Analyzing the Genetic Basis of Alpine Adaptive Dwarfism in Aquilegia jonesii

Diego Orellana, Jason Johns, and Scott Hodges
Ecology Evolution, and Marine Biology

Abstract:
Plants have been evolving for millions of years to best fit a range of environments. One such extraordinary case is alpine plants, which grow in extremely harsh conditions. To adapt in these conditions alpine plants have adopted several unique traits, one of which is dwarfism. Dwarfism is a highly sought-after trait in agriculture due to its ability to forego common problems such as lodging, and research has been done in several model agricultural species such as rice and corn, revealing candidate genes for its mechanism. However, very little work has been done in alpine adapted ecological systems. The genus Aquilegia has a well-researched and annotated genome, making it an important ecological and evolutionary model. Our focal species for this project is Aquilegia jonesii, the most alpine adapted columbine, holding many of the classic traits seen in alpine plants, including extreme dwarfism. This summer we analyzed second generation (F2) hybrid crosses between A. jonesii and another columbine variety, A. coerulea 'Origami', which exhibit a gradient of intermediate phenotypes and genotypes between the two parents. We have measured leaf area (a proxy for dwarfism) on 97 of the F2 plants, giving us an estimate of the distribution of phenotypes for the F2 population, which is representative of the distribution of genotypes. We have prepared genomic DNA libraries for each of these 338 individuals to analyze areas of the genome associated with the dwarf phenotype using a Quantitative Trait Locus (QTL) analysis in the near future.

Methods:
Genotyping:
We isolated DNA from leaf tissue from each individual in our F2 population of 338 plants. We prepared whole genome libraries for each individual using the iGenomx Riptide kit. We sent these DNA libraries to UC Davis to be sequenced on a NovaSeq6000 to return genomic DNA from each individual, which we will use in the coming months to perform a QTL analysis.

Phenotyping:
We used leaf area as a proxy for dwarfism. To calculate leaf area, we took a representative leaf from each individual and dissected it. The dissected leaf is then mounted on a sheet of paper, scanned, and processed in ImageJ which calculates the area of each segment of the leaf blade. These data are used to perform the QTL analysis, correlating genotype with phenotype.

Introduction:
To find the genetic basis of adaptive alpine dwarfism, I will be using Aquilegia jonesii (blue limestone columbine) as a model organism. A. jonesii is an ideal model due to its ability to thrive in harsh alpine environments, withstand strong and consistent winds, cold and fluctuating temperatures, intense UV radiation, and low carbon dioxide pressure. To adapt, A. jonesii has evolved to become dwarf, have waxy leaves, and have stomata on its upper leaf surface, traits that many alpine plants share (Körner 1999). A. jonesii is the most alpine adapted columbine, making it unique among its genus. We crossed A. jonesii to A. origami, a non-dwarf variety of Aquilegia, and used chromosomal recombination in second generation hybrids to produce a gradient of phenotypes and genotypes between the parents so that we can map the dwarfism phenotype to the region(s) of the genome that code it. Here we report the distribution of the dwarf phenotype in 97 of the F2s, quantified by leaf area. In the coming months we will phenotype the rest of the F2s and use their genomic data to perform a QTL analysis.

Discussion/Future Directions:
Leaf area is widely distributed among the F2 population, representing a range of phenotypes between the parents, A. jonesii and A. coerulea 'Origami'. This phenotypic range is evidence that the loci responsible for the leaf area trait have segregated in our F2 population. Because the variation is continuous, and not restricted within discrete values, this suggests that leaf area may be a genetically complex trait. Going forward, we will measure leaf are for the remaining F2 individuals, as well as measure other proxies for dwarfism such as leaf stalk length and overall plant height. Once we receive the genomic DNA of our entire population, we will map dwarfism to the Aquilegia genome. This allows to see what regions of the genome are correlated with dwarfism with a QTL analysis.

Acknowledgements
Special thanks to Jason Johns and Scott Hodges for allowing me to work in their lab and being patient with me through the worst and best of times. Thank you to the EUREKA! Program for allowing me to undergo this wonderful experience. I am sincerely grateful I got to spend this summer doing something I have a newfound passion for.