Novel markers for assessing *Wolbachia* genome dynamics in the cherry fruit fly

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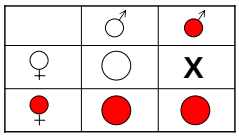
Cytoplasmic Incompatibility

- The endosymbiotic α-proteobacterium *Wolbachia pipientis* infects up to 76% of all insect species and is maternally inherited.
- It is a powerful manipulator of host reproduction. The most important phenotype is cytoplasmic incompatibility (CI) causing embryonic mortality in matings of of uninfected females with infected males.



Wolbachia and *R. cerasi*

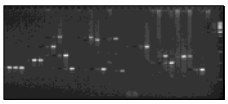
- In the 1970ies, crossing experiments [1] revealed division of *Rhagoletis cerasi* (Diptera, Tephritidae) into two geographic complexes with unidirectional incompatibility.
- It was shown that two *Wolbachia* strains *w*Cer1 and *w*Cer2, distinguishable in the *wsp* gene sequence, are present in *R. cerasi*, with the latter one inducing CI [2].
- Currently the *w*Cer1 single infection is maintained in Eastern Europe. Central and Southern populations are double infected.
- Several more strains have been identified in Sicilian populations.

wsp

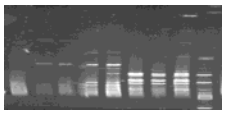
- The *Wolbachia* Surface Protein *wsp* gene is highly dynamic and therefore a broadly used marker for *Wolbachia* detection and strain typing [3].
- While *w*Cer2 induces strong CI in *R. cerasi*, at least three other *Wolbachia* strains with identical *wsp* sequence isolated from other insect species do not induce CI [4].
- Hence, the *wsp* marker has limited potential for separating closely related *Wolbachia* strains and is not suitable for predicting the CI phenotype.

Alternative markers

- *w*Mel contains at least 50 copies of transposable **insertion sequences (IS)** [6]. We developed a PCR strategy to monitor IS polymorphism and mobility [5].
- In addition, 63 sites with **Variable Number of Tandem Repeats (VNTRs)** exist in the *w*Mel genome [5]. These sites show high potential to discriminate strains similar in *wsp*.
- **Ankyrin repeats (ANK)** code for cell cycle proteins that are hypervariable among *Wolbachia* strains [7]. They are candidate markers for CI predicition. We are using the most informative ANK primer sets.



VNTR-141 screening: strain specific PCR fragments obtained from *Rhagoletis* and *Drosophila* samples; the size depends on the number of 141 bp repeat units, ranging from one to seven.



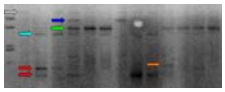
6% PAGE gel with PCR fragments obtained in the IS5 display of flies infected with different *Wolbachia* strains. The IS5 insertion polymorphism generates a strain-specific fingerprint pattern.

Low titer infections

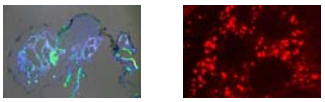
- Conventional PCR approaches estimated a rate of 16 to 22% of infected insect species [8], but the use of long PCR and proof-reading enzymes reveald rates up to 76% [9]. Strains may be in low titer generally, in certain tissues or during specific ontogenic stages of the insect.
- We apply sensitve PCR and hybridization techniques for reliable low titer detection.
- The titers of different strains in multiinfected insects may vary in orders of magnitudes. We develop qRT-PCR applications to quantify strain specific *Wolbachia* copy numbers.

Transinfection experiments

- Long-term coevolution of *Wolbachia* and its host leads to high transmission, low fitness cost and low levels of CI.
- Thus, by microinjection of the endosymbiont into a new, uninfected host, low transmission, high fitness cost and high CI levels should be expected.
- We will test this prediction by artificial infection of *Drosophila* and rearing over many generations.
- Transinfection also segregates single infected fly lines, in which the novel *w*Cer strain's ability to induce CI will be tested.



DGGE analysis of *Drosophila* species. Marked bands were cut out, cloned, sequenced and identified by BLAST analysis, resulting in affiliations to *Wolbachia*, *Pseudomonas* and *Acetobacter* and to *Drosophila* host 18S rDNA.



left: cross section of a *D. paulistorum* female. Organ specific accumulation of *Wolbachia* is visualized by green staind *wsp* antibody.
right: FISH stained *Wolbachia* (red) in *Drosophila* ovarioles

Microbial diversity

- Many insects live in relationship with endosymbiotic bacteria species
- These ‚hidden passengers' might be co-transferred in artificial *Wolbachia* transinfection, with unpredictable impact on the recipients fitness.
- Microbial diversity of *R. cerasi* populations is evaluated by denaturing gradient gel electrophoresis (DGGE) of 16S rDNA amplicons.
- Tissue specific bacteria detection is carried out by *wsp* immunostaining and fluorescent in situ hybridization (FISH).

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Genome dynamics

- Initial observations of the *Wolbachia* genome uncover unusual plasticity at many loci. We will evaluate to which extent the arrival of the endosymbiont in a new host and its establishment in the germ line will alter its genome.
- *w*Cer2 was sucessfully transferred to *D. simulans* in 2000 [10] and coevolved with its new host more than 200 fly generations.
- We will compare geomic maps of six independent transinfected lines with the original donor population in *R. cerasi*, using the marker sets described above.