## Abstract

The intracellular, cytoplasmically inherited bacteria are known to manipulate the host in different ways to disperse in the host population. These bacteria can affect host reproduction via cytoplasmic incompatibility, parthenogenesis induction, male killing and feminization of genetic males. Hosts may be infected with a single bacterium or co-infected with multiple bacteria strains. The coexistence of these symbionts leads to various interactions, which may be manifested in observed phenotypic effects and changes at the gene and gene expression levels. In this project, we focus on the interactions of *Wolbachia* (*W*) and *Spiroplasma* (*S*) co-occurring in quill mites (Acariformes: Syringophilidae).

As we have shown before, this group of arthropods is a promising target for endosymbiont research due to the large variety of W strains (three supergroups, including two previously unknown, detected in just a few mite species) and the possibility of coexistence with unique lineage of S. Further, since we usually observe a single population within one quill (female founder and offspring) with strongly disturbed sex ratio, we may be able to track the potential phenotypic effects of co-occurring endosymbionts, the dynamics of both bacteria and the efficacy of their vertical transmission. The observed composition of genetically unique strains of bacteria also prompts for in-depth research on their genomes. It all together makes quill mites a perfect subject to be tested like "in a laboratory tube" but under natural, non-artificially manipulated conditions.

The objectives of the proposed project are: *i*) to observe the frequency of single and multiinfection in mites; *ii*) to estimate the efficacy of vertical transmission of endosymbionts across all successive developmental stages (larva, protonymph, tritonymph and adult) and sexes (female/male); *iii*) to compare the fitness of mites (number of offspring in the quill) in noninfected populations with those infected with W only and both W and S strains; *iv*) to determine if there is a correlation between the sex ratio (presence and number of males) and absence/presence of a single/co-occurrence of both bacteria; *v*) to verify whether W/S reduces the abundance of the second symbiont in co-infected mite individuals; *vi*) to create a serie of W and S genomes; *vii*) to determine if W/S genome variants coexist in certain genetic combinations (compatible *vs* incompatible genomes).

We expect that the results of our research will expand our knowledge on the prevalence and dynamics of the spread of endosymbionts in mite populations. Further, it will enable unique insights into bacterial-induced phenotypic effects in the hosts (fitness, sex ratio). By investigating the genomic issues of the endosymbionts, the project will contribute to a better understanding of their origin, diversity and associations. The co-occurrence of both bacteria in quill mites is considered as a factor contributing to the acceleration the evolution of both symbionts, what would explain an observed high strain distinctiveness and diversity. This may cause that certain combinations of strains are compatible within the host individual and others are not compatible. This should be reflected in the mite's fitness and reproductive ability. Mutual evolutionary driving may result in symbiont gene wars targeted to manipulate the immune/regulatory pathways of the host and produce defensive compounds against competing symbionts.

Assessing the prevalence and genetic diversity of W and S in quill mites, recognition of their phenotypic effects, as well as examining the mechanisms of competition between bacteria are a crucial steps in understanding endosymbiont-mite interactions. Overall, our project will help in deciphering the ecology of intracellular symbionts and their hosts.