Report
Short Term Scientific Mission

COST Action 850
„Biocontrol Symbiosis“

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COST 850 WG1 Symbiosis Biology

Tissue tropism of *Wolbachia* infections in *Rhagoletis cerasi* and *Drosophila simulans* and experimental transfer of *Wolbachia* into *Ceratitis capitata*

Introduction

Wolbachia is an intracellular, maternally inherited α-proteobacteria occurring in arthropods and nematodes. *Wolbachia* manipulates host reproduction in several ways favouring thereby its own dispersal in host populations (Bourtzis & O´Neill 1998). A common phenotype of *Wolbachia* is cytoplasmic incompatibility (CI) (Hoffmann & Turelli 1997). CI is embryonic lethality which occurs when infected males mate with uninfected females whereas reciprocal crosses between infected females and uninfected males do have offspring. In CI, sperm of infected males is free of *Wolbachia,* but is modified during spermatogenesis by the infection in the father. Infected eggs are able to rescue the imprinted sperm. Besides CI, other phenotypes caused by *Wolbachia* infections were described: thelytokous parthenogenesis in wasps, feminization in isopods, male killing in different insect orders and modifications of fecundity and fertility (Stouthamer *et al*. 1999).

Strong levels of unidirectional CI were detected between European populations of the cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae) (Boller *et al*. 1976). All populations were found infected by two different strains of *Wolbachia*, *w*Cer1 and *w*Cer2. Individuals from one part of Europe were only infected by *w*Cer1 and individuals from the other part were superinfected by *w*Cer1 and *w*Cer2. CI occurred between the infected and superinfected populations suggesting that *w*Cer2 induces CI (Riegler & Stauffer 2002). *w*Cer2 was transferred experimentally to *Drosophila simulans*, a model organism for *Wolbachia* research, where it induced CI at a lower level than in its original host. Variation in the strength of CI was observed in the injected lines which could be due to differences in bacterial load or genetic variability in the transferred *w*Cer2 (Riegler *et al*. 2002).

Aims of the Short-Term Scientific Mission STSM

a) Characterisation of tissues infected by *Wolbachia* *w*Cer1 and *w*Cer2

The intention of the STSM at the Institute of Molecular Biology and Biotechnology, IMBB, FORTH, Crete, was to gain more information about tissue tropism and infection densities of the *Wolbachia* strains in the original host *R. cerasi* and the novel host *D. simulans*, as this might explain the differences found in the levels of CI.

Wolbachia-host associations have an unique tissue tropism. Infections are not restricted to the reproductive organs but are widely distributed in different host tissues. (Dobson *et al*. 1999). The distribution of the bacteria in the reproductive organs seem to be correlated with the phenotype they induce in the host species (Clark *et al*. 2002; Z Veneti & K Bourtzis, pers. comm.).

b) Infection experiment – transfer of *Wolbachia* between tephritid pest species

The Mediterranean fruit fly *Ceratitis capitata*, another tephritid of economic importance, was experimentally injected with *w*Cer1 and *w*Cer2. So far, infection experiments with *Wolbachia* strains from *Drosophila* species into *C. capitata* failed because of missing host acceptance (K Bourtzis, pers. comm.). *C. capitata* and *R. cerasi* are both members of the same dipteran family and *w*Cer1 and *w*Cer2 could therefore be better candidates for a transfer experiment.

Materials & Methods

Insect lines: *R. cerasi* from Austria, superinfected with *w*Cer1 and *w*Cer2
R. cerasi from Hungary, infected with *w*Cer1
both populations collected in 2001 by M Riegler
D. simulans infected with *w*Cer2
6 lines from the transfer experiment by Riegler *et al*. (2002)
uninfected *C. capitata*, wilde type population, hold at the IMBB

Immunostaining:

Ovaries and testes were dissected from infected and superinfected *R. cerasi*. The tissues were fixed onto slides and immunostained according to the protocols of Clark *et al*. (2002) and Z Veneti by using the *Wolbachia* specific antibody developed by Dobson *et al*. (1999). Embryos of *D. simulans* transinfected by *w*Cer2 were collected from egg laying plates. Fixation and immunostaining was done after dechorionation and removal of the vitelline membrane, according to the protocol of Z Veneti. The slides with the fixed organs and embryos were analysed by using a confocal microscope.

Microinjection experiment:

Eggs of infected and superinfected *R. cerasi* females were donors of the *w*Cer1 and *w*Cer2 infections. Eggs were dissected from females after maturation feeding according to Boller (1985). *Wolbachia* from the donor species was microinjected into embryos of *C. capitata* by transferring cytoplasm, according to the microinjection protocol of Poinsot *et al.* (1998). Receiver embryos were collected every 1-2 hours and dechorionated before microinjection.

Results & Discussion

Immunostaining:

Reproductive organs of *R. cerasi* were heavily infected by *Wolbachia*. *Wolbachia* was found in all parts of the testes. Within a developing cyst *Wolbachia* were localized in the proximal end, as described in Clark *et al*. (2002). In the following steps of spermatogenesis *Wolbachia* seemed to be shed of the spermatids, forming *Wolbachia* accumulations in the testes. *Wolbachia* free spermatids were found in the spermiduct. In the ovary, *Wolbachia* infections occurred at high densities in the germarium. During oogenesis, *Wolbachia* infections preferentially occurred around the nurse cells and the oocyte. A polarisation of the *w*Cer infections to the posterior part of the egg was observed, with a similarity to the *w*Mel an *w*Cof infections (Z Veneti, pers. comm.). Unfortunately, the chorion and the vitelline membrane were not successfully removed in order to see a polarisation of *w*Cer in embryos of *R. cerasi* and *D. simulans*. After a first glance it was not possible to detect a difference in the distribution and the number of *Wolbachia* in the tissues of infected and superinfected *R. cerasi*. However, a difference was expected as it was described in an analysis by electron microscopy (Blümel *et al*. 1991).

Microinjection experiment:

microinjections on 6 days (19-20/7 and 22-25/7); total of 48 slides with 70 embryos each (total of 3360)
ca. 2870 embryos injected with *w*Cer1&2 cytoplasm
ca. 490 embryos injected with *w*Cer1
8 females of *R. cerasi* used as donor, each female had between 10 to 20 eggs
339 injected *C. capitata* embryos hatched from the injections of the first 4 days (hatching rate of 15%); hatchings 3-5 days after the injection

Outlook

The immunostaining method is a powerful tool to study *Wolbachia*/host associations during the spermatogenesis and germline development. In *R. cerasi* it revealed a specialisation to parts of the ovaries, eggs and testes like it has previously been shown in *D. simulans* (Clark *et al*. 2002; Z Veneti, pers. comm.).
Another question of this project was to get an insight into transmission efficiency of *w*Cer in the cherry fruit fly. According to the ovary dissections *Wolbachia* transmission was complete. The *w*Cer2 association with *D. simulans* in the reproductive tissues still needs to be studied. An establishment of *w*Cer2 in *C. capitata*

would allow the comparison of *Wolbachia*/host associations of the same *Wolbachia* strain in three different host species.

It will be interesting to see whether *w*Cer will be established in *C. capitata*. Another transfer experiment showed that *w*Cer1 could not be established in *D. simulans*. *w*Cer2 was able to infect *D. simulans* but it would not maintain itself in the novel host. It was necessary to select for the infection in each generation (Riegler *et al*. 2002). An establishment of *w*Cer infections in *C. capitata* would furthermore allow a phenotypical analysis of the *w*Cer strains in a new host. A CI phenotype would be of a major research interest in this fruit fly species of economic importance.

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