminatory, or a reduction in foraging activity, reduce colony efficiency (Cole, 1986; Nonacs & Carlin, 1990; Nonacs, 1993; Heinze *et al.*, 1997). Colony-level costs alone may account for our findings, or may reinforce effects on the evolution of worker reproduction caused by split sex allocation.

Note that, within the study population, both classes of colony produced a mixed sex ratio (Chan *et al.*, 1999), whereas split sex ratio theory predicts at least one unisexual class (Boomsma & Grafen, 1990, 1991). This likely occurred due to errors made by workers in assessing their colony class (Ratnieks & Boomsma, 1997). However, even with monogynous colonies raising some males, the effect identified by the model should apply, because most queen-produced offspring replaced by a reproductive worker would still be females, which make up the majority of brood. If sex ratio effects do help contribute to the infrequency of worker reproduction detected in the study *L. acervorum* population, a prediction is that other *L. acervorum* populations that are almost entirely monogynous with unsplit sex ratios (e.g. Chan *et al.*, 1999), but in which costs of worker reproduction are likely to be similar, should have a higher frequency of worker-produced adult males.

The model we have developed potentially applies to other social Hymenoptera with sex ratios split by workers' relatedness asymmetry, a grouping with representatives widely spread across ants, bees and wasps (e.g. Queller & Strassmann, 1998). Male parentage among adult males has been genetically investigated in several species with facultative variation in kin structure (e.g. ants: Sundström *et al.*, 1996; Evans, 1998; Herbers & Mouser, 1998; Walin *et al.*, 1998; Foitzik & Heinze, 2000; bees: Paxton *et al.*, 2001, wasps: Arévalo *et al.*, 1998; Hastings *et al.*, 1998; Goodisman *et al.*, 2002; Henshaw *et al.*, 2002). In general, worker production of adult males was found to be absent or rare irrespective of colony kin structure or sex ratio (where investigated), even when workers were predicted to be reproductive on the basis of relatedness comparisons. In some cases (Sundström *et al.*, 1996; Walin *et al.*, 1998), this could have been due to sex ratio biasing by selective male destruction as suggested previously (Walin *et al.*, 1998; Foster & Ratnieks, 2001b). In other cases, as again suggested in previous studies (e.g. Arévalo *et al.*, 1998), it could be due to unmeasured costs of worker reproduction. However, few studies explicitly examined male parentage as a function of split sex ratios. In two exceptions, Evans (1998) and Herbers & Mouser (1998) found higher worker male-production in male-specialist than in female-specialist colonies of *Myrmica* ants, a finding that could have been consistent with our model's prediction of worker-produced males being present only in the colony class with low relatedness asymmetry under some circumstances (see 'Model results').

Male parentage has previously been investigated in detail among both male eggs and adult males in just a single species with facultative variation in kin structure, the wasp *Dolichovespula saxonica* (Foster & Ratnieks, 2000). In this case, as predicted from relatedness comparisons alone, workers in colonies with a singly mated queen produced a high frequency of adult males whereas there was worker policing of worker-laid male eggs in colonies with a polyandrous queen. The possible effect of split sex ratios on the workers' fitness from worker reproduction was not investigated in this study. Therefore, investigations in other species of the model we have proposed remain to be undertaken. It is nonetheless apparent that testing the predictions of kin selection theory with respect to male parentage when sex ratios are split is more complex than previously thought. This is important because the same species whose facultative variation in colony kin structure makes them likely to exhibit split sex ratios are also the ones likely to be chosen for within-population studies of male parentage (see Introduction). Studies that measure the costs of worker reproduction are evidently also required. Finally, it is clear (cf. Walin *et al.*, 1998; Foster & Ratnieks, 2001b) that, for a full understanding of social evolution, different types of kin-selected phenomena require simultaneous consideration.

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