STUDY OF TRICHOME HEREDITY IN *PHASEOLUS VULGARIS* (BLACK BEAN) CULTIVARS
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PROJECT SUMMARY

A. ABSTRACT
Phaseolus vulgaris, the common bean, is a primary source of nutrients, especially protein, for many human cultures and populations. Trichomes have been recognized and studied as both chemical and structural defense characters in many plant populations, including the common green bean. However, the inheritance and maintenance of trichomes within populations remains unexamined. We hypothesize (Aim 1) that the amount of trichome cover and trichome architecture (shape) will vary between populations, (Aim 2) that the amount of trichome cover shows a polygenic inheritance pattern, (Aim 3) and that the shape of trichomes (hooked or straight) shows a more discrete heritability pattern. These conclusions would be useful in suggesting cultivar selection for trichome cover as a natural method of defense in *Phaseolus vulgaris* populations.

B. PUBLIC HEALTH RELEVANCE
Phaseolus vulgaris is a primary economic and agricultural feature across many temperate climates. It is native to North and Central America, but has been also cultivated in Europe. Developing countries, including ones supported the U.S. have supported *Phaseolus vulgaris* crop establishment in developing countries as a high-protein crop. The black bean has as much as 2.6 grams of protein per tablespoon, which makes it comparable to meat without the energy investment and dilution involved in feeding animals. Black beans can be used for a high-protein alternative for flour (Simons, 2015). The black beans are just one member of *Phaseolus vulgaris*, along with pinto beans and the common green bean. Dry beans are used for nutritional aid to other countries and underprivileged individuals by many first-world countries, including the U.S. If we can establish bean crops/cultivars in these areas, the inhabitants could gain a sustainable high-protein food source.

We have selected cultivars for their protein content and size. The beans have one wild relative thought to be the relative for all the other species. However, the selection for larger, more nutritious beans may increase the risk of herbivorous insect attack. Unless natural defenses, like trichomes, are also artificially selected for, cultivars in the U.S., Europe, and even in developing countries may be susceptible and become pesticide dependent. A better understanding of trichomes as a heritable defense in *Phaseolus vulgaris* may educate judgments for the artificial selection of trichomes in common bean cultivars. Trichomes have been analyzed for their defense benefits in crops including raspberries (Graham, 2014), strawberries(Gonzalez-Dominguez, 2015), and maize (Abdala Roberts, 2014). Understanding the black bean's defense heritability of the leaf trichomes may increase productivity and global human health impact of the cultivar.

C. QUALIFICATIONS

In the summer of 2015, I worked with Stacy Endriss on a DDIG project at Colorado State University characterizing the divergence in trichome defense between North American and European *Verbascum thapsus* (common mullein), which looked at several morphological measures of defense in the two populations including trichome cover and trichome length. We analyzed trichome divergence in terms of the plant's invasive history because it is indigenous to Europe and was introduced to North America about three centuries ago. I have also worked in Marinus Pilon's laboratory that studies the nutrient requirements of *Arabidopsis* . I have a Bachelor of Science in Biological Sciences from Colorado State University with an emphasis on genetics, cellular and molecular biology.

RESEARCH PLAN

A. SPECIFIC AIMS

Our overall goal is to characterize the inheritance of trichomes in *Phaseolus vulgaris L.* as a defense system and make suggestions regarding the maintenance of the trichomes in cultivars.

Specific Aim 1: Analyze trichome cover and shape on the leaves in numerous *Phaseolus vulgaris* populations.

Rationale: Different populations display different trichome densities and shapes (hooked, straight). Trichomes are inherited defense characteristics that display genotypic variation that corresponds to these phenotypic differences.
Hypothesis: The bean plants should show small amounts of variation within a population and large amounts between cultivars, with a high F-correlation relative to their own variety and low correlation relative to others.
Objective 1: Grow 100 plants from each of the populations in a greenhouse. Measure trichome density and characterize trichome shape.

Specific Aim 2: Investigate the mode of inheritance of trichome density.

Rationale: Density is likely a polygenic trait with some quantifiable heredity.
Hypothesis: The bean plants should show small amounts of variation within a population and large amounts between populations, with a high F-correlation relative to their own population and low correlation relative to other populations. Heredity should be low within the population due to lack of phenotypic variation and high between populations due to variability between populations.
Objective 3: Cross populations with extreme phenotypes (lowest density and highest density) and analyze heritability.

Specific Aim 3: Investigate the mode of inheritance of trichome shape.

Rationale: Hooked and straight trichomes are probably single loci traits with variability in expression based on dominance or copy number genotypic effects. The trichome shape should have a measurable pattern of heritability.

Hypothesis: Since hooked and straight are binary phenotypes, I would expect some sort of dominant interaction.
Objective 3: **(3A)** Cross trichome shape heterozygotes and estimate variation and heritability components. **(3B)**Test trichome shape for independence with trichome density.

B. SIGNIFICANCE

The wild relative of the common green bean, *Phaseolus vulgaris L. var. aborigineus*, morphologically diverges from its cultivar relatives (Hoc et al., 2006). Since it is a cultivar, growers select for color, size, and shape as well as localized pest resistance and pod pubescence, among other traits (Rana et al., 2015). Cultivars also show variation among straight, hooked, and glandular trichomes (Dahlin et al. 1992). Trichomes are an important component of the interplay between mechanical and chemical defenses on bean specimens (Ballhorn, 2013) and the type and density of trichomes affects herbivore resistance (Ojeda, 2013). For example, differential density in hooked trichomes has been shown to affect resistance to the potato leafhopper in *Phaseolus vulgaris* (Pillemar et al, 1978). Understanding the genetic inheritance of pubescence as well as the genetic models they follow under field conditions would offer insight on how to select cultivar lineages and maintain variation within populations.

C. RESEARCH STRATEGY

Collect beans from 10 distinct lineages from a seed bank, selecting for trichome shape when possible (ideally, 5 hooked and 5 straight trichome populations). Use SSR genetic markers (Sarikamis et al., 2009) to characterize relationships between populations to establish expected divergence and relatedness based on developed markers.

Grow sample population plants (~100/cultivar, ~1000 total) in a greenhouse to establish a baseline measurement with low levels of environmental variance.

Photograph old and young leaves mid-season through a microscope. Estimate the amount of trichome cover using ImageJ and compare same-aged leaves' trichome density data.

Determine the variation within the trichome density, and graph. Look for means and extremes. Proceed according to Appendix A: if there are extreme phenotypes (low trichome density and high trichome density) within populations, we will cross the populations with the extreme phenotypes. We would cross the plants, particularly the lowest density and highest density pubescent plants (for 1000 or more offspring). Propogate the crossed beans and measure trichome densities. Try to analyze through mid-parent regression. If the trichome densities are continuous, with no extremes (Appendix A), then we would perform all 55 pairwise comparison crosses among the black bean cultivars and do midparent regressions among the comparisons.

We will also compare trichome architecture between black bean cultivars based on the photographs of mid-season leaves and microscopic analyses. We will cross parents with hooked and straight phenotypes to establish a line of hooked-straight heterozygotes. We would cross the heterozygotes to measure heritability. If the filial generation shows discrete traits (hooked or straight), as preliminary data indicates, the dominance will be measurable. If the filial generation shows mixed (plants with both hooked and straight trichomes), we should be able to estimate heritability based on proportions of hooked and straight trichomes.

D. INNOVATION

We should be able to simplify an accurate heritability estimate by using a common garden model, so environmental variation should be close to zero. Thus we can use $2pqa^2 = V_a$ and $(2pqd)^2 = V_D$ to estimate heredity with the simplification that p and q are 0.5, which means we essentially need means and deviations to estimate the heritability. The use of SSR would add baseline correlations for overall genetic analysis.

E. SCOPE:

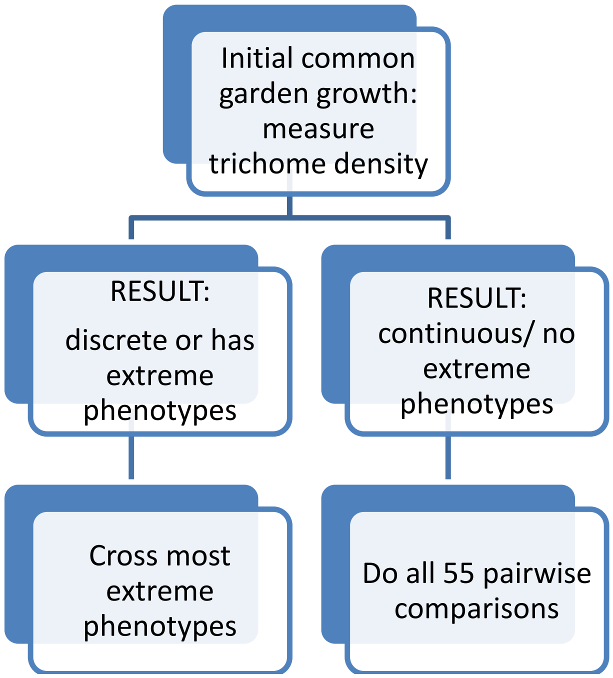
This study analyzes the physical characteristics of the trichomes as a measure of defense. It is beyond the scope of the experiment to analyze biochemistry of trichomes and thus the heredity of those defenses. Although it is possible that pubescence and protein content are also co-dependent, it is also beyond the scope of this study to test the relatedness of these two traits.

As an extension of our experiment, we could use field studies in local environment to estimate environmental variance in conjunction with greenhouse-grown plants. This would allow us to look at deviation in this measure of defense among the environments in which these black bean cultivars are usually propagated.

Furthermore, the particular mechanical-chemical defense interplay is not fully understood, and biochemical tests could be run to further understand the necessity of trichomes is *Phaseolus vulgaris* cultivars.

Appendices

Appendix A: Experimental Flow of Determining Trichome Density Inheritance



Appendix B: Preliminary Experiment Budget

Item	Reasoning	Budget
PI Salary	$30,000 per year for three years for propagation, crossing, measuring, and analyzing	$90,000
Beans	Purchasing bean specimens from the seed bank	$500
Growth space and maintenance	$5,000 per year for three years for propagation and harvesting	$15,000
Genetic tests	SSR genetic testing on 1,000+ individuals (~100 per cultivar)	$15,000
Other lab equipment and maintenance	Three years sustained use and replacement of materials	$10,000
ESTIMATED TOTAL		$130,500

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