

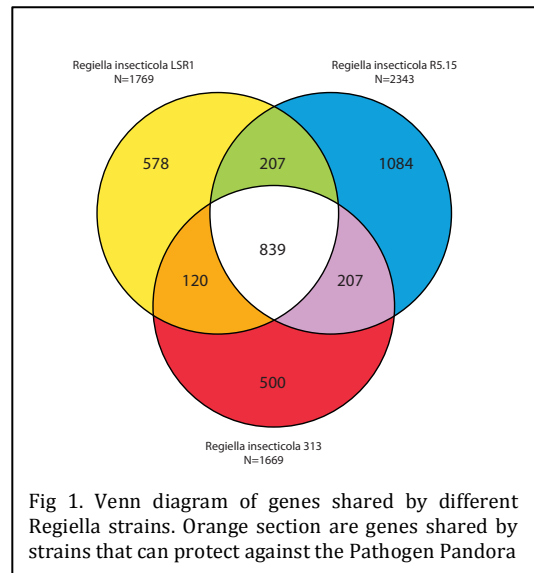
## Summary description of the project objectives

This project aimed to 1) identify genes involved in the bacterial symbiont *Regiella insecticola*'s ability to protect its insect hosts against the fungal pathogen *Pandora*, and 2) characterize the ecological interactions between *Regiella* and its aphid hosts, and then 3) explore the phylogenetic distribution of ecological traits under which the protective function have been gained and lost.

## Main results achieved thus far

### *Identifying genes involved in symbiont-mediated protection*

We sequenced the genome of a *Regiella* strain known to protect against the pathogenic fungus *Pandora* and one that does not protect against the fungus. These genomes were assembled and annotated for gene function and then combined with an additional published *Regiella* genome that protects host insects against the pathogen. We used a comparative genomic analysis to identify gene complexes only present in protective strains as candidate genes involved in the protective phenotype. 1,842 protein-encoding genes were identified, 1,093 were core genes and 120 of these genes were shared by *Regiella* strains that protect from the fungal pathogen that are absent in non-protective strains (Fig 1). Within these genes we found evidence of secretion machinery (e.g. Type I and III secretion systems), membrane transporters and excreted exotoxins that have been implemented with pathogenesis and therefore may be involved in defense against the pathogen. We are currently writing these results into a manuscript on the genetic mechanisms of fungal defense in symbiotic bacteria.

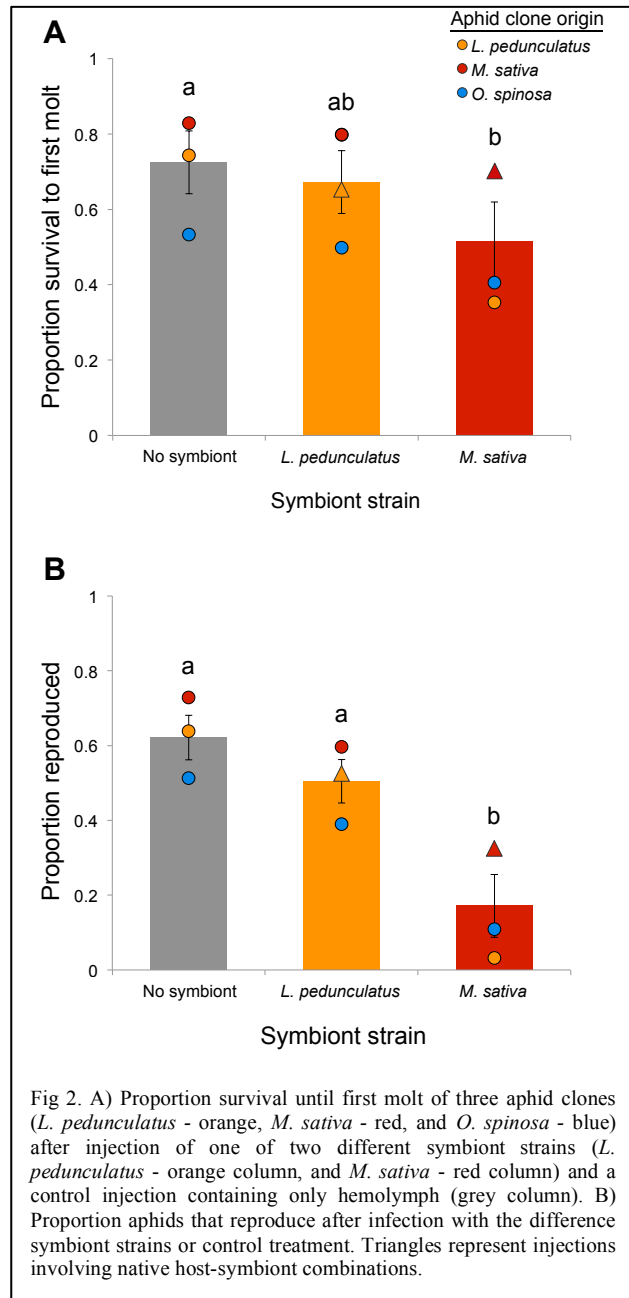


### *Characterize interactions between symbiont and aphid hosts*

We collected live aphids from the field known to harbor the symbiont *Regiella insecticola* and other symbiont species. In total 11 aphid species were brought into the lab for culturing and experimental manipulations. We established clonal lines that carried symbiont lineages of interest. Test aphids were fed on selective antibiotics to cure them of their facultative symbionts, while retaining the primary symbiont *Buchnera*. Interestingly, when we cured the aphids of their facultative

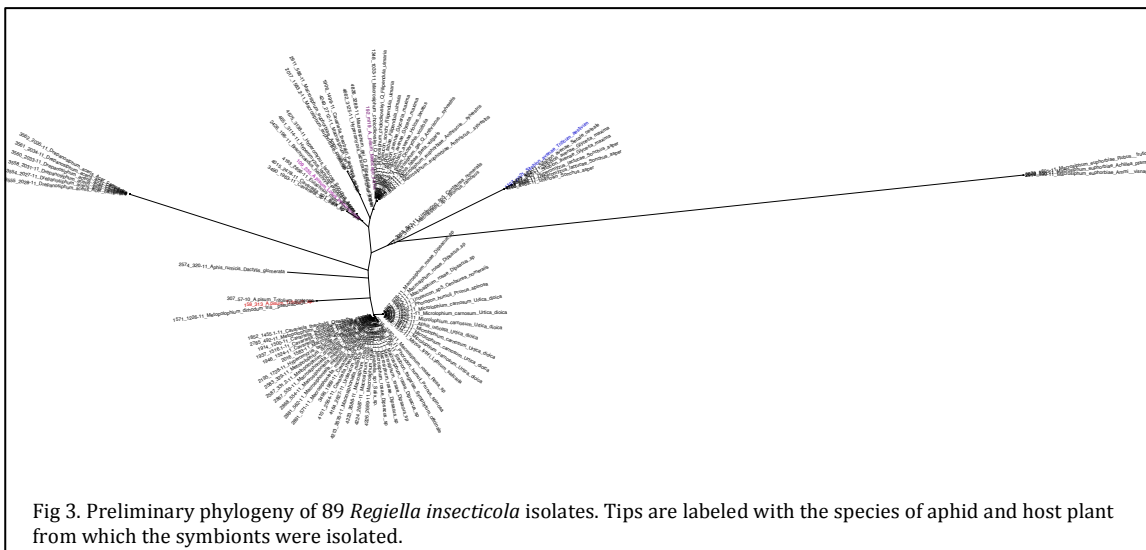
symbionts, several of the aphid species could no longer develop and reproduce. This suggests these aphid species have developed a co-obligate relationship with these normally facultative symbionts in addition to their obligate symbiont Buchnera. Evolving a co-obligate relationship with two symbionts is very rare in aphids (it is only known in 1 aphid species, *Cinara cedri*). We replicated the curing with clonal lines of these aphids and compared the growth and reproduction of these manipulated aphids to uncured control clones to provide evidence that the facultative symbionts are “required” for aphid reproduction. These results are being written into a manuscript on symbiont “domestication” and are being used as the foundation for a new research avenue on how symbiotic bacteria transition to permanent obligate associations with hosts, such as in the formation of the mitochondria and chloroplast.

In aphids where we were able to perform reciprocal transfections we discovered something remarkable. We demonstrate that certain symbiont strains can cause high host mortality and inhibit offspring production when introduced to aphid hosts other than those from their original population (Fig 2). Therefore these presumed mutualistic symbionts could actually be highly pathogenic when transferred to non-native hosts. These results demonstrate that insects and their facultative symbionts can become co-adapted to each other and host-symbiont genotype interactions represent an important barrier to sharing of these bacteria between different insects. This is a key discovery as it demonstrates a mechanism that will limit the spread of symbionts, and adaptive traits they carry, to certain aphid lineages. These results have been written into a manuscript and were recently submitted to the journal *Evolution*.



### *Comparative phylogenetic analysis of protective symbionts*

We performed Multi-Locus Sequence Typing (MLST) on *Regiella* isolates from field collected samples. Three MLST genes have been amplified from 89 *Regiella* isolates collected. We are currently completing the MLST sequencing and have built a preliminary phylogeny (Fig 3). Once completed we will map the ecological characteristics across tips of the phylogeny to gain a better understanding of how ecology shapes the genetic structure of this protective symbiont.



### **Expected final results and potential impact**

This project has generated genomic and ecological data on the biology of protective symbioses in insects. The results will be disseminated in four manuscripts, one of which was submitted to the top journal in Evolutionary Biology. These results will directly benefit the scientific community by providing knowledge on how symbionts interact with different species of hosts, gaining an understanding of why normally mutualistic microbes sometimes become pathogenic to their hosts, as well as providing a better understanding of the genomic basis of symbiont-mediated defensive functions.