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The impacts of defensive symbionts and host plants on fitness and population dynamics of pea aphids, *Acyrthosiphon pisum*

By

Ehiole Akhirome

Adviser: Nicole Gerardo, Ph.D.

Department of Biology, Emory University

_____Jacobus de Roode, Ph.D._____
Committee Member

_____Jason Hammonds, Ph.D._____
Committee Member

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ABSTRACT

The impacts of defensive symbionts and host plants on fitness and population dynamics of pea aphids, *Acyrthosiphon pisum*

By Ehiole Akhirome

Populations are constantly changing in response to many factors including predation, parasitism, disease, competition, and the environment. In many animals and plants, these fluctuations may be impacted by interactions with beneficial microbial partners. Pea aphids, *Acyrthosiphon pisum*, have evolved to host endosymbiotic bacteria that confer increased resistance to various parasites and predators. Here, I explore how both the pea aphid genotype and the bacterial endosymbiont, *Regiella insecticola*, influence the fitness of aphids under alternative ecological conditions, namely in the presence competition, in the presence of pathogens, and using alternative host plants. Previous studies have demonstrated that aphids hosting *Regiella* have higher survival than aphids without *Regiella* when infected with *Pandora neoaphidis*, an aphid-specific, entomopathogenic fungus. However, ecological surveys indicate that only 16% of aphid populations harbor this beneficial symbiont. To determine if there are competitive costs to harboring *Regiella* in the absence of a fungal infection, I conducted population cage experiments on aphid strains with and without *Regiella*. Although there were fecundity increases when hosting *Regiella*, I found that there was no significant cost or benefit during competition. In contrast, I found that competition between aphids of different genotypes with similar reproductive rates in the absence of competition led to one aphid strain consistently outcompeting the other. Pea aphids are also able to utilize a range of host plant species, but some aphid strains are adapted to using certain host plants but not others. Because the host plant supplies the nutrients and comprises a major part of the external environment for the aphids, I wanted to determine if the host plant affects aphid resistance to *Pandora*. I monitored the survival of infected aphids on fava bean, red clover, and crimson clover. While host plant did not affect resistance to infection, I did find that genotype influenced the aphids' performance on crimson clover and fava bean. These findings show how host genotype, symbiotic partners, and external biotic factors, including competition and host plants, can interact with one another to shape aphid population dynamics.

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INTRODUCTION

PART I: Populations and Competition

The wondrous variety we see in nature also plays a defining role in shaping the evolution of populations. Described by Charles Darwin in *On the Origin of Species*, variation allows species to respond to other organisms and changes in the environment. His theory of evolution by means of natural selection emphasizes that slight variations in inherited genes and phenotypes within a species will affect each individual's success in its current conditions. As such, success or fitness is highest for individuals that survive to reproduce and contribute the most offspring to the next generation (Darwin 1872). Even the slightest survival advantages conferred to a variant and its offspring will cause those advantageous traits to spread through a population (Haldane 1937). The fitness of those traits is dependent on a variety of factors including the individual's genotype, intra- and interspecific interactions, and interactions with habitat (Frankham 1996). However, many times it is unclear what factors affect fitness (Schwaegerle *et. al.* 1991). Work by Gregor Mendel in the late 1800s proved that a majority of phenotypic traits are the result of genes inherited from ancestors. When genetic variation in these traits impact life history traits, they also influence fitness measures (Werner *et. al* 1977). Understanding key factors that affect fitness provides a powerful tool for predicting population dynamics in various scenarios (Gaillard *et. al* 2000).

Studies in the *Daphnia pulex-pulicaria* complex and many other systems show that genotypic variation among populations impacts fitness early or late in life. In this particular study, daphnia from a pond-lake environmental gradient were assessed for various life history traits including fecundity, survival, and age of peak reproductive value. In laboratory conditions, there were substantial differences in fecundity between strains (Dudycha *et. al.* 1999). Those

with the highest lifetime fecundity in the laboratory were expected to contribute most to subsequent populations. However, there were drastic differences in the timing of reproduction. For strains from the pond habitats, where pond evaporation occurs and temperature is variable, rate of reproduction was much faster and life span was shorter. The opposite phenomenon was evident for those from the more permanent conditions of the lake habitat, suggesting a tradeoff between future and current reproduction. This dichotomy is an example of life history tradeoffs, which means improving one fitness trait leads to a detriment in another fitness related trait (Reznick 1985). Life history strategy emphasizes that no organism can be most fit everywhere because selective pressures determine the optimal traits in each environment. Life history tradeoffs highlight the important role that external selective pressures play in determining which traits are important for fitness in different environments.

Evolution is guided not only by abiotic environmental factors, but also by biological interactions. Competition is a type of interaction that occurs between and within species when there is a limited supply of common resources and organisms struggle to better access those resources (Birch 1957, Darwin 1872). Every species has a role in its ecosystem called a niche (Elton 1927). Zoologist G. Evelyn Hutchinson (1957) established that when a niche contains a full range of nutrient resources and habitat in the absence of competitors, this is considered a fundamental niche. However, in nature, this is almost never the case; competitors limit the range of resources and force organisms into a tighter range of resources to which they are more adapted —the realized niche (Case & Gilpin 1974). Because competition acts within niches, organisms can be in competition with conspecifics or different species that occupy that same or similar sympatric niche.

Intraspecific competition, occurring between members of the same species, is heavily density dependent. Ecosystems have a relatively constant set of available resources, thus limiting the number of organisms that they can support. The intrinsic limit to population growth, the carrying capacity, carries critical biological and socioeconomic implications for competition (Seidl *et. al.* 1999, Malthus 1798). As the number of individuals increase, the availability of resources decreases until it reaches zero, then individuals begin to die off or birth rates decline until the amount of available resources can again support the population. These resources are not only limited to nutrients and habitat, but also include access to mates (Frank 1987, Nadler 1988). Traits that give individuals a competitive advantage are selected for, and competition can increase diversity in populations if individuals with alternative traits are selected for in order to reduce competition (Schluter 2000). In this way, natural selection forces conspecifics into constant competition, selecting traits to improve resource acquisition, retain habitat, and secure mates, all of which can increase the fitness and survival of descendants.

Gause's law of competitive exclusion emphasizes that two or more sympatric, sexually isolated populations filling the same ecological niche cannot be perfectly equal and one species or population within a species will force the other to extinction (Gause 1934, Hardin 1960). Therefore, selection pressures on interspecific members of the niche will narrow the fitness optima of each species and move those optima further apart through adaptive radiation (Ross 1957). This phenomenon is critical for coexistence and maintenance of diversity in many systems (Schoener 1983, Linnell *et. al.* 2000, Chesson 2000). Due to this strong selective pressure, competition has strong evolutionary implications in shaping populations.

The selective pressure of competitive interactions acts via three main mechanisms: interference, exploitative, and apparent competition. Interference competition involves direct

action of one species to decrease the fitness of a competitor; this can involve actions such as predation, overgrowth, territoriality, undercutting, and allelopathy (Schoener 1983, Amarasekare 2002). These actions require energy expenditure in order to improve fitness, and sometimes involve mortality risks as seen in kleptoparasitic spiders stealing prey from aggressive spiders (Whitehouse 1997). Exploitative competition is a more indirect form of competitive interactions. In this process, one interacting species is superior at accessing and utilizing one or more common resources and this greatly limits net resources available to the competitor (Case & Gilpin 1974). Tilman *et. al.* (1982) and Wedin & Tilman (1993), using phytoplankton and plant communities, demonstrated that different ambient nutrient concentrations are exploited and utilized more efficiently by some species and thus the ratios of available resources has direct implications on competitive dominance.

Competition can also occur without direct action or nutrient limitation through apparent competition. Pioneered by Thomas Park (1948) in *Tribolium* flour beetle populations, apparent competition occurs when the presence of either species leads to a reduced population density for the other species at equilibrium because of an indirect link mediated by the effects of a common parasite, pathogen, or predator (Park 1962, Holt 1977, Holt & Pickering 1985). Park, using two species of flour beetle (*T. castaneum* & *T. confusum*), was able to reverse the outcomes of competition by introducing a common parasite, *Adelina tribolii*. Parasite- and pathogen-mediated competition can place strong selective pressure on competitive interactions (Grosholz 1992). If one competitor acquires a higher level of resistance to infection, that becomes "a powerful competitive weapon" and could lead to a rapid decline in the other population (Haldane 1949). A common example in humans applies these phenomena to epidemic Old World diseases —small pox, measles etc. — that ravaged Native American populations upon the arrival of European

settlers in colonial times (Crosby 1972). Predators can also have similar impacts on dynamics in neighboring populations. Nettle aphids (*Microlophium carnosum*) studied on plants near grass plots with a grass aphid (*Rhopalosiphum padi* L.) outbreak suffered reduced population density and produced fewer alate dispersers (Müller & Godfray 1997). The grass aphid outbreak, induced by grass fertilization, recruited Coccinellidae (ladybugs) to the area and increased predation on the nettle aphids leading to the decline, not evidenced in control colonies of nettle aphids. Understanding how interactions across and between trophic levels influence populations is key for ecological models and can lend insight into various fields from conservation to epidemiology (Daszak *et. al.* 2000).

PART II: Pea aphid population dynamics

The pea aphid

Population dynamics are constantly under the influence of a number of factors, including predation, parasitism, symbiosis, disease, and competition. Studying these factors is essential for understanding how natural selection acts in the wild. Aphids present themselves as an excellent model for studying population dynamics. The insect family Aphididae contains over

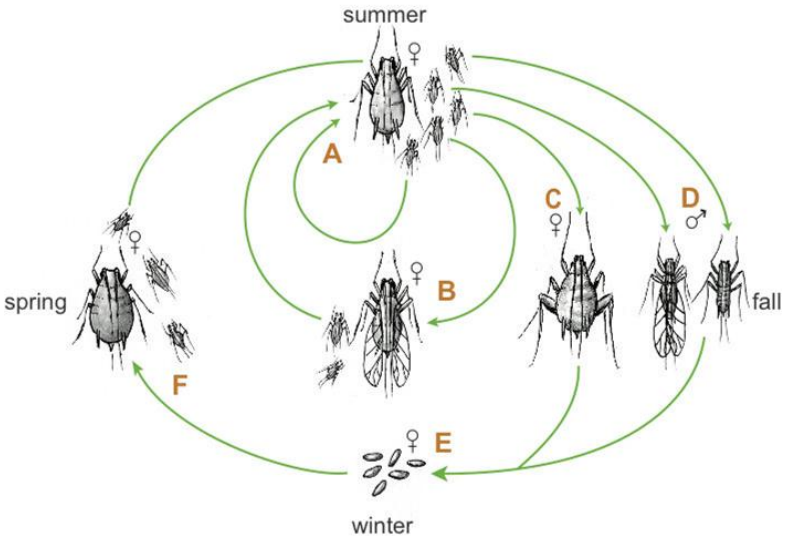

Figure 1. The pea aphid life cycle.
Illustration by Nancy Lowe

4000 species of aphid that are cosmopolitan in temperate regions across the globe (Von Dohlen *et. al.* 2000). Species of aphid are able to utilize a very broad range of plant hosts (Dixon 1977). As a phytophagous insect, they derive their nutrition from the phloem of their plant hosts (Febvay *et. al.* 1999). The pea aphid, *Acyrthosiphon pisum*, is similar to many aphids in the Aphididea. As illustrated in Figure 1, they exhibit a seasonal, polymorphic life cycle that involves asexual female parthenogenetic reproduction in summer months until light cycles change in autumn, and then male and female sexual morphs arise, mate and produce eggs that hatch into asexual females in spring (Dixon 1977, Moran & Dunbar 2006). Pea aphids also exhibit winged dimorphism mediated by exposure to the aphid alarm pheromone (*E*)-*β*-farnesene (EBF); this allows dispersal from crowded plants or areas of high predation (Kunert *et. al.* 2005). Apart from their life histories, symbioses within the aphid system provide another interesting focus for many microbial interactions.

Pea aphids, along with a majority of the aphids in the Aphididae, host the obligate endosymbiont *Buchnera aphidicola* (Buchner 1965). This mutualistic association has persisted in aphids through vertical transmission for nearly 200 million years (van Ham *et. al.* 2003) and has resulted in an obligatory symbiotic relationship between the aphid and bacterium (Brisson & Stern 2006). The aphid receives essential amino acids from *Buchnera* and the bacterium receives specialized housing in bacteriocytes in the aphid hemocoel (Price *et. al.* 2011). Loss of this endosymbiont through chance failure in transmission or clearance with antibiotics results in severely diminished performance, sterility, and death in the aphids (Koga *et al.* 2003).

Apart from this primary symbiosis, *A. pisum* may also contain one for more facultative bacteria in the family *Enterobacteriaceae*. These bacteria provide their aphid hosts with protection against a range of external pressures and risks. *Hamiltonella defensa* protects against

parasitoids, *Serratia symbiotica* against heat stress, and *Regiella insecticola* against fungal infection. *Regiella* also increases aphids' ability to utilize clover as a host plant in parts of its range (Oliver *et. al.* 2005, Montllor *et. al.* 2002, Scarborough 2005, & Tsuchida *et. al.* 2004). These three secondary endosymbionts are inherited vertically, like *Buchnera*. Because of the benefits they provide, facultative symbionts could serve as a powerful competitive weapon in populations. However, frequencies of secondary symbionts infection in surveyed pea aphid populations show a surprisingly low prevalence of infection ~15% for the three species (Oliver *et. al.* 2010), suggesting that there are costs associated with maintaining these symbiotic bacteria (Vorburger *et. al.* 2011).

Aphid competition

Because of their intimate association with microbial symbionts, aphid competition is mediated both through traits conferred through the aphids' genotype and through the presence of symbionts. In terms of symbionts, Oliver *et. al.* (2008) conducted an experiment to examine how the mutualistic symbiont, *H. defensa,* altered competitive interactions within pea aphid populations. The results indicated that in the presence of parasitoid wasp infection, the frequency of those aphids with the protective endosymbiont, *H. defensa* went to fixation; however, in the absence of parasitism, the uninfected aphids grew to a majority while the *Hamiltonella*-infected aphids were maintained at a basal level. This example of parasitoid-mediated apparent competition affirms the key role symbionts have on aphid population dynamics.

Other symbionts may also mediate apparent competition and thus influence aphid population dynamics. *Regiella* confers substantial protective effects against infection from *Pandora neoaphidis,* an aphid-specific entomopathogenic fungus, shown in Figure 2.

Scarborough *et al.* (2005) used several pea aphid strains to compare the survival rates with and without the symbiont. *R. insecticola* infection significantly protected against fungal infection: aphids with *Regiella* had higher survival rates and drastically reduced sporulation frequencies as compared to aphids without *Regiella.* Given this, it is possible that, in the presence of fungal pathogens, *Regiella* would increase the competitive advantage if its host aphid. However, this has not been tested, and it is unknown whether there is a cost to harboring *Regiella* in the absence of fungal infection.

Beyond apparent competition, symbionts may impact exploitative competition by altering the ability of aphids to utilize alternative host plants as a resource. For instance, Japanese pea aphids from vetch (*Vicia sativa*) transfected with *R. insecticola* exhibited increased fecundity and survival compared to *Regiella*-free aphids, when given white clover (*Trifolium repens*) as a host plant (Tsuchida *et. al.* 2004). *Regiella* infection allowed aphids to have equal fecundity on both vetch and clover, while symbiont negative groups saw a 50% fecundity reduction on clover.

Figure 2. *Pandora neoaphidis* infection in a) sporulating green peach aphid and b) pea aphid *Pandora* cadavers, and ring of deposited conidia.

Simon *et al.* (2003) determined that pea aphids collected from clover plants harbored *Regiella* at a substantially higher rate than aphids from other plants, supporting the hypothesis that, on clover, *Regiella* could increase the competitive advantage of aphids.

Host plant exploitation, however, is likely not only mediated through symbiont effects. Groups of pea aphids form distinct genetic clusters associated with their preferred host plant; aphids from each group exhibit maximum performance on their preferred host plant (Ferrari *et. al.* 2006, 2008). Phytophagous insects require that a suitable host plant provide them with essential amino acids and carbohydrates (Karley *et. al.* 2002). The proper nutrient profile in a host plant is critical for supporting healthy, viable aphids (Sandström & Pettersson 1994). Host plants could also play important roles in defense against infection. Certain plants have been shown to alter key antioxidant protein and enzyme concentrations in pea aphids (Lukasik *et. al.* 2011). Plants that modulate or even accentuate antioxidant production during critical times of infection may help combat pathogens in adapted aphids. Some host plants could also contain compounds that help combat infection. Monarch butterfly larvae that feed on a cardenolide-rich host plants, for example, decrease the virulence of *O. elektroscirrha* parasite infection (de Roode *et. al.* 2008). In other instances, the plant structure may affect the rates of infection. In a study that compared *Pandora* infection rates on two pea plant morphs (*Pisum sativum sativum*) with normal and low epicuticular wax levels, 4-fold higher proportions of aphids were killed by *Pandora* on plants with low wax levels (Duetting *et. al.* 2003). The lower wax content allowed more conidia to adhere to the leaves thereby promoting higher rates of contact with the aphids. Likewise, the pubescent stems of some clover species may also provide added surface area for conidia and over time could increase selection for fungal resistance on these host plants. The

mechanisms for many of these interactions are unknown, but it is clear that there are important fitness consequences for aphids on their various host plants.

My study

This thesis work focuses on how genotype, bacterial symbionts, and other environmental factors modulate pea aphid fitness and impact population dynamics. In part one, I study competitive interactions between alternative aphid genotypes in the absence of symbionts. In part two, I shift focus to the impact of the symbiont *Regiella* on competitive interactions. In part three, I ask whether host plant affects fungal resistance. This comprehensive approach facilitates a more complete understanding of the complex forces mediating intraspecific competition.

METHODS

Insect and Plant Rearing

Plants were grown in a greenhouse and watered three times a week. Plants used in the population cages were grown for 3-4 weeks to a height of 30-35cm; plants used in all other experiments were grown for 2-3 weeks to 7-12cm in height and were covered in cup cages. The cup cages are plastic containers with a vented, mesh top placed over the plant to contain aphids and allow air exchange. Due to their height, the fava bean (*Vicia faba*) plants used in the population cages were tied to 30cm bamboo stakes for support. I grew 10-15 sprouts/pot of crimson clover (*Trifolium incarnatum*) and red clover (*Trifolium pratense*) for 2-3weeks to a height of 5-8cm for use in fungal infection and fitness assays. Pea aphids are from the Gerardo lab stock and have been maintained in lab culture for several years. All aphids and experiments were housed in incubator chambers 20° Celsius, with a 16L:8D light cycle.

Competition between alternative genotypes in the absence of facultative symbionts

Fecundity measurement. In order to understand how reproduction rates and affect fitness in competitive interactions, we quantified the reproduction rates of two aphids strains (LSR1-O and G3) that did not harbor facultative symbionts. One adult aphid was placed on a 2-3 week old fava bean plants (*Vicia faba*) and allowed to reproduce until death. Offspring were counted then removed every two days; this eliminated crowding and subsequent deterioration of both aphid and plant health. This process was repeated for 20 individuals per aphid genotype. This allowed me to generate a baseline measurement of the intrinsic growth rate of each aphid strain before introduction into population cages.

Population cages. To mimic natural conditions of competition, I used large, mesh population cages to enclose fava fava bean plants, which were watered with 100mL of filtered water before insertion in to the cages. Cages, shown in Figure 3, were 60cm×60cm×60cm PVC pipe enclosure stored in a mesh bag to restrict aphid movement but allow light and air to enter freely. In the cages, I introduced two *Acyrthosiphon pisum* strains (LSR1 & G3) that differ in genotype, but do not harbor a secondary, facultative symbiont. I introduced 20 adults per strain in each cage (40 total adults to begin the experiment). I evenly distributed aphids on each of the four plants in the cage. New plants were cycled in/out every 4 days, and cages were stored a uniform distance from a light source. Plants were added to the cages two at a time to allow aphids to move to the new plants. Plants in the cage were watered with 100mL of filtered water as needed. I set up two of these cages with both aphid lines. For controls, I maintained two cages for each aphid strain in the absence of the other strain (40 adults of one genotype) and counted those to monitor population growth without the effects of the other aphid strain. For all cages, the total number of adults on the plants were counted every two days. LSR1 and G3 are pink and green aphid lines respectively, allowing me to distinguish aphids of each line.

Figure 3. Population cages containing staked fava bean plants.

Competition mediated by *Regiella insecticola*, a facultative symbiont

Fecundity measurement. I assessed reproduction rates and fecundity, as described above, here for genotypes 5A and LSR1 (also referred to as strains 5AO and LSR1-O, respectively, to mark the lack of symbionts). Each strain in this experiment was used in conjunction with its *Regiella*-infected counterpart, 5AU and LSR1-Ri (the U and Ri portions designating *Regiella* symbiont infection).

Population cages. These cages were set up and conducted as described above in the previous section. The strains in the *Regiella* population cages were the same genotype, but half were infected with the facultative, secondary endosymbiont *Regiella insecticola*. Thus, I used strains 5AO and 5AU in the same cages, and LSR1 and LSR1-Ri in the same cages. There were two cages for each combination. A sample of 40 adults was removed from population cages every 10 days to screen for *Regiella* by PCR. Sampling was done randomized by selecting adult aphids from different areas on the plant, and ten adults from each plant. Sampling had little effect on the intensity of competition because 40 adults was always <10% of the total cage population.

***Regiella* screen and PCR protocol.** I screened 20 of the 40 sampled aphids at each time point for the presence of *Regiella*. DNA was extracted using DNEasy Blood and Tissue Kit (Qiagen) or Bender buffer DNA extraction protocol (See Appendix A for extraction protocol). PCR reactions were performed on 96 well reaction plates, for 80 reactions at once. Using MasterTaq™ PCR Kit from 5Prime, I diluted DNA samples to 20 ng/μL and used primers U1279F (forward) and 35R (reverse) in the PCR reaction (Russell & Moran 2005). Ten-microliter reactions were performed with the following concentrations: 2.8 μL H_2O, 2 μL TaqMaster PCR Enhancer, 1 μL PCR Buffer + Mg^{2+}, 0.2 μL dNTPs, 1 μL of each primer, 2 μL of DNA, and 0.1 μL of MasterTaq Polymerase. I ran PCR products on a 1.5% agarose gel at

90V, stained the gel for 20 minutes in ethidium bromide solution, and scored the presence of the *Regiella*-specific PCR product on the gel.

Host plant effect on *Pandora* infection

Experimental design. To determine if the pea aphid host plant modulates resistance to *Pandora neoaphidis*, I conducted two separate experiments using fava bean, crimson clover, and red clover as host plants. Using the aphid strains 5AO and BP14, which lack secondary endosymbionts, I wanted to determine whether pea aphids reared on a different host plant exhibit a difference in *Pandora* resistance. Here I raised all mothers on fava bean, transferred them as 10-day old adults to one of the host plant species, and allowed them to reproduce for 24 hours on that plant. These offspring were raised to adulthood, infected, and replaced on the same host plant species. I changed the actual plant after infection to maintain consistent plant conditions and placed 5 aphids in a single plant pot. I infected 105 10-day old adult aphids per aphid strain with *Pandora* spores (35 aphids per host plant, with an equal number of control samples) and placed them on one of three host plants. Next, I wanted to determine if feeding on a different host plant only *during* the period of infection affects *Pandora* resistance. I grew all aphids on fava bean. I infected 60 10-day old adult aphids per aphid strain with *Pandora* spores (with an equal number of control samples) and placed them on one of three host plants, four aphids per plant (20 aphids per host plant per treatment). For both experiments, I scored these aphids for survival and sporulation for the ten days following infection.

***Pandora neoaphidis* fungal culture**. I infected aphids with lab cultured strains of the aphid specific entomopathogenic fungus, *Pandora neoaphidis*. Cultures of *P. neoaphidis* were grown on SDAEY agar plates for 2-3 weeks from USDA ARS Collection of Entomopathogenic

Fungal Cultures. I induced sporulation by subculturing the fungus from SDAEY plates onto 60×15mm tap–water agar plates (TWA) 20 hours prior to the exposure treatment in order to induce conidiogenesis by depriving the fungus of nutrients.

Fungus infection survival assay. I placed clean slide covers into the bottom half of small petri dishes. On the top of each dish, I placed a plastic vial that had been cut to be about an inch and a half for the exposure chambers. Each plastic vial had Insect Slip on the sides to prevent the aphids from escaping the exposure chamber. I inverted sporulating fungus subcultures over each exposure chamber. After 30 minutes, I checked for spores on the coverslip at the bottom of each exposure chamber. Once it was evident that the fungi were releasing spores into the chambers, I placed 20-25 pre-reproductive (10-day old) adult aphids in a 60×15mm petri dish and labeled these by genotype. I suspended the sporulating fungus over the aphids inside the exposure chamber for 2 hours. I used plain tap water agar plates for infection controls. I moved the aphids to the plants by placing the petri dish onto the dirt next to the plant stalks (aphids will climb up onto the plants without having to disturb any fungal spores). To increase humidity, I placed closed top cup cages over each plant to restrict air movement. Additionally, I added water to the bottom of trays in which the plant pots were stored to water dry soil as needed. I maintained the relative humidity near 100% for 4 days, then changed to vented cup cages to revert to ambient humidity. Infection of an individual aphids was typically visible within 4-7 days post-infection (Figure 2). I monitored aphid survival daily and scored for sporulation status at death.

Genotype × Host Plant interaction

In order to determine whether some genotypes are better suited for certain host plants, I reared aphids of seven unique genotype on both crimson clover and fava bean plants and measured the time to the first reproductive event and the number of offspring produced in the 12 days following that. This allowed me to assess the fitness of each genotype when utilizing these two host plants. I placed ten-day-old mothers, raised on fava bean, onto either crimson clover or fava bean plants for 24 hours and allowed them to produce between five and ten offspring on each plant. I removed the adult after 24 hours and at day 8 culled the number of aphids to five per plant. I began checking for reproduction daily until the first reproductive event; then I recorded fecundity counts for the next 12 days. By day 12, most aphids have produced the majority of their offspring. The study consisted of seven fava bean and crimson clover plants for each genotype; 35 aphids per host plant species. I took fecundity counts for the total offspring produced by all five aphids on the plant and removed the counted offspring from the plants every two days. I accounted for deaths during the experiment by subtracting the deaths from the number of samples after that time point.

Statistical Analyses

For the fecundity measurements, I used repeated measures ANOVA; for the LSR1-O and G3 population control cage data, I used paired t-tests, repeated measures ANOVA and generalized linear models with quasi-poisson error to account for over-distribution. I did not perform any analyses on the sampled data in the LSR and 5A *Regiella*-mediated competition cages. I applied ANOVA and a Cox Proportional Hazard Survival Analysis to evaluate the data from the fungus infection survival assay. The data from the genotype by host plant experiment

were analyzed by repeated measures ANOVA and significant differences between the age to reproduction and fecundity on each host plant was determined using Tukey's Honestly Significant Difference test. All analyses were conducted in R version 2.13.1.

RESULTS & DISCUSSION

Competition in the absence of facultative symbionts

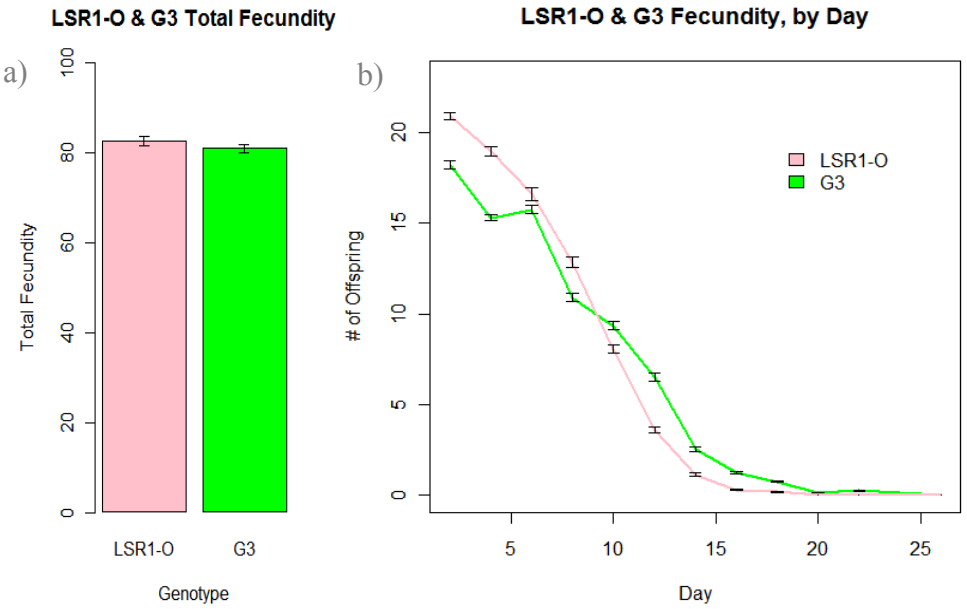

Figure 4. Fecundity measurements of the LSR and G3 strains a) for average lifetime fecundity and b) reproduction recorded every two days (n=20). Error bars indicate SEM.

Fecundity measurement of aphids without facultative symbionts. In the absence of competition, the mean total fecundity for LSR1-O (mean=82.6) and G3 (mean=80.95) strains were similar (ANOVA, $F_{1,38}$=0.0691, p=0.79, n=20, Figure 4a). The fecundity by day, an estimation of reproduction rate, in LSR1-O and G3 strains were nearly identical (ANOVA, $F_{1,22}$=0.002, p=0.97, Figure 4b). The fecundity rates in both LSR1-O and G3 showed a constant downward slope. Although the overall reproduction rates are nearly identical, it is interesting to note that LSR1-O reproduction rates are higher than G3 in the first ten days, then that relationship reverses in the following ten days.

Population cages assessing competitive dynamics of aphids without facultative symbionts. In the presence of competition, the LSR1-O and G3 strains in the population cages exhibited different reproduction rates from predictions in the fecundity trials. In both population cages with the two genotypes, LSR1-O outcompeted G3 and reached fixation at the end of the experiment (Figure 5a). The same pattern occurred in both competition. However, in both control cages G3 reached significantly higher densities than LSR1-O (paired t-test, p=0.038). During the

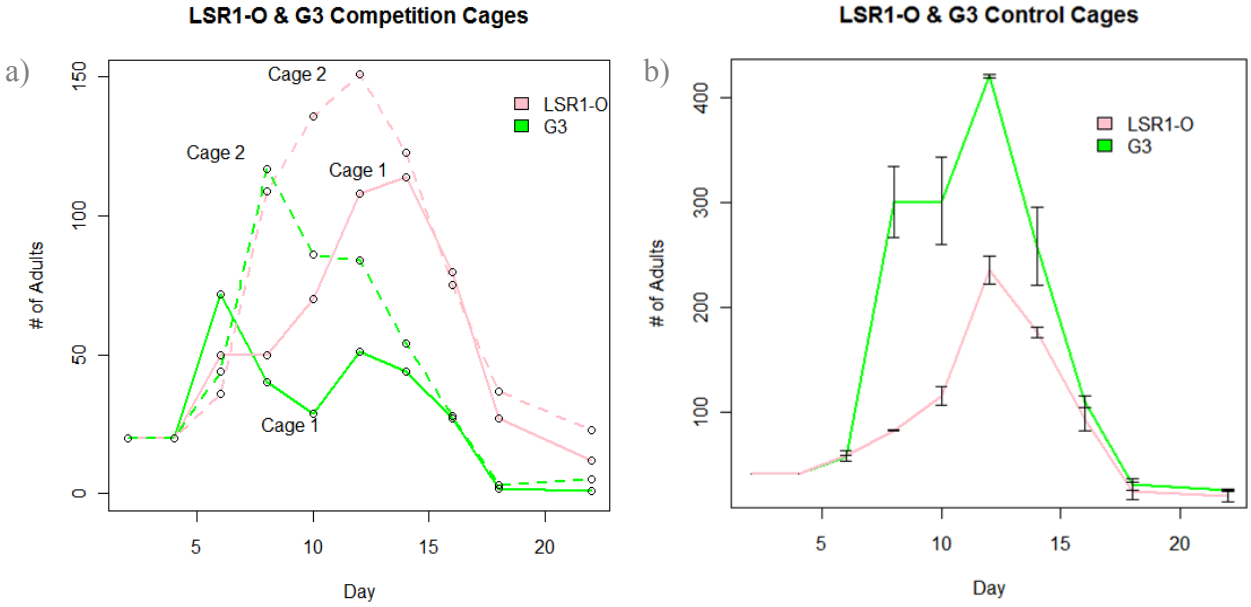

Figure 5. Dynamics of competition in population cages without facultative symbionts. a) Competition cages (n=2) b) one-strain control cages (n=2 per genotype). Error bars indicate SEM.

course of the experiment, it was noted that a large proportion of the G3 were found off the plants (therefore not counted) in the competition cages. Figure 5 shows that the presence of the LSR1-O strain elicits reduced reproduction and crowding tolerance in an otherwise highly fecund G3 strain.

Due to the similar fecundity of LSR1-O and G3 in the absence of competition (Figure 4), I hypothesized that the cage populations would reach a dynamic equilibrium that would allow

coexistence. However, that was not the case because LSR1-O outcompeted G3 and nearly went to fixation. This phenomenon signals that there are genotypically determined factors, apart from fecundity, that impact competitive fitness. It is possible that some pea aphid genotypes produce more or less stress hormone, EBF, in crowded situations. EBF typically causes aphids to drop from the plant. Alternatively, some genotypes may show stronger behavioral responses to EBF. That may be the case in these population cages. While counting, I observed that G3 could be found off the plants in the competition cages, wandering around inside the cage, but in the control cages G3 reached tremendous densities on the plants. It is possible that, if the G3 genotype naturally produces less EBF in response to crowding, then they would naturally be sensitive to the higher amounts of EBF released by a competing aphid strain, such as LSR1-O. This can be tested with behavioral assays studying the responses to EBF for a range of pea aphid strains. Knowing whether there is variation will allow further testing to determine the underlying mechanisms.

Competition mediated by *Regiella insecticola*, a facultative symbiont

Fecundity measurement of aphid strains with and without *Regiella*. Infection with *Regiella* resulted in significant fecundity increases in both strains. The *Regiella*-infected strain, LSR1-Ri (mean=100.6), on average produced significantly more offspring than the symbiont-free, LSR1-O (mean=87.2; ANOVA, $F_{1,37}$=5.9535, p=0.0196, Figure 6a). The *Regiella*-infected strain 5AU (mean=105.6), also produced significantly more offspring than the symbiont-free 5AO (mean=96.9; ANOVA, $F_{1,38}$=10.816, p=0.0022, Figure 7a). Although data were collected every two days, I needed to pool data into four day increments because of a missed count day in the LSR1-Ri group. The fecundity measured by day (Figure 6b, 7b) for both 5AO (ANOVA, $F_{1,22}$=0.046, p=0.83) and LSR1-O (ANOVA, $F_{1,10}$=0.0444, p=0.84) genotypes were not

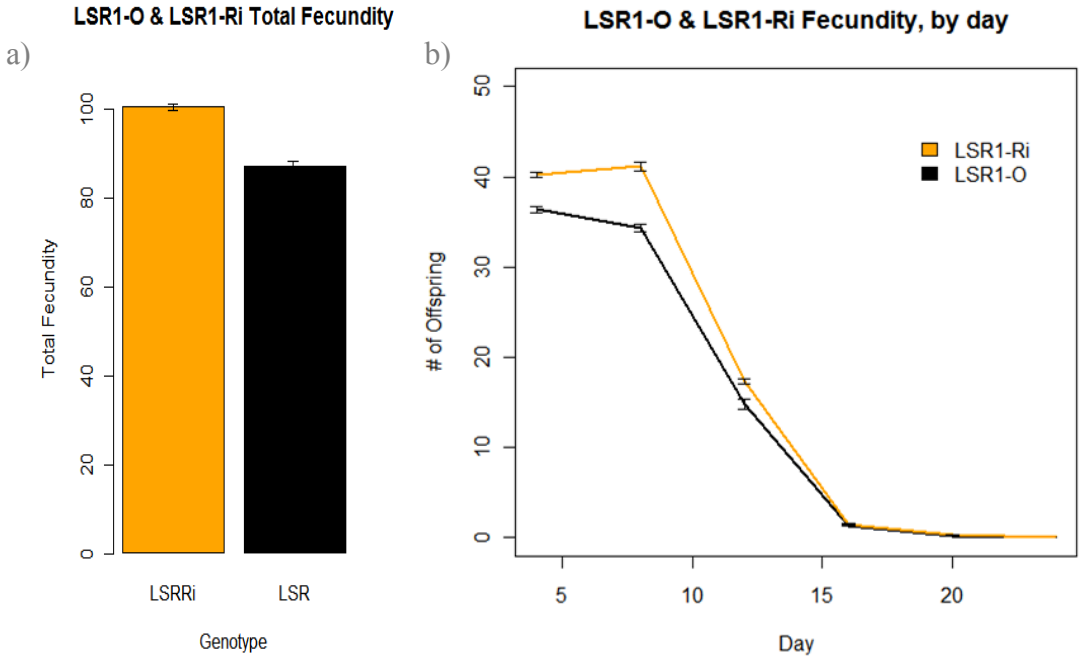

Figure 6. Fecundity measurements of the LSR1-O and LSR1-Ri strains a) for average lifetime fecundity and b) reproduction recorded every four days (n=20). Error bars indicate SEM.

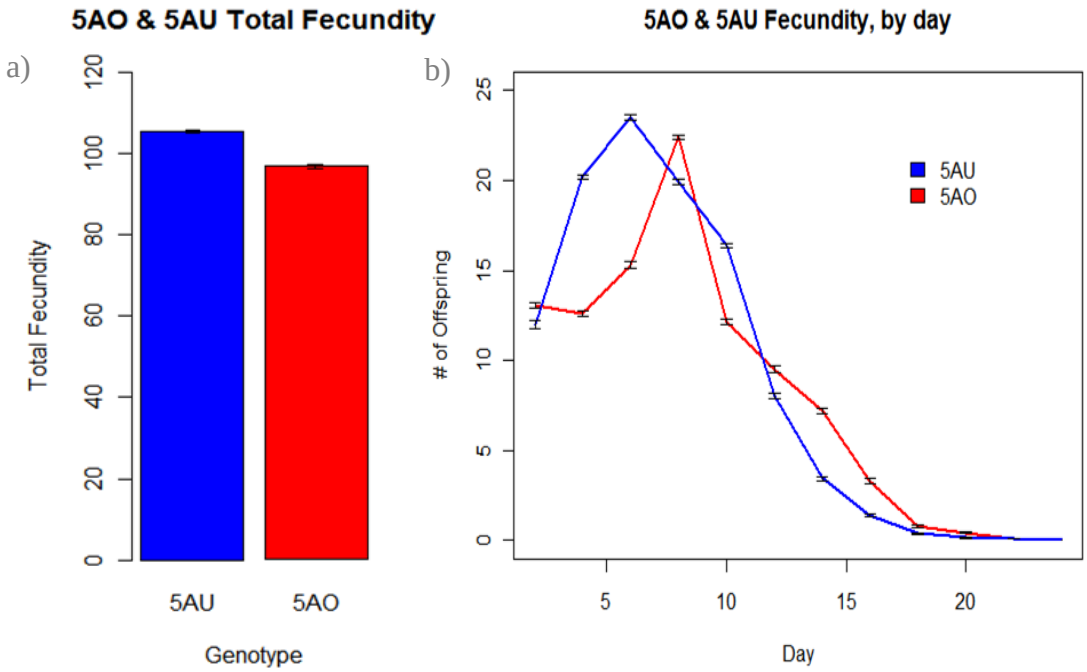

Figure 7. Fecundity measurements of the 5AO and 5AU strains a) for average lifetime fecundity and b) reproduction recorded every two days (n=20). Error bars indicate SEM.

significantly different from the *Regiella* infected, but LSR1-Ri, on average, did produce more offspring daily than LSR1-O. 5AU began reproduction at a higher rate than 5AO, and the daily reproduction values varied over the course of the experiment.

Population cages assessing the impact of *Regiella* on competition. I hypothesized that the elevated fecundity and reproduction rates observed in pea aphids with *Regiella* would translate to fitness advantages in competitive environments. *Regiella* infection, however, did

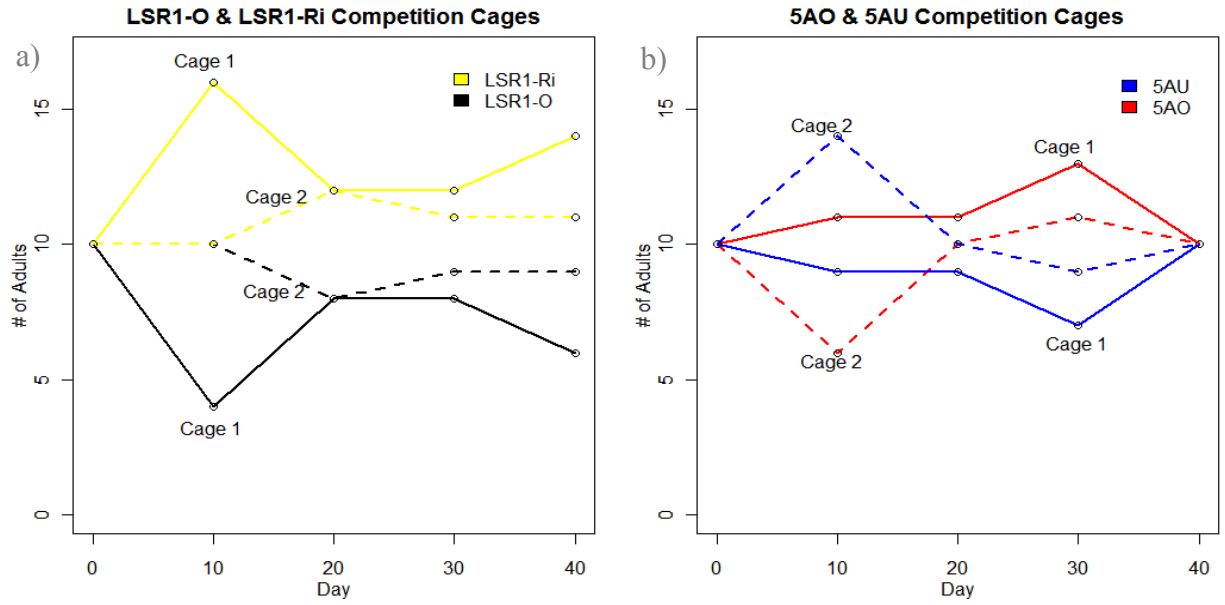

Figure 8. *Regiella* infection from the two competition cages in the absence of *Pandora* fungal infection. a) Number of LSR1-O and LSR1-Ri and b) 5AO and 5AU strains during competition (n=20).

little to alter competitive dynamics in the cage for both aphid strains (Figure 8). Separately for both the 5A and LSR1 genotypes, I pooled 20 samples from each cage replicate (n=40) and calculated frequency of *Regiella* infection together. LSR1-Ri frequency in the cage populations was near 60% for the duration of the experiment, ending at a final frequency of 62.5% for *Regiella*-infected individuals. 5AO and 5AU frequencies oscillated around 50% during the course of the experiment, and both ended at 50% at day 40.

Even though *Regiella* infection conferred increased fecundity and reproduction rates, it appears that was not sufficient for those aphids to gain competitive dominance in the population cage. Based on the absence of fixation of *Regiella* in natural aphid populations, the initial hypothesis was that there would be both a fecundity cost and reproductive cost to harboring *Regiella* in the absence of fungal infections, but that does not seem to be the case. Instead of a fecundity cost, there was a significant increase in reproduction. Further, those same *Regiella*-infected aphids were able to maintain an equilibrium, if not an advantage, with the symbiont-free aphids in the competition cages. Other pea aphid secondary symbionts, namely *S. symbiotica* and *H. defensa*, are known to harbor costs in environments where they do not give a competitive advantage. While no studies to date have stipulated a cost to maintaining *Regiella*, it may be that we have yet to examine the right factors. It is possible that *Regiella* performs poorly on a certain set of host plants or under other ecological conditions not tested here, but the costs are not readily evident otherwise. It may also be that maintaining *Regiella* requires that aphids have a constant food supply. Studies have shown that pea aphids with higher reproductive investment have low resistance to starvation (Kouame & Mackauer 1992), so the fecundity enhancement associated with *Regiella* infection may be quite detrimental in starvation situations.

Host plant effect on *Pandora* infection

Fungus infection survival assay. Both of the *Pandora* survival assays resulted in weak infection frequencies and control aphids had generally low survival, therefore treatment (fungus/control) was not a significant factor in the Cox Proportional Hazard survival analysis (ANOVA, df=5, p=0.23, 0.97, Figures 9a. & 10a.) and was removed from the minimal model. Although fungus infections did not significantly impact survival, both aphid genotypes showed overall better performance on fava bean. BP14 performed relatively well on all host plants, while 5AO only performed well on fava bean. In Figure 9, aphids were reared on the host plant prior to infection, genotype significantly affected survival (ANOVA, df=5, p<0.00001, Figure 9b.) on all host plants. There was a significant Genotype × Host Plant interaction (ANOVA, df=3, p<0.00001, Figure 9b.) indicating that BP14 performed better than 5AO on both clover host

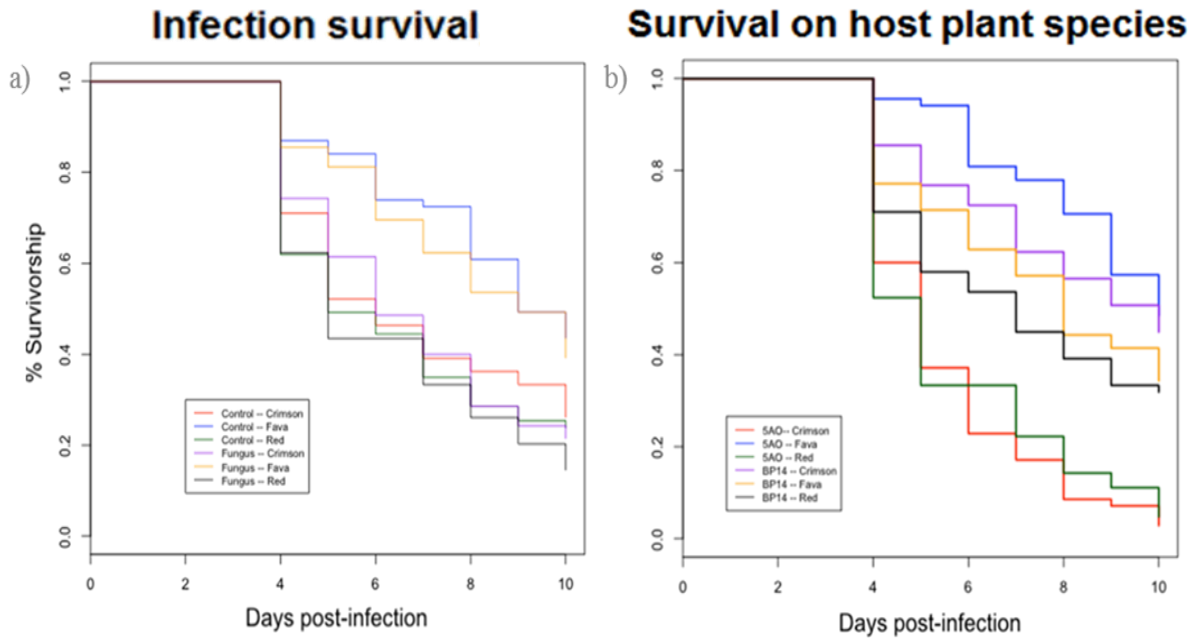

Figure 9. Aphids lived on the specified host plant species from birth and were placed on the same plant species after the fungus infection. a) Survival was monitored for ten days after exposure to *Pandora* conidia, and resulted in a low infection frequency. b) Survival data for both fungus and control treatments were pooled to show survival of the aphid genotypes on each host plant. (n=70)

plants but not on fava bean. In the following experiment (Figure 10), when aphids were reared on a common host plant (fava bean) then transferred to alternative hosts after the fungal infection, the same trend occurred here (ANOVA, df=3, $p<0.00001$, Figure 10b.), but it appears that 5AO survival on red clover declined substantially after the first 4 days.

The significant Genotype × Host Plant interaction suggests that genotype helps define which host plants the aphids are able to use. While the underlying genetic mechanism is unknown, research on this issue can lend insight into the genetic factors that impact fitness on different host plants. This has many ecologically important ramifications, especially in the context of agriculture, because a majority of the agriculturally significant aphid pests are polyphagous or host-alternating species (Dixon 1977). Since aphids derive a bulk of their nutrition from the host plant, it is very likely that performance on these host plants impacts the aphids' immunity and resistance to pathogens.

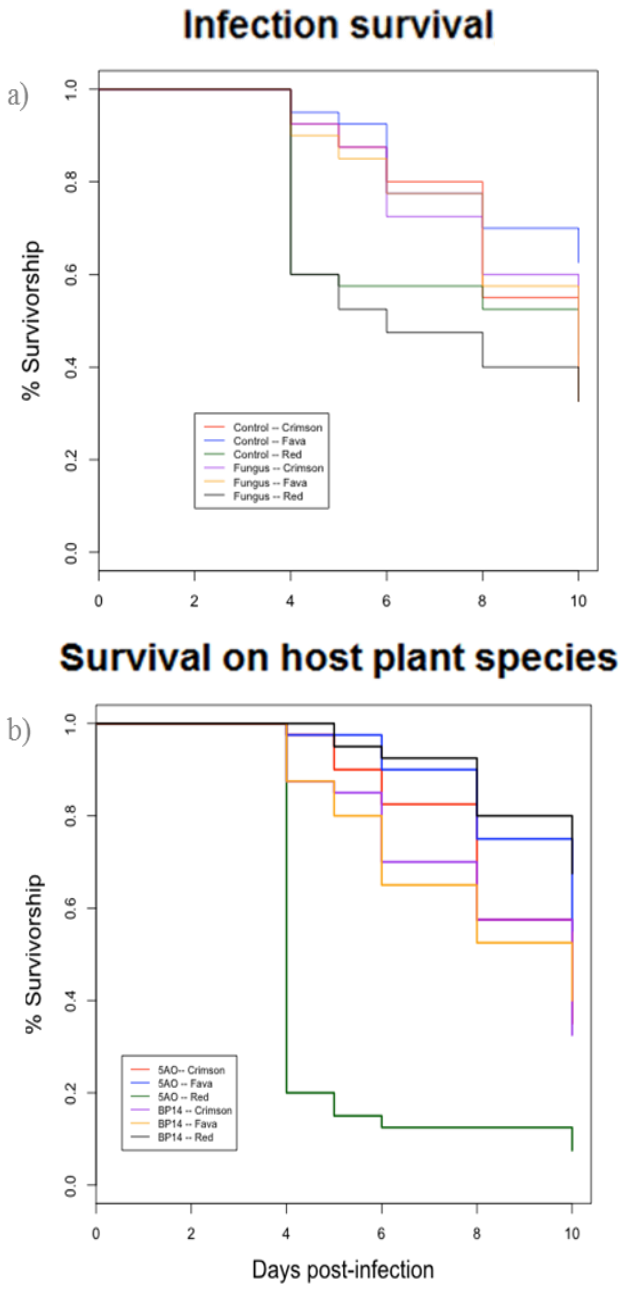

Figure 10. Aphids were reared on fava bean, then moved to the specified host plant species after the fungus infection. a) This experiment also resulted in a low infection frequency. b) See Figure 9b. caption. (n=40)

I intend to repeat these fungal infections with stronger infection frequencies to better address the link between diet and immunity in aphids. As aphids change host plants, they can happen upon a less suitable host plant and may find themselves more susceptible to infection. However, if the opposite occurs, aphids may experience reduced mortality from infection and be able to reproduce with fewer checks to their increase. De Roode *et. al.* (2008) shows that monarch butterflies can substantially reduce the virulence of their parasite by using a different host plant during times of infection, thus highlighting the impact that host plants have on parasite resistance for monarch butterflies. Host plant switching for aphids in similar scenarios involving infection may modulate the spread of disease within a population and affect dynamics at the community level.

Genotype × Host Plant interaction

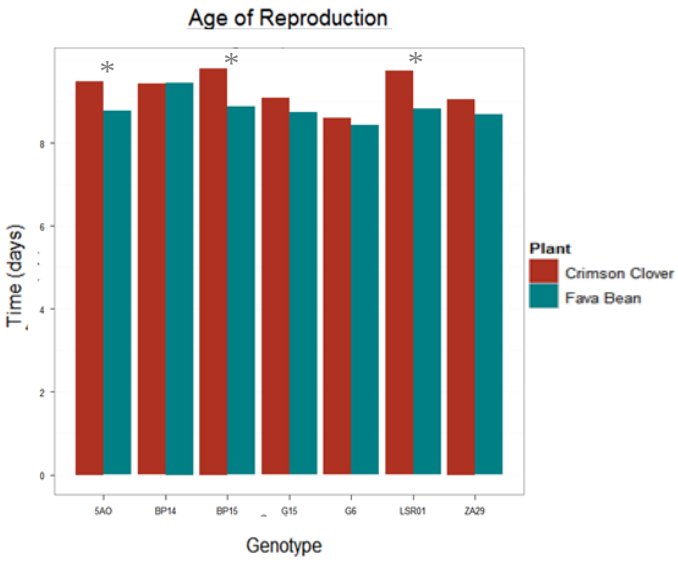

Figure 11. Aphids reared on each host plant were monitored every 24 hours for the first reproductive event to estimate the development time (n=35). * indicates significantly different fecundities. SEM error bars not shown, all SE< 0.2 days.

The above experiments indicated that in two aphid lines, there was a significant interaction between the aphid genotype and host plant species, which influenced aphid fitness. In a follow-up experiment utilizing seven different aphid lines, host plant species again significantly affected the development times for the aphids (Figure 11; ANOVA, $F_{1,84}$=92.23, p<0.00001). On average, aphids developed faster (i.e., began

reproducing faster) on fava bean than on crimson clover. However, strain BP14 did average slightly faster development on crimson clover. Genotype also had a significant effect on development times (Figure 11; ANOVA, $F_{6,84}$=23.62, p<0.00001), indicating that the development rates varied by genotypes. The interaction between genotype and host plant was significant here as well (Figure 11; ANOVA, $F_{6,84}$=7.59, p<0.00001). Genotypes 5AO, BP15, and LSR1-O appear to be adapted for faster maturation on fava bean. The reproduction during the first 12 days varied by genotype, which had a significant effect on fecundity (Figure 12; ANOVA, $F_{6,84}$=3.23, p=.007). Strains 5AO, G15, LSR1-O, and ZA29 produced substantially more offspring on fava bean. The other strains showed only minor fecundity increases when on fava bean. G6 was the only strain to average slightly more offspring on crimson clover, although non-significant (Tukey's HSD, p_{adj}=1). Host plant strongly influenced the amount of reproduction (Figure 12; ANOVA, $F_{1,84}$=118.0, p<0.00001). All strains, except G6, reproduced more on fava bean than on clover. There was a significant genotype × host plant interaction (Figure 12; ANOVA, $F_{1,84}$= 11.6, p<0.00001), such that strains LSR1-O and ZA29, which performed well on fava bean, tended to do poorly on crimson clover. However, the strains that performed poorly on fava bean (BP14 & G6) relative to the other strains, performed moderately well on crimson clover in comparison to those that did very well on fava bean. There was a negative correlation between age of reproduction and fecundity (i.e., aphids with longer development times produced fewer offspring; see Appendix B).

Time to first reproduction and fecundity measurements have been used before (Oliver *et. al.* 2008) as a method to estimate overall aphid fitness. In my studies, those factors are very strong predictors of performance and estimators for fitness. Although a majority of aphid strains in this study performed well on fava bean, which is suggested to be the universal pea aphid host plant (Sandström and Petterson 1994), G6 and BP14 strains performed equally well in both fitness measurements on either host plant (see Appendix C for performance measures graphs grouped by host plant). This may be indicative of a few possibilities: G6 and BP14 are best adapted to using clover and are able to use the more amiable host plant, fava, just as well; or BP14 and G6 may have sacrificed specialization on a particular host plant for increased flexibility in host plant choice. While there is little evidence to suggest the latter, numerous studies and phylogenetic analyses have demonstrated that aphid groups form specialties on the eight known groups of aphid host plants (Simon *et. al.* 2003, Ferrari *et. al.* 2006 & 2008, Peccoud *et. al.* 2009). Symbiosis with *Regiella* allows aphids to utilize a larger set of host plants, particularly clover species. The mechanism behind this interaction between the aphid genotype, host plants, and microbial partners is not well understood (Leonardo 2004). This confirms the need for more studies, such as this one, that address these factors from different perspectives. Building this knowledge base will not only allow application to aphid-specific

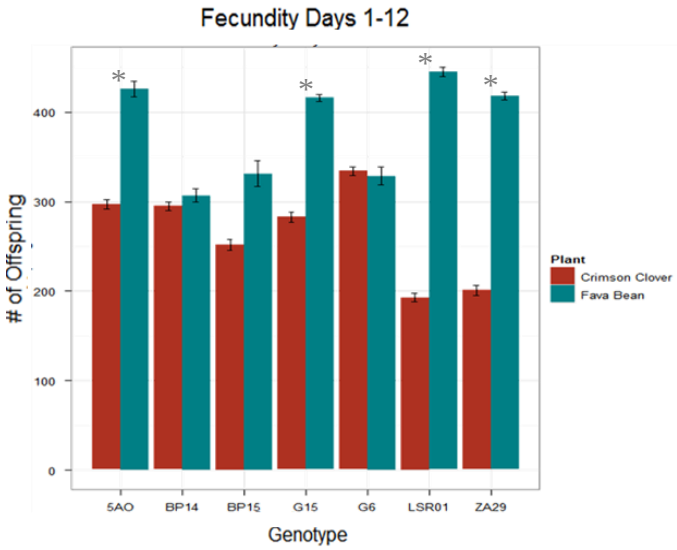

Figure 12. Total fecundity averages from 5 aphids during the first twelve days of reproduction (n=7). *indicates significantly different fecundities.

problems and strategies for bio-control of this invasive agricultural pest, but it will cultivate an understanding of the microbial and environmental interactions that shape all populations.

CONCLUSIONS

English poet William Cowper accurately stated that, "variety's the very spice of life." Although he was inferring a more humanistic interpretation, the variety or dynamic biotic and abiotic conditions evident in nature also play a defining role in shaping how populations change over time. During the course of my work, it became readily apparent that genotype, symbiotic partners, and the external pressures of competition and habitat are some of the key arbiters of evolution by natural selection. In the *A. pisum* model system, I was able to test these different factors for their impact on population dynamics. In all the experiments executed in this work, genotype variation consistently indicated differences in performance that translate to changes in population dynamics. Different aphid genotypes showed variety in performance on crimson clover and fava bean. This highlights the importance of an individuals' genetic ancestry as a determinant for traits adapted to utilizing each host plant. If genetics alone do not provide the blueprint for success on a host plant, partnerships with symbiotic partners may enhance performance. In the case of the pea aphid symbiotic partner *Regiella*, symbioses can provide competitive advantages in populations facing stresses from external biotic factors, such as novel host plants and fungus pathogen infections. Because there are expected costs to maintaining such advantages, these relationships could prove to be a liability in the absence of those stresses, as seen in other host-symbiont interactions. However, the measures in my study showed no competitive cost to individuals hosting *Regiella*, thus exhibiting the curious effects that symbioses can have on population dynamics.

FUTURE DIRECTIONS

Further research on the previously discussed topics could follow a several paths. In the experiments addressing competition in the absence of facultative symbionts, I found that one aphid strain, LSR1-O consistently outcompetes the G3 strain. The fitness advantage seen here may only apply on the fava bean host plant. Additional studies could address the competitive interaction on another host plant to determine if fitness is mediated by a genotype adaptation to a host plant. Another experiment may investigate whether aphid genotypes vary in sensitivity to EBF, a stress hormone, and the impact that the response may have on fitness. In the case of *Regiella*-mediated competition, I initially hypothesized that there would be a cost to harboring the symbiont compared to an uninfected clone when fungal infections were absent. Since that did not prove to be true, one possible test is to determine if other factors, such as starvation, play a role in determining which strains are successful. To determine the effects of starvation, a test could measure the fecundities and survival of *Regiella*-infected and uninfected clones after periods of starvation. Host plants were also significant factors in all the experiments conducted in this thesis work. The genotype × host plant interaction may also impact resistance to fungal pathogens. With the knowledge of this interaction, pairwise fungal infections of those genotypes on crimson clover and fava bean could show patterns of resistance among genotypes, host plants, or both; differences in each genotype's response to infection could studied via analyses of gene expression and cellular responses to infection.

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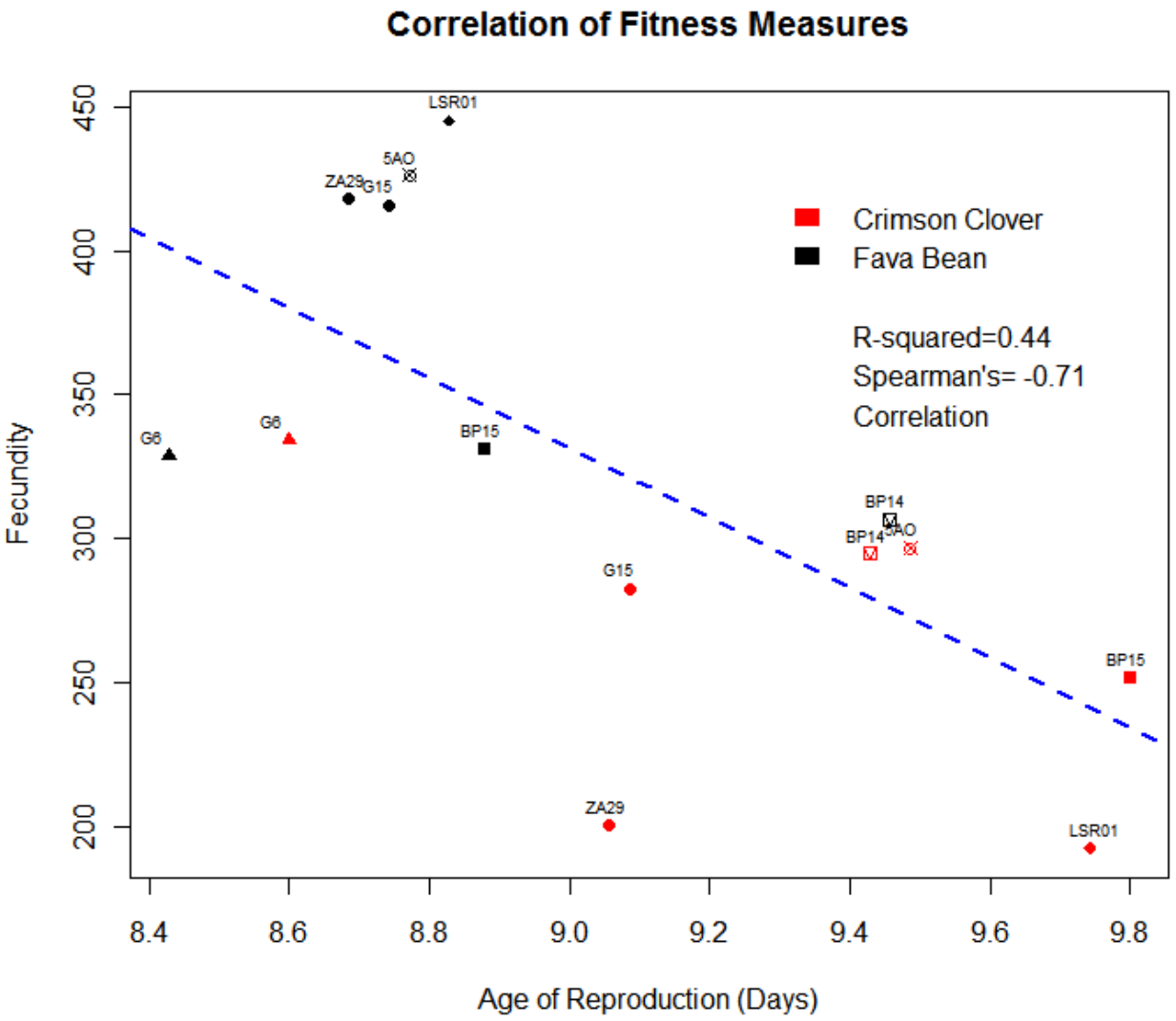
APPENDIX A:

Bender buffer DNA extraction. I separated aphids individually into 1.5mL Eppendorf tubes and froze those tubes in liquid nitrogen. I pulverized each adult aphid into a fine powder using a sterile pestle, then added 100 µL of Bender Buffer to each tube and homogenize with a new pestle. I added 2.5 µL of Proteinase K, then incubated at 65°C for 2 hours on a shaking heating block, set to at least 700 rpm. While tubes were warm, I added 14 µL of 8M KoAc. I stored the samples on ice for at least 1 hour and at maximum 24hours, then centrifuged each sample at 13,000 RPM for 15 minutes to precipitate cellular debris. I transferred the supernatants to new tubes and added a multiple of 100 µL of cold 100% EtOH to achieve ~70% EtOH concentration to effect maximal DNA precipitation. After letting them sit for 5 minutes at room temperature, I precipitated the DNA at 13,000 RPM for 15 minutes. I decanted the supernatant, washed each sample twice with 200 µL of 70% EtOH, and centrifuged at 13,000 RPM for 5 minutes between each wash. Finally, I added 200 µL of 100% EtOH and centrifuged the samples for 5 minutes, decanted the supernatants and let the DNA pellet dry. I resuspended the pellet in 25-50 µL of H_2O.

APPENDIX B:

Correlation of fitness measures during the genotype × host plant interaction experiment. There is a negative correlation between the time until reproduction and fecundity. Hence, the aphids that develop slower also produce less offspring. (Linear regression model, R^2 =.44, $F_{1,12}$=9.38, p=.0099; Spearman's rank correlation, ρ= -0.71, p=0.00597).



APPENDIX C:

Supplemental data for the genotype × host plant interaction experiment. When genotypes were grouped by host plant species, it showed increased performance on fava bean for the majority of the aphid genotypes. On average, aphids exhibited both shorter times to reproduction and higher fecundity on fava bean.