

Deleterious *Wolbachia* in the ant *Formica truncorum*

T. Wenseleers^{1*}†, L. Sundström² and J. Billen¹

¹Zoological Institute, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

²Department of Ecology and Systematics, Division of Population Biology, PO Box 17, FIN-00014 Helsinki, Finland (liselotte.sundstrom@helsinki.fi)

Wolbachia is a maternally inherited bacterium that may manipulate the reproduction of its arthropod hosts. In insects, it is known to lead to inviable matings, cause asexual reproduction or kill male offspring, all to its own benefit, but to the detriment of its host. In social Hymenoptera, *Wolbachia* occurs widely, but little is known about its fitness effects. We report on a *Wolbachia* infection in the wood ant *Formica truncorum*, and evaluate whether it influences reproductive patterns. All 33 colonies of the study population were infected, suggesting that *Wolbachia* infection is at, or close to, fixation. Interestingly, in colonies with fewer infected workers, significantly more sexuals are produced, indicating that *Wolbachia* has deleterious effects in this species. In addition, adult workers are shown to have significantly lower infection rates (45%) than worker pupae (87%) or virgin queens (94%), suggesting that workers lose their infection over life. Clearance of *Wolbachia* infection has, to our knowledge, never been shown in any other natural system, but we argue that it may, in this case, represent an adaptive strategy to reduce colony load. The cause of fixation requires further study, but our data strongly suggest that *Wolbachia* has no influence on the sex ratio in this species.

Keywords: *Wolbachia*; sex ratio; selfish cytoplasmic elements; social insects; fitness

1. INTRODUCTION

Wolbachia are alpha proteobacteria that occur in the reproductive organs of various arthropods (O'Neill *et al.* 1997; Werren 1997; Stouthamer *et al.* 1999). Like mitochondria, which are phylogenetic relatives of *Wolbachia* (Sicheritz-Ponten *et al.* 1998; Lang *et al.* 1999; Muller & Martin 1999), they are only transferred vertically from mother to offspring. A unique aspect of *Wolbachia* is that they manipulate host reproduction in ways that enhance their transmission to future generations. For example, *Wolbachia* induces parthenogenesis in parasitoid wasps (Stouthamer 1997), feminizes genetic males in isopods (Rigaud 1997) and selectively kills male offspring in *Acraea* butterflies, a *Tribolium* beetle and a *Drosophila* fruit fly (Jiggins *et al.* 1998; Hurst *et al.* 1999, 2000; Fialho & Stevens 2000; Jiggins *et al.* 2000; Stevens *et al.* 2001). This results in highly female-biased host broods, thereby enhancing the spread of the exclusively maternally inherited *Wolbachia*.

Aside from sex-ratio distortion, *Wolbachia* may also cause reproductive incompatibility ('cytoplasmic incompatibility' or CI) whereby males infected with *Wolbachia* effectively sterilize uninfected females upon mating (Rousset & Raymond 1991; Hoffmann & Turelli 1997). The consequent reduction in local competition benefits *Wolbachia* clones present in neighbouring females (Frank 1997). CI may cause partial, to complete, reproductive failure in diploids (Hoffmann & Turelli 1997), but exclusive male production in haplodiploids (Saul 1961; Breeuwer & Werren 1993; Reed & Werren 1995; Perrot-Minnot *et al.* 1996; Vavre *et al.* 2000).

Wolbachia and other selfish cytoplasmic elements have been intensively studied in the light of their potential to cause unusual sex ratios in solitary Hymenoptera (Saul 1961; Breeuwer & Werren 1993; Hurst 1993; Reed & Werren 1995; Hurst *et al.* 1996; Perrot-Minnot *et al.* 1996; Stouthamer 1997). By contrast, studies on sex-ratio variation in social Hymenoptera have centred almost exclusively on the Trivers & Hare (1976) queen-worker conflict theory. In their seminal paper, Trivers & Hare (1976) pointed out that, owing to haplodiploidy, females are three times more related to full sisters than to brothers. Hence, workers should favour female-biased broods, against the interests of their mother queen. Empirical evidence, collected mostly from ants, shows that population-wide sex ratios are indeed usually female-biased, and closer to the worker optimum than to that of the queen (reviewed in Bourke & Franks 1995; Crozier & Pamilo 1996; Queller & Strassmann 1998; Chapuisat & Keller 1999). A few studies have also directly demonstrated that workers bias the sex ratio through fratricide (Passera & Aron 1996; Sundström *et al.* 1996; Chapuisat *et al.* 1997). Nevertheless, this does not exclude the possibility that sex ratios in social Hymenoptera are also under the influence of maternally transmitted symbionts (Wenseleers *et al.* 1998; Chapuisat & Keller 1999). Indeed, *Wolbachia* has recently been shown to occur widely in ants (Wenseleers *et al.* 1998; Jeyaprakash & Hoy 2000; Van Borm *et al.* 2001; Wenseleers 2001), making this possibility all the more intriguing.

We provide, to our knowledge, the first comprehensive study of patterns of *Wolbachia* infection in a social insect, the wood ant *Formica truncorum*. Our aims are threefold. (i) We analyse the prevalence of *Wolbachia* across different colonies, cohorts, castes and sexes. (ii) We test for plausible effects, such as CI or an influence on sex allocation. (iii) We test whether *Wolbachia* causes any deleterious

* Author for correspondence (t.wenseleers@sheffield.ac.uk).

† Present address: Laboratory of Apiculture and Social Insects, Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

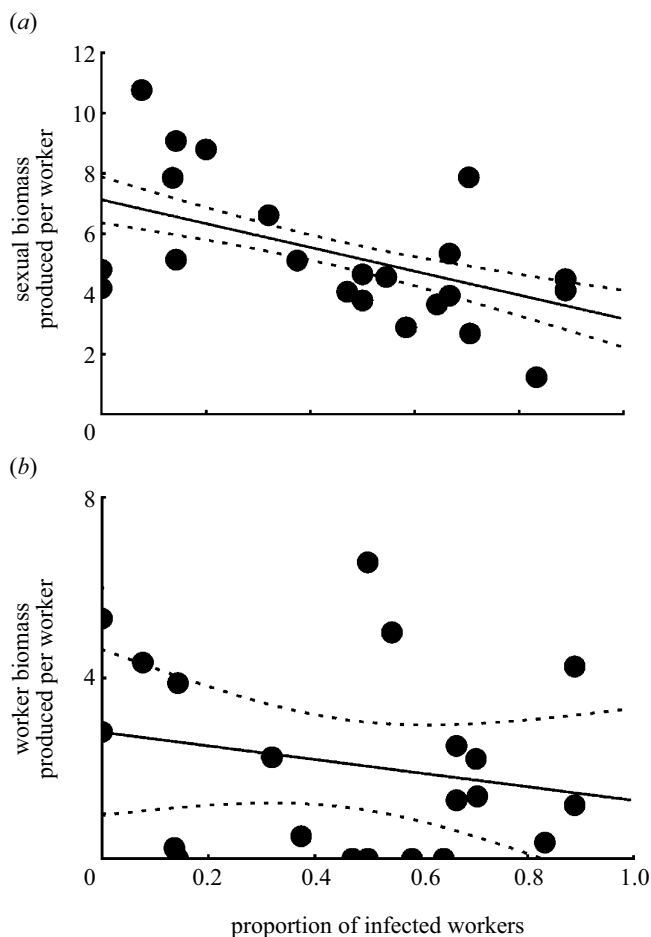


Figure 1. *Wolbachia* infection in workers reduces colony efficiency. Colonies with a higher fraction of infected workers produce less sexual biomass per worker (a) ($\beta = -0.56$, $F_{1,20} = 9.02$, $p < 0.01$, adjusted $R^2 = 28\%$). The per capita production of new workers, however, is unaffected by *Wolbachia* infection (b) ($\beta = -0.28$, $F_{1,20} = 1.66$, $p > 0.05$, adjusted $R^2 = 3\%$).

effects on its host in terms of reduced reproductive output of colonies. A direct negative impact on host fecundity has been demonstrated in a few solitary insects (Hoffmann *et al.* 1990; Min & Benzer 1997; Fleury *et al.* 2000). In social insects, however, deleterious effects may also appear as an indirect consequence of a metabolic load on the workers, which would result in a drop in colony productivity.

2. MATERIAL AND METHODS

(a) Study species and sampling

The study population, located in the archipelago of southern Finland, has been studied for more than 10 years (Sundström 1993, 1994, 1995a,b; Sundström & Ratnieks 1998; Walin *et al.* 1998; Sundström & Boomsma 2000). Thus, there are extensive background data on queen number and mating frequencies, as well as colony productivity and sex ratios. All colonies in this population have a single queen, which is mated to one or more (usually two) males (Sundström 1993, 1994). Queens regularly live for more than 10 years and all colonies have been headed by the same queen based on repeated genotyping of workers over several years (L. Sundström, unpublished data). Colonies typically contain 1000–10 000 workers and the production of both worker and sexual biomass tends to be in the same range (figure 1). Workers facultatively bias sex ratios in response to

Table 1. Summary of *Wolbachia* infection rates across different castes based on a screening of 33 colonies.

	% infected (N)	95% confidence intervals
sexuals		
males	95 (105)	89–98%
young queens	94 (191)	90–97%
workers		
pupae	87 (158)	81–91%
adults	45 (387)	40–50%

queen mating frequency so that colonies headed by singly-mated queens specialize in female production and those headed by multiply-mated queens specialize in male production (Sundström 1994, 1995b). The population-wide sex ratios are female-biased and coincide with the worker optimum of 63% female investment (Sundström 1994, 1995b; Sundström & Ratnieks 1998).

Most of the data for this study were collected in 1997 (table 1), when sex-investment ratios, colony size (number of adult workers), sexual production and worker production were estimated by mark recapture, as described in Sundström (1995b) and Sundström & Ratnieks (1998). The data were also enhanced with worker samples from additional colonies of other years (table 1). These were either older colonies that had died before 1997, or were newly founded in later years. From each colony up to 30 workers, young queens and males were collected and stored in 99% ethanol for subsequent DNA analysis (see Appendix A). Dry weights of males (6.6 ± 0.8 mg, $N = 200$, Sundström (1995a)), queens (8.7 ± 0.5 mg, $N = 200$, Sundström (1995a)) and workers (4.6 ± 0.7 mg, $N = 55$) were used to express colony production in biomass.

(b) Detection of *Wolbachia* infection

A PCR-based assay based on specific amplification of the *Wolbachia* *ftsZ* gene was used to detect *Wolbachia* in single ants. Amplification with the *Wolbachia*-specific primer pair *FtsZFT2* (5'-GAA-GGT-GTG-CGA-CGT-ATG-CG-3'-*FtsZRT3* 5'-CTG-ACT-TGA-GTA-GCC-AAA-ATT-GC-3' (Grasso *et al.* 2000) gives a fragment of ca. 755 bp when *Wolbachia* is present, but no fragment when absent or below detection threshold. Extractions of infected males of *F. truncorum* amplified up to a template dilution of 1 : 1000 relative to the concentration used in the PCRs. This means that, unlike in some other studies (Jeyapakash & Hoy 2000), we did not encounter any sensitivity problems and we should also have been able to detect weak infections, as the detection threshold was below 1/1000th of that of a typical infected male. Infection percentages mentioned hereafter refer to the percentage of the samples for which *Wolbachia* density fell below the detection threshold. To exclude errors owing to failure during amplification we re-amplified all negative samples twice. We also amplified a 555 bp fragment of the host *18S rDNA* gene in a separate reaction (using the insect-specific *18SF1* 5'-TTG-GAG-GGC-AAG-TCT-GGT-GC-3'-*18SR1* 5'-ACT-TCG-GCG-GAT-CGC-TAG-CT-3' primer pair, Grasso *et al.* (2000)), to exclude the possibility of detection failures owing to poor DNA quality. Samples for which the host gene did not amplify were discarded (in total seven samples were discarded). Primer stocks were always kept lyophilized in single-use subaliquots, to prevent them from degrading.

DNA was extracted by boiling single individuals in 500 μ l of

a 10% Biorad Chelex 100 resin solution for 30 min. The samples were centrifuged and stored at -20°C prior to use. PCR amplification reactions were carried out in 15 μl reaction mixtures consisting of 0.5 μM of each *FtsZ* primer (0.02 μM for the 18S *rDNA* primers to make the amplification sensitivity of the host gene match that of the *Wolbachia* gene), 0.2 mM of each dNTP, 1.5 mM MgCl_2 , 1.5 μl template, 0.3 U of Taq polymerase (AmpliTaq, Perkin Elmer Cetus) and 1 \times enzyme buffer supplied by the manufacturer. Each reaction mixture was overlaid with ca. 20 μl of mineral oil. For both the *FtsZ* and 18S *rDNA* gene, PCR was performed on a Biometra TGradient 96 Thermoblock with initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of 94°C for 30 s, 63°C for 1 min and 72°C for 2 min, and a 10 min terminal extension step at 72°C . Five millilitres of the reaction mixture were electrophoresed with a 100 bp DNA ladder size standard (GibcoBRL) on 1% agarose minigels. DNA bands were visualized by ethidium bromide staining and gels were digitized on a Pharmacia Biotech ImageMaster VDS gel documentation system. Known infected *F. truncorum* samples and ddH₂O were used as positive and negative controls.

(c) Cloning and sequencing

The *wsp* gene, which is more variable than *ftsZ* (Zhou *et al.* 1998; Van Meer *et al.* 1999), was used to determine the phylogenetic position of the *Wolbachia* found in *F. truncorum*. Amplification was performed using the primer pair *wsp81F-wsp691R* (Braig *et al.* 1998) and the resulting PCR product was cloned into a T-tailed vector using Invitrogen's TA-cloning kit. Plasmids were then isolated using the QIA-prep Spin plasmid kit (Qiagen) and sequenced on a Li-Cor sequencer. The *wsp* sequence obtained has been deposited in GenBank under accession number AF326978.

(d) Statistical procedures

The statistical significance of differences in infection rates among different colonies and castes was assessed using a generalized linear model (GLZ). Because infection status, the dependent variable, could only take the values 0 (absent) or 1 (present), a binomial error structure and logit link function was used. Binomial confidence intervals on the average proportions of infected workers and sexuals were calculated according to the formulae in Spiegel (1961). The effects of *Wolbachia* infection on sex ratio and colony productivity were tested using multiple regression or general linear models (GLM). The supplementary samples, not collected in 1997, were only used to assess the fraction of infected colonies in the population; in the other analyses only the data from 1997 were used. All statistical analyses were carried out using STATISTICA 5.5 (Statsoft 1995).

3. RESULTS

(a) Phylogenetic affiliation

Sequencing showed that the *F. truncorum* *Wolbachia* belongs to the A supergroup, the most common of one of three supergroups that occur in insects (O'Neill *et al.* 1997; Vandekerckhove *et al.* 1999), and primarily associated with incompatibility phenotypes (O'Neill *et al.* 1997). Its closest relative is a *Wolbachia* strain found in the braconid wasp *Asobara tabida* (GenBank accession no. AF124859), but with a 7.8% sequence difference the genetic distance is sufficient to warrant its placement in a novel *Wolbachia* subgroup, here termed 'Trunc' (the arbi-

trary criterion of Zhou *et al.* (1998) is greater than 2.5% sequence divergence).

(b) Infection incidence in different castes and colonies

All 33 colonies in the study population were infected by *Wolbachia* (see Appendix A and table 1 with summary figures). Intracolony infection rates for male (95%) and female (94%) sexuals did not differ significantly (GLZ, $p > 0.05$; table 1). Interestingly, adult workers had significantly lower infection rates (45%) than worker pupae (87%) (GLZ, $p < 0.000\ 0001$), while worker pupae and young queens had similar infection rates (GLZ, $p > 0.05$). Only when data from males and queens were pooled, was the infection rate of worker pupae significantly lower than that of sexuals (GLZ, $p = 0.015$; table 1). Infection rates of males, queens and workers also differed significantly across colonies (GLZ, $p < 0.0001$, except in the queens versus males analysis, where $p < 0.05$).

(c) Effects on sex ratio and colony fitness

The similar infection rates of males and queens show that *Wolbachia* does not influence sex-ratio patterns directly. However, the possibility of an indirect influence mediated by changes in worker behaviour remains. For instance, workers may kill males in excess of the fraction predicted under their colony-specific sex-ratio optimum. We tested for such an effect by regressing the sex ratio on the proportion of infected workers, using colony size and number of mates (to correct for the effect of facultative worker biasing of the sex ratio, Sundström (1994)) as cofactors. A GLM, however, demonstrates that there is no evidence for such an effect (table 2). As in previous analyses (Sundström 1994, 1995b), only the number of males the queen has mated with correlates with the sex ratio (table 2).

Within-colony infection incidence in workers may also affect fitness parameters such as colony productivity. A regression analysis shows that this is indeed the case, as colonies with a high fraction of infected workers produced less sexual biomass per adult worker ($\beta = -0.56$, $F_{1,20} = 9.02$, $p < 0.01$, adjusted $R^2 = 28\%$, figure 1a). The β coefficient value of approximately -0.5 implies that colonies in which all workers were infected were approximately half as efficient as colonies in which workers were completely clear of infection. However, the fraction of infected workers had no effect on the production of new workers ($\beta = -0.28$, $F_{1,20} = 1.66$, $p > 0.05$, adjusted $R^2 = 3\%$, figure 1b).

(d) Test for an incompatibility effect

A simple test for CI in social insects is to compare the production of uninfected queens from infected colonies with the frequency of uninfected colonies in the population overall. If *Wolbachia* causes CI, uninfected founding queens mated to infected males should be eliminated from the population (Wenseleers *et al.* 1998; Van Borm *et al.* 2001). As 6% of the virgin queens in the population were uninfected ($N = 191$), approximately 2 out of 33 colonies are expected to be uninfected if *Wolbachia* does not cause CI. In the whole study population, however, no uninfected colonies were found. Unfortunately, whether this deficiency of uninfected colonies is due to a CI effect is inconclusive, since the observed pattern is still within the limits of sampling error (one-sided binomial test, $p = 0.14$).

Table 2. Test statistics of two general linear models that show that colonies containing more infected workers (model 1) or worker pupae (model 2) do not produce a more female-biased sex ratio. (IW and IWP, proportion of infected workers and worker pupae; MATES, number of males the queen has mated with). Abbreviations: SS, sum of squares; MS, mean squares.

	SS	d.f.	MS	F	p
model 1: sex ratio on IW + colony size + MATES					
IW	0.11	1	0.11	0.85	0.37
colony size	0.09	1	0.09	0.69	0.42
MATES	0.63	1	0.63	4.88	0.04
error	2.31	18	0.13		
model 2: sex ratio on IWP + colony size + MATES					
IWP	0.27	1	0.27	2.99	0.10
colony size	0.25	1	0.25	2.81	0.11
MATES	0.61	1	0.61	6.83	0.02
error	1.33	15	0.09		

4. DISCUSSION

Our data show that *Wolbachia* occurs throughout a natural population of the ant *F. truncorum*, a model system in sex-ratio research (Sundström 1994, 1995b; Sundström & Ratnieks 1998; Sundström & Boomsma 2000). Contrary to expectation, however, it was found that *Wolbachia* does not influence sex allocation. (i) All of the colonies in the study population were infected, despite marked variation in the sex ratio produced by different colonies (Sundström 1994, 1995b). (ii) A direct effect, through male killing or parthenogenesis induction, could be excluded, because infection rates were similar in males and queens. In either case (male killing or parthenogenesis), the infection rate should be lower in males than in females (O'Neill *et al.* 1997; Stouthamer 1997; Jiggins *et al.* 1998, 2000, 2001; Hurst *et al.* 1999, 2000; Fialho & Stevens 2000; Van Borm *et al.* 2001). (iii) *Wolbachia* infection did not appear to affect the sex-allocation decisions of individual workers, since the proportion of infected workers or worker pupae did not correlate with the sex ratio produced by each colony. Thus, all the evidence indicates that *Wolbachia* has no influence on the sex ratio in this species (Sundström 1994, 1995b; Sundström & Ratnieks 1998; Sundström & Boomsma 2000).

Interestingly, colonies with more infected workers had a lower *per capita* sexual productivity. This suggests that *Wolbachia* has deleterious effects on colony function. Deleterious effects have also been shown in other insects (Hoffmann *et al.* 1990; Min & Benzer 1997; Fleury *et al.* 2000), but in these species the situation is unique because the costs apparently arise indirectly via a metabolic load on the workers. In mechanistic terms, it is possible that *Wolbachia* reduces the locomotory performance of workers, as demonstrated in the parasitoid wasps *Leptopilina heterotoma* (Fleury *et al.* 2000). Admittedly, the causality between infection incidence and productivity is hard to infer from field studies such as this one, and the result is possibly ambiguous. For example, if colonies with better resources lose their infection more readily, a correlation between infection incidence and productivity may arise as a by-product of a correlation with environmental conditions and resource abundance. Indeed, resource abundance has been shown to correlate with colony size (Deslippe & Savolainen 1995). This effect, however, was factored out in the present study because the analysis was

based on productivity per worker rather than total production. Therefore, the interpretation that *Wolbachia* infection truly reduces worker performance remains the most plausible one.

A second major result was that adult workers were found to have lower infection rates (45%) than worker pupae (87%) or virgin queens (94%). This could either mean that workers lose their infection over life, or that infected workers experience greater mortality than uninfected ones. However, the latter hypothesis seems unlikely for two reasons. (i) If the difference in infection rate were due to mortality, one would expect a positive relationship between worker infection rate and colony fitness, since less-infected colonies, i.e. colonies where more infected workers die, should tend to be less productive. This is contrary to the relationship we found. (ii) A shift in infection rate from 87% to 45% would require that approximately half of all workers are eliminated—perhaps too heavy a burden on the colony to be very realistic. Therefore, loss of infection seems the most probable explanation for the low infection rates of adult workers. In addition, such infection clearance would have adaptive benefits, since colonies with low infection rates were found to produce more sexuals. Loss of infection from the sterile worker caste to boost colony productivity is akin to the idea that germline symbionts (and their host) benefit from clearance in somatic tissue if that improves individual fitness (Frank 1996). In this respect, our study documents the prediction that heritable symbionts have no interest in being sterile workers, as no transmission to future generations can occur through them.

Are other interpretations possible for the low infection rates of adult workers? Jeyaprakash & Hoy (2000) reported that detecting weak *Wolbachia* infections may be difficult using standard PCR methods. In our case, however, low PCR sensitivity cannot account for the low infection rate of adult workers, since *Wolbachia* could be amplified from infected samples at template dilutions of up to 1:1000, which included worker pupae samples. Nevertheless, it does remain possible that *Wolbachia* is lost from adult workers due to non-adaptive factors. For example, infection clearance could also be a by-product of ovarian regression, as often occurs across the lifespan of individual worker ants (Hölldobler & Wilson 1990). If *Wolbachia* is restricted to ovarian tissue (as was shown by

Cheng *et al.* (2000), but see Dobson *et al.* (1999) and Min & Benzer (1997)), then ovarian degeneration might cause adult workers to lose their infection. However, in *F. truncorum*, *Wolbachia* also occurs abundantly in other tissues (e.g. nerve tissue: T. Wenseleers, unpublished data), and newly eclosed *F. truncorum* workers have only weakly developed ovaries (H. Helanterä, personal communication). Hence, ovarian regression alone is unlikely to account for loss of infection in adult workers. Alternatively, *Wolbachia* might be eliminated from workers because of exposure to harsher environmental conditions, such as higher temperatures, which have been shown to affect *Wolbachia* density in other insects (O'Neill *et al.* 1997). Irrespective of the actual way in which *Wolbachia* is cured from the ants, however, our study stands as the first demonstration, to our knowledge, of *Wolbachia* infection clearance in a natural system.

Given that *Wolbachia* occurs throughout the whole

population, it is clear that it must have evolved an efficient means of transmission. Unfortunately, based upon our data, the exact mechanism of infection remains unknown. An indirect test for CI (Hoffmann & Turelli 1997) proved inconclusive, and *Wolbachia* did not seem to cause female biased sex ratios. Clearly, laboratory experiments will need to be carried out to elucidate the mechanism by which *Wolbachia* has become established in this species.

In conclusion, this study provides what is, to our knowledge, the first evidence for (i) *Wolbachia* having a deleterious effect on colony performance in a social insect, and (ii) partial mitigation of such costs by infection clearance in workers. The outcome represents the best interests of both host and symbiont, as both parties benefit from increased colony productivity. These observations provide a solid basis for future experimental work that could elucidate the causal pathways and the means by which *Wolbachia* has come to infect this ant species.

APPENDIX A

Wolbachia infection rates across different castes in the 33 colonies screened.

colony	year of collection	% of males (N)	caste			
			% of young queens (N)	% of sexuals (total N)	% of worker pupae (N)	% of adult workers (N)
brsk1	1997		86 (8)	86 (8)	100 (6)	14 (14)
brsk2	1997	100 (8)	100 (4)	100 (12)	100 (5)	55 (11)
brsk5	1997	100 (2)	100 (9)	100 (11)	100 (2)	58 (12)
jsk1	1997	100 (5)	100 (4)	100 (9)	67 (6)	38 (8)
jsk2	1997	100 (6)		100 (6)		50 (24)
jsk4	1997					26 (27)
kh2	1997		100 (4)	100 (4)	100 (4)	20 (10)
kh5	1997		63 (8)	63 (8)	100 (6)	14 (22)
kh6	1997		100 (8)	100 (8)	86 (7)	67 (15)
msk1	1997		71 (7)	71 (7)	100 (7)	67 (21)
msk11	1997		100 (8)	100 (8)	100 (2)	89 (9)
msk12	1997	100 (8)		100 (8)	100 (7)	89 (9)
msk13	1997		100 (7)	100 (7)		47 (17)
msk4	1997	78 (9)	86 (14)	83 (23)	100 (3)	50 (16)
msk5	1997	63 (8)	75 (4)	67 (12)	92 (12)	64 (14)
msk6	1997	89 (9)		89 (9)	17 (6)	8 (13)
msk7	1997	100 (9)		100 (9)	43 (7)	83 (6)
msk9	1997		100 (9)	100 (9)	100 (8)	70 (27)
rh1	1997		90 (21)	90 (21)		14 (14)
rh2	1997		89 (9)	89 (9)	100 (5)	71 (17)
sh6	1997	100 (4)	100 (16)	100 (20)	84 (19)	0 (26)
sh7	1997	100 (9)		100 (9)	50 (30)	32 (25)
sh8	1997	100 (2)	100 (7)	100 (9)	100 (7)	0 (14)
brsk6	1998				100 (9)	
brsk7	2000	100 (9)		100 (9)		
jsk5	1998		100 (9)	100 (9)		
jsk6	1998		100 (9)	100 (9)		
msk10	1992		100 (9)	100 (9)		
msk14	2000		100 (9)	100 (9)		
sh2	1991	100 (9)		100 (9)		
sh3	1994	100 (8)		100 (8)		
sh4	1993					100 (8)
sh5	1993		100 (8)	100 (8)		0 (8)
Overall (total N = 841)		95 (105)	94 (191)	95 (296)	87 (158)	45 (387)

We are grateful to Paul Stevens and Steven Van Borm for their help in the laboratory, to Laurent Keller and Francis Ratnieks for their comments, and to Minttu Hannonen, Lauri Saarikoski, Kalle Trontti and Heikki Helanterä for their aid in the field and sharing unpublished data. This work was funded by the EU 'Social Evolution' and INSECTS TMR networks, and by grants from the Flemish Fund for Scientific Research (FWO-Vlaanderen, grant no. 2.0014.94 to J.B. and T.W.), the University of Helsinki and the Academy of Finland (grants no. 42725 to L.S. and 46661 to the Spatial Ecology programme).

REFERENCES

- Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants*. Princeton University Press.
- Braig, H. R., Zhou, W., Dobson, S. L. & O'Neill, S. L. 1998 Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *J. Bacteriol.* **180**, 2373–2378.
- Breeuwer, J. A. J. & Werren, J. H. 1993 Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics* **135**, 565–574.
- Chapuisat, M. & Keller, L. 1999 Testing kin selection with sex allocation data in eusocial Hymenoptera. *Heredity* **82**, 473–478.
- Chapuisat, M., Sundström, L. & Keller, L. 1997 Sex-ratio regulation: the economics of fratricide in ants. *Proc. R. Soc. Lond. B* **264**, 1255–1260. (DOI 10.1098/rspb.1997.0173.)
- Cheng, Q., Ruel, T. D., Zhou, W., Moloo, S. K., Majiwa, P., O'Neill, S. L. & Aksoy, S. 2000 Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. *Med. Vet. Entomol.* **14**, 44–50.
- Crozier, R. H. & Pamilo, P. 1996 *Evolution of social insect colonies. Sex allocation and kin selection*. Oxford University Press.
- Deslippe, R. J. & Savolainen, R. 1995 Sex investment in a social insect: the proximate role of food. *Ecology* **76**, 375–382.
- Dobson, S. L., Bourtzis, K., Braig, H. R., Jones, B. F., Zhou, W., Rousset, F. & O'Neill, S. L. 1999 *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem. Mol. Biol.* **29**, 153–160.
- Fialho, R. F. & Stevens, L. 2000 Male-killing *Wolbachia* in a flour beetle. *Proc. R. Soc. Lond. B* **267**, 1469–1474. (DOI 10.1098/rspb.2000.1166.)
- Fleury, F., Vavre, F., Ris, N., Fouillet, P. & Bouletreau, M. 2000 Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma*. *Parasitology* **121**, 493–500.
- Frank, S. A. 1996 Host control of symbiont transmission: the separation of symbionts into germ and soma. *Am. Nat.* **148**, 1113–1124.
- Frank, S. A. 1997 Cytoplasmic incompatibility and population structure. *J. Theor. Biol.* **184**, 327–330.
- Grasso, D. A., Wenseleers, T., Mori, A., Le Moli, F. & Billen, J. 2000 Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. *Ethol. Ecol. Evol.* **12**, 309–314.
- Hoffmann, A. A. & Turelli, M. 1997 Cytoplasmic incompatibility in insects. In *Influential passengers. Inherited microorganisms and arthropod reproduction* (ed. S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 42–80. Oxford University Press.
- Hoffmann, A. A., Turelli, M. & Harshman, L. G. 1990 Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* **126**, 933–948.
- Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Berlin: Springer.
- Hurst, G. D. D., Jiggins, F. M., von der Schulenburg, J. H. G., Bertrand, D., West, S. A., Goriacheva, I., Zakharov, I. A., Werren, J. H., Stouthamer, R. & Majerus, M. E. N. 1999 Male killing *Wolbachia* in two species of insect. *Proc. R. Soc. Lond. B* **266**, 735–740. (DOI 10.1098/rspb.1999.0698.)
- Hurst, G. D. D., Johnson, A. P., von der Schulenburg, J. H. G. & Fuyama, Y. 2000 Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. *Genetics* **156**, 699–709.
- Hurst, L. D. 1993 The incidences, mechanisms and evolution of cytoplasmic sex-ratio distorters in animals. *Biol. Rev.* **68**, 121–194.
- Hurst, L. D., Atlan, A. & Bengtsson, B. O. 1996 Genetic conflicts. *Q. Rev. Biol.* **71**, 317–364.
- Jeyaprakash, A. & Hoy, M. A. 2000 Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. *Insect Mol. Biol.* **9**, 393–405.
- Jiggins, F. M., Hurst, G. D. D. & Majerus, M. E. N. 1998 Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male killing bacterium. *Heredity* **81**, 87–91.
- Jiggins, F. M., Hurst, G. D. D., Dolman, C. E. & Majerus, M. E. N. 2000 High-prevalence male-killing *Wolbachia* in the butterfly *Acraea encedana*. *J. Evol. Biol.* **13**, 495–501.
- Jiggins, F. M., Bentley, J. K., Majerus, M. E. N. & Hurst, G. D. D. 2001 How many species are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common by intensive sampling. *Proc. R. Soc. Lond. B* **268**, 1123–1126. (DOI 10.1098/rspb.2001.1632.)
- Lang, B. G., Gray, M. W. & Burger, B. 1999 Mitochondrial genome evolution and the origin of eukaryotes. *A. Rev. Genet.* **33**, 351–397.
- Min, K. T. & Benzer, S. 1997 *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc. Natl Acad. Sci. USA* **94**, 10 792–10 796.
- Muller, M. & Martin, W. 1999 The genome of *Rickettsia prowazekii* and some thoughts on the origin of mitochondria and hydrogenosomes. *BioEssays* **21**, 377–381.
- O'Neill, S. L., Hoffmann, A. A. & Werren, J. H. 1997 *Influential passengers. Inherited microorganisms and arthropod reproduction*. Oxford University Press.
- Passera, L. & Aron, S. 1996 Early sex discrimination and male brood elimination by workers of the Argentine ant. *Proc. R. Soc. Lond. B* **263**, 1041–1046.
- Perrot-Minnot, M. J., Guo, L. R. & Werren, J. H. 1996 Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: effects on compatibility. *Genetics* **143**, 961–972.
- Queller, D. C. & Strassmann, J. E. 1998 Kin selection and social insects. *Bioscience* **48**, 165–175.
- Reed, K. M. & Werren, J. H. 1995 Induction of paternal genome loss by the paternal-sex-ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): a comparative study of early embryonic events. *Mol. Reprod. Dev.* **40**, 408–418.
- Rigaud, T. 1997 Inherited microorganisms and sex determination of arthropod hosts. In *Influential passengers. Inherited microorganisms and arthropod reproduction* (ed. S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 81–101. Oxford University Press.
- Rousset, F. & Raymond, M. 1991 Cytoplasmic incompatibility in insects—why sterilize females? *Trends Ecol. Evol.* **6**, 54–57.
- Saul, G. B. 1961 An analysis of non-reciprocal cross incompatibility in *Mormoniella vitripennis* (Walker). *Zeitschr. Vererbungs.* **92**, 28–33.
- Sicheritz-Ponten, T., Kurland, C. G. & Andersson, S. G. E. 1998 A phylogenetic analysis of the cytochrome *b* and cytochrome *c* oxidase I genes supports an origin of mitochondria from within the Rickettsiaceae. *Biochim. Biophys. Acta* **1365**, 545–551.
- Spiegel, M. R. 1961 *Theory and problems of statistics*. Schaum's Outline Series. New York: McGraw-Hill.

- StatSoft 1995 *STATISTICA for Windows (computer program manual)*. Tulsa, OK: StatSoft, Inc. See <http://www.statsoft.com>.
- Stevens, L., Giordano, R. & Fialho, R. F. 2001 Male-killing, nematode infection, bacteriophage infection, and virulence of cytoplasmic bacteria in the genus *Wolbachia*. *A. Rev. Ecol. Syst.* **32**, 519–545.
- Stouthamer, R. 1997 *Wolbachia*-induced parthenogenesis. In *Influential passengers. Inherited microorganisms and arthropod reproduction* (ed. S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 102–124. Oxford University Press.
- Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. 1999 *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *A. Rev. Microbiol.* **53**, 71–102.
- Sundström, L. 1993 Genetic population structure and socio-genetic organization 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**, 266–267.
- Sundström, L. 1995a Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. *Behav. Ecol.* **6**, 132–139.
- Sundström, L. 1995b 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. & Boomsma, J. J. 2000 Reproductive alliances and posthumous fitness enhancement in male ants. *Proc. R. Soc. Lond. B* **267**, 1439–1444. (DOI 10.1098/rspb.2000.1161.)
- Sundström, L. & Ratnieks, F. L. W. 1998 Sex ratio conflicts, mating frequency, and queen fitness in the ant *Formica truncorum*. *Behav. Ecol.* **9**, 116–121.
- 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.
- Van Borm, S., Wenseleers, T., Billen, J. & Boomsma, J. J. 2001 *Wolbachia* in leafcutter ants: a widespread symbiont that may induce male killing or incompatible matings. *J. Evol. Biol.* **14**, 805–814.
- Van Meer, M. M., Witteveldt, J. & Stouthamer, R. 1999 Phylogeny of the arthropod endosymbiont *Wolbachia* based on the *wsp* gene. *Insect Mol. Biol.* **8**, 399–408.
- Vandekerckhove, T. T., Watteyne, S., Willems, A., Swings, J. G., Mertens, J. & Gillis, M. 1999 Phylogenetic analysis of the 16S rDNA of the cytoplasmic bacterium *Wolbachia* from the novel host *Folsomia candida* (Hexapoda, Collembola) and its implications for wolbachial taxonomy. *FEMS Microbiol. Lett.* **180**, 279–286.
- Vavre, F., Fleury, F., Varaldi, J., Fouillet, P. & Bouletreau, M. 2000 Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. *Evolution* **54**, 191–200.
- Walsh, L., Sundström, L., Seppä, P. & Rosengren, R. 1998 Worker reproduction in ants—a genetic analysis. *Heredity* **81**, 604–612.
- Wenseleers, T. 2001 Conflict from cell to colony. PhD thesis, Katholieke Universiteit Leuven, Belgium.
- Wenseleers, T., Ito, F., Van Borm, S., Huybrechts, R., Volckaert, F. & Billen, J. 1998 Widespread occurrence of the micro-organism *Wolbachia* in ants. *Proc. R. Soc. Lond. B* **265**, 1447–1452. (DOI 10.1098/rspb.1998.0456.)
- Werren, J. H. 1997 Biology of *Wolbachia*. *A. Rev. Entomol.* **42**, 587–609.
- Zhou, W., Rousset, F. & O'Neil, S. 1998 Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc. R. Soc. Lond. B* **265**, 509–515. (DOI 10.1098/rspb.1998.0324.)

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.