Testing genotype x genotype interactions between a fungal pathogen and protective insect symbionts in the field

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Background/Question/Methods:

Vertically transmitted symbiotic microorganisms in animal hosts can promote protective phenotypes against pathogens. However, the degree of protection against pathogens is dependent on pathogen and symbiont genotypes. Results from laboratory studies indicate that strains of *Regiella insecticola*, a bacterial symbiont of pea aphids (*Acyrthrosiphon pisum*), vary in host protection against strains of the fungal pathogen, *Pandora neoaphidis*. However, it is unknown how symbiont genetic diversity at the population-level is impacted by pathogen infection in a natural population. Therefore, our objective was to characterize the level of host protection conferred by different symbiont and pathogen genotypes in a natural population of pea aphids.

We hypothesized that (i) rare symbiont genotypes are more protective against the pathogen than common genotypes, and (ii) certain pathogen genotypes will evade symbiont protection (i.e. breakthrough infections). We collected aphids in Spring 2021, measured survivorship, and screened for symbionts using PCR. Aphids harboring the symbiont *Regiella* were Sanger sequenced to determine if *Regiella* genotypes protect or result in breakthrough infections by *P. neoaphidis.* We used low-coverage, whole genome sequencing to determine if breakthrough infections are explained by genetic variability in the pathogen.

Results/Conclusions:

Nearly 20% of collected aphids (119 of 630) were killed by *Pandora neoaphidis* and resulted in a sporulating cadaver. Aphids harboring the protective symbiont *Regiella* were less likely to be killed by the pathogen than aphids without protective symbionts. Despite this symbiont-mediated protection, 17 of 109 (15.6%) aphids harboring *Regiella* were still killed by the pathogen. We found nine SNPs within a 474-bp region of the murE gene, which we used to genotype *Regiella*. All field-collected strains were distinguished into two clades, with the dominant clade (clade II) representing 101 of our 109 samples. We found a similar frequency of infections by *P. neoaphidis* in both clades: 16 out of 101 for clade II (15.8%) and 1 out of 8 for clade I (12.5%). Due to the imbalanced sample size, we could not rigorously test for differences in protection between the dominant and rare *Regiella* genotypes. Collections in Spring 2022 will target clade I *Regiella*-harboring aphids. Future analyses will determine if breakthrough infections result from genetic variability in the pathogen or are a product of symbiont-pathogen genotype interactions. These results will help clarify the maintenance and evolution of pathogen and protective symbiont genetic diversity within natural host populations.