The bacterial endosymbiont *Wolbachia* in the invasive cherry fruit fly *Rhagoletis cingulata* (Diptera, Tephritidae)

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Abstract: Wir berichten über zwei *Wolbachia* Isolate in einer europäischen Population der amerikanischen Kirschfruchtfliege *Rhagoletis cingulata*. Die Isolate *w*Cin1 und *w*Cin2 wurden durch Amplifikation, Klonierung und Sequenzierung des *Wolbachia surface protein* (*wsp*) Gens identifiziert. Eine phlyogenetische Analyse der *wsp* Region ergab, dass *w*Cin1 und *w*Cin2 ident mit *w*Cer1 und *w*Cer2 sind, welche in der Europäischen Kirschfruchtfliege, *R. cerasi*, gefunden wurden. Potentieller horizontaler *Wolbachia* Transfer und mögliche Folgearbeiten werden diskutiert.

Key words: *Rhagoletis*, *Wolbachia*, invasive species

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Introduction

Wolbachia is a gram negative endosymbiotic bacterium found in up to 65% of insect species (Hilgenboecker & al. 2008). Infections have been detected in all major orders of insects and some other arthropod taxa (Werren & al. 2008). Although its main path of transmission is transovarial through the cytoplasm of host eggs *Wolbachia* is supposed to occasionally jump horizontally among species (Baldo & al. 2008). *Wolbachia* can change the reproductive traits of its hosts to enhance colonization of the germline and vertical transmission (Werren & al. 2008). Cytoplasmic incompatibility (CI) is the most common phenotype in insects. It leads to embryonic death of fertilized eggs when infected males mate with uninfected females, while matings between infected males and females are compatible. This results in a reproductive advantage of infected over uninfected females and leads to increased infection rates in host populations over generational cycles (Hoffmann & Turelli, 1997).

Based on extensive single pair crossing experiments, Boller & al. (1976) concluded that populations of the European cherry fruit fly, *Rhagoletis cerasi*, are divided into two geographic complexes which exhibit unidirectional incompatibility. Blümel & Russ (1989) detected Rickettsia Like Organisms (RLOs) in the ovaries of individuals in all populations. By applying PCR techniques with *Wolbachia* specific primers, Riegler & Stauffer (2002) detected two different *Wolbachia* strains, *w*Cer1 and *w*Cer2 in cherry fruit fly populations. Transinfection experiments with *w*Cer2 revealed complete CI in the Mediterranean fruit fly, *Ceratitis capitata* and cage experiments demonstrated that *Wolbachia*-induced CI could be used as a tool for population control (Zabalou & al. 2004).

It has recently been reported that the American cherry fruit fly, *R. cingulata*, is present in Europe. So far, the species has been found in Austria, Germany, Hungary, Slovenia and Switzerland (Boller 2000, Daniel & Wyss 2007, Egartner & al. 2008, EPPO 2006, EPPO 2007a, EPPO 2007b, Vogt & al. 2009). *R. cingulata* has a similar biology as *R. cerasi* with the likely exception of required higher cumulative temperatures for *R. cingulata* pupae to reach maturity and delayed emergence of adults from the soil (Vogt & al. 2009). *R. cingulata* is a serious pest in cherries in Northeast American regions (Bush 1966, Rothwell & al. 2006).

Here we investigated *Wolbachia* infections in *R. cingulata* from a German population. We discuss potential horizontal *Wolbachia* transmission between *R. cingulata* and *R. cerasi*, as both species might co-occur in the same cherries. The *Wolbachia* detection was carried out by PCR using *wsp* primers and subsequent cloning and sequencing of the amplicons.

Materials & Methods

R. cingulata flies were collected from yellow sticky traps in Heidesheim, Germany, in 2008 and stored in absolute ethanol at -20°C. DNA of two individual flies was extracted using the Sigma GenElute Mammalian DNA extraction Kit following the protocol of the manufacturer. DNA was eluted in 50 µl TE (10 mM Tris, 1 mM EDTA, pH=8.0) and stored at -20°C. All PCR reactions were performed on a 2720 thermal cycler (Applied Biosystems) in a total volume of 10 µl containing: 1x Mg-free buffer (Fermentas), 2 mM $MgCl_2$, 100 µM dNTPs, 0.2 µM of each primer, 0.25 U Taq polymerase (Fermentas) and 0.8 µl template DNA. Cycling conditions for universal *wsp* amplification using the primers wsp81F and wsp691R (Braig & al. 1998) were 95°C for 2 min followed by 35 cycles at 94°C for 30 sec, 55°C for 45 sec, 72°C for 1 min and a final extension at 72°C for 15 min. For cloning, a 0.8 µl aliquot of PCR product was ligated into the pTZ57R vector of the InstaClone PCR cloning kit (Fermentas) according to the instructions of the manufacturer. The ligated plasmids were used for transformation of competent JM109 *E. coli* cells and after overnight growth white colonies were picked and transferred to liquid LB medium. Insert size was determined by PCR with M13 vector primers and plasmid DNA was extracted by alkaline lysis. Sanger sequencing was performed by a commercial provider. Retrieved sequences were edited manually, aligned using ClustalX (Thompson & al. 1997) and compared with *Wolbachia* sequences from GenBank by BLAST analysis.

Results

PCR with the *Wolbachia* specific primers resulted in positive amplicons in the two analysed German individuals. These two amplicons were cloned and 21 plasmids were sequenced. Sequence analysis revealed that both individuals were infested by two *Wolbachia* variants which were named *w*Cin1 and *w*Cin2. A BLAST search and subsequent alignment revealed that *wsp* sequences of *w*Cin1 and *w*Cin2 are identical to those from *w*Cer1 and *w*Cer2 detected in *R. cerasi*.

Discussion

We report about two *Wolbachia* sequence variants in the American cherry fruit fly *R. cingulata*. *wsp* of *w*Cin1 and *w*Cin2 are identical to *w*Cer1 and *w*Cer2 detected in *R. cerasi*. This suggests a horizontal strain transfer between the two cherry fruit fly species. To further test this hypothesis we will need to characterise more loci of the *w*Cin isolates by Multi Locus Strain Typing (MLST) as described by Baldo & al. (2006) and compare with the MLST loci of *w*Cer. This characterization will give deeper insight into the genomes of the strains and might reveal differences between the Cin and the Cer-strains. In order to interpret the direction of transfer it has to be tested whether American populations of *R. cingulata* are also infected by *w*Cin1 and *w*Cin2.

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