Evolution of phenotypic plasticity in the pea aphid *Acyrthosiphon pisum*

Background: Phenotypic plasticity is a notable and widespread source of phenotypic diversity, allowing organisms to respond to varying environments on a short timescale. In the most extreme cases, an environmental cue can signal for the production of two completely distinct, complex phenotypes. Phenotypic plasticity, and its underlying mechanisms, are known to evolve over time, but the nature of this evolution is currently not well understood at either a genetic or an ecological level. Understanding the evolution of phenotypic plasticity is important because phenotypic plasticity plays an essential role in fitting organisms' phenotypes to their environments,[1] and may even be a source of evolutionary novelty via genetic accommodation.

I propose to study the evolution of phenotypic plasticity in the pea aphid, *Acyrthosiphon pisum*. This species exhibits a striking wing dimorphism in females. Wingless females invest heavily in fecundity and maturation speed at a cost to dispersal ability, while winged females invest in dispersal at a cost to fecundity and maturation speed (also, note that dispersal may be risky, as a dispersing aphid must locate a new suitable host plant in order to succeed). The wing polyphenism is transgenerational, meaning that offspring phenotypes depend on the mother's environment: mothers produce winged daughters at a higher rate in response to high density (crowded) conditions.

There is considerable variation in the rate of winged offspring production between pea aphid lineages. In fact, the "species" is composed of at least 15 host races which have specialized on different host plants over the past ~500,000 years.[2] We have discovered that some host races are composed entirely of 'high-inducing' lineages (i.e., mothers produce many winged offspring in response to a crowding signal), while other host races are composed of 'low-inducing' lineages (i.e., mothers produce few winged offspring in response to a crowding signal), or a mix of high- and low-inducing lines (Fig. 1). The source of these differences is not clear. Below, I propose a series of experiments to assess the genetic basis of variation in the pea aphid wing polyphenism both between and within host races, and discover the ecological factors that are driving these differences.

Significance: Phenotypic plasticity allows organisms to dynamically alter their phenotypes in order to succeed in different environmental conditions. An understanding of the genetics and ecology that underlie plasticity and its evolution will reveal the way in which plasticity may arise and may be modulated through time. Pea aphids are an excellent system in which to study the evolution of phenotypic plasticity because (1) the plasticity is adaptive and should be subject to natural selection, and (2) the host races are ideal for comparative work, allowing contrasts to be made within and between incipient species in order to dissect the variation at different time scales.

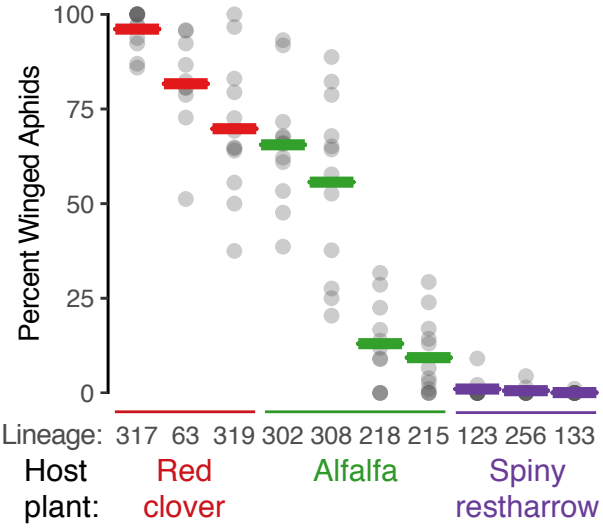

Figure 1. Production of winged offspring in response to a crowding signal varies widely among host races. Numbers along the x-axis indicate pea aphid lines. Each line is color-coded according to its host race, and points indicate individual biological replicates. Note that the alfalfa host race includes both high- and low-inducing lineages.

Aim 1: Characterize the functional role and evolutionary history of genes thought to be involved in the pea aphid wing polyphenism. Previous transcriptomics work that I performed in this system indicated a core set of six genes that are differentially regulated in response to a crowding signal in many pea aphid lineages (Parker, **Driscoll** et al in prep). However, many of these genes are poorly characterized. My first goal will be to uncover the functional role and evolutionary history of these prospective plasticity-related genes. Approach: I will use RNA interference (RNAi) to knock down expression of candidate genes in crowded aphids and establish whether these genes can modulate the crowding response (i.e., wing induction). I will also employ sequence similarity comparisons and phylogenetics to characterize the evolutionary history of candidate genes. Preliminary work suggests that the evolutionary history of many of these genes may be complex, including horizontal gene transfer[3] and gene duplication events.

Aim 2: Determine the genetic architecture of variation in phenotypic plasticity in pea aphids. I will apply a forward genetics approach to discover the specific genetic variants that contribute to variation in phenotypic plasticity between pea aphid lineages. Approach: I will screen numerous lines from the alfalfa host race and identify at least 50 high-inducing and 50 low-inducing lines. I will then genotype pooled DNA samples from the high- and low-inducing lines using whole-genome short-read sequencing in order to produce a high-density association map. This will allow me to confidently identify genomic regions that contribute to the phenotypic difference between high-inducing and low-inducing lines, and understand the genetic architecture of plasticity in this system. In addition, I will assess whether implicated genomic regions coincide with the location of any of the candidate genes previously indicated by transcriptomics work (see aim 1 above), or suggest alternative candidate genes.

Aim 3: Investigate the ecological basis of host race differences in wing induction. In order to understand why higher or lower wing induction should be adaptive for different host races, I will examine the connection between wing induction and host plant ecology. My preliminary analysis suggests that high-inducing host races tend to be those found on crop plants. This could result from differences in the likelihood of successful dispersal in the context of a dense crop field vs. a more patchily distributed natural habitat. Alternatively, the pattern could result from differences in habitat persistence (e.g., due to crop rotation). Approach: To test these hypotheses, I will first thoroughly characterize the variation in wing induction among host races. I will collect additional aphid lines from 5 well-studied host races, as well as 2-4 host races not previously studied, and quantify wing induction in these lines. I will then examine these races' host plants to assess patch size, persistence from year to year, and (for crop plants) farming practices. As the pea aphid host races occur in western Europe, I will build on our past collaboration with Jean-Christophe Simon and Denis Tagu of the University of Rennes throughout my work for this aim.

Broader impacts: The proposed work will allow me to introduce many young aspiring scientists to real-life research. I am currently working with two undergraduate students on projects related to the above proposed work, and intend to involve several more undergraduates in the work for each of these aims over the next few years. I also co-lead a weeklong research-based biology course for marginalized high school students each summer, and use my research as inspiration for experiments the students can try. In addition, I strive to build a strong scientific community among my peers: I share computational skills with my colleagues by leading R programming workshops, and encourage inclusivity in my community through a Women in Biological Sciences (WIBS) support/activism group that I co-founded last year. I will continue to pursue these and other outreach and community-building activities throughout my graduate career.

References [1] West-Eberhard, M (2003). Oxford University Press. [2] Falazova, V et al (2019). bioRxiv doi: https://doi.org/10.1101/769133. [3] Parker, BJ and Brisson, JA (2019). Curr Biol 29:12.