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In haplodiploid insect species, thelytokous parthenogenesis may be associated with bacterial endosymbionts that manipulate their host reproduction. Such an association was tested in the highly invasive genus *Megastigmus*, within which thelytokous parthenogenesis is much less frequent than arrhenotoky, which is the classical way of reproduction in chalcid insects.

## SCREENING FOR REPRODUCTION-ASSOCIATED ENDOSYMBIONTS AMONG *MEGASTIGMUS* SPECIES

Specific primers were used for the screening of *Wolbachia* (*wsp*), *Cardinium* (16S rDNA), *Rickettsia* (16S rRNA), and *Arsenophonus* (23S rDNA) endosymbionts in seven thelytokous and three arrhenotokous *Megastigmus* species. PCR amplification with *Wolbachia* primers was successful in all of the seven thelytokous *Megastigmus* species while no reaction occurred using *Cardinium*, *Rickettsia* and *Arsenophonus* primers. None of the three arrhenotokous species tested allowed amplification whatever the primer (Table 1).



TABLE 1. Detection tests of endosymbionts associated with host reproduction manipulation in 8 thelytokous and 3 arrhenotokous *Megastigmus* species.

Species (N)	Reproduction	Infection %			
		<i>Wolbachia</i>	<i>Cardinium</i>	<i>Arsenophonus</i>	<i>Rickettsia</i>
<i>M. rosae</i> (30)	thelytoky	100	0	0	0
<i>M. aculeatus</i> (43)	thelytoky	100	0	0	0
<i>M. brevicaudis</i> (1)	thelytoky	100	not tested	not tested	not tested
<i>M. pinsapinis</i> (60)	thelytoky	100	0	0	0
<i>M. suspectus</i> (65)	thelytoky	100	0	0	0
<i>M. pictus</i> (30)	thelytoky	100	0	0	0
<i>M. pistaciae</i> (30)	thelytoky	100	0	0	0
<i>M. alba</i> n.sp. (5)	thelytoky	100	not tested	not tested	not tested
<i>M. spermotrophus</i> (30)	arrhenotoky	0	0	0	0
<i>M. rafni</i> (30)	arrhenotoky	0	0	0	0
<i>M. schimitscheki</i> (30)	arrhenotoky	0	0	0	0

## ASSOCIATION BETWEEN THELYTOKY AND *WOLBACHIA* ENDOSYMBIONTS

Both *Wolbachia* infection and progeny sex ratios were compared between non- and antibiotic-treated females of *M. pinsapinis*. Seven-day tetracycline treatments of *M. pinsapinis* females resulted in a loss of *Wolbachia* infection and in a return to an arrhenotokous mode of reproduction as they mainly produced a male progeny using asexual reproduction (Table 2).



TABLE 2. Effects of antibiotic treatments on the infection of *M. pinsapinis* females by *Wolbachia* and their male production following asexual reproduction. (Control = sugared water ; Antibiotic = Tetracycline (2<sup>nd</sup>°) + sugared water)

Treatment mode	Treatment duration (in days)	Infection % by <i>Wolbachia</i> after treatment (N females)	Frequency of males in the progeny ± S.E. (N larvae)
Control	3	100 (38)	0 (127)
	7	100 (44)	0 (112)
Antibiotic	3	100 (42)	0 (109)
	7	0 (45)	95.4 ± 2.6 (132)

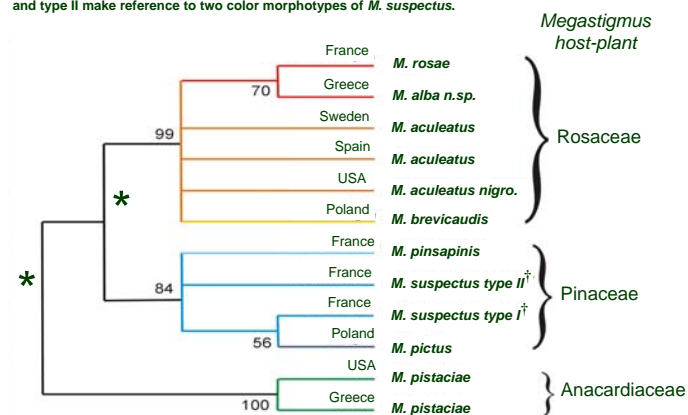
## PHYLOGENY OF *WOLBACHIA* STRAINS INFECTING THELYTOKOUS *MEGASTIGMUS* SPECIES

The sequences of the *Wolbachia* *wsp* fragments found in the different thelytokous *Megastigmus* species were used to assess the evolutionary trajectories of their association. Molecular phylogeny of *Wolbachia* variants paralleled the observed radiation of *Megastigmus* species with respect to host-plant families (Pinaceae, Rosaceae and Anacardiaceae), but no significant divergence in *Wolbachia* sequences was found within host-plant families (Figure 1).



Figure 1. Phylogenetic relationships between sequences of the *Wolbachia* *wsp* fragments found in thelytokous *Megastigmus* species.

A neighbor-joining algorithm was used and numbers above branches indicate bootstrap support from 1000 replicates. A star (\*) indicates that sequences significantly diverged between groups (>3.5% of sequence divergence). † Type I and type II make reference to two color morphotypes of *M. suspectus*.



## CONCLUSIONS

Thelytokous parthenogenesis in *Megastigmus* species may be associated with *Wolbachia* infection. Phylogenetic analyses indicate a possible partition of the studied *Wolbachia* strains in three clades that fit the host-plant structuration of their insect host. This suggests that horizontal transfers of *Wolbachia* may have occurred between *Megastigmus* species exploiting closely related host-plants. This may raise critical questions within the context of biological invasions in this insect genus, which frequently generate novel cases of sympatry and thus novel ecological connexions that may influence the spread of *Wolbachia*.