Reproductive alliances and posthumous fitness enhancement in male ants

Liselotte Sundström[1,2]* and Jacobus J. Boomsma[2,3]

[1]*Department of Ecology and Systematics, Division of Population Biology, University of Helsinki, PO Box 17, FIN-00014 Helsinki, Finland*
[2]*Department of Ecology and Genetics, Institute of Biological Sciences, University of Aarhus, DK-8000 Aarhus C, Denmark*
[3]*Department of Population Ecology, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen East, Denmark*

Ants provide excellent opportunities for studying the evolutionary aspects of reproductive conflict. Relatedness asymmetries owing to the haplodiploid sex determination of Hymenoptera create substantial fitness incentives for gaining control over sex allocation, often at the expense of the fitness interests of nest-mates. Under worker-controlled split sex ratios either the reproductive interests of the mother queen (when workers male bias the sex ratio) or the father (when workers female bias the sex ratio), but never that of both parents simultaneously, are fulfilled. When workers bias sex ratios according to the frequency of queen mating, males which co-sire a colony have a joint interest in manipulating their daughter workers into rearing a more female-biased sex ratio. Here we show that males of the ant *Formica truncorum* achieve such manipulation by partial sperm clumping, so that the cohort-specific relatedness asymmetry of the workers in colonies with multiple fathers is higher than the cumulative relatedness asymmetry across worker cohorts. This occurs because a single male fathers the majority of the offspring within a cohort. Colonies with higher average cohort-specific relatedness asymmetry produce more female-biased sex ratios. Posthumously expressed male genes are thus able to oppose the reproductive interests of the genes expressed in queens and the latter apparently lack mechanisms for enforcing full control over sperm mixing and sperm allocation.

Keywords: reproductive conflicts; kin selection; social insects; male strategies; sex allocation; *Formica*

1. INTRODUCTION

Colonies of social insects comprise groups of related individuals united by the collective interest of maximizing their inclusive fitness through the production of reproductive individuals over the life span of the colony. Nevertheless, individuals within colonies may have different interests regarding the type of offspring to raise (Trivers & Hare 1976; Bourke & Franks 1995; Crozier & Pamilo 1996). Further complexity is added by between-colony differences in the frequency of queen mating and number of queens per colony (Bourke & Franks 1995; Crozier & Pamilo 1996). As a result, the relatedness asymmetry (the difference in the relatedness of workers to sisters versus brothers) will vary, being lower than 3:1 in colonies with multiple, related queens or multiply mated queens. Under these conditions, split sex ratio theory (Boomsma & Grafen 1990, 1991) predicts that those colonies with a high relatedness asymmetry will produce female-biased sex ratios and those with a low relatedness asymmetry will produce male-biased sex ratios. Empirical tests have repeatedly confirmed these predictions showing that the workers control sex allocation and produce the sex of highest kin value to them (e.g. Queller *et al.* 1993; Chan & Bourke 1994; Sundström 1994; Evans 1995; Sundström *et al.* 1996).

When workers in low-asymmetry colonies predictably male bias sex ratios in response to the frequency of queen mating they also increase the reproductive fitness of their mother queen (Ratnieks & Boomsma 1995), but the

males which co-sired the same colony may face complete reproductive failure (Boomsma 1996). This is because, owing to arrhenotoky, males never father sons and so their fitness is zero when their daughter workers only raise new workers and males. Relatedness-induced, split sex ratios therefore induce a reproductive conflict between queens and males regarding mating frequency and post-mating sperm use (Boomsma 1996).

How is this queen–male conflict regarding mating frequency resolved? Males of social Hymenoptera typically die soon after their nuptial flight, whereas the queens in some ant species may live up to 30 years (Pamilo 1991*a*). The sperm received during a single nuptial flight lasts the entire life span of the queen (Höll-dobler & Wilson 1990). Although population-wide operational sex ratios may be female biased (Sundström 1994, 1995) males are unlikely to encounter many females during their limited life span because their nuptial flights are brief and individual females are only available during a short time. Therefore, avoiding non-virgin females altogether may not be an option for males.

When male ants come to share paternity in a colony founded by a multiply mated queen, they have two ways in which they can posthumously increase their fitness. First, a model developed by Boomsma (1996) predicts that 'second-male continence' may evolve under intermediate, population-wide, mating frequencies. In this scenario males which are second in a mating sequence are predicted to transfer only small amounts of sperm so that the relative relatedness asymmetry of the ensuing colony increases above the critical threshold for producing females. Second, males may evolve sperm clumping so

that the use of the sperm of single males varies significantly across cohorts of diploid offspring (Trivers & Hare 1976; Crozier & Brückner 1981; Boomsma 1996; Boomsma & Ratnieks 1996). In the extreme case, males would alternate in fathering clutches of offspring. This would lead to a short-term, queen-mating frequency of one and, hence, a maximal average relatedness asymmetry within cohorts, despite a low relatedness asymmetry across cohorts. However, even if sperm clumping is only partial, it will still increase male fitness if an increased short-term relatedness asymmetry causes workers in colonies headed by multiply mated queens to invest more in females than predicted from the split sex-ratio theory (Boomsma 1996). It could therefore pay for males to cooperate and alternate their paternity across cohorts of diploid offspring in order to increase the relatedness asymmetry within single cohorts.

We analysed comparative data on paternity skew across species of *Formica* ants in a previous study (Boomsma & Sundström 1998) and showed that the average paternity skew in half-sib colonies (i.e. those headed by multiply mated queens) covaries with the frequency of multiple mating in the population. In this paper, we present, to the authors' knowledge, the first analysis of temporal variation in the paternity skew in colonies headed by multiply mated queens in a population of ants where relatedness-induced, split sex ratios are known to occur. Furthermore, we assess the degree to which workers in half-sib colonies adjust the sex ratio in response to subtle differences in relatedness asymmetry, the prediction being that colonies with higher paternity skew and, thus, higher relatedness asymmetry produce more female-biased broods. Finally, we compare the fitness of males which sire a colony alone with that of males which co-sire a colony. Our data come from nine colonies of the ant *Formica truncorum* headed by doubly or triply mated queens. The colonies were surveyed over a period of ten years and the relative paternity contributions were assessed for the separate cohorts of worker broods.

2. MATERIAL AND METHODS

(a) *Study species and samples*

In a Finnish population of the ant *F. truncorum*, the colonies have a single queen and the sex ratios are split in accordance with the frequency of queen mating. As a result colonies headed by singly mated queens mainly produce females, whereas those headed by multiply mated queens mainly produce males (Sundström 1994, 1995; Sundström & Ratnieks 1998). Based on the year-to-year consistency of worker genotypes, all colonies were inferred to be headed by the same queen over the entire study period.

After hibernation, the queens lay eggs from early May to early June. An early-laid fraction of these eggs gives rise to new sexuals which eclose in mid-July, whereas a later fraction gives rise to workers which eclose approximately two weeks after the sexual brood. No within-year differences in the patriline contribution to sexual versus worker brood were found in a previous study on this population (Keller *et al.* 1997). Marking experiments (Sundström 1995; L. Sundström, unpublished data) have shown that workers normally hibernate only once, so adults alive in June–July eclosed the previous summer.

Samples for electrophoresis were collected together with data on the colony sex ratios and total sexual production. Worker pupae were collected in most years except in 1993 and 1995 when adults were collected the following June. Only adults were genotyped in one colony (Jsk 2) as the allozyme locus αGpdh, which was diagnostic for discriminating between patrilines in this particular colony, only stained in adults. One colony (Rh 1) produced diploid males. This was because the queen and one of the fathers carried identical alleles at the sex-determining locus causing 50% of the diploid individuals to have a male phenotype. Including this colony in the analyses may have biased the results, so we omitted it from all analyses except for the analysis of the variation in paternity shares between cohorts which is not affected by the 50% reduction in one patriline across all cohorts.

An unbiased estimate of paternity representation requires there being no differential mortality between patrilines. To validate this assumption, we also collected adult workers from all colonies over the years 1994–1998. The genotype frequencies of adults collected in June–July were compared to those among pupae in the previous year. This allowed us to track changes in the genotype frequencies within single cohorts of workers, which would most probably be due to differential survival of patrilines, e.g. during hibernation.

Data available from previous studies (Sundström 1994, 1995; Sundström & Ratnieks 1998) on the colony sex ratios and total sexual production were augmented with data from three more years (1997–1999). In total, 35 colonies were found during the entire study period and on average 24 colonies were alive in each year. Thirty-three of these colonies were genotyped (30–100 individuals per year and colony). The overall proportion of multiply mated queens was 39%, whereas the population-wide effective mating frequency (taking into account unequal paternity shares) across years was 1.29 ± 0.05 ($n = 8$). The colony sex ratios were determined from a sample of 20–100 pupae and the total sexual production was assessed as described in Sundström (1995).

(b) *Genotyping procedures*

Horizontal starch gel electrophoresis was used to separate alleles at four different polymorphic allozyme loci (αGpdh, Idh, Pgk and Aco) as described in Sundström (1993) and Seppä (1992). An additional combined analysis of the allozyme and microsatellite data (three loci, FL12, FL20 and FL21) (Chapuisat 1996) for the colonies assessed as doubly mated led to the detection of two triply mated queens (L. Sundström, unpublished data). Previously, three other triply mated queens had been identified. One of those colonies had died and is not included here and in the other two the third fathers were found at very low frequencies in one year only and have not been found since. In addition, the proportion of offspring fathered by the third male was very small in the newly found, triply mated colonies (two out of 22 in Mskl1 and two out of 15 in Sh6). Since the microsatellite data were available for only one year we have combined the rare third patrilines with those from which they were indistinguishable with allozyme data.

(c) *Statistical procedures*

The heterogeneity in the paternity contributions between years was tested with a *G*-test for each colony separately. The paternity skew ($= \Sigma p_i^2$) was calculated following Pamilo (1993) with a correction for a finite sample size, such that

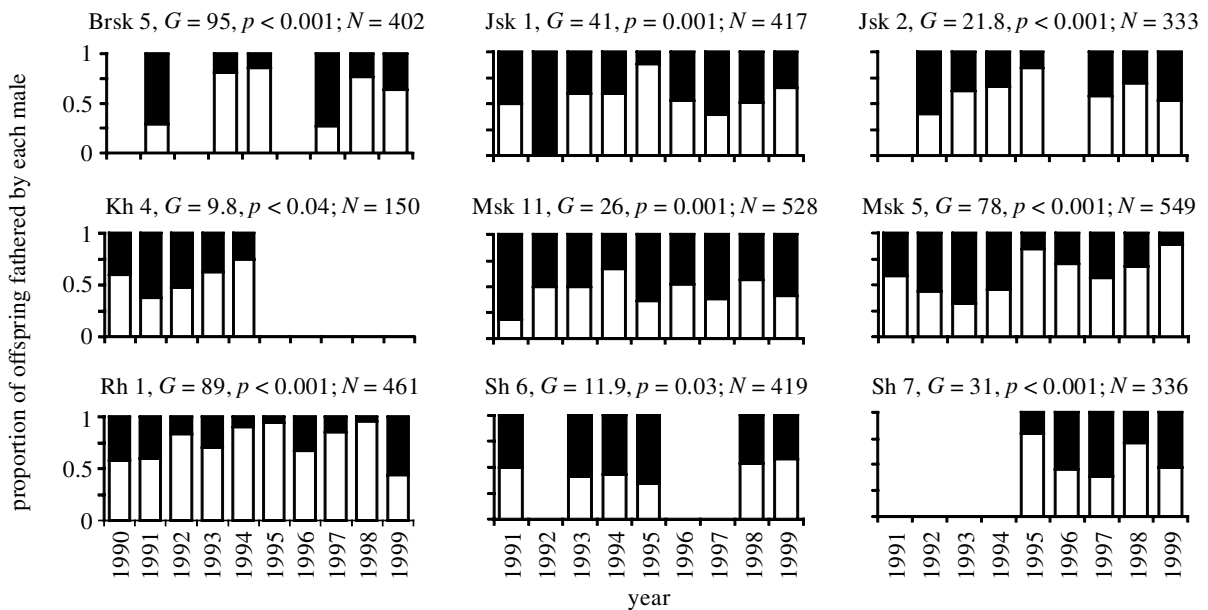$$\sum p_i^2 = \left(\mathcal{N} \sum y_i^2 - 1 \right)/(\mathcal{N} - 1), \tag{1}$$

Figure 1. Cohort-specific paternity shares for the nine study colonies. The nest code, *G*-values, significance and total number of workers analysed are given above each panel. The majority male across cohorts and, in cases Msk11 and Sh6, the certain single patriline is shown in white.

where y_i is the proportion of offspring fathered by the *i*th male and N is the number of individuals genotyped. The probability of not detecting third fathers was extremely low due to the large number of marker loci (seven) and their high variability (the probability of two males carrying an identical genotype at all loci was 1×10^{-5}).

The variance in the paternity skew (S) across cohorts was tested by calculating the deviation (D) from a homogeneous distribution across years for each colony, then averaging D over all colonies and finally constructing the 99% confidence intervals to test for a deviation from zero.

$$D = (S_{mean} - S_{pooled})/S_{pooled}, \qquad (2)$$

where S_{mean} is the paternity skew averaged across cohorts of the same colony and

$$S_{pooled} = \left[\left(\sum p_1 \right)/x \right]^2 + \left[\left(\sum p_2 \right)/x \right]^2, \qquad (3)$$

where Σp_1 and Σp_2 are the paternity shares of fathers 1 and 2 summed across the cohorts of a colony and x is the number of cohorts. The calculation of D is analogous to that for testing the allele frequency variance between subpopulations with Wright's (1951) *F*-statistics.

The total production of reproductives did not differ significantly between colonies headed by singly versus multiply mated queens (Sundström & Ratnieks 1998) and worker reproduction is absent in this species (Walin *et al.* 1998). Thus, simplifying the general inclusive fitness expressions of Pamilo (1991*b*) and Boomsma & Grafen (1991), male fitness can be expressed as

$$W_{m_i} = f_i/n_i F, \qquad (4)$$

where the subscript *i* refers to a specific father or his colony, *f* is the proportional investment in reproductive females in the focal colony, *F* is the population-wide proportional allocation to reproductive females and *n* is the effective number of fathers sharing paternity in focal colony *i*. Equation (4) was used to compare the fitness of males siring a colony alone with that of males co-siring a colony using the population-wide *F*-values for each year.

Finally, the association between relatedness asymmetry and sex allocation was tested with a model I linear regression analysis having ascertained that the residuals of the regression analysis were normally distributed (Wilk–Shapiro rankit plot, $r = 0.97$).

3. RESULTS

We found no evidence of differential survival between patrilines within the same colony. The patriline representations among worker pupae in one year and among adult workers the next year were practically identical in most cases with χ^2-values ranging from 0 to 6.18 (see electronic Appendix A at The Royal Society Web site). We found a significant deviation between the pupal and adult genotype frequencies in only one case (Jsk 1) ($\chi^2 = 6.18$ and $p = 0.01$), but this significance disappeared after a Bonferroni correction ($\alpha' = \alpha/k = 0.005$, where k is the number of tests). We therefore used adults collected in June–July in order to complement missing pupal cohorts for the years 1993 (all colonies) and 1995 (colonies Jsk1, Msk11, Msk5, Rh1 and Sh7).

We found significant between-cohort variance in the patriline representations in all nine colonies (figure 1). The average (mean ± 99% CI) magnitude of variation in the paternity for the majority male, expressed as the coefficient of variation (CV), was $31 \pm 9\%$ ($n = 8$) (see electronic Appendix A) (colony Rh1 excluded from now on). The average paternity skew of 0.57 ± 0.04 (mean ± 99% CI) was also significantly higher than the pooled paternity skew of 0.51 ± 0.01, so that D was estimated as 0.10 ± 0.06 (electronic Appendix A). Hence, the variance in the paternity skew across years was higher than expected for a homogeneous distribution.
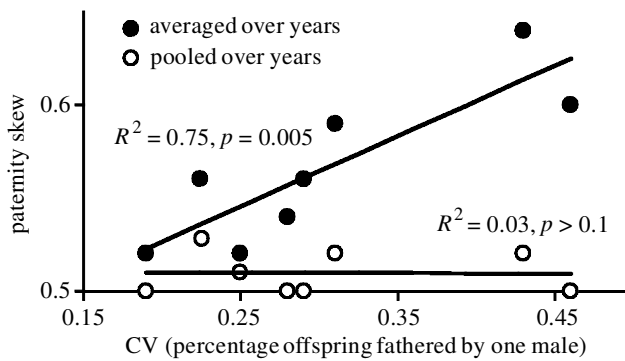
Figure 2. The relationships between the variance in paternity skew (given as the coefficient of variance, CV), the average and the pooled paternity skew.
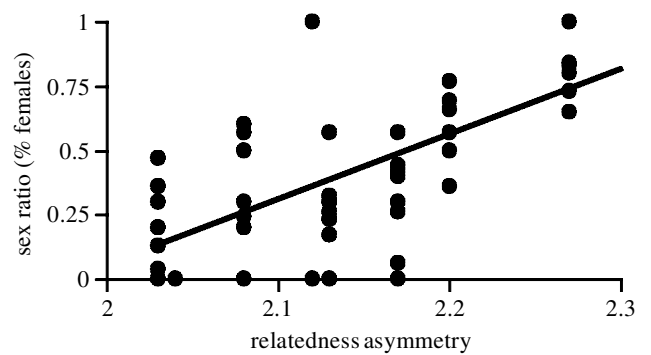

Figure 3. The relationship between the colony-specific average relatedness asymmetry and yearly sex ratios ($r^2 = 0.40$, $F_{1,7} = 31$ and $p < 0.001$).

Table 1. *Population-wide averages for sexual production, sex ratio, the fraction of females produced and male fitness for colonies headed by singly versus multiply mated queens (colony Rh 1 included).*

(Male fitness was calculated using numerical sex ratios.)

	1992[a]	1994[a]	1995[a]	1997	1998	1999	grand	t	d.f.	p
number of reproductives										
single mated	4182 ± 1879	1539 ± 1006	3584 ± 2278	3831 ± 2780	3171 ± 2344	3299 ± 2911	326 ± 8922	-0.48	5	> 0.1
double mated	4030 ± 2632	3152 ± 1204	2303 ± 1967	3848 ± 2216	3343 ± 1531	3972 ± 878	3441 ± 661			
sex ratio[b]										
single mated	0.82 ± 0.22	0.73 ± 0.39	0.74 ± 0.32	0.72 ± 0.33	0.76 ± 0.32	0.60 ± 0.45	0.73 ± 0.08	5.9	5	0.002
double mated	0.22 ± 0.30	0.22 ± 0.26	0.51 ± 0.38	0.41 ± 0.35	0.52 ± 0.38	0.42 ± 0.35	0.40 ± 0.05			
percentage of all females[c]										
all colonies	62	46	75	62	67	49	60 ± 11			
single mated	75	74	79	83	74	72	76 ± 3	15.75	5	< 0.0001
double mated	25	26	21	17	26	28	24 ± 4			
male fitness										
single mated	1.28 ± 0.41	1.43 ± 0.97	0.96 ± 0.46	1.11 ± 0.58	1.08 ± 0.52	1.19 ± 0.93	1.18 ± 0.16	9.31	5	0.0002
double mated	0.22 ± 0.22	0.18 ± 0.25	0.32 ± 0.26	0.30 ± 0.27	0.37 ± 0.27	0.39 ± 0.36	0.30 ± 0.08			

[a] Includes all colonies in the population and therefore differs from averages given in Sundström & Ratnieks (1998).
[b] Investment ratios.
[c] The operational sex ratio calculated from the total number of females and males produced in the population.

The average skew (S_{mean}) increased linearly with the variation in the paternity skew (figure 2). This suggests that a high average skew and, thus, a high relatedness asymmetry across cohorts is found specifically in colonies which show a high degree of alternation in paternity. The pooled paternity skew (S_{pooled}) is close to 0.5 throughout the range of observed variances and we found no correlation between the pooled skew and the variation in the skew (figure 2). This suggests that the males mating with the same queen transfer approximately equal amounts of sperm.

Analysis of covariance (Sokal & Rohlf 1995) on the arcsine-transformed data for the cohort-specific relatedness asymmetry (assessed from pedigree data) and sex ratio revealed significantly different intercepts between colonies ($F_{7,43} = 5.8$ and $p < 0.0001$), as well as significantly different slopes between colonies ($F_{7,36} = 4.2$ and $p = 0.002$). Because the slopes were different we could not use an ANCOVA to test for the effects of the colony-specific, yearly relatedness asymmetries on the sex ratios.

The fact that the slopes differed significantly between groups suggests that the colony-specific, yearly sex ratios did not track the year-to-year variance in the relatedness asymmetries.

However, we found a significant positive association between the average colony-level relatedness asymmetry and the mean proportional investment in females ($r^2 = 0.40$, $F_{1,7} = 31$ and $p < 0.001$, corrected for repeated sampling of the same colony following Sokal & Rohlf (1995, pp. 476–486)) (figure 3). Hence, colonies headed by multiply mated queens with a higher average relatedness asymmetry (S_{mean}) owing to a higher average paternity skew produced a more female-biased brood.

The fitness of males fathering each of the two classes of colony (singly and multiply mated) is directly proportional to the fraction of daughters they contribute to the mating pool (equation (4)). On average $76 \pm 3\%$ (mean $\pm 99\%$ CI) ($n = 6$ years) of all females in the population were produced by colonies headed by singly mated queens, whereas $24 \pm 4\%$ of all females were produced by

colonies headed by multiply mated queens (table 1). Taking the relative mating success of these daughters into account, equation (4) yields an almost fourfold (1.18 : 0.3 = 3.9) fitness differential in favour of single fathers (table 1).

4. DISCUSSION

To the authors' knowledge, this is the first study to quantify the fitness of male ants in the case where workers facultatively bias sex ratios in response to the frequency of queen mating. The results show that males which sire a colony alone have a fourfold fitness advantage compared to those which co-sire a colony. This is a slight underestimate because the third males were not considered separately. Approximately 80% of this fitness loss is due to facultative sex-ratio biasing by workers in response to the frequency of queen multiple mating, whereas only 20% is due to sharing paternity. Hence, the fitness of colony fathers largely depends on the sex-ratio preferences of their worker daughters.

The fitness of males which co-sired a colony was above that expected from the split sex-ratio theory. Given that 39% of the queens in the study population were multiply mated, a 10% female investment would be expected according to the pure split sex-ratio model of Boomsma & Grafen (1991), but the average observed investment in females was 40% (table 1). The magnitude of the difference between the observed and expected average sex ratios of the multiply mated class of colony is too large to be explained under a pure split sex-ratio scenario given relatedness asymmetry classes of 3:1 (the colonies with singly mated queens) and 2.13:1 (the colonies with multiply mated queens). However, quantitative predictions for split sex ratios show that shifts in the observed magnitude can occur if workers make errors in assessing the relatedness asymmetry of their colony and if selection has fine-tuned the sex allocation responses to compensate for this (Ratnieks & Boomsma 1997).

The present results confirm previous suggestions (Ratnieks & Boomsma 1997) that there are limitations to the accuracy with which *F. truncorum* workers can assess small differences in relatedness asymmetry because the sex ratios did not track the yearly fluctuations in the paternity skew. This is not surprising considering that each worker is exposed to three different cohorts of siblings: the workers which reared them, their own cohort and the siblings they help rear. Therefore, the cues available to the workers for assessing small changes in colony kin structure are highly noisy and, thus, the best solution is probably to respond to some running average relatedness asymmetry rather than imprecise estimates of relatedness asymmetry at any given moment.

The results show that males mated to the same queen are represented by fairly equal amounts of sperm in the spermatheca. This is not consistent with the conditional second-male continence hypothesis (Boomsma 1996) in which second males are predicted to transfer only small amounts of sperm. However, this lack of fit is not surprising since one of the assumptions of the second-male continence hypothesis was error-free assessment of the relatedness asymmetry causing a stepwise shift from all-male to all-female broods. Both the 30% sex-ratio

deviation discussed above and the observed gradual increase in female bias with increasing average relatedness asymmetry (figure 3) reject this assumption. Another explanation for the second-male continence hypothesis not being substantiated could be that males cannot discriminate between virgin and mated queens.

Instead, the present results are consistent with some degree of sperm clumping combined with fairly equal, long-term representation of males among colony offspring. This is demonstrated by both the colony-wise *G*-tests for heterogeneity in the paternity shares across cohorts and by the overall heterogeneity in the paternity skew which yielded a *D*-value different from zero. Sperm clumping as a mechanism of enhancing male fitness does not depend on the assumption of an error-free assessment of relatedness asymmetry (Boomsma 1996). It only requires that workers respond to small increases in relatedness asymmetry by producing more female-biased sex ratios. This condition is fulfilled, as workers of *F. truncorum* respond to differences in relatedness asymmetry in a gradual way rather than by a step function (figure 3). Hence, males apparently actively but posthumously prevent their sperm from mixing with other sperm in the spermatheca, thus increasing their fitness because their daughter workers rear a more female-biased sex ratio.

However, the alternation of paternity was far from complete (figure 1), suggesting that queens oppose this male strategy by actively mixing the sperm. Active sperm mixing is in the evolutionary interests of the queens (Ratnieks & Boomsma 1995; Boomsma 1996; Sundström & Ratnieks 1998) as colonies with equal paternity are predicted to rear a (male-biased) sex ratio closer to the queen optimum (Boomsma & Grafen 1991). Hence, the current outcome probably reflects a manifest conflict between queens and males over sperm use (Boomsma 1996). The results suggest a compromise between complete sperm clumping (the joint interests of the males mated to a queen) and complete sperm mixing (the queen's interests), where some queens are more successful than others in mixing sperm and, thus, having more male-biased sexual broods. The mechanism creating sperm clumping may be simple—semen may contain specific chemicals with a heritable component which attracts sperm with a similar chemical profile. To our knowledge no such studies have been performed, but the present results suggest that this may be a fruitful avenue of research.

Other studies have looked for variation in paternity contributions over time in *Vespula* (Ross 1986) and *Polistes metricus* (Metcalf & Whitt 1977), but found no differences between cohorts. In annual species this is not surprising because the entire sexual brood is produced in a single cohort so that no opportunities for alternation in paternity arise. In perennial species, such as honeybees, sexuals are produced repeatedly but, given the high mating frequencies (seven to 20) (Estoup *et al.* 1994; Moritz *et al.* 1995), the average male would gain little by sperm clumping. Nevertheless, partial sperm clumping has also been demonstrated in honeybees (Laidlaw & Page 1984). Relatedness-induced, split sex ratios have not been reported in honeybees implying some other explanation for sperm clumping or that split sex ratios also occur in this species after all.

In conclusion, our results present evidence for an operational queen–male conflict over mating and sperm use as a consequence of split sex ratios and worker-controlled sex allocation. The results also suggest that males which co-sire a colony may, apart from being competitors exposed to as-yet unknown forces of sexual selection, also cooperate in dividing paternity across cohorts in order to increase their joint fitness to a surprising extent. The reason for the unexpected magnitude of male control through their sperm may be that, once the long-term shares of paternity are settled during the sperm storage process directly after mating, all males mated to the same queen gain by sperm clumping. Hence, such male alliances can work successfully against the interests of queens, although the latter have control over sperm storage.

REFERENCES

Boomsma, J. J. 1996 Split sex ratios and queen–male conflict over sperm allocation. *Proc. R. Soc. Lond.* B **263**, 697–704.

Boomsma, J. J. & Grafen, A. 1990 Intraspecific variation in ant sex ratios and the Trivers–Hare hypothesis. *Evolution* **44**, 1026–1034.

Boomsma, J. J. & Grafen, A. 1991 Colony-level sex ratio selection in the eusocial Hymenoptera. *J. Evol. Biol.* **3**, 383–407.

Boomsma, J. J. & Ratnieks, F. L. W. 1996 Paternity in eusocial Hymenoptera. *Phil. Trans. R. Soc. Lond.* B **351**, 947–975.

Boomsma, J. J. & Sundström, L. 1998 Paternity patterns in *Formica* ants. *Behav. Ecol. Sociobiol.* **42**, 85–92.

Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants*. Princeton University Press.

Chan, G. L. & Bourke, A. F. G. 1994 Split sex ratios in a multiple-queen ant population. *Proc. R. Soc. Lond.* B **258**, 261–266.

Chapuisat, M. 1996 Characterization of microsatellite loci in *Formica lugubris* B and their variability in other ant species. *Mol. Ecol.* **5**, 599–601.

Crozier, R. H. & Brückner, D. 1981 Sperm clumping and the population genetics of Hymenoptera. *Am. Nat.* **117**, 561–563.

Crozier, R. H. & Pamilo, P. 1996 *Evolution of social insect colonies, sex allocation and kin selection*. Oxford University Press.

Estoup, A., Solignac, M. & Cornuet, J.-M. 1994 Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. *Proc. R. Soc. Lond.* B **258**, 1–7.

Evans, J. D. 1995 Relatedness threshold for the production of female sexuals in colonies of a polygynous ant, *Myrmica tahoensis*, as revealed by microsatellite DNA analysis. *Proc. Natl Acad. Sci. USA* **92**, 6514–6517.

Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Berlin: Springer.

Keller, L., Sundström, L. & Chapuisat, M. 1997 Male reproductive success: paternity contribution to queens and workers in *Formica* ants. *Behav. Ecol. Sociobiol.* **41**, 11–15.

Laidlaw, H. H. & Page, R. E. 1984 Polyandry in honey bees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships. *Genetics* **108**, 985–997.

Metcalf, R. A. & Whitt, G. S. 1977 Intra-nest relatedness in the social wasp *Polistes metricus*. A genetic analysis. *Behav. Ecol. Sociobiol.* **2**, 339–351.

Moritz, R. F. A., Kryger, P., Koeniger, G., Koeniger, N., Estoup, A. & Tingek, S. 1995 High degree of polyandry in *Apis dorsata* queens detected by DNA microsatellite variability. *Behav. Ecol. Sociobiol.* **37**, 357–363.

Pamilo, P. 1991a Life span of queens in the ant *Formica exsecta*. *Insectes Sociaux* **38**, 111–121.

Pamilo, P. 1991b Evolution of colony characteristics in social insects. I. Sex allocation. *Am. Nat.* **137**, 83–107.

Pamilo, P. 1993 Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity* **70**, 472–480.

Queller, D. C., Strassmann, J. E., Solis, C. R., Hughes, C. R. & DeLoach, D. M. 1993 A selfish strategy of social insect workers that promotes social cohesion. *Nature* **365**, 639–641.

Ratnieks, F. L. W. & Boomsma, J. J. 1995 Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. *Am. Nat.* **145**, 969–993.

Ratnicks, F. L. W. & Boomsma, J. J. 1997 On the robustness of split sex ratio predictions in social Hymenoptera. *J. Theor. Biol.* **185**, 423–439.

Ross, K. G. 1986 Kin selection and the problem of sperm utilization in social insects. *Nature* **323**, 798–800.

Seppä, P. 1992 Genetic relatedness of worker nestmates in *Myrmica ruginodis* (Hymenoptera: Formicidae) populations. *Behav. Ecol. Sociobiol.* **30**, 253–260.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*, 3rd edn. New York: W. H. Freeman & Co.

Sundström, L. 1993 Genetic population structure and sociogenetic organistaion in *Formica truncorum* (Hymenoptera; Formicidae). *Behav. Ecol. Sociobiol.* **33**, 345–354.

Sundström, L. 1994 Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* **367**, 266–268.

Sundström, L. 1995 Sex allocation and colony maintenance in monogyne and polygyne colonies of *Formica truncorum* (Hymenoptera: Formicidae); the impact of kinship and mating structure. *Am. Nat.* **146**, 182–201.

Sundström, L. & Ratnieks, F. L. W. 1998 Sex ratio conflicts, mating frequency and queen fitness in *Formica* ants. *Behav. Ecol.* **9**, 116–122.

Sundström, L., Chapuisat, M. & Keller, L. 1996 Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. *Science* **274**, 993–995.

Trivers, R. L. & Hare, H. 1976 Haplodiploidy and the evolution of the social insects. *Science* **191**, 249–263.

Walin, L., Sundström, L., Seppä, P. & Rosengren, R. 1998 Worker reproduction in ants—a genetic analysis. *Heredity* **81**, 604–612.

Wright, S. 1951 The genetical structure of populations. *A. Eugenics* **15**, 323–354.

An electronic appendix to this paper can be found at ⟨http://www.pubs.royalsoc.ac.uk⟩.