

Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*

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In hymenopteran societies, workers are not always sterile, and may produce parthenogenetically either males (arrhenotoky) or females (thelytoky). Thelytoky however is exceptional, and has been recorded in only four ant species. Here we provide evidence for worker thelytoky in an additional species, the harvesting ant *Messor capitatus* (Latreille) (Hymenoptera Formicidae Myrmicinae). Two orphaned colonies produced a large amount of worker offspring during 10 months in the laboratory. Dissections showed that reproduction was performed by workers and not by mated worker-like individuals (ergatoids). In some parasitoid wasps, parthenogenetic reproduction is caused by the maternally transmitted bacterium *Wolbachia*. Using a PCR-based assay we showed that *Wolbachia* can not be involved in parthenogenesis induction in this species. Finally, we point out reasons for the low *Wolbachia* susceptibility of parthenogenetic ant species.

KEY WORDS: worker reproduction, thelytoky, arrhenotoky, *Messor capitatus*, *Wolbachia*, social insects.

INTRODUCTION

It is clear that the common picture of hymenopteran societies as being composed of fertile queens and sterile workers can no longer be upheld. Frequently, workers produce some of the males in the colony (reviewed by FLETCHER & ROSS 1985, BOURKE 1988, CHOE 1988, CROZIER & PAMILO 1996; for recent genetic evidence see EVANS 1998). More exceptionally, they may also produce diploid female eggs, through amphigonic (reviewed by PEETERS 1997) or parthenogenetic reproduction (thelytoky) (BOURKE 1988, CHOE 1988, CROZIER & PAMILO 1996). In ants, thelytoky has been recorded in only four species: in *Cataglyphis piliscapa* (LENOIR et al. 1988, where it occurs under orphaned conditions, and in *Pristomyrmex pungens*: ITOW et al. 1984; TSUJI 1988, 1990), *Cerapachys biroi* (TSUJI & YAMAUCHI 1995), and *Platythyrea punctata* (SCHILDER et al. 1999a, 1999b) where it is an obligate phenomenon. Although the factors promoting amphigonic worker reproduction and worker thelytoky have largely been discussed separately (gamergate reproduction: PEETERS

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1997; thelytoky: LENOIR & CAGNIANT 1986, MORITZ 1989, GREEFF 1996), GADAGKAR (1997) argues for a more integrated approach. In his view, selection for solitary reproduction leads to reproductive totipotency.

One factor that potentially detracts from such an approach is that parthenogenesis need not be adaptive from the perspective of the nuclear genes. In particular, in chalcidoid and cynipoid parasitoid wasps the maternally transmitted parasite *Wolbachia* has been shown to be responsible for parthenogenetic reproduction (reviewed by STOUTHAMER 1997). *Wolbachia* manipulates these wasps to produce broods of mostly females to guarantee its spread and survival. In addition, *Wolbachia* may also cause three other reproductive alterations in arthropods: feminisation, male killing or sterilisation of uninfected females (reviewed by STOUTHAMER et al. 1999). Recent studies have shown these bacteria are present in 15% of all insect species (WERREN et al. 1995), and in 50% of Indo-Australian ant species (WENSELEERS et al. 1998).

The aims of this paper are twofold. First, we report on worker thelytoky in the harvesting ant *Messor capitatus* (cf. the preliminary report of GRASSO et al. 1998). Secondly, we show that in *Messor capitatus*, thelytoky has evolved independently from *Wolbachia* and point out reasons for the low *Wolbachia* susceptibility of parthenogenetic ant species.

MATERIALS AND METHODS

Messor collection, laboratory conditions and observations

The ants were collected from two colonies near Brescia (Northern Italy) and were used to set up two queenless worker groups, each consisting of 350 adult workers. At the time of collection these colony fragments did not contain any eggs or larvae. The worker groups were kept in plaster nests at 25 °C, a relative humidity of 70% and a constant 12:12 photoperiod and were observed daily. They were fed with seeds, freshly killed insects and honey; water was provided ad libitum. Thirty workers of each colony were dissected under distilled water to verify the state and features of their ovaries.

PCR-based *Wolbachia* screening

Sampling and DNA extraction

Messor capitatus material was collected from the two thelytokous laboratory colonies and from natural colonies of Berceto (province of Parma) and Rome, and was subsequently preserved in ethanol 95%. DNA was extracted by boiling the abdomen of single individuals in 600 µl 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

Based on Genbank deposited *Wolbachia* *ftsZ* sequences (WERREN et al. 1995, SCHILTHUIZEN & STOUTHAMER 1998), a generic and two strain specific primer pairs were developed for *Wolbachia* specific *ftsZ* amplification. For maximum sensitivity and reliability, a two step PCR assay was used. The generic primer pair FtsZFT2 5'-GAA GGT GTG CGA CGT ATG CG-3' – FtsZRT3 5'-CTG ACT TGA GTA GCC AAA ATT GC-3' was used in a first amplification round resulting in a 751-762 bp fragment depending upon strain. One µl of 1:1000 diluted reaction product served as a template for a second multiplex PCR. There we combined the FtsZFT2 forward primer with the specific reverse primers FtsZRTA1 5'-CTC TGA GTC TTC

GCT CTG ACT TAT AGG-3' and FtsZRTB2 5'-ACT CTT TCG TTT GTT TGC TCA GTT G-3' for the simultaneous amplification of a 591 bp and 670 bp stretch of the *Wolbachia ftsZ* gene of strains A and B respectively (Fig. 1). Results have shown that these primer pairs are specific for *Wolbachia*, as they amplify all ant *Wolbachia* (WENSELEERS et al. 1998), but fail to amplify other members of the alpha proteobacteria. In addition, we checked the DNA quality of all samples through amplification of 18S rDNA of the host (for details see WENSELEERS & BILLEN 2000). Samples for which this control PCR failed were discarded.

All PCR amplification reactions were carried out in a 15 µl reaction mixture consisting of 0.5 µM of each Ftsz primer (1 µM of the FtszFT2 primer in the multiplexed PCR), 0.2 mM of each dNTP, 1.5 mM MgCl₂, 3 µl template, 0.3 U of *Taq* polymerase (AmpliTaq, Perkin Elmer Cetus) and 1 x enzyme buffer supplied by the manufacturer. Each reaction mixture was overlaid with 20 µl of mineral oil. For both the *ftsZ* and 18S rDNA gene, PCR was performed on a Biometra TRIO-Thermoblock 48 with initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of 94 °C for 30 sec, 60 °C for 1 min and 72 °C for 2 min. Five µl of the reaction mixture from the last PCR step was electrophoresed with a 100 bp DNA ladder size standard (GibcoBRL) on 1% agarose minigels. DNA bands were visualised by ethidium bromide staining and gels were digitised on a Pharmacia Biotech ImageMaster[®] VDS gel doc system. *Gnamptogenys menadensis* (A strain infected, from Karaenta, Sulawesi), *Diplolepis rosae* (B strain infected, from Aix-en-Provence, France), a *Monomorium* sp. (infected by both strains, from Kebun Raya Bogor, West Java), and ddH₂O were included as positive and negative controls in every amplification in order to discriminate between an experimental failure of amplification and lack of infection (Fig. 1).

RESULTS

Thelytoky

One month after orphanage (25 and 33 days for the first and second group, 29 ± 5.6 days on average), both worker groups started to lay eggs. These eggs hatched and matured into workers after an average development time of $55 (\pm 2.8)$ days. No males were produced during this initial period. Subsequently, the egg-laying activity increased, and a large amount of brood was produced in the following 10 months, all of which eclosed into workers. For example, 8 months after initial orphanage, 191 and 150 larvae were present in colony 1 and 2 respectively, and when these were reared in a subfragment of the colony, they all developed into workers. After this initial 10 month period however, we also started to record some large larvae in both colonies that developed into males. The production of males

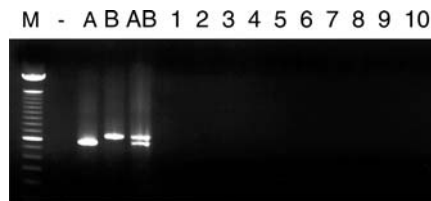


Fig. 1. — Gel showing absence of *Wolbachia* infection in *Messor capitatus*. Lane M: 100 bp size marker; -: negative control (ddH₂O); A: *Gnamptogenys menadensis* A strain infection; B: *Diplolepis rosae* B strain infection; AB: *Monomorium* sp. double A+B strain infection; 1-2: *Messor capitatus* workers from a first thelytokous colony collected at Brescia (Italy); 3: male from the same colony; 4-5: *Messor capitatus* workers from a second thelytokous colony collected at Brescia (Italy); 6: a male from that colony; 7-8: *Messor capitatus* workers from a colony collected at Berceto (Italy); 9-10: *Messor capitatus* workers from a colony collected at Rome (Italy).

became gradually predominant so that in the following months only male offspring was present. During the final 2 months of observation, a few tens of males were reared, while a few hundred workers had been reared by these colonies in the preceding months.

Dissection of adult workers showed that 78% of them had well-developed functional ovaries (consisting of two ovarioles each) without spermatheca. This confirms that unmated workers were the reproductively active individuals and that worker production did not result from the presence of mated worker-like reproductives (ergatoid queens; moreover ergatoids have not been recorded in any *Messor* species, PEETERS 1991).

Wolbachia screening

Ten workers and three males from each of the laboratory colonies and 10 workers from each of the two field colonies were examined for *Wolbachia* infection using a PCR assay, but none turned out to be infected (Fig. 1). The correct amplification of all positive controls indicates true lack of infection and not a failure in amplification (Fig. 1). In addition, the correct amplification of the nuclear 18S rDNA gene indicates that the quality of the DNA extractions should have been sufficient to detect *Wolbachia* if it had been present.

DISCUSSION

The results of our research show that, under certain conditions, workers of *M. capitatus* produce female offspring by thelytokous parthenogenesis. In the laboratory, a large amount of workers were produced for almost a year, allowing both colonies to flourish in absence of the mother queen. Dissections showed that the reproductively active individuals in the colony did not have a spermatheca, excluding the possibility that female eggs were produced by mated worker-like reproductives. So far, strong evidence for worker thelytoky has been gathered in five social hymenopteran species only: the Cape Honey Bee (see references in MORITZ & HABERL 1994) and four ant species: *Cataglyphis piliscapa* (LENOIR & CAGNIANT 1986), *Platythyrea punctata* (HEINZE & HÖLLDOBLER 1995; SCHILDER et al. 1999a, 1999b), *Pristomyrmex pungens* (ITOW et al. 1984) and *Cerapachys biroi* (TSUJI & YAMAUCHI 1995).

Our research also confirms a study by DELAGE (1968), in that *M. capitatus* workers can under certain conditions produce males in queenless colonies (in our case after prolonged housing in the laboratory). Hence, workers of this species can be considered deuterotokous, being able to generate both male and female offspring parthenogenetically. Worker male production under orphaned conditions has previously also been observed in *Messor ebeninus* and *M. semirufus* (CHOE 1988). As noted by BOURKE (1988), this is what would be expected from kin selection theory: when the mother queen dies, male production is the workers' only option left to increase their fitness. In thelytokous species, like *M. capitatus*, where workers have the option to produce either sex, the situation is more complex. The observed switch from female to male production especially needs explaining. One interpretation is that after queen loss, workers would try to rear a replacement queen, but once a source of female siblings had been established they would switch to producing their own males. The fact that only workers eclosed in the laboratory could then be seen as an artefact, since caste development is generally under complete environ-

mental control (HÖLLDOBLER & WILSON 1990). Rearing of replacement queens as a factor favouring facultative worker thelytoky has support from the parthenogenetic cape bee, since it is the only honey bee race where replacement queens can be reared only through thelytokous worker reproduction (R.F.A. MORITZ pers. comm.).

To gain insight into the evolutionary factors that could promote female parthenogenesis in *M. capitatus*, we also tested the possibility of reproductive manipulation by the maternally transmitted bacterium *Wolbachia*. The results of our PCR-based screening however show that none of the examined samples were infected, thus excluding the possibility of *Wolbachia* involvement in the parthenogenetic reproduction of *Messor capitatus*.

The fact that *Wolbachia* does not seem to be involved in parthenogenesis induction in this ant species, nor in any of the other thelytokous ant species (WENSELEERS & BILLEN 2000) may not be surprising. Parthenogenesis inducing *Wolbachia* generally make their host produce completely homozygous diploid offspring, which in ants would result in the exclusive production of sterile diploid males (STOUTHAMER 1997 and references in WENSELEERS & BILLEN 2000). But why should a *Wolbachia* with another phenotype not occur in any of the parthenogenetic social Hymenoptera? First, it is easy to see that a *Wolbachia* causing mating incompatibility cannot spread in parthenogenetically reproducing species since they do not mate. Second, a male killing *Wolbachia* that distorts the sex ratio would spread with greater difficulty in a species where thelytokous reproduction has already lead to a highly female biased sex ratio because it becomes impossible for *Wolbachia* to do any better. These may be the prime reasons why thelytokous social Hymenoptera never seem to be infected with *Wolbachia* (WENSELEERS & BILLEN 2000).

What selective forces may have favoured thelytoky in *Messor capitatus* remain enigmatic however. Based on the fact that thelytoky in ants seems to be a derived character, occurring patchily in different subfamilies (BOURKE 1988, CHOE 1988, HEINZE & HÖLLDOBLER 1995, TSUJI & YAMAUCHI 1995, CROZIER & PAMILO 1996), GADAGKAR (1997) interpreted thelytoky as a partial reversal of social evolution. Future empirical work along these lines might prove fruitful.

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