Parasitoids as potential vectors for *Wolbachia* transfer

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Alle Gelegenheit, glücklich zu werden, hilft nichts,
wer den Verstand nicht hat, sie zu nutzen.
Johann Peter Hebel

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Abstract

Wolbachia is an obligate intracellular bacteria found in many arthropods. *Wolbachia* manipulates the reproduction system, most commonly by Cytoplasmic Incompatibility (CI). The European cherry fruit fly, *Rhagoletis cerasi*, is infected with five different *Wolbachia* strains and *w*Cer2 induces unidirectional CI between northern European females and southern European males. The invasive Eastern cherry fruit fly, *R. cingulata*, was detected to be infected with two *Wolbachia* strains *w*Cin1 and *w*Cin2 ident to *w*Cer1 and *w*Cer2, respectively, based on a MLST screening. However, the American *R. cingulata* populations were not infected with *w*Cin1, suggesting a recent horizontal *Wolbachia* transfer from *R. cerasi* to *R. cingulata* since *R. cingulata* arrived in Europe. A potential vector of such a horizontal transfer could be parasitoids.

The main aim of this thesis was to search for the presence of *Wolbachia* in the two cherry fruit fly parasitoids, *Psyttalia rhagoleticola* and *Utetes magnus*. These species are common parasitoids of *R. cerasi* and are supposed to infect also *R. cingulata.* This study should reveal if a horizontal transfer from *Wolbachia* strains between the two cherry fruit fly species happened. Species were defined by DNA barcoding as well as by morphological analyses by a braconid specialist..The species were described as *Psyttalia rhagoleticola* and *Utetes magnus*.

A PCR with *Wolbachia* specific *wsp* primers showed that all *P. rhagoleticola* individuals were infected with *w*Rha2. This sequence was ident to *w*Cin2 (=*w*Cer2), however, MLST analysis did not confirm identity to these *Rhagoletis* strains. Nested PCR with special primers amplifying for *w*Cer1 resulted in the detection of *w*Rha1 ident to *w*Cin1 (=*w*Cer1). Here no MLST products were obtained.

In *U. magnus* one specimen *w*Mag1 ident to *w*Cin1 (=*w*Cer1) was detected and in a few individuals *w*Mag2 a strain belonging to the *Wolbachia* supergroup B was detected. Here no MLST products were obtained.

This master thesis found new *Wolbachia* strains in *P. rhagoleticola* and *U. magnus* but could not prove that these strains are ident to the ones detected in *R. cingulata*

and *R. cerasi*. Thus horizontal *Wolbachia* transmission by parasitoids could not be confirmed by this study.

Zusammenfassung

Wolbachia ist ein obligat intrazellulär lebendes Bakterium und wurde bisher in vielen Arthropoden gefunden. *Wolbachia* manipuliert die Vermehrung seiner Wirte z.B. durch zytoplasmatische Inkompatibilität (CI).

Die Europäische Kirschfruchtfliege, *Rhagoletis* cerasi, ist mit fünf verschiedenen *Wolbachia* Stämmen infiziert, wobei *w*Cer2 für unidirektionale CI zwischen nordeuropäischen Weibchen und südeuropäischen verantwortlich ist. In der invasiven Amerikanschischen Kirschfruchtfliege, *R. cingulata,* wurde eine Infektion von *w*Cin1 und *w*Cin2 gefunden. Diese Stämme sind aufgrund von genetischen MLST Untersuchungen, identi zu *w*Cer1 und *w*Cer2. Allerdings waren amerikanischen *R. cingulata* Populationen nicht mit *w*Cin1 infiziert, was auf einen horizontalen *Wolbachia* Transfer von *R. cerasi* auf *R. cingulata* hindeutet. Dieser horizontale Transfer könnte durch Parasitoide geschehen sein.

Das Hauptziel dieser Diplomarbeit war die Suche nach *Wolbachia* in den zwei Kirschfruchtfliegen-Parasitoiden *Psyttalia rhagoleticola* und *Utetes magnus.* Diese Arten sind häufige Parasitoide von *R. cerasi* und könnten auch invasive *R. cingulata befallen.* Diese Arbeit sollte zeigen ob es zu einem horizontaler Transfer zwischen den zwei Kirschfruchtfliegen-Arten gekommen ist.

Vor dieser Analyse wurden die zwei Parasitoide taxonomisch bestimmt. DNA Barcoding sowie morphologische Analysen durch einen Braconiden Spezialisten wurden durchgeführt, um die Art zu bestimmen, wobei das genetische Screening zwei verschiedene Arten identifizierte. Die Arten wurden in Folge aufgrund morphologischer Merkmale als *Psyttalia rhagoleticola* und *Utetes magnus* beschrieben.

Eine PCR mit *Wolbachia* spezifischen *wsp* Primern ergab, dass alle *P. rhagoleticola* Individuen von *w*Rha2 befallen waren. Diese Sequenz war identisch mit *w*Cin2 (=*w*Cer2), jedoch konnte eine MLST Analyse die Identität zu diesen *Rhagoletis* Stämmen nicht bestätigen. Nested PCR mit speziellen *w*Cer1 Primer ergab eine

Infektion von of *w*Rha1 identisch mit *w*Cin1 (=*w*Cer1). Hier wurden keine MLST Produkte erhalten.

In einem *U. magnus* Individuum wurde *w*Mag1 aufgrund der wsp Analyse identifiziert. *w*Mag1 war auf diesem Locus ident zu *w*Cin1 (=*w*Cer1). In weitern Individuen wurde *w*Mag2 gefunden, ein Stamm, welcher phylogenetisch zu der *Wolbachia* Supergroup B zugeordnet werden konnte. Bei *U. magnus* wurden keine MLST Produkte erhalten.

In dieser Masterarbeit wurden neue *Wolbachia* Stämme in *P. rhagoleticola* und *U. magnus* gefunden, jedoch konnte nicht belegt werden, dass diese Stämme mit jenen von den beiden Kirschfruchtfliegenarten ident sind. Deswegen konnte horizontaler *Wolbachia* Transfer durch Parasitoide nicht bestätigt werden.

Index of Abbreviations

A	Adenosine
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
C	Cytosine
°C	Degree Celsius
CI	Cytoplasmic incompatibility
cm	Centimetre
*cox*A	Cytochrome c oxidase, subunit I
DNA	Deoxyribonucleic acid
dNTP	2´-deoxyribonucleoside-5´-triphosphate
*fbp*A	Outer surface protein
ftsZ	Cell division protein
G	Guanosine
g	Gram
*gat*B	Glutamyl-tRNA-(Gln)-amidotransferase
*hcp*A	Conserved hypothetical protein
IIE	Incompatible Insect Technique
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
lacZ	Gene encoding for the enzyme β-galactosidase
LB	Lysogeny broth
M	Molar
m	Milli
MgCl2	Magnesium chloride
min	Minute(s)
MLST	Multilocus sequence typing
n	Nano
NJ	Neighbour Joining Method
NaOH	Sodium hydroxide
PEG	Polyethylenglycole
PCR	Polymerase chain reaction
RLOs	Rickettsia-like organisms

RNase	Ribonuclease
rpm	Rounds per minute
sec	Second(s)
SOC	"Salt optimized + carbon"
spp.	Subspecies
T	Thymidine
TAE	Tris[aminomethyl]aminoethane
Taq	*Thermus aquaticus*
TE-Buffer	Tris-EDTA-Buffer
T_M	Melting temperature
U	Unit
UV	Ultraviolet (light)
wAjap	*Wolbachia* variant from *Asobara japonica*
*w*Alb	*Wolbachia* variant from *Aedes albopictus*
*w*Ana	*Wolbachia* variant from *Drosophila ananassae*
*w*Atab	*Wolbachia* variant from *Asobara tabida*
*w*Au	*Wolbachia* variant from *Drosophila simulans* (Australia)
*w*Bac	*Wolbachia* variant from *Bactericera cockerelli*
*w*Bm	*Wolbachia* variant from *Brugia malayi*
*w*Bor	*Wolbachia* variant from *Drosophila borealis*
*w*Cal	*Wolbachia* variant from *Calyptratae sp.*
*w*Cer	*Wolbachia* variant from *Rhagoletis cerasi*
*w*Chl	*Wolbachia* variant from *Chloropidae sp.*
*w*Cin	*Wolbachia* variant from *Rhagoletis cingulata*
wCneg	*Wolbachia* variant from *Ceutorhynchus neglectus*
*w*Dia	*Wolbachia* variant from *Diabrotica barberi*
*w*Mag	*Wolbachia* variant from *Utetes magnus*
*w*Mel	*Wolbachia* variant from *Drosophila melanogaster*
*w*Mono	*Wolbachia* variant from *Monomorum chinense*
*w*Ngir	*Wolbachia* variant from *Nasonia giraulti*
*w*Nlon	*Wolbachia* variant from *Nasonia longicornis*
*w*Nvi	*Wolbachia* variant from *Nasonia vitripennens*
*w*Pom	*Wolbachia* variant from *Rhagoletis pomonella*
*w*Pro	*Wolbachia* variant from *Protocalliphora sialia*

*w*Qua	*Wolbachia* variant from *Drosophila quinria*
wOvu	*Wolbachia* variant from Eusomus ovulum
wRha	*Wolbachia* variant from *Psyttalia rhagoleticola*
*w*Sim	*Wolbachia* variant from *Drosophila simulans*
*w*Spt	*Wolbachia* variant from *Drosophila septentriosaltans*
*w*Wil	*Wolbachia* variant from *Drosophila willistoni*
wsp	*Wolbachia* surface protein
*wsp*81F	Primer for amplifying *wsp*
*wsp*691R	Primer for amplifying *wsp*
X-Gal	5-bromo-4-chloro-3-indolyl- β-D-Galactopyranoside

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1.   Introduction

1.1.   The genus *Wolbachia*

Bacterial endosymbionts are grouped in primary and secondary symbionts. Primary symbionts are considered to have beneficial relationships since they are usually required for host survival and reproduction. Often they contribute the hosts diet with essential nutrients. In contrast, secondary symbionts are often facultative symbionts from the host's view and often have a shorter coevolutionary background with a species (Moran & Baumann 2000). The impact of secondary symbionts on host fitness depends on the environment and can be beneficial in one environment and negative in another (Haine 2008).

Wolbachia are maternally inherited intracellular endosymbiotic bacteria (Werren 2008) and are secondary symbionts. The genus *Wolbachia* was first described in the mosquito *Culex pipientis* (Diptera) and was named *Wolbachia pipientis* (Hertig & Wolbach 1924; Hertig 1936). *Wolbachia* belongs to the alpha-2 subdivision of the Proteobacteria, forming a monophyletic group closely related to intracellular bacteria of the genera *Ehrlichia, Anaplasma, Rickettsia* and *Cowdria* (Bourtzis 2008) based on 16 rDNA analysis. *Wolbachia* is a group of intracellular bacteria that encloses species with parasitic, mutualistic and commensalistic relationship with its hosts. These bacteria are not cultivable and live in the cells of their hosts and are transmitted vertically during the host reproduction. The endosymbiont *Wolbachia* got important due to its enormous abundance, the effects on their hosts i.e. manipulation of the reproductive system of arthropods and as potential applicants in pests and disease vector control of invertebrates (Bourtzis 2008). These bacteria are common in insects and also found in mites and in filarial nematodes (Sironi et al. 1995; Bandi et al. 1998).

The infection rate of *Wolbachia* of all insect species is estimated at about 20% (West et al. 1998; Werren et al. 1995; Werren & Windsor 2000). Hilgenboecker et al. (2008) estimate by a screening of 20 different studies, that 66% of all insect species are infected by *Wolbachia*, because the percentage of infected species is based on the distribution of infection levels among species. They discovered a so called "most or few" infection pattern, which means that either most or just few individuals of a species are infected by *Wolbachia*.

Due to 16S ribosomal sequences and other sequence information *Wolbachia* spp. is divided into supergroups (A, B, C, D, F, H and K) (Casiraghi 2005; Baldo & Werren 2007). The arrangement of the groups is in a constant change. Lately two new groups were found: M (Simoes et al. 2011) and I (Haegeman et al. 2009). In contrast to other Rickettsiales all supergroups are monophyletic, and contain phylogenetically related strains (Baldo & Werren 2007). Supergroup C and D are commonly found in nematodes from the subgenera Filarioidea. All other six supergroups are primarily found in arthropods, in which supergroup A and B are most common (Werren 2008). *Wolbachia* has developed different mechanisms of host reproduction manipulations in their hosts to favour their own dispersal (Riegler & O'Neill 2006; Werren 1997; Werren 2008). The transmission of *Wolbachia* is transovarial through the cytoplasm of the host egg like mitochondrial DNA. This is the reason why *Wolbachia* manipulates the host's reproduction to increase the rate of female offspring. Through reproduction manipulations *Wolbachia* promotes its own spread in a population by encouraging the production of female progeny and simultaneously by reducing the reproduction of uninfected females (Werren 2008). If infected females produce more daughters on average to uninfected females, the symbiont is able to spread in a population unless the total progeny is reduced (White et al. 2010; Werren 1997).

The most common mechanism is Cytoplasmic Incompatibility (CI). Further *Wolbachia* can induce thelytokous parthenogenesis, feminisation and male killing (for review see Werren et al. 2008).

1.1.1.   Cytoplasmic Incompatibility CI

CI is the most widespread effect that *Wolbachia* have on their hosts. CI results in embryonic death of fertilized eggs if infected males mate with uninfected females. This incompatibility is unidirectional, as matings between infected males and females are fully compatible (O'Neill and Karr 1990; Merçot et al. 1995) (Figure 1.1).

Figure 1.1 Left: unidirectional CI, the *Wolbachia* infected specimens are labelled red and the uninfected specimens are white. Right: Bidirectional CI, mating of males infected with *Wolbachia* strain I (green) and females infected by *Wolbachia* strain II (red) results in CI in both directions.

In order to understand the theoretical background for the uni- and bidirectional CI the "mod-resc" model has been suggested (Werren 1997). The model assumes that the bacteria have two different functions that together cause the compatibility type of the *Wolbachia* strain. First the modification (mod) function, which is expressed in the male germ line and "imprints" sperm. The rescue (resc) function which is expressed in the egg makes sure that the sperm imprinting is relieved (Werren 1997; Bourtzis et al. 1998; Apostolaki et al. 2011).

If a male and a female harbour *Wolbachia* strains with different "mod-resc" systems, then this results in bidirectional CI (Werren, 1997; Charlat et al. 2004; Bourtzis 2008). This incompatibility type was first described by O'Neill & Karr (1990) in *Drosophila simulans*. Bordenstein et al. (2001) showed with the closely related species *Nasonia longicornis* and *Nasonia girauli* and *Nasonia vitripennis* that bidirectional CI leads to reproductive isolation between the wasps. CI can act as post-zygotic barrier between individuals or populations with different infections status. Subsequent *Wolbachia* can reduce the gene flow and therefore play an important role in speciation (Bordenstein et al. 2007).

The expression of CI depends on the regulated *host-Wolbachia* strain combination and can range from a few to 100% embryonic incompatibility. *Wolbachia* infections can spread and persist in nature by replacing uninfected populations through the mechanisms of CI (Bourtzis 2008). Turelli & Hoffmann (1991) showed that an uninfected Californian population of *D. simulans* was totally invaded during the 1980 by a CI introducing *Wolbachia* strain. The infection was spreading more than 100km

per year and the population became almost completely infected within three years. The infection carried a specific mtDNA haplotype that was rare or absent before in the uninfected Californian populations (Turelli & Hoffmann 1992).
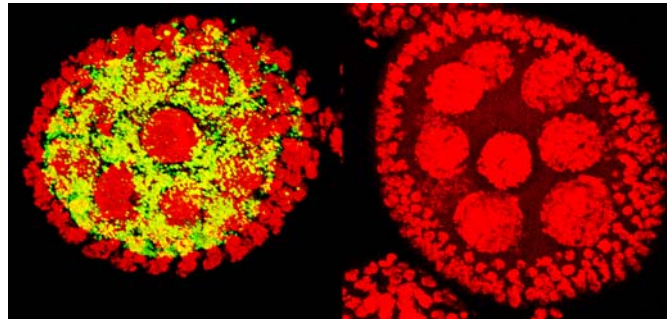

Figure 1.2 Fruit fly ovaries showing *Wolbachia* infections. http://www.rochester.edu/College/BIO/labs/WerrenLab/Wer renLab-*Wolbachia*Research.html

For the study of *Wolbachia*-host interactions three major surface proteins have been identified and are used for screenings: *wsp*, *wsp*A und *wsp*B (Braig et al. 1998; Zhou et al. 1998). *Wsp* is divided into four hypervariable regions (HVRs) and is highly polymorph (Baldo et al. 2006) and susceptible to recombination (Werren & Bartos 2001). Hence this locus is not ideal for phylogenetic characterisation of *Wolbachia*, but nevertheless a good marker for *Wolbachia* detection (Arthofer et al. 2009a).

Therefore an additionally typing system to the *wsp* genes was developed. The Multilocus Sequence Typing (MLST) uses different housekeeping genes for strain typing characterisation (Maiden et al. 1998). Baldo et al. (2006) used the five housekeeping genes *gat*B, *cox*A, *hcp*A, *fts*Z, *fbp*A to locate *Wolbachia* in the *w*Mel genome of *Drosophila melanogaster*. These five loci are very broad dispersed across the *w*Mel genome of *D. melanogaster* (Figure 1.3). *Wolbachia* strains with similar *wsp* sequences often have different MLST alleles (Baldo et. al. 2006). The MLST sequences can be submitted to the MLST database (http://pubmlst.org/*Wolbachia*/). However, MLST needs hosts with individual and not multiple strains of supergroups. Arthofer et al. (2011) introduced a novel approach, the Allele Intersection Analysis (AIA). AIA allows a correct assignment of different strains of MLST alleles in multiple infected host species without the need of artificial strain segregation (Arthofer et al. 2011).
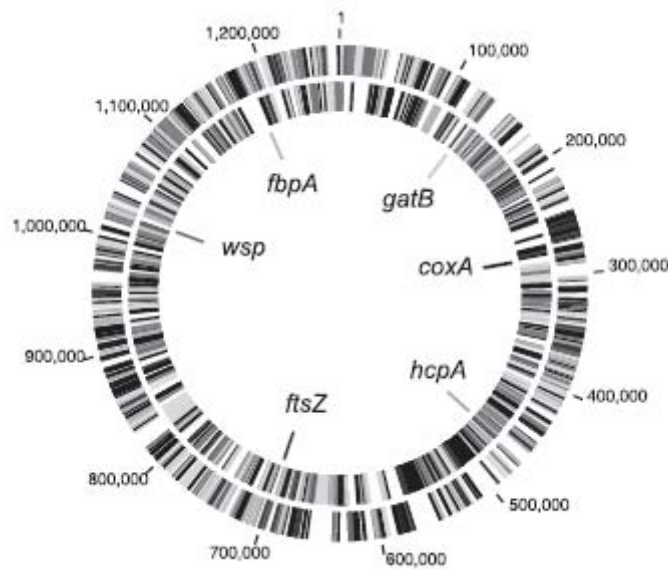
Figure 1.3 The *w*Mel genome with the five MLST loci and the locus of the *wsp* (Baldo et al. 2006).

1.1.2.   CI inducing *Wolbachia* in plant - and disease protection

The effects of *Wolbachia* on reproduction and host fitness i.e. CI have made *Wolbachia* interesting for their potential as a novel and environmental friendly bio-control agent (Bourtzis 2008; Ahantarig & Kittayapong 2011). Even long before the mechanisms of CI had been determined, Larven (1959) had recognised the potential of that mechanism to control mosquitoes. Boller & Bush (1974) and Boller et al. (1976) applied a tool under the name "Incompatible Insect Technique" (IIT) for pest population control. IIT could be described as the use of the mechanism of *Wolbachia*-induced CI for the control of populations of pest insects (Bourtzis & Miller 2006). IIT is in a way analogous to the "Sterile Insect technique" (SIT) where male insects are irradiated before their release. The main problem of the effective practical use of IIT exists in the mass rearing of *Wolbachia* infected males (Bourtzis 2008). A major problem for *Wolbachia* based strategies was the ability to transfer *Wolbachia* to novel hosts (Brelsfoard & Dobson 2009). But new studies overcame this obstacle by demonstrating transfers into novel hosts by microinjections of cytoplasm into embryos. Zabalou et al. (2004) evidenced in trans-infection experiments with the Mediterranean fruit fly, *Ceratitis capitata* that the *Wolbachia* strain *w*Cer2 is able to

induce complete CI and in cage experiments they demonstrated that *Wolbachia*-induced CI could be used for population control. Recently, Zabalou et al. (2009) transferred a CI-inducing *Wolbachia* strain to a genetic sexing strain of the Mediterranean fruit fly *Ceratitis capitata.* This strain carries a selectable *temperature sensitive lethal (tsl)* marker which is used for the production of only males in SIT programmes. The transferred *Wolbachia* induced high levels of CI even after the temperature treatment.

Further, *Wolbachia* proved useful also in disease protection. Dengue fever is a major health problem in the tropics and more than 50 million people are currently estimated to be infected with the Dengue virus. The transmission vector of this disease is the mosquito species *Aedes aegypti.* CI-inducing *Wolbachia* was used to control the mosquito populations. Walker et al. (2011) transferred the CI-inducing *w*Mel to *A. aegypti* and reduced the Dengue virus load of the mosquitoes. They also showed that the *w*Mel strain block transmission of Dengue viruses. Hoffmann et al. (2011) showed in field trials that the release of *w*Mel mosquitoes displaced the Dengue-virus infected *A. aegypti* in Australia.

1.2. *Wolbachia* in the tephritids *Rhagoletis cerasi* and *Rhagoletis cingulata*

The European cherry fruit fly, *Rhagoletis cerasi,* is an economically important agricultural pest (Fimiani 1983; Fischer-Colbrie & Busch-Petersen 1989). In crossing studies unidirectional incompatibility between northern and southern European populations was demonstrate and the existence of two geographic complexes was proposed (Boller & Bush 1974; Boller et al. 1976). Riegler & Stauffer (2002) found an infection of two *Wolbachia* strains named *w*Cer1 and *w*Cer2. Crossing studies between single-infected *w*Cer1 and double-infected *w*Cer1 & *w*Cer2 individuals showed that *w*Cer2 is inducing CI.

Arthofer et al. (2009a) detected three other *Wolbachia* stains *w*Cer3- *w*Cer5 in *R. cerasi*, however, the phenotype of those was not studied.

In North America the Eastern cherry fruit fly, *R. cingulata* is a serious pest in sweet and sour cherries as well as in the wild cherry *Prunus avium* and also olives (Weems 2001). *R. cingulata* was introduced in Europe and was detected the first time in

Switzerland in 1983 (Merz 1994). Since then, *R. cingulata* was also found in Germany (Lampe et al. 2005), Italy (EPPO 1996), the Netherlands (EPPO 2004) Slovenia (EPPO 2007) and in Austria (Egartner et al. 2010). The biology of *R. cingulata* and *R. cerasi* is very similar, both having a univoltine life cycle whereas. *R. cingulata* has its flight climax three to four weeks later needing a higher temperature sum for emergence from pupae (Daniel & Wyss 2009; Lampe et al. 2005).

Schuler et al (2009) discovered that *R. cingulata* from German populations are infected by two *Wolbachia* strains, named *w*Cin1 and *w*Cin2. These strains were ident to *w*Cer1 and *w*Cer2, respectively, based on *wsp* and MLST analyses (Schuler et al. 2009). Schuler et al. (personal communication) screened also American *R. cingulata* populations, however, these individuals were only *w*Cin2 (=*w*Cer2) infected. It is likely that European *R. cingulata* obtained *w*Cin1 (=*w*Cer1) in Europe by horizontal transfer.

Theoretically, horizontal transfer happens either by cannibalism or parasitoids having the two species as common hosts. Horizontal transfer by cannibalism could occur during the larval stage, when *R. cerasi* and *R. cingulata* develop in the same fruit. As the two fruit flies evolved in separately the host marking pheromones might not cause deterrence. But a horizontal *Wolbachia* transfer by cannibalism from *R. cerasi* to *R. cingulata* is certainly rare because of the different biology. Thus, the horizontal transfer by parasitoids is more likely.

1.3.   Horizontal transfer of *Wolbachia* through parasitoids

Even though *Wolbachia* normally undergoes a vertical transmission through the maternal line of its host population (Hoffmann et al.1990), phylogenetic studies showed that similar strains could also be found in phylogenetically differing host taxa which in some cases are ecologically associated (Baldo et al 2006; Cordaux et al. 2001). This disagreement of phylogenetic relationships between host and *Wolbachia* makes it likely that these bacteria cross barriers horizontally (Baldo et al. 2008). Horizontal transmission is the infectious transfer of symbionts among unrelated hosts (Vautrin & Vavre 2009). Different factors must be fulfilled, for horizontal transmission

to occur which can be considered as a sequence of filters *Wolbachia* has to pass through (Vavre et al. 2003; Vavre 2009).

It is still necessary to establish the clear processes of horizontal transmission and the potential vectors. One way is to track *Wolbachia* among hosts by checking the geographical distribution of genetically closely related *Wolbachia* strains (Jiggins et al. 2002; Vavre 1999; Baldo et al. 2006). Another way is to investigate the *Wolbachia* and their hosts DNA diversity. Some studies showed evidence for disequilibrium, infected and uninfected populations were carrying different mitotypes, which shows that *Wolbachia* strain and mitochondrial haplotypes were not correlated (Bordenstein et al. 2001).

Wolbachia strains of the three closely related species *Nasonia longicornis*; *Nasonia girauli* and *Nasonia vitripennis* were independently acquired by horizontal transfer (Bordenstein & Werren 2007). Horizontal transfer was also detected in nematodes (Casiraghi et al. 2001; Casiraghi et al. 2005). Experiments have shown that *Wolbachia* indeed can establish new infections when transferred to a new host. This was observed between parasitoid *Trichogramma* wasps (Huigens et al. 2000; Huigens et al. 2004; Grenier 1998). Horizontal transfer was accomplished in experimental microinjection studies by transferring *Wolbachia* into native hosts, intra- and interspecifically (Braig et al. 1994; Heath et al. 1999; Zabalou et al. 2004; Riegler et al. 2004). That shows that natural *Wolbachia* transfer between species is possible. Some of the best vectors of horizontal transmission of *Wolbachia* are insect parasitoids, which contain a quarter of fall insect species and have an enormous range of host taxa (Werren et al. 1995). Host-parasitoid relations where parasitoids develop in their insect hosts before killing them are long-lasting interactions between insect species which are ideal conditions for horizontal transfers (Godfray 1994). Vavre et al. (1999) showed that hymenopteran parasitoids of frugivorous *Drosophila* are notably susceptible to *Wolbachia* and that the parasitoids are more susceptible to *Wolbachia* infections than *Drosophila* are. *Wolbachia* was experimentally transmitted from *Drosophila simulans* to the parasitic wasp *Leptopilina boulardi* with a success rate of 0.7%, showing that the transfer occurs during parasitism. This infection was lost in following generations, suggesting that there are resistance mechanisms to *Wolbachia* infections which could not be passed (Heath et al. 1999).

The parasitoids have two ways they could acquire *Wolbachia* from their host either at the larval stage inside or outside the host's body or when they feed their hosts. There

are even some symbionts which manipulate the superparasitism behaviour of their host to increase their horizontal transmission (Varaldi et al. 2003).

The transfer from parasitoids to hosts is very difficult and does not occurs as often as the transfer from the host to the parasitoid, because the hosts can't survive when they are parasitized and therefore they are not able to transmit the bacteria to their offspring (Vavre et al. 1999). But not each parasitation is successful. Some hosts are able to mount an immune response leading to encapsulation of the wasp egg.

Wolbachia infected *Drosophila simulans* showed to have a reduction in their ability to encapsulate eggs of the parasitoid wasp *Leptopilina heterotoma*. *Wolbachia* uninfected larvae of *D. simulans* achieved a 19,9% higher encapsulation rate compared to the *Wolbachia* infected larvae. But *Wolbachia* infections also reduce the ability of the parasitoids to guard its eggs against the immune response of the host. The *Wolbachia* infected parasitoids expiated on average 22.6% more encapsulation of their eggs in comparison to the *Wolbachia* uninfected parasitoids (Fytrou et al. 2006).

Parasitoids frequently engrave with their ovipositor into a host but do not lay an egg. Due the ovipositor is normally covered in sensillae it seams likely that the parasitoid rejects after discovering that the host is unsuitable (Godfrey 1994).

The hosts are maybe used both for ovipositions and as a food source. Host feeding is defined as the consumption of host tissue by the adult female parasitoid and has been observed in more then 140 species belonging to 17 hymenopteran families (Jervis & Kidd 1986; Burger et al. 2004). Parasitoids show this behaviour to obtain necessary nutrients for egg production. Host-feeding reduces the quality of the host for the developing larva (Jervis & Kidd 1986; Giron et al. 2002). But some species are unable to lay eggs until they have host-fed at least once. Some studies suggest that at least one part of the function of host feeding is to regain the resources used by the parasitoid in maintenance and host searching (Collins et al 1981; Sandlan 1979). *Nasonia vitripennis* (Braconidae) does host feeding and decides according to the amount of nutrient extracted from the host if they do host feeding (Rivero & West 2005).

1.3.1. The braconid parasitoids *Psyttalia rhagoleticola* and *Utetes magnus*

Psyttalia rhagoleticola and *Utetes magnus* belong to the subfamily Opiinae, which is one of the largest subfamilies in the family of Braconidae. The parasitoids of this family are koinobiont endosymbionts of cyclorrhaphous Diptera (Hoffmeister 1992). Koinobiontic endosymbionts are normally host specialists.which have precise physiological adaptations to a specific host. They have to defeat the host's immune system as they do not kill their host immediately and permit their hosts to grow after the oviposition. A lot of host modifications and ways of preventing encapsulation are common in these relationships (Godfray 1994).

The detection of the host larvae is aided by antennation (the movement of the antennal sensilla) over the fruit surface. The parasitoid behaves different in the near of the host and start to an erratic walk across the fruit surface. This change in the behaviour of the parasitoids shows their ability to locate the fruit fly maggots in the cherry chemically by their sensillae. After the location, the female wasp inserts its ovipositor through the fruit flesh into the larvae and lays its eggs in them (Rousse et al. 2006; Ero 2009; Ero et al. 2009). The wasps also inject chemical substances during the ovipositions to weaken the host's immune system (Wharton 1997). These chemical substances create one of the reasons for the high level of specialisation of the parasitoids with their main hosts. The higher the specialisation level, the lower the immunological response of the host.

The genus *Psyttalia* (Braconidae: Opiinae) contains several species which are considered to be useful for the biological control of fruit flies. The main host of *Psyttalia rhagoleticola* is *R. cerasi*. *P. rhagoleticola* was counted originally to the genus *Opius.* Fischer (1972) who described this species placed it in the subgenus *Psyttalia*. Subsequently the suborder became an independent genus after studies of Wharton (1987). The combination *P. rhagoleticola* (Sachtleben 1934) was first used by Wharton (1988). Belokobylskij (2003) suggested that *Psyttalia carinata* is a senior synonym of *P. rhagoleticola*. *P. rhagoleticola* is a specialised endoparasitoid of late larval instars of the cherry fruit flies *R. cerasi*. Especially in Austria it's the most important parasitoid of this species (Hoffmeister 1992).

In 1984 *P. rhagoleticola* was introduced in Canada to control the apple maggot *Rhagoletis pomonella*. They successfully parasitized larvae of *R. pomonella*. The parasitoid was still found in 1985, which shows its ability to survive the winters there (Monteith 1971). The appearance of this species ranges from Western Europe to Kazakhstan. *P. rhagoleticola* parasites additionally different tephritids like *Carpomya vesuviana*, *Rhagoletis basiola*, *Rhagoletis batavia*, *Rhagoletis berberidis*, *Rhagoletis flavicincta*, *Rhagoletis lycopersella* (Kandybina 1977), *Rhagoletis pomonella* (Monteith 1972), *Myoleja lucida* (Kandybina 1977; Fry 1987; Hoffmeister 1992) and also *Rhagoletis cingulata* (Köppler pers. comm.).

Riegler (2002) described a *Wolbachia*-infection of *P. rhagoleticola* ident to *w*Cer2 in *R. cerasi* based on *wsp*. It indicates that a horizontal transfer of *w*Cer2 between the host species *R. cerasi* and the parasitoid could have occurred. Therefore it is possible that *P. rhagoleticola* is also infected with a *w*Cer1 strain in low titre and therefore a possible vector for a horizontal *Wolbachia* transfer between the two fruit flies *R. cerasi* and *R. cingulata*. A more careful screening with different loci and also with different techniques that allow a more sensitive *Wolbachia* screening was applied in this study.

U. magnus is also a koinobiont parasitoid which is described to parasite the *R. cerasi*, but the main host of this parasitoid is *Rhagoletis alternata* (Hoffmeister 1992). In comparison to *P. rhagoleticola* the flight period of *U. magnus* is two weeks later. This parasitoid hatched from a *R. cingulata* population of Germany (Dresden) and was defined as *Utetes magnus* (by Maximilian Fischer). The later flight time of this parasitoid correlates well with the later flight peak of *R. cingulata* (Hoffmeister 1992; Vogt et al. 2009)

Fischer (1958) originally described this species as *Opius magnus*. It was transferred from *Opius* to *Utetes* by Wharton (1997). *Utetes* had been admitted as a subgenus of *Opius* by Fischer (1972), and later changed to a generic rank by Wharton (1988). *Utetes sayanicus* (Tobias 1977) is a junior synonym (Fischer 1984).

1.4.　　　DNA barcoding of insects

DNA barcoding is a method for rapid species identification based on standardized DNA sequences. This can be done by comparing a short sequence of a standard part of the genome to a set of reference taxa of barcode sequences of known identity. The concept of DNA barcoding is based on the fact that most eukaryote cells have mitochondrial organelles which contain plasmid like DNA. Mitochondrial DNA (mtDNA) has a very fast mutation rate. This results in high variation of mtDNA sequences between species (Herbert et al. 2003). The mitochondrial cytochrome c oxidase subunit I (COI) gene is a common loci for animals and other eukaryotes (Former et al. 1994). The short sequences of DNA (400-800bp) are able to characterise each species (Savolainen et al. 2005). The sequence data of the species is stored in the Genbank (http://www.ncbi.nlm.nih.gov/).

2.   Aims

The main aim of this thesis was to search for the presence of *Wolbachia* in the two cherry fruit fly parasitoids, *Psyttalia rhagoleticola* and *Utetes magnus*. These species are common parasitoids of the European cherry fruit fly, *Rhagoletis cerasi,* and the Eastern cherry fruit fly, *R. cingulata.* The main question was if a horizontal transfer from *Wolbachia* strains between the two cherry fruit fly species happened by these parasitoids. Before that analysis the two parasitoids had to be taxonomically determined.

Thus the aims of that thesis can be listed as followed:

2.1.   Species verification of two barconids using DNA barcoding and morphology

DNA barcoding was done with mitochondrial COI primers and a BLAST search (Altschul et al. 1990) was performed with the obtained sequences. A BLAST search enables the comparison of the query sequence with sequences from the Genbank that resemble the query sequence above a certain threshold. Further, specimens were given to Dr. Maximilian Fischer (Natural History Museum, Vienna – former curator of the hymenoptera collection) who helped to identify species where no voucher sequence was found in the Genbank.

2.2.   Screening of the parasitoids for *Wolbachia*

The *Wolbachia* screening was done by PCR using general *wsp* primers (Braig et al. 1998). Cloning was performed to detect the presence of more sequence types. Nested PCR was applied to clarify if low titre *Wolbachia* strains are present. For a closer strain typing, *Wolbachia* was characterized by MLST markers i.e. five housekeeping genes (Baldo et al. 2006). Sequences were phylogenetically characterized by retrieving the most similar sequences from the Genbank.

3.   Material and methods

3.1.   Locations of collections

Braconid adults from four different localities were collected: six individuals from Vienna (AT), five from Brno (CZ), five from Ingelheim (GER) and five from Dresden (GER). These individuals were obtained and stored in ethanol at -20°C. The population from Ingelheim (GER) hatched from *Rhagoletis cingulata* whereas the population from Dresden (GER) hatched from *R. cingulata.* Both GER populations were provided by Kirsten Köppler (JKI, Dossenheim).

Figure 3.1 Map of locations from where specimens were obtained. Red dots represent the three *P. rhagoleticola* populations and the blue dot represents the *U. magnus* population.

3.2.   DNA extraction

The DNA extraction of the individuals was done with a DNA Miniprep SIGMA KIT (Appendix 1). The parasitoids were put in Eppendorf tubes, 180 µl lysis solution was added and afterwards homogenized with care. Then 20µl SIGMA proteinase K was added to this and put for 10 min in a heating block at 55°C. Afterwards 20 µl RNase was added, incubated then for 2 min and 200 µl lysis solution subsequent was added. After 10 min incubation at 70°C, 200 µl of absolute ethanol was appended to the samples. The whole solution was then transferred onto binding columns of the Sigma Kit. After two washing steps with the washing solution provided by the kit, the DNA was eluted in 100 µl elution buffer.

3.3.   DNA barcoding with mitochondrial primers

The COI primer LCO1490 paired with HCO 2198) were used in 20 µl reactions containing: 1x NH$_4$ buffer, 2mM MgCl$_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,4 U Taq polymerase (Fermentas) and 2 µl of template DNA. Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 94°C for 2 min, followed by 5 cycles at 94°C for 30 sec, 45°C for 90 sec and 72°C for 1 min and another 35 cycles at 94°C for 30 sec, 51°C for 1 min and 72°C for 1 min and a final extension at 72°C for 5 min. For sequencing amplicons were sent to Eurofins (Ebersberg, Germany) where they were purified and sequenced with an automated sequencer.

3.4.   PCR with general *wsp* primer

The *Wolbachia surface protein wsp* located in the outer membrane of *Wolbachia* (Braig et al. 1998) proved to be an excellent candidate for strain typing different *Wolbachia* strains as well as for fine-scale phylogeny of *Wolbachia* strains. *wsp* 81F and *wsp* 691R developed by Braig et al. (1998) were applied using 20 µl reactions containing: 1x NH$_4$ buffer, 2mM MgCl$_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,4 U Taq polymerase (Fermentas) and 2 µl of template DNA. Amplification was performed

in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 94°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 sequencing DNA was sent to Eurofins.

Hotstart DNA-polymerases are modified versions of *Taq* DNA polymerase with hot start capability. The advantage of hotstart DNA-polymerases are that the extension time is shorter and the polymerase is more robust. The PCR program is shorter and works well with small amounts of template. The reactions were accomplished in 20 µl containing: 1x GC-reaction buffer (Peqlab), 100 µM dNTPs, 0,2 µM of each primer, 0,4 U KAPAHiFi^{TM} DNA-polymerase (Peqlab) and 2 µl of template DNA.

Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 95°C for 5 min, followed by 40 cycles at 98°C for 20 sec, 56°C for 15 sec, 72°C for 30 sec; and a final extension at 72°C for 5 min. For sequencing amplicons were sent to Eurofins (Ebersberg, Germany) where they were purified and sequenced with an automated sequencer.

3.5.   PCR with MLST

PCR analysis was performed with five housekeeping genes *gat*B*, cox*A*, hcp*A, *fts*Z and *fbp*A of *Wolbachia* (Baldo et al. 2006).

The PCR reactions were accomplished in 20 µl reactions: 1x NH$_4$ buffer, 2mM MgCl$_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,1 or 0,2 U Taq polymerase (Fermentas), 1 or 2 µl of template DNA and sterile water.

Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C for 30 sec, 55°C for 45 sec and 72°C for 1 min; and a final extension at 72°C for 15 min. With the primer *fts*Z which didn't work at a TM of 55°C PCR, a TM of 50°C led to better success. For sequencing amplicons were sent to Eurofins (Ebersberg, Germany) where they were purified and sequenced with an automated sequencer.

Table 3.3.1 MLST primers described by Baldo et al. (2006). The table is taken from this publication – here also the *wsp* locus is listed.

Cluster category[a]	Locus code (*w*Mel)	Gene	Product	Primer Designation	Primer Sequence (5′–3′)	Gene length (bp)[b]	Amplified nucleotide range (bp)[b]	MLST fragment size (bp)
α-*Proteobacteria*	WD_0146	*gatB*	Glutamyl-tRNA(Gln) amidotransferase, subunit B	gatB_F1	GAKTTAAAYCGYGCAGGBGTT	1,425	421–891	369
				gatB_R1	TGGYAAYTCRGGYAAAGATGA			
Escherichia coli	WD_0301	*coxA*	Cytochrome *c* oxidase, subunit I	coxA_F1 coxA_R1	TTGGRGCRATYAACTTTATAG CTAAAGACTTTKACRCCAGT	1,551	491–977	402
Escherichia coli	WD_0484	*hcpA*	Conserved hypothetical protein	hcpA_F1 hcpA_R1	GAAATARCAGTTGCTGCAAA GAAAGTYRAGCAAGYTCTG	741	91–605	444
Escherichia coli	WD_0723	*ftsZ*	Cell division protein	ftsZ_F1 ftsZ_R1	ATYATGGARCATATAAARGATAG TCRAGYAATGGATTRGATAT	1,197	274–798	435
Rickettsiales	WD_1238	*fbpA*	Fructose-bisphosphate aldolase	fbpA_F1 fbpA_R1	GCTGCTCCRCTTGGYWTGAT CCRCCAGARAAAAYYACTATTC	900	241–749	429
Wolbachia	WD_1063	*wsp*	Outer surface protein	wsp_F1 wsp_R1	GTCCAATARSTGATGARGAAAC CYGCACCAAYAGYRCTRTAAA	714	85–688	546

3.6. Nested PCR

The nested PCR consists of two consecutive PCRs whereat at the second PCR another primer pair is used. The first PCR uses the general *wsp* primers 81F and 691R (Braig et al. (1998). The second PCR was performed with the template from the first PCR reaction and primers *wsp*226F and *spec*1R (Arthofer et al. 2009a)

The first PCR reactions were accomplished in 10 µl reactions containing: 1x NH_4 buffer, 2mM $MgCl_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,1 U Taq polymerase (Fermentas) and 1 µl of the template DNA. Finally a drop of petroleum was added.
Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 94°C for 2 min, followed by 15 cycles at 94°C for 30 sec, 55°C for 45 sec and 72°C for 1 min; without a final extension.

The second PCR reactions were accomplished in reaction volumes of 10 µl for the infection screening or in 20 µl for post-PCR sequencing containing: 1x NH_4 buffer (Fermentas), 2mM $MgCl_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,2 U Taq polymerase (Fermentas), 1 to 2 µl of template DNA and sterile water.
Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 94°C for 2 min, followed by 30

cycles at 94°C for 30 sec, 60°C for 45 sec and 72°C for 1 min; with a final extension time of 7 min. For sequencing amplicons were sent to Eurofins (Ebersberg, Germany) where they were purified and sequenced with an automated sequencer.

3.7.   Visualisation of PCR reactions

For detection, 5 µl amplicon products were loaded in a submarine horizontal gel system using a 1x TAE running buffer. The gels had a 1,5 to 2% agarose concentration and 0,5 µg/ml ethidium bromide was added to the gels. The gels were visualised via a UV transilluminator. Pictures were done with a Sony MVC-FD83 digital camera. At the end of the thesis GelRed® (Biotium) was used. For detection 1,5 to 2 µl of DNA fragment was loaded onto the submarine horizontal gel system using a 1x TAE running buffer. The agarose concentration was 1,5 % and 5 µl GelRed® was added to a 100 ml solution.

3.8.   Cloning

For cloning a TA cloning kit (Fermentas) was used. The exact procedures are listed in Appendix II. An aliquot of 0,8 µl of a PCR product was mixed with 1,4 µl H_2O, 0,1 µl of the vector pTZ57R (InstarClone PCR, Fermentas), 0,3 µl polyethylenglycol (PEG3350), 0,3 µl of T4 buffer and 0,1 µl T4 ligase for cloning. The tubes were held constantly at 15°C over night. JM109 *E. coli* cells were used for the transformation, which were stored in a freezer at -80°C. These cells were placed on ice for 20 minutes. Then 50 µl of the cells were added to each sample. After carefully vortexing they were placed on ice for another 20 min. After heating the samples for 50 sec at 42°C, 950 µl of a SOC-media was added and placed at 37°C in a conditioning cabinet for 60 min. The samples were centrifuged for 5 min at 4°C at 3.000 rpm. Most of the supernatant was taken off and the bacteria were discarded in an autoclavable glass. For the Blue White Colony Screening, 3200 ml of the remaining substrate was smoothed out on Petri dishes with agar containing 100 µg/ml ampicillin, 160 µg/ml x-Gal and 48 µg/ml IPTG. The plates were stored at 37 degrees over night. On the following day the plates were controlled for white bacteria colonies. The white colonies were marked and transformed with a toothpick to a test tube containing a

lysogeny broth (LB) medium (Sambrook et al. 1989). A PCR with the M13 primers was done. The PCR reactions were accomplished in 20 µl reactions: 1x NH_4 buffer, 2mM $MgCl_2$, 100 µM dNTPs, 0,2 µM of each primer, 0,2 U Taq polymerase (Fermentas), 2 µl of template DNA and sterile water. Amplification was performed in a 2720 thermal cycler (Applied Biosystems) with following program: an initial denaturation step at 96°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 54°C for 30 sec and 72°C for 45 sec; and a final extension at 72°C for 5 min. A gel electrophoresis showed which bacteria contained plasmids with PCR products and which ones did not. For sequencing DNA was sent to Eurofins.

3.9.   Phylogenetic analysis of the sequences

Retrieved sequences were edited with BioEdit (Hall 1999) and aligned with ClustalX (Thompson et al. 1997). The Basic Local Alignment Search Tool BLAST compares a query sequence with data from the Genbank and identifies sequences resembling the query sequence above a defined threshold (Altschul et al. 1990).

For tree construction in MEGA4 (Tamura et al. 2007) both distance and parsimony method were used. In the trees shown in the thesis a neighbour-joining (NJ) algorithm with Kimura-2-parameter distances (Kimura 1980) was applied. Bootstrap analysis was done with 1000 replicates.

4. Results & Discussion

The main aim of my master thesis was to obtain more insight if the braconid parasitoids hatched from cherry fruit fly populations are infected with *Wolbachia* strains ident to *w*Cin1 (=*w*Cer1) or *w*Cin2 (=*w*Cer2). In that case the braconids would be potential vectors for the horizontal transfer of these *Wolbachia* strains from *Rhagoletis cerasi* to *Rhagoletis cingulata*. The braconids were collected from four different populations and had to be determined taxonomically with the help of DNA barcoding and morphological analysis, first.

Figure 4.1 Left: *P. rhagoleticola* collected in Austria (picture), Czech Republic and Germany identified by DNA barcoding. Right: *U. magnus* from Dresden identified by M. Fischer (NHM Vienna).

4.1. DNA barcoding of the two braconid parasitoids

Adult specimens from both groups were brought to the former curator of the hymenopteran collection of the Natural History Museum, Vienna, Dr. Maximilian Fischer. He clearly identified the specimens from Dresden, GER as *U. magnus* a species which M. Fischer described the first time in 1958 (Fischer 1958).
Psyttalia rhagoleticola is morphologically almost ident to *P. concolor*, another parasitoid on cherry fruit fly species. As our specimens were just stored in ethanol a closer determination was not possible.

As braconids are difficult to determine morphologically both parasitoids were also determined by DNA barcoding. The PCR analysis with the mitochondrial primer was applied to the 21 individuals from the four European populations.
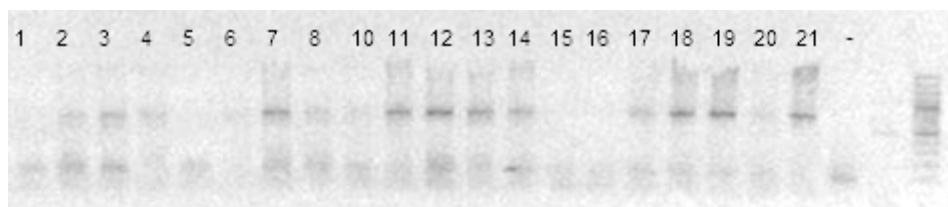

Figure 4.2 PCR was applied onto 21 specimens from four different populations and 13 revealed positive amplicons: lane 2-4 AT; lane 8, 11 CZ; lane 12-14 Ingelheim (GER); lane 17-19, 21 Dresden (GER). The last lane is the negative control done with sterile water.

The thirteen positive PCR products were sequenced, edited and aligned. The three *Psyttalia* populations (AT, CZ and Ingelheim GER) were ident except one mutation at position 405 (Figure 4.3). The mutation proved not to be population specific. The *U. magnus* specimens were all ident and differed from the *Psyttalia* sequences by almost 2% or 95-96 bp (Figure 4.4.) (Appendix III).
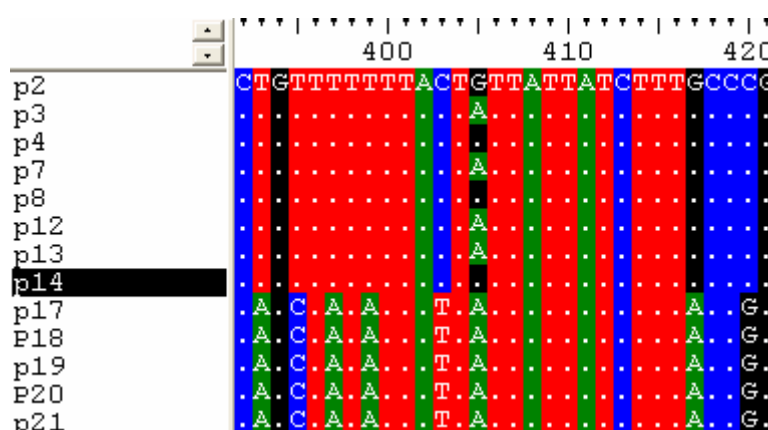

Figure 4.3 Alignment of the mitochondrial sequences obtained by direct sequencing showing two group of sequences – the ones from AT, CZ and Ingelheim GER (2-14) and the five sequences from Dresden GER (17-21).

The consensus sequences of the two parasitoids were analysed by the Genbank – BLAST search (Altschul et al. 1990) and the first group (2-14 from Figure 4.3) was identified as *Psyttalia* sp. The second consensus sequence was clearly identified as a braconid sequence, however, no voucher sequence could be detected. The most similar sequence was an unknown sequence (AAQ2936) identified as Hymenoptera sp. (Figure 4.4.). The *P. concolor* sequence retrieved from the Genbank was different compared to our sequence. Because *P. rhagoleticola* is the main parasitoid of the cherry fruit fly, Mr. Fischer agreed that this makes it very likely that the other species is *P. rhagoleticola.*

The phylogenetic analysis resulted in a monophyletic group for *P. rhagoleticola* and the *Psyttalia* retrieved from the Genbank. With a genetic distance of 0,8% *U. magnus* is related with the sequence of the unknown species Hymenoptera sp. (AAQ2936). The next related sequence was the one of *Dacnusa* species with 0,13% (Appendix III). However, only few of the more than 1500 Opiinae species (Wharton et al. 1997) have a voucher sequence in the Genbank.

The study on the two braconids shows that DNA barcoding can support the taxonomic workflow by fast identification of unknown specimens in case voucher sequences are available. However, if voucher sequences are not available detection until the species level is impossible without the help of morphology and comparison of the neotypes specimens from collections – like it was the case for *U. magnus*. In poorly studied taxonomic groups, DNA barcoding can be performed before conventional taxonomic work to quickly sort specimens into genetically divergent groups (Hajibabaei et al. 2007). DNA barcoding could be a helpful and completive tool to traditional taxonomy especially when the sample material is of low quality or in an ontogenetic stage which is hardly definable.
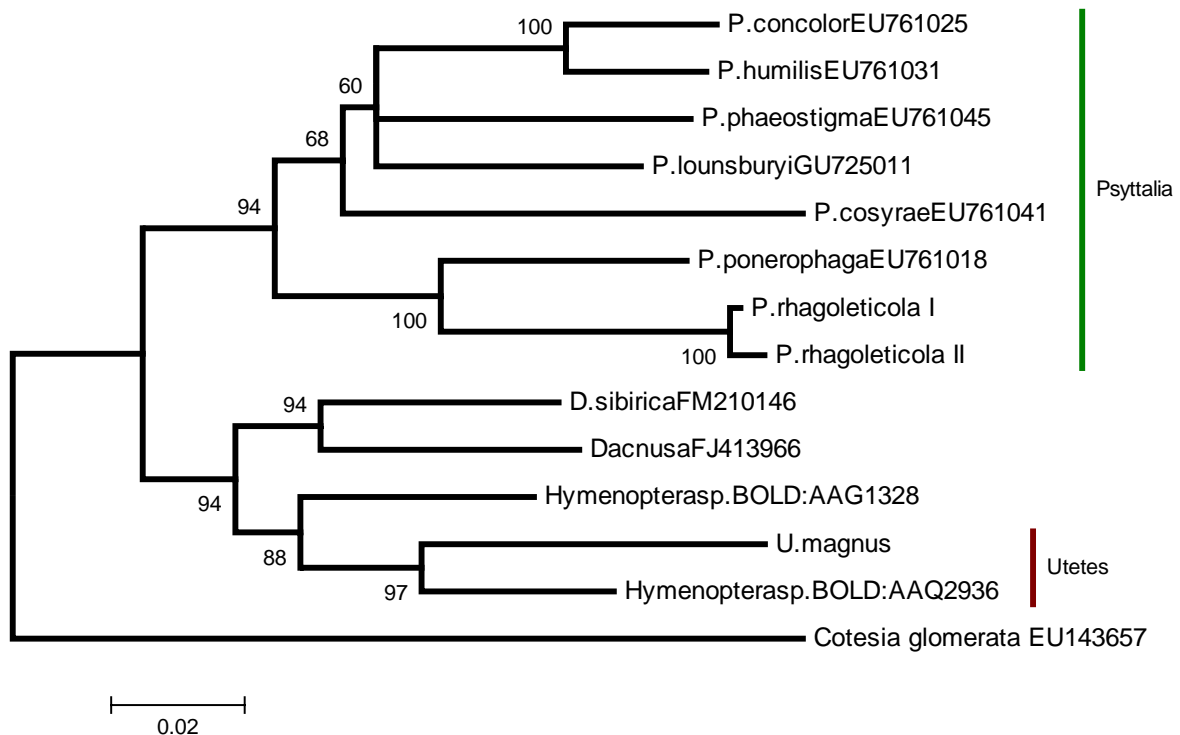
Figure 4.4 Phylogenetic analysis of the COI sequences of the three consensus sequences and sequences retrieved from the Genbank after a BLST search. Analysis was done with Neighbour Joining method using Kimura-2-parameter distances. Bootstrap analysis (nodes) was done with 1000 replicates.

4.2. Detection and characterization of *Wolbachia* in *P. rhagoleticola*

The PCR of the *wsp* gene was amplified with *wsp*81F *and wsp*691R (Braig et al. 1998) and all *P. rhagoleticola* showed positive amplicons.
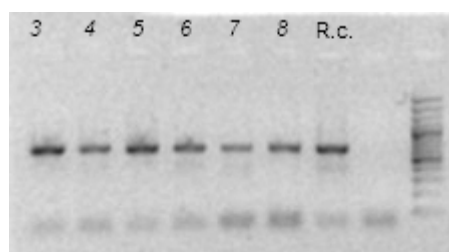

Figure 4.5 Agarose gel showing positive amplicons for *P. rhagoleticola* from AT (lane 3,4,5,6) and CZ (lane 7,8); positive control was DNA from *R. cerasi* (R.c.) after PCR with the *wsp* primers. Lane 9 is the negative control and 10 the 100bp ladder.

The amplicons of *P. rhagoleticola* were sequenced, edited and aligned. Further some *wsp* products of *P. rhagoleticola* were cloned PCR products were ligated into a vector and transformed into competent *Escherichia coli* cells. Plasmid DNA of four clones were sequenced. All sequences and all sequences obtained by direct sequencing of the PCR amplicons from *P. rhagoleticola* were 100% ident (Figure 4.6). A BLAST search revealed that these sequence types were ident to *w*Cin2 (=*w*Cer2) and consequently named *w*Rha2.
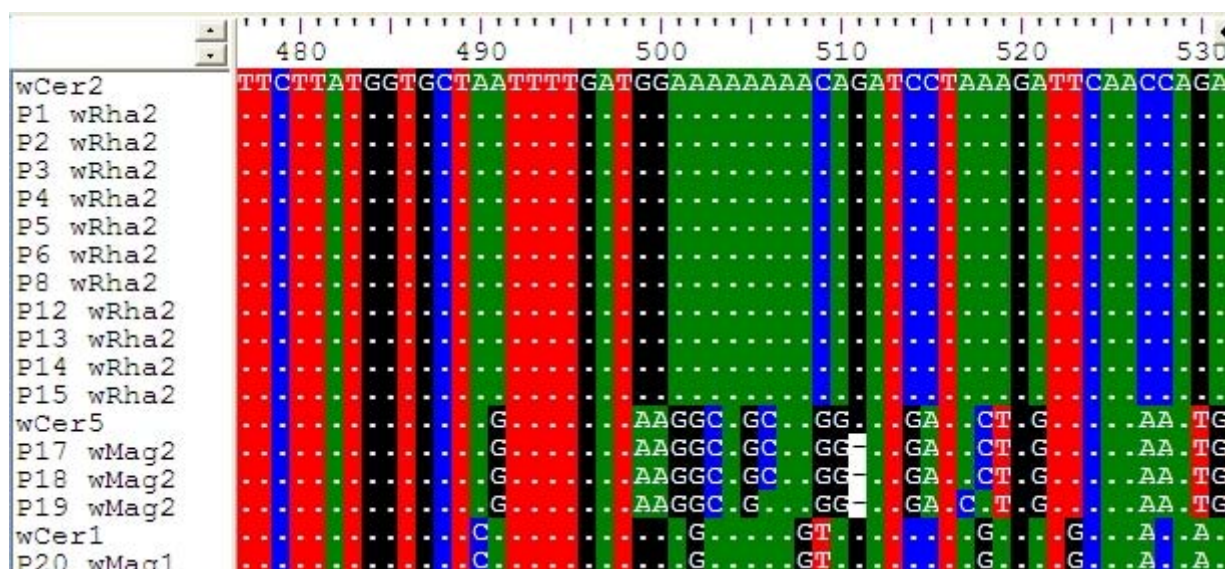

Figure 4.6 Alignment of the *wsp* sequences obtained by direct sequencing, showing the *w*Rha2 strains of *P. rhagoleticola* (P1-15), the three *w*Mag2 sequences of *U. magnus* (P17-19) and one *w*Mag1 (P20) sequence of *U. magnus*.

Wolbachia is known to infect insects in very low densities, difficult to detect by conventional PCR. Arthofer et al. (2009b) analysed *Pityogenes chalcographus* (Coleoptera, Scolytinae) by nested PCR, which lowered the detection limit from 10^{-2} ng plasmid DNA to 10^{-7} ng. In our study nested PCR was applied to enable the detection of the possible low titre strains *w*Cin1 (=*w*Cer1). Nested PCR was performed with strain specific primers. This PCR showed positive amplicons for five individuals of *P. rhagoleticola* (Figure 4.7). Sequences of these amplicons revealed that *P. rhagoleticola* are infected additionally with a second strain *w*Rha1 (=*w*Cin1 = *w*Cer1) based on the *wsp* gene. The PCR products were also cloned and also these sequences revealed identity to *w*Rha1..
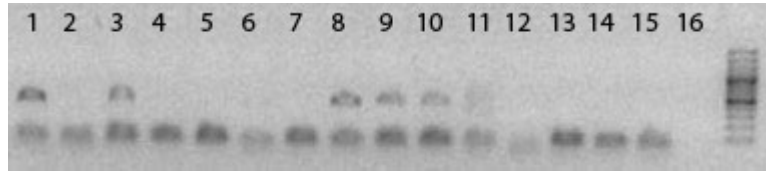
Figure 4.7 Agarose gel showing positive amplicons for the nested PCR product of AT [1,3] and CZ [8-10]. Lanes 12 - 16 are negative controls.
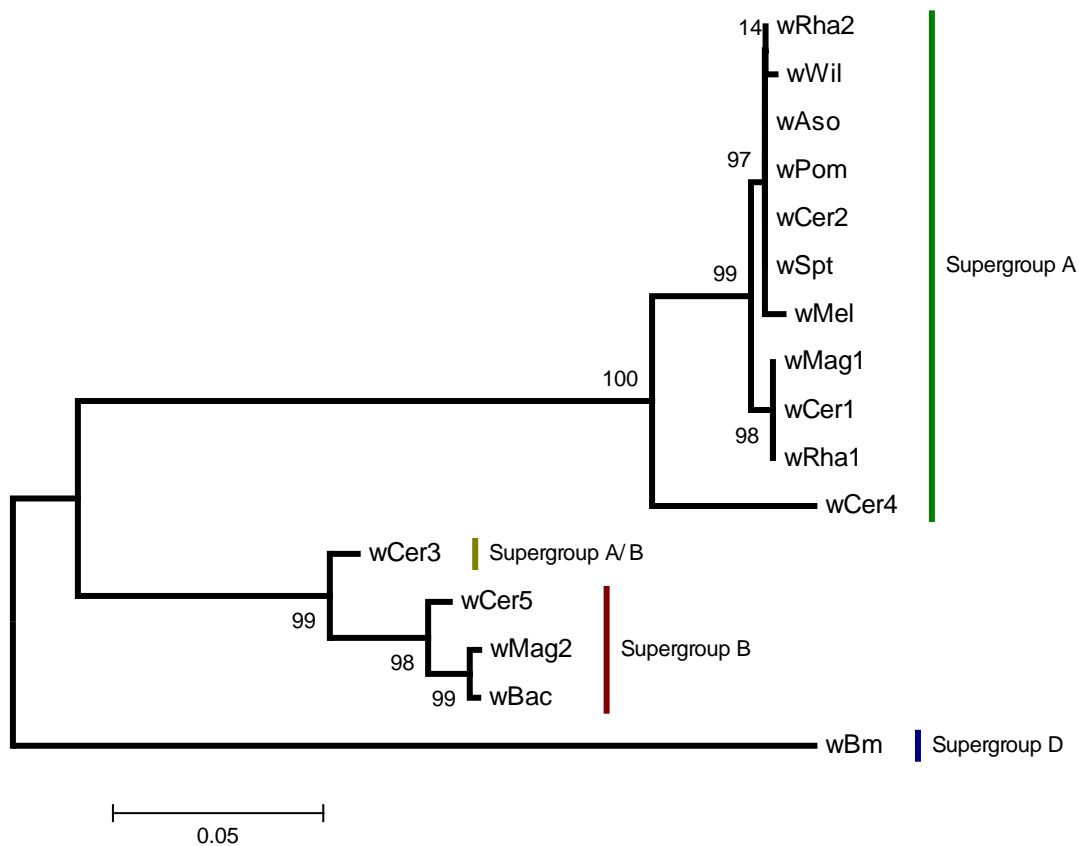
Figure 4.8 Phylogenetic analysis of the sequence data of *wsp* of *w*Rha1 and *w*Rha2 of *P. rhagoleticola* and *w*Mag1 and *w*Mag2 of *U. magnus.* Further, *w*Cer1-5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank were used. Analysis was done with Neighbour Joining method using the *wsp* sequences. Bootstrap analyses (numbers on the nodes) were done with 100 replicates. *w*Cer= *R. cerasi*, *w*Wil *Drosophila willistoni* (AY620229), *w*Spt= *D.septentriosaltans* (AY620209), *w*Atab= *Asobara tabida* (AY581191), *w*Mel= *D. innubila* (AY552553), *w*Bac= *Bactericera cockerelli* (AY971917), *w*Bm= *Brugia malayi* (AY527202), *w*Pom= *Rhagoletis pomonella* (HQ333157)

The NJ tree based on the *wsp* gene revealed that *w*Rha1 has a genetic distance to *w*Rha2 of 0,08% and shows 10 mutations in the 556 bp fragment. *w*Cer2 and *w*Rha2 are 100% ident (Figure 4.8; Appendix III). These strains belong to the A supergroup according to clustering patterns of 16S-DNA and the genes *fts*Z and *wsp* (Werren et al. 1995, Bandi et al. 1998, Zhou et al. 1998, Lo et al. 2002,Werren et al. 2008).

To confirm the results obtained by the *wsp*, *w*Rha2 was also characterized by the five MLST housekeeping genes (Baldo et al. 2006). Between 8 and 12 *P. rhagoleticola* specimens were sequenced for each of the five loci (Figure 4.9, 4.10). The *fts*Z-primers showed weak bands with an annealing temperature (T_M) of 55°C showed weak results (Figure 4.11) and only a T_M of 50°C resulted in positive amplicons (data not shown).

Figure 4.9 Agarose gel showing the amplicons for *P. rhagoleticola* for the locus *Cox*A Lane AT (1-6) and Czech (7-8).
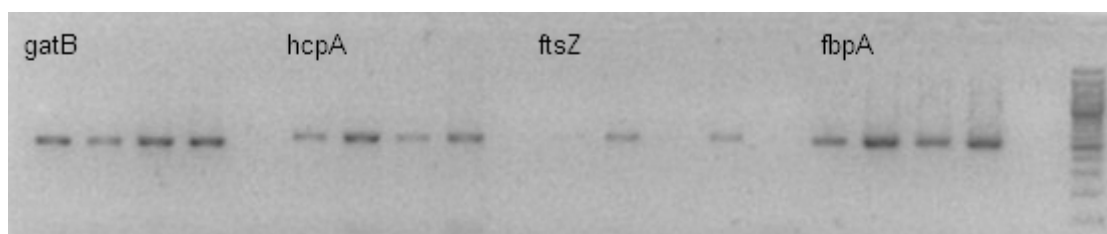
Figure 4.10 Agarose gel with the five MLST loci of *P. rhagoleticola* (Table 1.1). The *fts*Z loci didn't work well with the T_M of 55°C. This locus was successfully amplified with a T_M of 50°C. All other primers amplified bands strong enough to be sequenced.
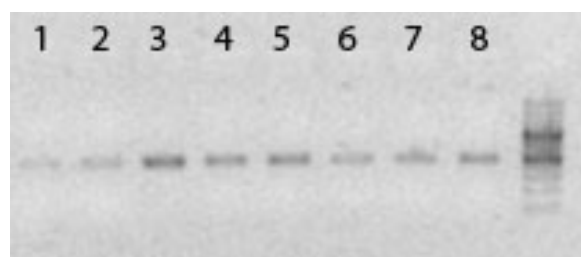
*- gat*B

Figure 4.11 Phylogenetic analysis of the sequence data of *gat*B of *w*Rha1 and *w*Rha2 of *P. rhagoleticola*, *w*Cer1, *w*Cer2, *w*Cer4, *w*Cer5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank. Analysis was done with Neighbour Joining method using he gat*B* sequences. Bootstrap analyses (numbers on the nodes) were done with 1000 replicates. *w*Qua= *Drosophila quinria* (JF764003), *w*Cneg= *Ceutorhynchus neglectus* (HQ602878),*w*Ana= *D. ananassae* (EF611906), *w*Alb= *Aedes albopictus* (DQ842416), *w*Bor= *D. borealis*(FJ41547), *w*Ngir= *Nasonia giraulti* (DQ842442), *w*Sim= *D. simulans* (DQ842432)

The NJ tree based on the *gat*B gene revealing *w*Rha2 is most closely related to *w*Ngir of the parasitoid wasp *Nasonia giraulti* (DQ842442) with 0,08% (Figure 4.11). The distance to *w*Cin1 (=*w*Cer1) is 0,39% and to *w*Cer2 0,22% (Appendix III).

*- cox*A

Figure 4.12 Phylogenetic analysis of the sequence data of *cox*A of *w*Rha1 and *w*Rha2 of *P. rhagoleticola*, *w*Cer1, *w*Cer2, *w*Cer4, *w*Cer5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank. Analysis was done with Neighbour Joining method using he *cox*A sequences. Bootstrap analyses (numbers on the nodes) were done with 1000 replicates.
*w*Cer= *R. cerasi*, *w*Mel= *Drosophila melanogaster*, *w*Nlon= *Nasonia longicornis*(FJ390239)*, w*Ana= *Drosophila ananassae* (EF611963), *w*Vit= *Nasonia vitripennis* (DQ842296), *w*Mono= Monomorium chinense (EU127564), *w*Cal= *Calyptratae* sp. (EU126244), *w*Sim= *D. simulans* (DQ842285), wAjap= *Asobara japonica (*FM872332)

The NJ tree based on the *gat*B gene revealed *w*Rha2 is most closely related to *w*Vit

of the parasitoid wasp *Nasonia vitripennis* (DQ842296) with 0,05% (Figure 4.12). The

distance to *w*Cin1 (=*w*Cer1) is 0,13% and to *w*Cer2 0,19% (Appendix III).
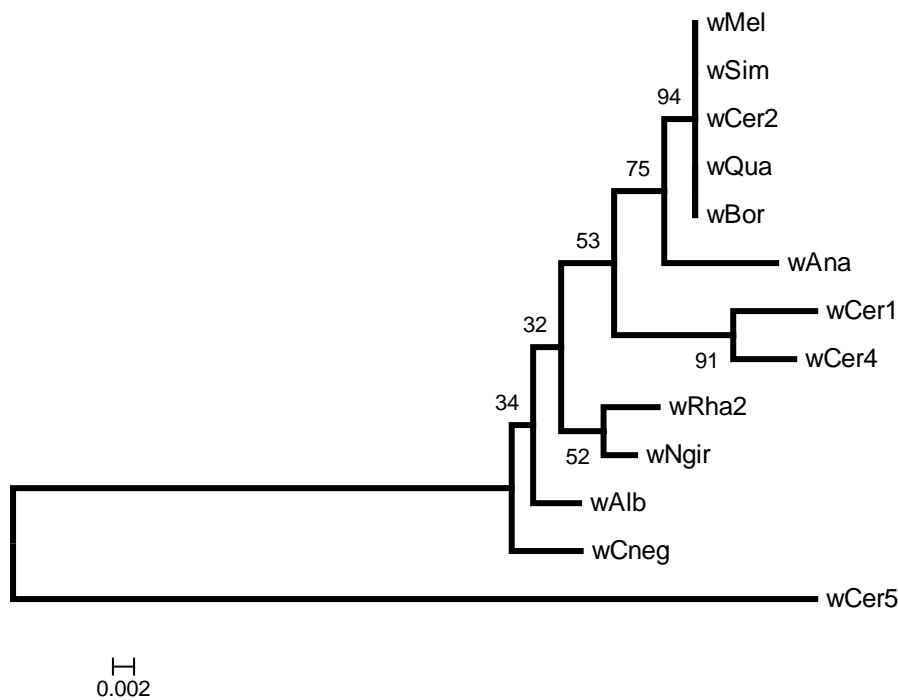
- *hcp*A

Figure 4.13 Phylogenetic analysis of the sequence data of *hcp*A of *w*Rha1 and *w*Rha2 of *P. rhagoleticola*, *w*Cer1, *w*Cer2, *w*Cer4, *w*Cer5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank. Analysis was done with Neighbour Joining method using he *hcp*A sequences. Bootstrap analyses (numbers on the nodes) were done with 1000 replicates.
*w*Cin= *Rhagoletis cingulata*, *w*Cer= *R. cerasi*, *w*Mel= *Drosophila melanogaster* (AE017196)*,* *w*Nvi= *Nasonia vitripennis* (DQ842407), *w*Ngir= *Nasonia giraulti* (DQ842405), *w*Cal= *Calyptrata* muscoid fly (EU126301), *w*Bor= *Drosophila borealis* (FJ415472), *w*Ovu= *Eusomus ovulum* (GU111709), *w*Atab= *Asobara tabida* (FM872341), *w*Ana= *Drosophila ananassae* (EF611978)

The NJ tree based on the *gat*B gene revealed *w*Rha2 is ident to *w*Cal of *Calyptrata* muscoid fly (EU126301) (Figure 4.13). The distance to *w*Cin1 (=*w*Cer1) is 0,27% and to *w*Cin2 (=*w*Cer2) 0,24% (Appendix III).

- *fts*Z

The NJ tree based on the *gat*B gene revealed *w*Rha2 is with a genetic distance of 0,02% related to *w*Cer4, *w*Pro from *Protocalliphora sialia* (DQ266423) and *w*Ajap *from Asobara japonica (*FM872334) (Figure 4.14). The distance to *w*Cin1 (=*w*Cer1) is 0,23% and to *w*Cin2 (=*w*Cer2) 0,16% (Appendix III).
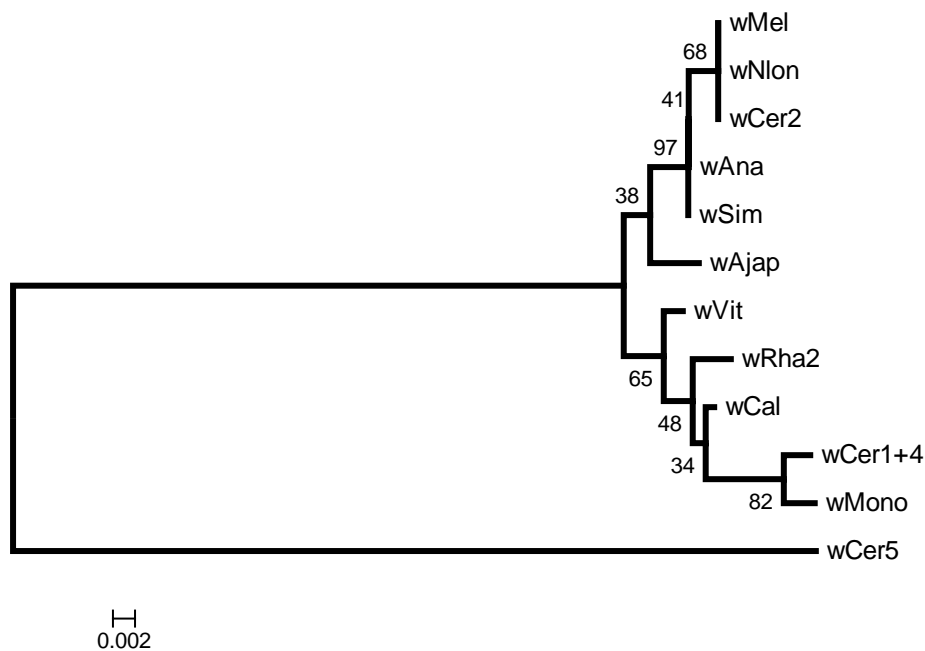
Figure 4.14
Phylogenetic analysis of the sequence data of *fts*Z of *w*Rha1 and *w*Rha2 of *P. rhagoleticola*, *w*Cer1, *w*Cer2, *w*Cer4, *w*Cer5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank. Analysis was done with Neighbour Joining method using he *fts*Z sequences. Bootstrap analyses (numbers on the nodes) were done with 1000 replicates.

*w*Cer = *R. cerasi*, *w*Sim= *Drosophila simulans* (EF423735), *w*Dia= *Diabrotica barberi* (AY136554), wAtab= *Asobara tabida* (FM872339), wAu= *D. simulans* (AY227739), *w*Mel= *Drosophila melanogaster* (AE017196), *w*Ajap *Asobara japonica (*FM872334), *w*Ana= *D. ananassae* (EF611883), *w*Pro= *Protocalliphora sialia* (DQ266423).
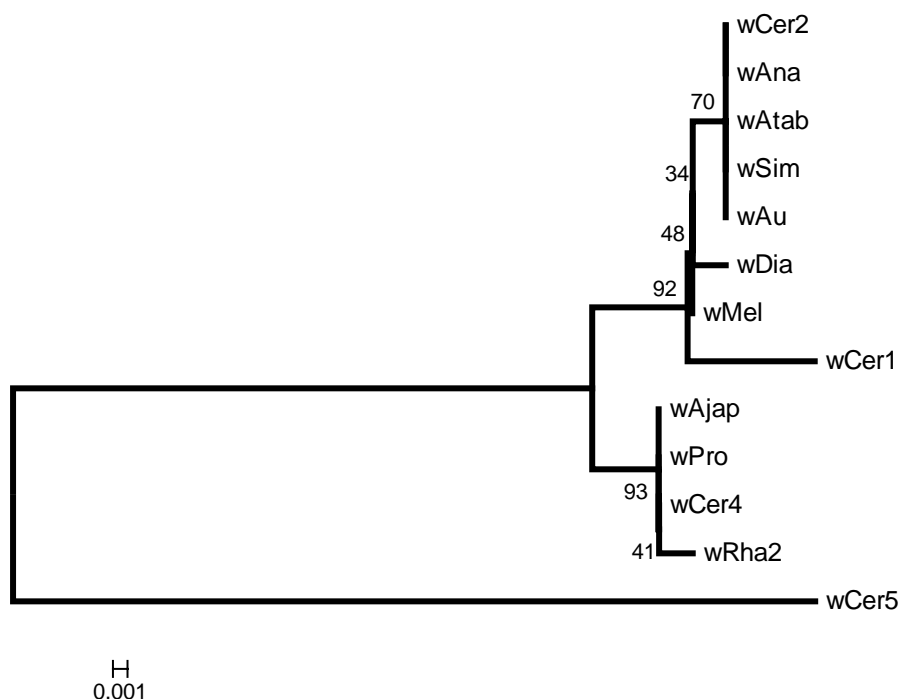
- *fbp*A
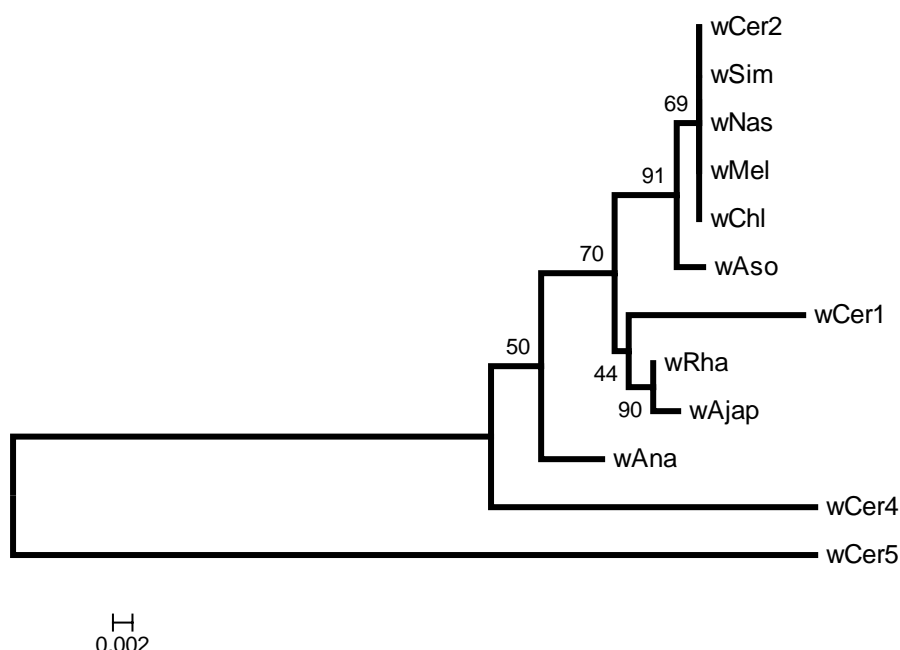
Figure 4.15 Phylogenetic analysis of the sequence data of *fbp*A of *w*Rha1 and *w*Rha2 of *P. rhagoleticola*, *w*Cer1, *w*Cer2, *w*Cer4, *w*Cer5 of *R. cerasi* and other *Wolbachia* strains retrieved from the Genbank. Analysis was done with Neighbour Joining method using he *fbp*A sequences. Bootstrap analyses were done with 1000 replicates and are the numbers above the node.
*w*Cer= *R. cerasi*, *w*Mel= *Drosophila melanogaster* (AE017196), *w*Sim= *D. simulans* (DQ842358) *w*Nvi= *Nasonia vitripennis* (DQ842370), *w*Chl= *Chloropidae sp.* (EU126395), *w*Ana= *Drosophila anassae* (EF611894), *w*Ajap= *Asobara japonica* (FM872333), *w*Atab= *Asobara tabida* (FM872338)

The NJ tree based on the *gat*B gene revealed *w*Rha2 with a genetic distance of 0,02% compared to *w*Cer4 (Figure 4.15). The distance to *w*Cin1 (=*w*Cer1) is 0,21% and to *w*Cin2 (=*w*Cer2) 0,13% (Appendix III).

The analyses of *P. rhagoleticola* with the five MLST loci revealed in all loci mutations compared to *w*Cin2 (=*w*Cer2) and *w*Cin1 (=*w*Cer1). Thus the strain found in parasitoids did not match with those found in its hosts. Either *w*Rha1 and *w*Rha2 are from an unknown occasional host species (Carton et al. 1986) or the *Wolbachia* transmission occurred long ago and then diverged or got lost by the initial host (Vavre 1999).

4.3. Detection and characterisation of *Wolbachia* in *U. magnus*

The five specimens from the population of *U. magnus* showed no visible amplicons with the standard PCR program. However, the hotstart PCR resulted in four positive amplicons (data not shown). These sequences revealed that one specimen of *U. magnus* was infected with a sequence type ident to *w*Cin1 (=*w*Cer1), consequently named *w*Mag1.

The sequence type of the three other *U. magnus* specimens was different compared to the sequences found in *P. rhagoleticola* but similar to *w*Cer5 from *R. cerasi* (Figure 4.6), a strain belonging to the *Wolbachia* supergroup B. This sequence was named *w*Mag2.

The *U. magnus* sequence chromatograms showed ambiguous peaks (Figure 4.7). The presence of double peaks in a sequence is a hint of presence of more than one *wsp* sequence (Jiggins et al. 2002). In the one sample with the *w*Mag1 sequence the lower peaks were those of *w*Mag2 sequences whereas the lower peaks of the other three *w*Mag2 sequences were almost ident to *w*Cin1 (=*w*Cer1) and *w*Cin2 (=*w*cer2) (data not shown).
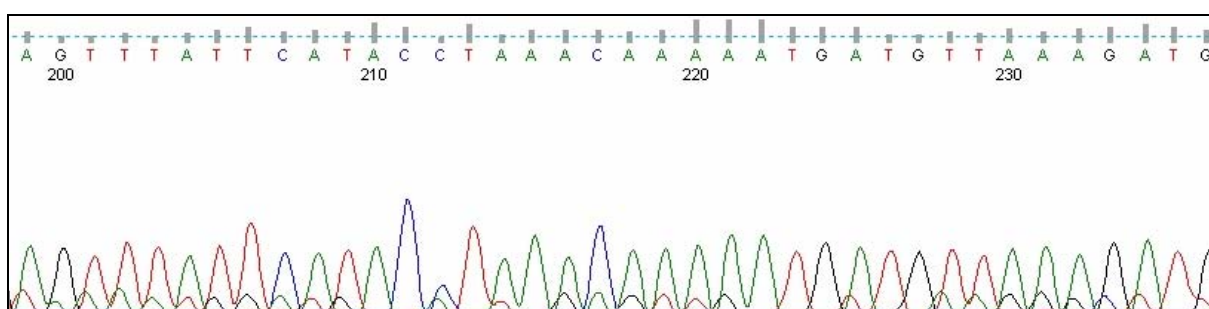

Figure 4.16 Sequence of *U. magnus* with double peaks. Analysis revealed *w*Mag2 and *w*Mag1.

The NJ tree based on the *wsp* gene revealed that *w*Mag2 is most closely related to *w*Bac of *Bactericera cockerelli* (AY971917) with genetic distance of 0,04% (Appendix III). The distance to *w*Cer1 is 0,39% and to *w*Cer2 0,22% at this locus.

In *U. magnus* two sequence types *w*Mag1 and *w*Mag2 based on *wsp* were detected. PCR with hotstart DNA polymerase (Peqlab) showed that one individual was infected with *w*Mag1 ident to *w*Cin1 (=*w*Cer1) and the other three individuals with *w*Mag2 similar to *w*Cer5 from *R. cerasi* were infected. The *w*Mag2 sequence type was phylogenetically different as it belongs to the *Wolbachia* supergroup B. All four chromatograms of the *U. magnus* specimens showed a lot of double peaks. According to our results *U. magnus* seems to be double infected with a *w*Mag2 and *w*Mag1.

In further studies it would be necessary to characterize the *w*Mag1 strain by cloning and additional by MLST loci. Additionally also nested PCR with specific *w*Cer1 primers needs to be done to look for low titre infections.

4.4   Conclusions

To prove horizontal *Wolbachia* transmission, cage studies are proposed with *w*Cer1 uninfected *R. cingulata* flies and *w*Cer1 infected *R. cerasi* flies. Uninfected parasitoids should be put into the cages and if the progeny of *R. cingulata* is infected with *w*Cer1 after the end of the experiment horizontal transmission would be proven.

This master thesis found new *Wolbachia* strains in *P. rhagoleticola* and *U. magnus* but could not prove that these strains are ident to the ones detected in *R. cingulata* and *R. cerasi*. Thus horizontal *Wolbachia* transmission by parasitoids could not be confirmed by this study.

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5.   Appendix

Appendix I Working Protocols

<u>- Extraction after SIGMA kit</u>

- pipette 180 µl of lysis solution T (B-6678) in a 1,5 ml Eppendorf tube
- add the insect specimen and mince with the drill – put the samples on ice
- add 20 µl of proteinase K
- vortex (ca. 15 sec.) and put on the heating block at 55°C/450 rpm for 2 hrs
- add 20 µl RNase and let tubes stand for 2 min at room temperature
- add 200 µl of lysis solution C (B-8803)
- vortex carefully and incubate at 70° for 10 min
- during incubation prepare the tubes and columns - add 500 µl column preparation
- Solution to the column and spin at 13.000 rpm for 1 minute
- discard flow- through and put column back into the same tube
- add 200 µl absolute ethanol to the sample
- vortex for 15 sec
- transfer the samples to the binding columns (approx. 650 µl)
- spin at 8.000 rpm for 1 min
- discard tube with flow-trough and put column in a fresh tube
- add 500 µl of wash solution
- spin at 8.000 rpm for one minute
- discard flow-trough and put column back into the same tube
- add 500 µl wash solution
- spin at 13.000 rpm for 3 minutes
- discard flow-through and put column back into the same tube
- spin again for 1 min at 13.000 rpm to get rid of any remaining alcohol
- put column in a fresh tube
- add 50 µl of elution solution and let column stand for 5 minutes
- spin at 8.000 rpm for 1 min
- store DNA in the fridge

Appendix II Cloning

Day 1

- mix PCR product with 1,4 µl H2O, 0,1 µl ptZ57R, 0,3 µl PEG3350, 0,3µl T4 Buffer, 0,1 µl T4 ligase
- add 0,8 µl DNA
- incubate over night

Day 2

- thaw 35 µl competent cells per reaction on ice
- pre-cool ligation reaction mixtures on ice in 0.5 ml reaction tubes
- add competent cells to the ligations
- incubate on ice for 20 min
- heat shock bacterial suspensions in a 42 °C hot water bath for 50 sec
- put reactions back on ice for 1-2 min immediately
- add 300 µl of SOC medium were added to each tube
- incubate at 37 °C for 1-2 hrs
- prepare LB-Amp plates in the meantime: plate 40 µl X-Gal (20 mg/ml) and 40 µl IPTG (24 mg/ml) on each plate with a Drigalski spatula
- plate transformation reactions on the plates
- incubate upside down over night at 37 °C

Day 3

- transfer 0.5 ml up to 2 ml of overnight E. coli cultures into 1.5 ml reaction tubes.
- tip with a sterile toothpick
- transfer into Eppendorf tubes (containing master mix for PCR) and a cap-o-test vial containing 3 ml LB broth containing 50 µg/ml ampicillin.
- vials were incubated at 37 °C overnight under vigorous shaking (180 – 200 rpm).

Day 4

- transfer 0.5 ml up to 2 ml of overnight E. coli cultures into 1.5 ml reaction tubes.
- pellet cells by centrifugation: 10.000 rpm, 4 min
- discard supernatant and re-suspend pellets in 100 µl resuspension solution
- add 1µl RNase
- incubate for 2-5 min
- add 200 µl NaOH-SDS
- vortex at 1.400 rpm
- add 150 µl ice cold Kac-solution, vortex 10 sec
- put samples 5 min on ice
- centrifuge 5 min on 4°C at 15.000 rpm
- pipette supernatant in a new tube
- add 900 µl EtOH and vortex carefully
- incubate for 2 min and centrifuge for 5 min at 4°C on 15.000 rpm
- discard flow-through and dry the tube
- add cold 70% EtOH vortex carefully and centrifuge for 5 min at 4°C on 15.000 rpm
- discard ethanol and air-dry pellets for approximately 2 hours
- re-suspend pellet in 10 mM Tris

LB broth (Sambrook et al. 1989)

Per Litre

Bacto-tryptone	10.0 g
Bacto-yeast Extract	5.0 g
NaCl	10.0 g
Agar	15.0 g

Adjust the pH to 7,0 with 5N NaOH, adjust the volume of the solution to 1 litre with deionized H_2O and sterilised by autoclaving.

Appendix III Distances of genetic DNA data

COI

[1] #*P. lounsburyi*GU725011
[2] #*P. phaeostigma*EU761045
[3] #*D. sibirica*FM210146
[4] #*P. concolor*EU761025
[5] #*P. humilis*EU761031
[6] #*P. cosyrae*EU761041
[7] #*P. rhagoleticola_*I

[8] #*P. rhagoleticola_*II
[9] #*P. ponerophaga*EU761018
[10] #*U. magnus*
[11] #*Dacnusa*FJ413966
[12] #Hymenopterasp.BOLD:AAQ2936
[13] #Hymenopterasp.BOLD:AAG1328
[14] #*Cotesia glomerata*_EU143657

```
[     1     2     3     4     5     6     7     8     9    10    11    12    13    14 ]
[ 1]
[ 2]  0.088
[ 3]  0.132 0.132
[ 4]  0.089 0.105 0.142
[ 5]  0.084 0.089 0.138 0.044
[ 6]  0.105 0.135 0.178 0.119 0.119
[ 7]  0.128 0.145 0.153 0.130 0.130 0.134
[ 8]  0.132 0.149 0.157 0.134 0.134 0.138 0.007
[ 9]  0.132 0.124 0.148 0.119 0.127 0.128 0.084 0.082
[10]  0.167 0.165 0.136 0.182 0.189 0.202 0.174 0.178 0.176
[11]  0.132 0.130 0.074 0.155 0.153 0.163 0.157 0.161 0.151 0.128
[12]  0.151 0.140 0.105 0.155 0.155 0.176 0.165 0.169 0.144 0.080 0.107
[13]  0.124 0.120 0.087 0.144 0.134 0.171 0.155 0.159 0.138 0.101 0.097 0.086
[14]  0.213 0.229 0.198 0.224 0.228 0.231 0.219 0.224 0.217 0.224 0.211 0.200 0.206
```

Wsp

[1] #*w*Cer2   [7] #*w*Mel   [13] #*w*Mag2
[2] #*w*Pom   [8] #*w*Rha1   [14] #*w*Bac
[3] #*w*Rha2   [9] #*w*Spt   [15] #*w*Cer3
[4] #*w*Cer1   [10] #*w*Wil   [16] #*w*Bm
[5] #*w*Mag1   [11] #*w*Aso
[6] #*w*Cer4   [12] #*w*Cer5

```
[    1    2    3    4    5    6    7    8    9    10   11   12   13   14   15   16 ]
[ 1]
[ 2] 0,000
[ 3] 0,000 0,000
[ 4] 0,008 0,008 0,008
[ 5] 0,008 0,008 0,008 0,000
[ 6] 0,068 0,068 0,068 0,061 0,061
[ 7] 0,004 0,004 0,004 0,013 0,013 0,073
[ 8] 0,008 0,008 0,008 0,000 0,000 0,061 0,013
[ 9] 0,000 0,000 0,000 0,008 0,008 0,068 0,004 0,008
[10]  0,002 0,002 0,002 0,011 0,011 0,070 0,006 0,011 0,002
[11]  0,000 0,000 0,000 0,008 0,008 0,068 0,004 0,008 0,000 0,002
[12]  0,252 0,252 0,252 0,255 0,255 0,265 0,258 0,255 0,252 0,255 0,252
[13]  0,258 0,258 0,258 0,261 0,261 0,271 0,265 0,261 0,258 0,261 0,258 0,017
[14]  0,258 0,258 0,258 0,261 0,261 0,271 0,264 0,261 0,258 0,261 0,258 0,017 0,004
[15]  0,223 0,223 0,223 0,234 0,234 0,259 0,229 0,234 0,223 0,226 0,223 0,028 0,046 0,046
[16]  0,372 0,372 0,372 0,373 0,373 0,360 0,372 0,373 0,372 0,376 0,372 0,288 0,294 0,294 0,293
```

*gat*B

[1] #*w*Cer1   [6] #*w*Mel   [11] #*w*Bor
[2] #*w*Cer2   [7] #*w*Qua   [12] #*w*Gir
[3] #*w*Rha   [8] #*w*Neg   [13] #*w*Sim
[4] #*w*Cer4   [9] #*w*Ana
[5] #*w*Cer5   [10] #*w*Alb

```
[     1     2     3     4     5     6     7     8     9    10    11    12    13 ]
[ 1]
[ 2]  0,028
[ 3]  0,039 0,022
[ 4]  0,014 0,025 0,036
[ 5]  0,149 0,142 0,131 0,156
[ 6]  0,028 0,000 0,022 0,025 0,142
[ 7]  0,028 0,000 0,022 0,025 0,142 0,000
[ 8]  0,025 0,025 0,019 0,028 0,131 0,025 0,025
[ 9]  0,036 0,014 0,031 0,028 0,156 0,014 0,014 0,039
[10]  0,036 0,019 0,014 0,034 0,128 0,019 0,019 0,016 0,022
[11]  0,028 0,000 0,022 0,025 0,142 0,000 0,000 0,025 0,014 0,019
[12]  0,031 0,019 0,008 0,028 0,142 0,019 0,019 0,022 0,022 0,017 0,019
[13]  0,028 0,000 0,022 0,025 0,142 0,000 0,000 0,025 0,014 0,019 0,000 0,019
```

*cox*A

[1] #*w*Rha   [5] #*w*Mel   [9] #*w*Ana
[2] #*w*Cer1+4   [6] #*w*Sim   [10] #*w*Mono
[3] #*w*Cer2   [7] #*w*Nlo   [11] #*w*Cal
[4] #*w*Cer5   [8] #*w*Vit   [12] #*w*Jap

[1   2   3   4   5   6   7   8   9   10   11   12]
[1]
[2] 0,013
[3] 0,019 0,027
[4] 0,144 0,141 0,138
[5] 0,019 0,027 0,000 0,138
[6] 0,016 0,024 0,003 0,134 0,003
[7] 0,019 0,027 0,000 0,138 0,000 0,003
[8] 0,005 0,013 0,013 0,137 0,013 0,011 0,013
[9] 0,016 0,024 0,003 0,134 0,003 0,000 0,003 0,011
[10] 0,013 0,005 0,027 0,144 0,027 0,024 0,027 0,019 0,024
[11] 0,008 0,011 0,016 0,138 0,016 0,013 0,016 0,008 0,013 0,011
[12] 0,013 0,027 0,011 0,138 0,011 0,008 0,011 0,013 0,008 0,021 0,016

*hcp*A

[1] #*w*Cer1   [6] #*w*Rha   [11] #*w*Nvi

[2] #*w*Cer2   [7] #*w*Ovu   [12] #*w*Gir

[3] #*w*Cer4   [8] #*w*Tab   [13] #*w*Cal

[4] #*w*Cer5   [9] #*w*Ana

[5] #*w*Mel   [10] #*w*Bor

[1   2   3   4   5   6   7   8   9   10   11   12   13]

[1]

[2] 0,027

[3] 0,030 0,032

[4] 0,138 0,128 0,123

[5] 0,027 0,000 0,032 0,128

[6] 0,027 0,024 0,007 0,129 0,024

[7] 0,035 0,032 0,005 0,129 0,032 0,007

[8] 0,035 0,032 0,005 0,128 0,032 0,007 0,005

[9] 0,027 0,020 0,017 0,137 0,020 0,015 0,017 0,017

[10] 0,027 0,000 0,032 0,128 0,000 0,024 0,032 0,032 0,020

[11] 0,007 0,020 0,027 0,134 0,020 0,020 0,027 0,027 0,020 0,020

[12] 0,035 0,032 0,015 0,128 0,032 0,007 0,015 0,015 0,022 0,032 0,027

[13] 0,027 0,024 0,007 0,129 0,024 0,000 0,007 0,007 0,015 0,024 0,020 0,007

ftsZ

[1] #*w*Cer1	[6] #*w*Rha	[11] #*w*Sim
[2] #*w*Cer2	[7] #*w*Jap	[12] #*w*Dia
[3] #*w*Cer4	[8] #*w*Aso	[13] #*w*Pro
[4] #*w*Cer5	[9] #*w*Ana	
[5] #*w*Mel	[10] #*w*AU	

```
[     1    2    3    4    5    6    7    8    9    10   11   12   13 ]
[ 1]
[ 2] 0,011
[ 3] 0,021 0,014
[ 4] 0,107 0,104 0,099
[ 5] 0,009 0,002 0,011 0,102
[ 6] 0,023 0,016 0,002 0,102 0,014
[ 7] 0,021 0,014 0,000 0,099 0,011 0,002
[ 8] 0,011 0,000 0,014 0,104 0,002 0,016 0,014
[ 9] 0,011 0,000 0,014 0,104 0,002 0,016 0,014 0,000
[10]  0,011 0,000 0,014 0,104 0,002 0,016 0,014 0,000 0,000
[11]  0,011 0,000 0,014 0,104 0,002 0,016 0,014 0,000 0,000 0,000
[12]  0,011 0,005 0,014 0,104 0,002 0,016 0,014 0,005 0,005 0,005 0,005
[13]  0,021 0,014 0,000 0,099 0,011 0,002 0,000 0,014 0,014 0,014 0,014 0,014
```

ftsZ

*fbp*A

[1] #*w*Cer1   [5] #*w*Mel   [9] #*w*Ana

[2] #*w*Cer2   [6] #*w*Rha   [10] #*w*Nas

[3] #*w*Cer4   [7] #*w*Ajap   [11] #*w*Sim

[4] #*w*Cer5   [8] #*w*Aso   [12] #*w*Chl

[1   2   3   4   5   6   7   8   9   10   11   12]

[1]

[2] 0,028

[3] 0,064 0,056

[4] 0,159 0,152 0,166

[5] 0,028 0,000 0,056 0,152

[6] 0,021 0,013 0,053 0,152 0,013

[7] 0,023 0,015 0,056 0,156 0,015 0,003

[8] 0,028 0,005 0,056 0,155 0,005 0,013 0,015

[9] 0,037 0,023 0,037 0,152 0,023 0,015 0,018 0,023

[10] 0,028 0,000 0,056 0,152 0,000 0,013 0,015 0,005 0,023

[11] 0,028 0,000 0,056 0,152 0,000 0,013 0,015 0,005 0,023 0,000

[12] 0,028 0,000 0,056 0,152 0,000 0,013 0,015 0,005 0,023 0,000 0,000

Curriculum Vitae

Peter Kern

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Name   Peter Kern
Address   Martinstraße 19/27
                  1180 Wien, Austria
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Nationality   Germany
Date of birth   14. September 1985

Education

2006 – 2009   Study of BSc„Forestry and Environment"
                  („Waldwirtschaft und Umwelt") with minor
                  „Environmental Natural Sciences"
                  („Umweltnaturwissenschaften"), University of
                  Freiburg, Germany.

                  Degree awarded BSc in "Forestry and Environment"
                  September 2009)

2009 – 2011   Master Phytomedicine, Boku, University of Natural
                  Resources and Life Sciences, Vienna, Austria

                  Degree (Diplom-Ingenieur Phytomedicine)

Bachelor and Master thesis

2009   Compensation of iron and magnesium deficiency by
                  Thiachloprid 009SC.

2011   *Psyttalia rhagoleticola* (Hym., Braconidae): A
                  potential vector of *Wolbachia* transfer between
                  Rhagoletis cerasi and the invasive tephritid *R.
                  cingulata* in Europe

06/2002   Internship Natural History Museum Basel
                                               (Switzerland) Dr. Michael Brancucci

08/2007   Research about the ovary development of the
                                       butterfly, *Vanessa cardui* (Lepidoptera,
                                       Nymphalidae) – Institute of Forest Zoology (FZI),
                                       University of Freiburg (Germany)

04/2008 – 08/2008   Research about the new in Europe introduced
                                       insect pest *Cydalima perspectalis* (Lepidoptera,
                                       Pyraloidea) – FZI Freiburg

08/2008 – 10/2008   Studies about the occurrence of *Bois noir* in the
                                       vector *Hyalesthes obsoletus* (Homoptera: Cixiidae),
                                       host plants and in grapevine State Institute for
                                       Viticulture and Enology Freiburg -Ecology
                                       Department (Baden-Württemberg, Germany)

04/2009 – 06/2009   Internship Bayer CropScience Monheim,
                                       Group of Horticulture – Jürgen Hölters (Germany)

06/2009 – 09/2009   Bachelor thesis at Bayer CropScience
                                       ""Kompensation von Magnesium- und Eisenmangel
                                       an Pflanzen durch eine neue Insektizidformulierung
                                       aus der Wirkstoffklasse der Neonicotionoide "

08/2010 – 10/2010   Research about the Fire Blight resistance locus in
                                       Malus robusta - ETH Zurich Plant Pathology group
                                       Prof. Cesare Gessler (Switzerland)

Paper in preparation

Schuler H, Kern P, Arthofer W, Feder J, Egan S, Köppler K, Vogt H, Stauffer C:
    Horizontal *Wolbachia* Transfer between *Rhagoletis cerasi* and the invasive
    tephritid *R. cingulata* in Europe.

Poster

Kern P 2011 Parasitoide als potentielle Vektoren von den bakteriellen
    Endosymbionten *Wolbachia* zwischen zwei Kirschfruchtfliegenarten. 18th of
    May 2011, Celebration Hall of Boku, University of Natural Resources and Life
    Sciences, Vienna