First evidence of *Wolbachia* infection in the six toothed bark beetle *Pityogenes chalcographus* (Coleoptera, Scolytinae)

Wolfgang Arthofer[1], Dimitrios N. Avtzis[1], Markus Riegler[2], Wolfgang J. Miller[3] & Christian Stauffer[1]

[1] Institute of Forest Entomology, Forest Pathology & Forest Protection, Department of Forest & Soil Sciences, Boku, University of Natural Resources & Applied Life Sciences, Vienna, Austria
[2] School of Integrative Biology, The University of Queensland, St. Lucia QLD 4072, Australia
[3] Centre of Anatomy and Cell Biology, Medical University of Vienna, Austria

***Wolbachia pipientis* is an obligatory intercellular, maternally inherited member of the α-Proteobacteria. Classic PCR of the *Wolbachia* surface protein related *wsp* gene estimated 16 - 22% of all insect species to be infected with the endosymbiont [1]. The use of proofreading polymerases and long PCR enables the detection of low titer infections and indicates much higher prevalences up to 76% [2]. As a powerful manipulator of host's reproduction causing effects like feminization, male killing or cytoplasmic incompatibility, *Wolbachia* is not only of interest biasing mitochondrial haplotype distributions, but also as a potential biocontrol agent against insect pests [3].**

PCR detection

Long PCR of a 600 bp fragment of the bacteria's *wsp* gene gave positive results in 15.4% of 169 individuals from different localities all over Europe. Cloning and sequencing of a PCR product showed high homology to a Wolbachia strain isolated from the dipteran species *Tipula aino*.

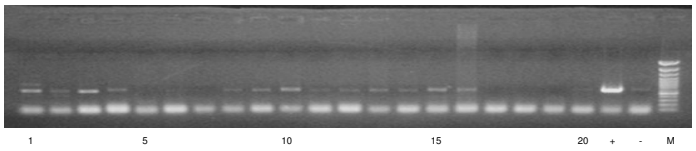

Fig 1. Gel photo of PCR products from 20 individuals of *P. chalcographus*. Note the much weaker bands obtained from the low titer infected beetle compared to the high titer positive control. Sensitive long PCR detection also revealed a weak infection in a *Drosophila simulans* specimen considered uninfected and used as negative control.

in situ hybridization

False positive results in PCR may be caused by parasitoids infected with *Wolbachia* and co-extracted with the beetle's DNA. For direct evidence of *Wolbachia* in insect tissue we used a DIG labelled *wsp* specific probe and NBT/BCIP staining of dissected ovaries.

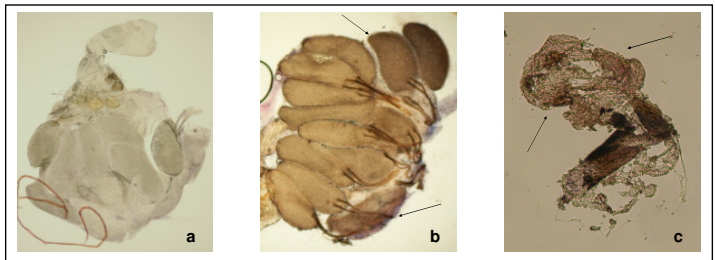

Fig 2. *in situ* hybridization of ovarial tissue. Accumulation of *Wolbachia* DNA is visualized as purple-brownish staining of ovarioles (arrows).
(a) uninfected *D. simulans* (b) high-titer infected *D. simulans* (c) *P. chalcographus*

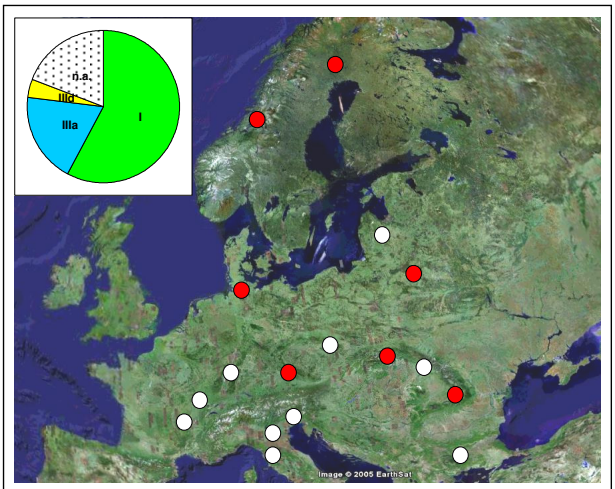

Fig 3. Distribution of *Wolbachia* in European *P. chalcographus* populations. Sampling sites are indicated by circles; red circles represent populations in which *Wolbachia* has been detected. The pie chart shows the assignment of positive individuals to mtDNA clades I, IIIa und IIId; n.a. not assigned

Wolbachia distribution

7 out of 17 screened populations contained infected individuals. These individuals belonged to three different phylogenetic clades revealed by mtDNA analysis [4]. We did not find any significant correlation between the infection pattern and the geographic or phylogenetic origin of the insects.

Conclusions

We present the first evidence of *Wolbachia* infections in a species with strongly diverged, sympatric mitochondrial lineages and partial crossing incompatibilites [5]. To further elucidate the endosymbiont's impact on the present population structure of *P. chalcographus*, further screening, controlled crossing experiments and identification of the *Wolbachia* phenotype are suggested.

References

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