

## Worker Reproduction in *Formica* Ants

Heikki Helanterä\* and Liselotte Sundström†

Department of Biological and Environmental Sciences, University of Helsinki, P.O. Box 65, FI-00014 Helsinki, Finland

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**ABSTRACT:** A potential tragedy of the commons arises in social-insect colonies where workers are fertile if egg-laying workers decrease their contribution to other tasks. We studied worker ovary development and egg laying in relation to kin structure, colony size, and the presence of a queen in nine species (11 populations) of *Formica* ants. Workers were highly fertile and laid eggs in the presence of a queen in five out of the seven species where egg samples were obtained. Worker fertility correlated neither with colony size nor with kin structure, which suggests that colony-level costs and efficiency of policing precede relatedness as the most important conflict determinant. We conclude that careful quantification of the costs of worker reproduction and policing is essential for inferences about the tragedy of the commons.

**Keywords:** social insects, *Formica*, kin conflict, worker reproduction, policing, polygyny.

The major transitions in evolution (Maynard Smith and Szathmary 1995) entail the integration of elements into cooperative collectives. For example, genes have united into chromosomes, cells into multicellular individuals, and individuals of, for example, naked mole rats and social insects such as ants, bees, wasps, and termites into societies. Such collectives represent different levels of selection (Lewontin 1970; Maynard Smith and Szathmary 1995; Leigh 1999; Reeve and Keller 1999), characterized by an impressive degree of cooperation. Nevertheless, conflicts are predicted to emerge within such collectives. This is because each element within a collective can increase its fitness within the group by exploiting the group resources

without contributing to the group output. As a result, group fitness declines, and the so-called tragedy of the commons occurs (Hardin 1968; Leigh 1999; Wenseleers and Ratnieks 2004). A trade-off between individual and collective interests applies to all cooperative systems where selfish exploitation of collective resources at a cost to the group is possible (reviewed by, e.g., Hammerstein [2003]; Sachs et al. [2004]). Examples include genes that increase their own transmission in meiosis at a cost to the genome as a whole (Hoekstra 2003), defectors in Prisoner's Dilemma games (Trivers 1971), and parasites that increase their virulence within a host at the expense of reduced transmission to new hosts (Frank 1996a).

Conflicts within groups can be alleviated and the tragedy of the commons can be overcome in two ways: if cooperators are related (Hamilton 1964, 1972) and/or through mutual policing (Ratnieks 1988; Frank 1995, 1996b, 2003; Wenseleers et al. 2004). Relatedness in terms of shared genes (Hamilton 1964; Grafen 1985; Queller 1994; Frank 1995, 1998) decreases selfish incentives because the costs of selfishness are carried by relatives and the inclusive fitness of the selfish actor thus diminishes with diminishing group performance (Frank 1995, 2003). Mutual policing can suppress selfish behavior and also select for cooperation in groups where relatedness alone is not sufficient to drive the evolution of cooperation. The lower the within-group relatedness and the costs of policing are, the more resources individuals will spend on policing each other (Frank 1995, 1996b). If selfish behavior is costly and its payoffs are prevented by policing, policing selects for self-policing, and the tragedy of the commons is alleviated (Ratnieks 1988; Frank 1996b, 2003; Wenseleers et al. 2004).

In haplodiploid-insect societies, reproductive conflicts arise wherever unfertilized workers are able to lay male-destined eggs. Each female is most closely related to her own sons and is thus predicted to reproduce based on kin value only. In species where workers are able to lay male-destined eggs, the conflict turns into a potential tragedy of the commons if reproductive workers invest less in other colony tasks. However, worker policing is expected to evolve when worker reproduction is costly or when the workers are, on average, more closely related to the sons of the queen(s) than to the sons of other workers (i.e.,

\* Corresponding author. Present address: Laboratory of Apiculture and Social Insects, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom; e-mail: h.helantera@sheffield.ac.uk.

† E-mail: liselotte.sundstrom@helsinki.fi.

when there is relatedness asymmetry toward the offspring of the queen). Conversely, the effects of colony kin structure on the conflict over worker reproduction depend on the presence of worker policing. If worker policing is absent (due to, e.g., prohibitive costs), the fertility of workers should increase with reduced relatedness (Bourke 1988; Wenseleers et al. 2004; Wenseleers and Ratnieks 2006a), whereas when worker policing is present, the relatedness asymmetry within colonies will dictate the outcome (Wenseleers and Ratnieks 2006a).

Regulation of worker reproduction by worker policing has been observed in all major groups of social Hymenoptera (bees, wasps, and ants; reviewed by Ratnieks and Wenseleers [2005]; Wenseleers and Ratnieks [2006a]). A comparative analysis of worker reproduction indeed shows that sons of workers are rarely reared when workers are more closely related to sons of queens, which is consistent with widespread worker policing (Wenseleers and Ratnieks 2006a). However, whether worker policing has actually led to self-restraint and conflict resolution has rarely been quantified (but see Wenseleers and Ratnieks 2006b). Self-restraint of workers has been considered a prerequisite for evolution of highly derived, complex eusocial systems (Bourke 1999).

We studied worker reproduction in *Formica* ants, a genus of highly derived social insects with highly variable colony size and queen number. Mature colonies range in size from fewer than 2,000 in *Formica fusca* (Savolainen 1990; Czechowski et al. 2002; personal observations by the authors) to as many as one million in *Formica polyctena* and *Formica aquilonia* (Rosengren et al. 1993). Queen number varies extensively, and consequently, within-colony relatedness varies between three-quarters in single-queen (monogyne) colonies and zero in multiqueen (polygyne) colonies (Rosengren et al. 1993; Sundström et al. 2005). Despite the common occurrence of polygyny, worker reproduction has rarely been studied in polygyne ants (for exceptions, see Evans 1998; Hammond et al. 2003; Helanterä and Sundström 2005). Importantly, the genus *Formica* includes species with unicolonial populations in which within-colony relatedness is virtually zero (Sundström et al. 2005). Under these circumstances, cooperative worker traits such as worker policing and self-restraint may no longer be favored by selection, and high levels of worker selfishness are expected (Queller and Strassmann 1998).

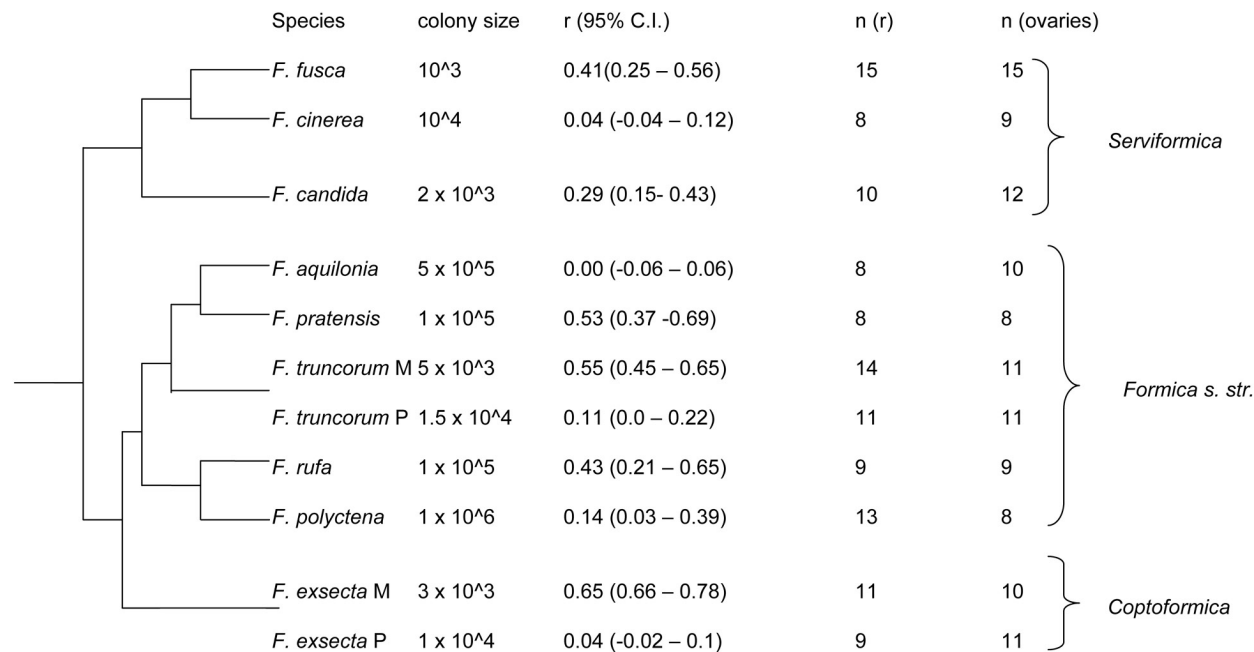
Workers of *Formica* species have functional ovaries (Gösswald 1989), but the extent of worker oviposition has not been quantified. Thus, it is not clear whether a tragedy of the commons exists in *Formica* or workers practice self-restraint. In *Formica*, worker oviposition and policing have been demonstrated only in *F. fusca*. Worker-laid eggs were found in more than half of the colonies, but very few worker-laid eggs are reared, because of worker policing

(Helanterä 2004; Helanterä and Sundström 2005). Thus, at least in this species, worker policing has not led to self-restraint. Our objectives here are to assess the level of worker selfishness in nine species of *Formica* ants and to test whether kin structure and colony size affect worker selfishness. Because data on efficiency of worker policing are not available in *Formica*, we test for the effects of both worker relatedness and relatedness asymmetry on worker fertility. Thus, when policing is weak or absent, we predict enhanced worker selfishness under low worker relatedness, whereas under efficient worker policing or self-policing, we predict enhanced worker selfishness under low relatedness asymmetry (Wenseleers et al. 2004; Wenseleers and Ratnieks 2006a, 2006b). We combine genetic data with assessments of worker ovary development and oviposition in queenright and orphaned colonies. Furthermore, variation in worker fertility is analyzed in relation to phylogenetic information.

## Methods

### *Species and Colony Samples*

We studied nine species of *Formica* collected from southern Finland and Sweden and spanning three subgenera, *Serviformica*, *Coptoformica*, and *Formica* s.s. (fig. 1). The phylogeny, average mature-colony sizes, worker relatedness, and numbers of colonies included in this study are shown in figure 1. In two of the species, the socially polymorphic *Formica exsecta* and *Formica truncorum*, both monogynous and highly polygynous populations were studied. Workers and queens were collected in the spring, before the onset of egg laying (early April–mid-May). At this time, queens and workers gather near the nest surface so that both queens and intranidal workers, which are likely to be the most fertile (Hölldobler and Wilson 1990), can easily be collected. In all these species, the great majority of workers hibernate only once, and so the workers collected were almost exclusively from the cohort born the previous summer. Care was taken to ensure that no eggs were transferred from the field colonies to the newly established laboratory colonies. For polygynous species, single-queen and orphaned laboratory colonies were established as described by Helanterä and Sundström (2005). For monogynous species, queens were not collected, either because they resided in nature conservation areas and queen removal would kill the colonies (*F. exsecta* and *F. truncorum*) or because the colonies were very large and finding the single queen was extremely difficult (*Formica rufa* and *Formica pratensis*). For these species, only orphaned laboratory nests were established, and the field colonies were used as sources of eggs and intranidal workers for dissections. Owing to their simple social structure,



**Figure 1:** Phylogeny of Palearctic *Formica* species (from Goropashnaya 2003), with average colony sizes, relatedness, and sample sizes for each species.

egg parentage could be determined based on their genotype. Twenty workers from each colony were stored in 95% ethanol for genetic analyses.

#### *Worker Fertility and Egg Laying*

To compare worker fertility in queenright and orphaned colony fragments, we collected workers for dissection once the first eggs appeared in the colonies. All worker samples comprised 15 workers collected from the brood area (in the lab colonies) or from the core of the mound (field colonies) to minimize the proportion of foraging workers in the sample. Workers were frozen at  $-20^{\circ}\text{C}$ , and their ovaries were later dissected under a light microscope. Workers with oocytes larger than 90% of the size of an egg in their ovaries were classified as fertile (Foster and Ratnieks 2001). Simultaneously with samples for dissections, we also collected eggs for parentage analysis from laboratory or field colonies as described above. In *F. rufa* and *F. pratensis*, we failed to obtain egg samples from queenright field colonies, although the same colonies did produce broods later in the season.

Adult workers (eight to 16 per colony) and eggs were genotyped at five or six microsatellite loci described by Chapuisat (1996; FL loci) and Gyllenstrand et al. (2002; FE loci; app. B). The genotype data on the adult workers were used to estimate the relatedness among the filial gen-

eration of females (app. A). The PCR protocols used are described by Hannonen and Sundström (2002), except for the annealing temperatures, which varied across loci and species (app. B). The PCR products were sized and visualized with a MegaBace automated sequencer and the software Fragment Profiler 1.0 (Amersham Biosciences). We genotyped 24 eggs from each colony and designated them as queen laid or worker laid based on alleles present in the eggs, the workers, and the queen (for details, see Helanterä and Sundström 2005). Because we did not have access to the queens in the monogynous field colonies, we deduced the queen genotype from worker genotypes, assuming the minimum number of colony fathers. Because worker-laid eggs may carry the same alleles as queen-laid eggs, we corrected the probability of worker-laid eggs for nondetection errors as described by Helanterä and Sundström (2005). Orphaned colonies were not sampled for eggs, but the colonies were checked for presence of worker-laid eggs and, later, larvae to ensure that the eggs were viable.

#### *Statistical Analyses*

**Phylogenetic Dependencies.** Because species do not necessarily represent independent data points, as a result of common ancestry (Felsenstein 1985), we corrected for possible phylogenetic dependencies in the data. First, we

tested which variables (colony size, relatedness among workers, relatedness asymmetry [app. A], proportion of fertile workers, and proportion of eggs laid by workers) were phylogenetically dependent, using the test of serial independence (Abouheif 1999) as implemented in the software Phylogenetic Independence 2.0 (Reeve and Abouheif 2003). Here we used the molecular phylogeny of Palearctic *Formica* ants by Goropashnaya (2003; fig. 1). Second, for the variables that showed significant phylogenetic dependencies—that is, fewer than 5% of the 1,000 randomized phylogenies gave a *C* statistic (Abouheif 1999) greater than that observed for the original phylogeny—we calculated the independent contrasts (Felsenstein 1985) using the “Contrast” module in the software package PHYLIP 3.6b (Felsenstein 2005). The correlations between independent contrasts of phylogenetically dependent traits, worker ovary development, and worker oviposition were then analyzed.

*Proportion of Fertile Workers and Presence of Worker-Laid Eggs.* To analyze determinants of worker ovary development, we used a linear model with the proportion of fertile workers as the response variable and species and treatment as the factors. Colony-specific values of relatedness among workers and relatedness asymmetry (app. A) were nested within species as covariates in two separate analyses. Colony size was omitted from this analysis because it was phylogenetically dependent and was not correlated with measures of worker fertility (see “Results”). Next, we tested for an association between the proportion of fertile workers and the proportion of worker-laid eggs within colonies, to assess whether the two measures gave congruent information regarding worker selfishness. Finally, to analyze determinants of worker egg laying, we used a generalized linear model with a binomial error structure, with presence/absence of worker-laid eggs in each colony as the response variable and species as the independent factor. Relatedness asymmetry and worker relatedness were included as covariates nested within species. All analyses were carried out using S-PLUS 6.1 (Insightful).

## Results

### Worker Fertility and Egg Laying

Mean ovary development across all species was high in both queenright and orphaned colonies (table 1; queenright:  $n = 114$ , mean [SD] proportion of fertile workers = 0.16 [0.14]; orphaned:  $n = 129$ , mean [SD] proportion of fertile workers = 0.22 [0.17]). Orphaned colonies had a significantly higher proportion of fertile workers than queenright ones (table 1). The highest ovary scores were found in the subgenus *Serviformica* (*Formica*

**Table 1:** Mean (SD) proportions of fertile workers in each species by treatment

<i>Formica</i> species <sup>a</sup>	Orphaned	Queenright
<i>F. fusca</i>	.32 (.17)	.18 (.17)
<i>F. cinerea</i>	.41 (.083)	.35 (.18)
<i>F. candida</i>	.26 (.14)	.16 (.14)
<i>F. aquilonia</i>	.067 (.077)	.06 (.06)
<i>F. pratensis</i>	.30 (.11)	.21 (.19)
<i>F. truncorum</i> M	.23 (.22)	.14 (.13)
<i>F. truncorum</i> P	.21 (.18)	.18 (.10)
<i>F. rufa</i>	.10 (.11)	.18 (.11)
<i>F. polyctena</i>	.08 (.11)	.06 (.08)
<i>F. exsecta</i> M	.23 (.10)	.19 (.09)
<i>F. exsecta</i> P	.18 (.14)	.14 (.14)

Note: Based on 15 workers per colony; numbers of colonies per species as in figure 1.

<sup>a</sup> M = monogynous; P = polygynous.

*cinerea*, *Formica fusca*, and *Formica candida*) in both treatments (table 1). In the queenright treatment, both social forms of *Formica exsecta* also showed high ovary development. Furthermore, in orphaned colonies, *Formica pratensis* workers frequently had developed ovaries.

Workers started to lay eggs within 3 weeks (mean = 19.1 days, SD = 8.3) in orphaned colonies in all species except *Formica aquilonia* and *Formica polyctena*. The fastest responses were found in *F. candida*, polygynous *Formica truncorum*, and *F. fusca*, where egg laying commenced within a week of queen removal. By contrast, workers of *F. aquilonia* and *F. polyctena* were virtually infertile. In all species where workers laid eggs, these also hatched into larvae and were thus viable. Because workers in orphaned *F. aquilonia* and *F. polyctena* colonies did not lay eggs in our laboratory colonies, it is highly unlikely that workers in queenright colonies would do so either; thus, queenright egg samples were not genotyped in these species.

The amplification success of eggs produced in queenright colonies was low (53% of the eggs did not amplify at any locus), probably because of their young age and the consequent low numbers of cells in eggs, as also had been found for *F. fusca* (Helanterä and Sundström 2005). The proportion of worker-laid eggs in a colony was not correlated with the proportion of informative alleles in the workers (i.e., alleles present in workers but not in the queen; Spearman's  $r = 0.03$ ,  $n = 38$ ,  $P = .84$ ). Nor was the proportion of worker-laid eggs correlated with the proportion of nonamplified samples in a colony (Spearman's  $r = -0.002$ ,  $n = 38$ ,  $P = .99$ ). Furthermore, within-colony worker relatedness correlated with neither the proportion of informative alleles (Spearman's  $r = -0.17$ ,  $n = 38$ ,  $P = .29$ ) nor the proportion of nonamplified samples (Spearman's  $r = -0.16$ ,  $n = 38$ ,  $P = .32$ ). Thus, our results were not biased by variation in information

content of loci or amplification success, and the information content of loci was independent of the kin structure of colonies. All the analyses below are based on eggs where at least one locus amplified. The sex ratios were female biased (mean proportion of females averaged over all colonies of all species = 0.65, SD = 0.23). On average, 29% (SD = 0.08) could not be unambiguously assigned to either sex or parentage (classified as “unknown” in table 2) because the queen and her male mate carried similar alleles at the amplified loci.

Among the queenright colonies, worker-laid eggs were found in all species in which eggs were genotyped, except the monogynous form of *F. truncorum* (table 2). The average proportion of worker-laid eggs (of all eggs, not only male eggs) across all colonies of all species was 8% (SD = 0.16), excluding *Formica rufa* and *F. pratensis*, where eggs were not obtained from queenright colonies and assuming that workers of *F. aquilonia* and *F. polyctena* did not lay eggs. Interestingly, the highest frequencies of worker-laid eggs were found in the polygynous *F. truncorum* (worker-laid eggs in 8/8 colonies, mean = 27%, SD = 0.15,  $n = 8$ ) and the polygynous *F. exsecta* (4/7 colonies, mean = 16%, SD = 0.15), whereas the lowest frequencies of worker-laid eggs were found in the monogynous forms of these species (*F. truncorum*: 0/7 colonies; *F. exsecta*: 1/8 colonies, mean = 7%, SD = 0.2). The difference between colony types in occurrence of worker egg laying was significant in *F. truncorum* (Fisher’s exact test, two-tailed  $P = .002$ ) but not in *F. exsecta* (Fisher’s exact test,  $P = .12$ ).

#### Determinants of Worker Reproduction

The proportion of fertile workers was independent of the phylogeny in both treatments (queenright:  $C = 0.006$ ,  $P = .53$ ; orphaned:  $C = 0.33$ ,  $P = .07$ ). Similarly, relatedness asymmetry was not correlated with the phylogeny ( $C = -0.3$ ,  $P = .35$ ). Colony size correlated with the phylogeny ( $C = 0.54$ ,  $P = .018$ ), such that all the five

largest average colony sizes were found within the *Formica* s.s. species group. However, the independent contrasts of colony size and proportions of fertile workers were not correlated in either of the treatments (queenright: Spearman’s  $r = -0.06$ ,  $n = 11$ ,  $P = .86$ ; orphaned: Spearman’s  $r = -0.4$ ,  $n = 11$ ,  $P = .22$ ). Similarly, the presence/absence of worker-laid eggs in queenright colonies was independent of the phylogeny ( $C = 0.012$ ,  $P = .53$ ), and the independent contrasts of colony size and proportion of worker-laid eggs were not correlated ( $r = 0.46$ ,  $n = 7$ ,  $P = .29$ ). This indicates that colony size is not associated with worker reproduction, and consequently, colony size was not included in further analyses. The number of independent data points is limited, and the power of these tests is consequently low, so the negative results have to be interpreted cautiously. The effect sizes (i.e., correlation coefficients  $r$ ) that we found ranged between  $-0.40$  and  $+0.46$ , which classify as medium to large sensu, for example, Jennions and Møller (2003). With respect to the correlation between the contrasts of colony size and ovary development of orphaned workers ( $r = -0.4$ ), the power to find a significant effect of this magnitude is 24% with  $n = 11$  and  $\alpha = 0.05$ . Similarly, with respect to the correlation between the contrasts of colony size and proportion of worker-laid eggs ( $r = 0.46$ ), the power to find a significant effect of this magnitude is 19% with  $n = 7$  and  $\alpha = 0.05$ . For these correlations, the lack of significant relationship might be due to low power, whereas the small effect size for the correlation of colony size and queenright ovary development ( $r = -0.06$ , with  $n = 11$ ) suggests that a strong relationship is unlikely (the power to find a significant effect of this magnitude is 4% with  $n = 11$  and  $\alpha = 0.05$ ).

Relatedness showed a phylogenetic pattern opposite to size, so that variation among closely related taxa was significantly greater than expected under a random distribution (relatedness:  $C = -0.38$ ,  $P = .002$ ). This is not surprising, because the monogynous and highly polygynous populations of *F. truncorum* and *F. exsecta* were en-

**Table 2:** Mean (SD) numbers and proportions of female, worker-laid (corrected), and unassigned eggs

<i>Formica</i> species <sup>a</sup>	Female eggs		Worker-laid males		Unassigned males		Unknown <sup>b</sup>	
	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion
<i>F. fusca</i>	12.6 (7.4)	.60 (.33)	2.5 (3.2)	.12 (.16)	.7 (2.0)	.04 (.09)	4.9 (4.1)	.25 (.23)
<i>F. cinerea</i>	5.7 (6.1)	.47 (.42)	1.0 (1.9)	.07 (.15)	1.3 (2.2)	.1 (.17)	3.3 (4.7)	.36 (.46)
<i>F. candida</i>	8.7 (2.4)	.81 (.15)	.56 (.98)	.05 (.07)	.14 (10.4)	.01 (.03)	1.6 (2.2)	.13 (.17)
<i>F. truncorum</i> M	7.4 (9.6)	.41 (.51)	0 (0)	0 (0)	9.1 (8.8)	.57 (.53)	.57 (1.5)	.02 (.06)
<i>F. truncorum</i> P	5.0 (3.0)	.43 (.22)	4.9 (3.5)	.27 (.15)	0 (0)	0 (0)	3.5 (2.1)	.30 (.17)
<i>F. exsecta</i> M	7.1 (8.1)	.42 (.45)	1 (2.8)	.07 (.2)	6.9 (7.3)	.49 (.45)	0 (0)	0 (0)
<i>F. exsecta</i> P	5.3 (3.5)	.69 (.17)	1.6 (2.2)	.16 (.14)	.42 (.77)	.06 (.10)	.83 (.86)	.12 (.12)

Note: Each egg sample consisted of 24 eggs per colony.

<sup>a</sup> M = monogynous; P = polygynous.

<sup>b</sup> Could not be unambiguously assigned to either sex or parentage.

tered as sister taxa within their respective clades. Nevertheless, the pattern remained significant even when the two social forms were combined, despite the reduced power (relatedness:  $C = -0.14$ ,  $P = .01$ ). Hence, patterns of kinship are not phylogenetically constrained in the genus *Formica*.

Given that neither relatedness nor relatedness asymmetry was phylogenetically constrained, we analyzed the influence of these factors with a generalized linear model incorporating species, treatment, relatedness, and relatedness asymmetry as described in "Proportion of Fertile Workers and Presence of Worker-Laid Eggs." Neither variation in relatedness asymmetry nor relatedness was associated with the proportion of fertile workers, although the differences among species were significant (table 3). Similarly, the presence/absence of worker-laid eggs differed significantly among species but was not associated with either relatedness asymmetry or relatedness (table 4). Nevertheless, the rate of worker oviposition and the degree of ovary development among the workers in the same colony were not correlated (Spearman's  $r = 0.19$ ,  $n = 33$ ,  $P = .237$ ).

### Discussion

Our results reveal frequent worker egg laying and considerable ovary development in queenright colonies of *Formica* ants, a derived group of social insects with large colonies. Workers had more developed ovaries in orphaned than in queenright colonies, suggesting self-restraint in queenright conditions, and oviposition commenced after queen removal in all but two species. Worker oviposition and ovary development were independent of the phylogeny and were not associated with relatedness, relatedness asymmetry, or colony size. Worker fertility was high compared to Hymenopterans studied previously, but whether the conflict indeed translates into a strong tragedy of the commons in *Formica* cannot be resolved until data on the actual costs of worker fertility are available.

Somewhat surprisingly, we found an incongruence between worker ovary development and worker oviposition; that is, worker ovary development was not correlated with worker egg laying in a colony. This suggests that additional factors may affect the outcome, and we envisage two phenomena to explain this. First, the estimated proportions of worker-laid eggs are minimum estimates, because some worker-laid eggs may have been policed before sampling. This is especially the case with the monogynous populations of *Formica truncorum* and *Formica exsecta*, where field colonies were used as egg sources and the eggs may have been older than those sampled from laboratory colonies (maximum age of 1 week). Second, *Formica* workers can lay unviable trophic eggs for nutritional purposes

**Table 3:** Results of the linear models testing effects of treatment (orphaned or queenright), species, and kin structure (relatedness asymmetry or worker relatedness, nested within species) on the proportion of fertile workers in a colony

	df	F	P(F)
Relatedness asymmetry (RA): <sup>a</sup>			
Treatment	1	7.9	.005
Species	10	7.3	<.00001
Species × treatment	10	1.2	.29
Species/RA	11	.53	.88
Treatment × (species/RA)	11	.2	.95
Residual	155		
Worker relatedness (rw):			
Treatment	1	8.4	.004
Species	10	7.84	<.000001
Species × treatment	10	1.30	.24
Species/rw	11	.89	.55
Treatment × (species/rw)	11	.90	.55
Residual	155		

<sup>a</sup> RA =  $r_{qm}/r_{wm}$ , where  $r_{qm}$  and  $r_{wm}$  are the relatedness of a worker toward the sons of the queen and other workers, respectively; see appendix A.

(Gösswald 1989), and our dissections did not differentiate between trophic and viable eggs. Thus, worker ovary development may overestimate worker fertility and must be interpreted with caution. Because trophic eggs contain no DNA and therefore do not amplify, they may partly contribute to the low amplification success of the eggs. However, both the worker-laid eggs found in queenright colonies and the rapid onset of extensive egg laying after colony orphaning show that a considerable fraction of the workers were laying viable eggs in the presence of the queen. Because both the efficiency of worker policing and the proportion of trophic eggs may vary between species, they might also mask the effects of relatedness and colony size on worker fertility, especially given the small sample size and low statistical power in the species-level analysis. Nonetheless, the presence of worker-laid eggs demonstrates that workers act selfishly in all but the most derived obligately polygynous species.

Both the proportions of fertile workers and the proportions of worker-laid eggs found in *Formica* are the highest reported in social Hymenoptera with morphologically distinct castes and large colonies (i.e., more than 1,000 workers; Bourke 1999; see, e.g., Ratnieks 1993; Foster and Ratnieks 2001; Foster et al. 2001; Halling et al. 2001; Oldroyd et al. 2001; Ratnieks et al. 2006). In all species except *Formica aquilonia* and *Formica polyctena*, the proportion of workers with developed ovaries was at least an order of magnitude higher than in, for example, *Apis* bees (0.1% in *Apis mellifera*; Ratnieks 1993) or *Vespula* wasps (1% in *Vespula vulgaris*; Foster and Ratnieks 2001). Studies of worker ovipositioning are few in any species, and only

**Table 4:** Results of the generalized linear model testing effects of species and kin structure (relatedness asymmetry or worker relatedness, nested within species) on the presence of worker-laid eggs in a colony (a binomial variable)

	df	Deviance	Residual df	Residual deviance	$P(\chi)$
Relatedness asymmetry (RA): <sup>a</sup>					
Null	...	...	55	75.0	...
Species	6	17.8	49	57.2	.007
Species/RA	7	7.8	42	49.4	.35
Worker relatedness (rw):					
Null	...	...	55	75.0	...
Species	6	17.8	49	57.2	.007
Species/rw	7	8.3	48	56.5	.30

<sup>a</sup> RA =  $r_{qm}/r_{wm}$ , where  $r_{qm}$  and  $r_{wm}$  are the relatedness of a worker toward the sons of the queen and other workers, respectively; see appendix A.

in the wasp genus *Dolichovespula* and in *Vespula rufa* have similar or higher frequencies of worker-laid eggs been reported (between 44% and 76% of male eggs; Foster and Ratnieks 2000; Foster et al. 2001; Wenseleers et al. 2005). In *A. mellifera*, 7% of male eggs are laid by workers (Visscher 1996), whereas we found 5% or more of all eggs to be worker laid in six out of the seven species where eggs were analyzed.

Our study is the first to report high levels of worker ovary development and ovipositioning in social Hymenoptera with multiqueen colonies. Hammond et al. (2003) showed incomplete self-restraint by workers in polygynous *Leptothorax acervorum*, but the proportions of worker-laid eggs they reported (<10% of male eggs) are considerably lower than those reported in this study. Among our study species, worker fertility was particularly high in the polygyne populations of *Formica cinerea*, *F. truncorum*, and *F. exsecta*. In these species, the polygyne populations frequently lack colony boundaries, and the within-colony relatedness is very close to or indistinguishable from zero (fig. 1; Sundström 1993; Brown and Keller 2000; Goropashnaya et al. 2001; Seppä et al. 2004; Elias et al. 2005). High worker fertility in these species is thus consistent with the prediction by Queller and Strassmann (1998) that worker selfishness increases in societies with zero relatedness. In contrast, the very low worker fertility in the obligately highly polygynous *F. aquilonia* and *F. polyctena* agrees with the prediction by Keller (1995) that worker sterility is a prerequisite for stable uniclonality. Further studies are thus called for to examine whether facultative polygyny is also more generally associated with worker selfishness and obligate polygyny with worker sterility.

Our results show that relatedness is not the sole determinant of social behavior (reviewed by, e.g., Griffin and West [2002] and recently discussed by Wilson and Hölldobler [2005]; but see Foster et al. 2006 for a warranted critique). Instead, actions in the genetic interest of indi-

viduals are weighed against the costs of such actions at the level of the colony. Hence, social actions evolve in a multilevel selection setting (Price 1970; Hamilton 1972; Frank 1998; West et al. 2007), and neither the within-colony relatedness benefits nor the colony-level costs and benefits of actions alone can fully predict or explain the evolution of social traits. As a part of the ongoing debate, the role of relatedness in explaining patterns of male production has also been under examination (Hammond and Keller 2004; Wenseleers and Ratnieks 2006a). Even though the larger data set showed that relatedness is an important determinant of worker reproduction (Wenseleers and Ratnieks 2006a), kin structure does not unequivocally explain variation in worker reproduction (Hammond and Keller 2004; see Wenseleers and Ratnieks 2006a for a comprehensive list of factors affecting evolution of worker policing). The lack of relatedness effects shown by our data is not, however, applicable to the context of either study, because the studies by Hammond and Keller (2004) and Wenseleers and Ratnieks (2006a) examined the outcome of the conflict (i.e., the presence of adult worker-produced males), whereas our interest is conflict expression (i.e., the presence of worker-laid eggs that may or may not be destroyed, *sensu* Ratnieks and Reeve 1992). Because the predicted effects of relatedness on worker fertility depend on the presence of worker policing (Wenseleers et al. 2004), selection on worker policing cannot be inferred from fertility patterns alone.

In addition to relatedness, colony-level costs of worker reproduction have especially been invoked as an important determinant of the variation in male production (e.g., Hammond and Keller 2004; Toth et al. 2004; Wenseleers and Ratnieks 2006a), and fertile workers have indeed been shown to be inefficient in other tasks (Ross 1985; Monnin and Peeters 1999; Martin et al. 2002; Hartmann et al. 2003). However, only a few studies have quantified the actual costs of worker reproduction (Cole 1986; Reeve

1991; Lopez-Vaamonde et al. 2003). Furthermore, low viability of worker-laid eggs may favor worker policing (Pirk et al. 2004; Helanterä et al. 2006; Nonacs 2006; but see Beekman and Oldroyd 2005) because a colony-level cost may ensue if resources are wasted on low-quality worker-laid eggs. Like all costs of worker reproduction, these should be included as one part in the balance between relatedness benefits and colony-level costs that determines the evolution of worker reproduction and policing.

Only recently has recognition dawned that nonlinearities in the cost functions that relate the level of selfishness to group fitness (i.e., colony success) can alleviate the tragedy of the commons despite selfishness (Foster 2004). For example, even if fertile workers fail to participate in risky tasks such as foraging and colony defense (Franks et al. 1990), they can still work in the brood area to the benefit of the colony. Hence, even high levels of worker fertility do not necessarily turn into an intense tragedy of the commons. Given the temporal division of labor in *Formica* colonies and the higher fertility of young indoor workers, this may well be the case. As a result, selection for both worker policing and self-policing can be relaxed (Frank 1995; Foster 2004; Wenseleers et al. 2004).

High levels of worker fertility are also consistent with the absence of worker policing, which may stem either from low costs of worker reproduction and a relaxed selection on policing or from strong constraints on policing, such as lack of information and therefore costly recognition errors (Ratnieks 1988; Ratnieks and Reeve 1992). Information on egg parentage may be poor in ants because recognition cues may be scrambled in egg piles (Peeters and Tsuji 1993; Nakata and Tsuji 1996; Monnin and Ratnieks 2001; but see D'Ettorre et al. 2006), in contrast to bees and wasps, where each offspring is reared in an individual cell. However, previous data suggest that worker-produced adult males are absent in *Formica*, which implies that worker policing does occur at some stage of brood development (Walin et al. 1998; Helanterä and Sundström 2005). Indeed, workers of *Formica fusca* are apparently able to discriminate against worker-laid eggs after they have been transferred into egg piles (Helanterä and Sundström 2005). Thus, low costs are more likely to be the ultimate explanation for high levels of worker fertility than constraints on policing.

To conclude, we would like to stress two things. First, studies of conflicts should not pay attention only to the conflict outcome, that is, the parentage of brood reared. The expression of selfish behavior has to be charted in full in order to draw conclusions regarding the group-level costs of conflict. Second, the more evidence accumulates to suggest that relatedness is not the exclusive determinant of conflict behavior, the more important it is to study other

determinants of kin-selected conflicts, such as efficiency of policing and costs of selfishness and policing.

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## APPENDIX A

### Calculation of Relatedness Asymmetry

If workers are able to police each other, investment in worker policing is predicted to increase with the relatedness asymmetry (RA). Conversely, because worker policing selects for worker acquiescence (Wenseleers et al. 2004), worker reproduction should consequently be the rarer the higher the relatedness asymmetry is. Following Bourke and Franks (1995), we calculated the relatedness asymmetry for each colony as

$$RA = \frac{rqm}{rwm},$$

where  $rqm$  and  $rwm$  are the relatedness of a worker toward the sons of the queen and of other workers, respectively. These relatedness coefficients are calculated from

$$rqm = \frac{1 + (Ne - 1)rq}{2Ne},$$

$$rwm = \frac{1 + 2/Me + (Ne - 1)rq}{4Ne},$$

where  $Ne$  is the effective number of queens (set to 1 in monogynous species),  $rq$  the relatedness among queens in a colony, and  $Me$  the effective mating frequency of queens. The number  $Ne$  was calculated for polygynous populations according to Ross (1993):

$$Ne = \frac{4rs - rq - 2rm1}{4rw - rq - 2rm1},$$

where  $rq$  is relatedness of queens,  $rw$  is the relatedness among females in the filial generation (i.e., workers), and  $rm1$  is the relatedness among the male mates of nest mate queens (assumed to be 0). The quantity  $rs$  is the relatedness among the offspring of a single queen and was calculated



as  $r_s = 1/2Me + 0.25$  (Ross 1993). The values of  $Me$  were obtained from the literature (table A1), except for *Formica cinerea*, *Formica pratensis*, *Formica polyctena*, and the monogynous *Formica exsecta* and *Formica truncorum*. For the monogynous colonies of *F. exsecta* and *F. truncorum*, the mating frequency of each colony queen was determined from the smallest number of matings needed to explain the worker genotypes observed in that colony. For *F. cinerea*, *F. pratensis*, and *F. polyctena*, no estimates were available, and so we used the average value of the estimates for the other species in the same subgenus. Relatedness among workers ( $rw$ ) was estimated from our microsatellite genotype data using the method of Queller and Goodnight (1989) and the software Relatedness 5.0 (Queller and Goodnight). The relatedness among nest mate queens was

assumed to equal that among the workers ( $rw = rq$ ) because data on contemporary queens were not available for the study populations and colonies.

For some species, there is evidence that relatedness among queens in a colony is higher than that of workers (*F. polyctena* [Rosengren et al. 1993], *Formica fusca* [Hannonen et al. 2004], and *F. exsecta* [Liautard and Keller 2001]). This should not bias our results, given that the difference is in the same direction in each colony, because we are using relatedness asymmetry only as a relative measure in our analyses. Indeed, in all species except *F. polyctena*, the mean relatedness asymmetry was less than 1 (i.e., workers are more related to sons of workers than to sons of queens), which might result from too-low estimates of  $rq$ .

**Table A1:** Components of relatedness asymmetry (RA) in *Formica* species

Species <sup>a</sup>	rw/rq	Mean RA		Source of Me
		Me	(SD)	
<i>F. fusca</i>	.48	1.19	.82 (.13)	Mean of the two populations studied by Hannonen et al. (2004)
<i>F. cinerea</i>	.04	1.11	.86 (.14)	Mean of estimates for other <i>Serviformica</i> species
<i>F. candida</i>	.27	1.03	.9 (.07)	Pamilo 1982
<i>F. aquilonia</i>	0	1.48	.96 (.14)	Pamilo 1993
<i>F. pratensis</i>	.53	1.44	.92 (.12)	Mean of estimates for other <i>Formica</i> s.s. species
<i>F. truncorum</i> M	.55/single queen	1.43	.78 (.14)	Sundström 1993
<i>F. truncorum</i> P	.025	1.43	.98 (.12)	Sundström 1993
<i>F. rufa</i>	.43	1.39	.90 (.14)	Ranta 2002
<i>F. polyctena</i>	.46	1.44	1.02 (.1)	Mean of estimates for other <i>Formica</i> s.s. species
<i>F. exsecta</i> M	.62/single queen	1.23	.84 (.17)	Sundström et al. 1996
<i>F. exsecta</i> P	.14	1.23	.95 (.14)	Sundström et al. 1996

<sup>a</sup> M = monogynous; P = polygynous. See appendix text for definitions of  $rw$  and  $rq$ .

APPENDIX B  
Loci Used in Microsatellite Analyses

**Table B1:** Primers with number of alleles, heterozygosities (He), and annealing temperatures for each species

<i>Formica</i> species <sup>a</sup>	FL12	FL20	FL21	FE13	FE16	FE17	FE19	FE21	FE37	FE42	FE49
<i>F. fusca</i> :											
Alleles (He)	5 (.61)	6 (.56)		7 (.75)		5 (.42)	4 (.24)	5 (.54)			
Temperature (°C)	55	55		55		50	55	50			
<i>F. cinerea</i> :											
Alleles (He)	3 (.39)	3 (.59)		3 (.56)		7 (.73)				3 (.24)	
Temperature (°C)	55	55		55		50				50	
<i>F. candida</i> :											
Alleles (He)			4 (.26)			6 (.77)	3 (.55)			3 (.57)	3 (.65)
Temperature (°C)			55			50	55			50	50
<i>F. aquilonia</i> :											
Alleles (He)				4 (.38)			4 (.58)	4 (.28)		2 (.51)	
Temperature (°C)				55			55	50		50	
<i>F. pratensis</i> :											
Alleles (He)	2 (.50)	6 (.76)	4 (.74)				5 (.75)				
Temperature (°C)	55	55	55				55				
<i>F. truncorum</i> M:											
Alleles (He)	3 (.65)		5 (.69)	2	2	2 (.46)	6 (.69)				
Temperature (°C)	55		55	50	50	50	50				
<i>F. truncorum</i> P:											
Alleles (He)			10 (.78)	10 (.8)					3 (.41)	6 (.63)	4 (.61)
Temperature (°C)			55	50					50	50	50
<i>F. rufa</i> :											
Alleles (He)	4 (.65)		4 (.65)				3 (.6)			2 (.15)	
Temperature (°C)	55		57				55			50	
<i>F. polyctena</i> :											
Alleles (He)			10 (.78)	10 (.8)					3 (.41)	6 (.63)	4 (.61)
Temperature (°C)			55	55					50	55	50
<i>F. exsecta</i> M:											
Alleles (He)			26 (.94)	13 (.71)					8 (.86)	6 (.81)	18 (.83)
Temperature (°C)			55	55					50	44 <sup>b</sup>	44 <sup>b</sup>
<i>F. exsecta</i> P:											
Alleles (He)			10 (.77)	10 (.81)					3 (.41)	6 (.63)	4 (.61)
Temperature (°C)			55	55					50	44 <sup>b</sup>	44 <sup>b</sup>

<sup>a</sup> M = monogynous; P = polygynous.<sup>b</sup> Multiplex PCR protocol.

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