

Scale of plasticity and local adaptation in *Boechera stricta* along an altitudinal gradient

Student: Sarah Caroline Daws

Mentor: Dr. Jill Anderson

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Abstract

As environments change, natural selection will increasingly favor plasticity, a mechanism by which organisms express variable phenotypes based on their abiotic or biotic environment. While these trends occur in sweeping macroenvironmental or coarse-grain patterns, this variation can also exist on an extremely fine-grain scale. By analyzing foliar plant traits in a reciprocal transplant experiment, we aimed to identify how the spatial scale of environmental variation drives patterns of plasticity. In this study, we asked if a native mustard (*Boechera stricta*) expressed phenotypic plasticity in response to abiotic and biotic variation at two spatial scales: the macro- and microenvironment. By investigating these questions, we made predictions about how environmental heterogeneity influences plant responses to natural climatic variation. We found that plant traits are determined by genetics, macro- and microenvironment (spatial scale), and the interaction between genotype and environment. Spatial scale of environmental heterogeneity affects plasticity of some foliar traits (e.g., specific leaf area) and life history traits (e.g., flowering phenology) through both abiotic and biotic factors. These fine scale differences in environmental factors such as soil moisture, species richness, and plant diversity can drive patterns of plasticity, and suggest a need for a more in depth environmental understanding of genotype by environment interactions. Our research suggests that environment plays a complex role in determining adaptation in plant populations through both ecological and evolutionary means. In further studies of plasticity, it is paramount to define both spatial scale as well as the abiotic and biotic heterogeneity of the ecosystem in order to best understand what controls patterns of plasticity.

Introduction

As environments change over time, natural selection will increasingly favor plasticity, a mechanism by which organisms express variable phenotypes based on their abiotic or biotic environment (Agrawal 2001). Although environments are changing through macroenvironmental directional trends including warming, many ecosystems are also experiencing progressively variable conditions (Walther 2002). This variability includes change in abiotic factors like soil moisture, temperature, and precipitation (Davidson 2006, Walther 2002). Additionally, biotic factors are changing through the introduction of novel species interactions as range and phenological shifts occur (Parmesan 2003, Root 2003). Coarse-grain shifts in climatic and phenological trends have been shown for ecosystems around the globe (Parmesan 2003, Parmesan 2007, Inouye 2008). These coarse-grain patterns do not always adequately describe fine-grain heterogeneity across a landscape. Fine-grain heterogeneity can exist for many reasons and can significantly affect plasticity and ultimately, persistence (Baythavong and Stanton 2010).

Through these trends, anthropogenic climate change has already significantly altered the distribution of species worldwide, and will continue to do so as warming continues to increase. Species across taxa have begun a great migration poleward and upward in elevation (Parmesan 2007, Parmesan 2003). While some plants are mobile and able to physically distribute to more suitable environments via high seed dispersal, some plants have limited dispersal ability and must find alternate ways to adjust to changing conditions in situ through plasticity or adaptation. Thus, for species with limited dispersal range, survival must come through local adaptation or phenotypic plasticity.

Many studies have found evidence of plasticity using reciprocal transplant experiments, and observed that plants have adapted multiple strategies to maintain optimum fitness in their home sites across broad environmental gradients (Baythavong 2011, Linhart and Grant 1996, Pratt and Mooney 2013). Typically, these studies on plasticity focus on the interaction between genotype and environment by reciprocally planting genotypes in different environments (Kaweki and Ebert 2004). Previous studies have made substantial progress in understanding how plasticity enables populations to survive climate disruption, however few of these experiments have addressed micro-scale environmental variation (Dudley 1996a, Donohue et al 2000, Stinchcombe 2004, Miner et al 2005, Wilczek et al 2009). Consequently, we know little about plasticity in response to natural variation in temperature, precipitation, and biotic conditions at the microenvironmental scale. In this experimental model, within-site variation is often treated as naturally occurring noise. However, this kind of analysis begs the question: at what scale is environment defined?

While some environments are topographically distinct from one another (i.e. geographically isolated ecosystems), other forms of environmental scale can be dictated by the researcher, and can affect the plasticity observed in experiments. Microenvironmental differences influence the evolution of phenotypic plasticity (Baythavong 2011). For example, Baythavong (2011) found that serpentine soils favor plasticity in plant traits more than in non-serpentine soils, as both soil environments are heavily interwoven across the landscape. Plasticity patterns were as heterogeneous as the environmental landscape. Here we investigate the scale of plasticity in subalpine rocky meadows near Gothic, CO.

Subalpine rocky meadows are heterogeneous both temporally and spatially. In the temporal frame, one can address both natural variations between seasons as well as long-term general trends. In the East River Valley of Colorado, significant seasonal variation causes changes in plant community. In 2011, the valley received only 50% of its average annual snowfall, and only 75% in 2012 (USDA NRCS Precipitation Report 2013). These temporal differences, compounded by shifting snowmelt date and variable summer temperatures and monsoons, can impact the amount of plasticity occurring from year to year. Moreover, these rocky meadows exhibit fine-grained spatial variation, with areas varying in slope, aspect, rockiness, moisture availability, and vegetative composition and cover. Minor topographical variation can alter both abiotic and biotic conditions, influencing fitness and patterns of phenotypic plasticity. However, environmental factors have different impacts on phenotype and phenology (Baythavong and Stanton 2010). Because of this variability, it is necessary to investigate at what scale or scales phenotypic plasticity occurs.

To address these questions, we tested a series of hypotheses using *Boechera stricta* (Brassicaceae), a forb native to the US Rocky Mountains. *B. stricta* is a short-lived perennial that inhabits a wide variety of environments and spans a broad elevational gradient (Rushworth 2011). Locally, it occurs in sites as low as Almont (~2500 m in elevation) and sites higher than Emerald Lake (~3600 m in elevation), providing a wide gradient to understand how the scale of environmental variation influences phenotypic plasticity by testing the hypotheses that (1) scale significantly influences plant response to genetic and environmental factors, (2) plants alter both phenological timing and foliar traits to cope with different environmental factors, and (3) plants respond to both abiotic and biotic factors. We hope to understand the role of fine-grain heterogeneity on plasticity based on spatial differentiation. Broadly, this study aims to compare plasticity to microenvironmental and macroenvironmental variation and identify the ecological and evolutionary importance of plasticity.

Methods

Study system

B. stricta is a primarily selfing perennial mustard with limited seed dispersal (Anderson et al 2011). Because it is not predominantly reliant on pollination for reproduction and exhibits phenotypic variation in different habitats, *B. stricta* is a model system conducive to understanding the role of scale and environment on phenotypic plasticity. To address our questions and hypotheses, I conducted both experimental research comparing microenvironmental scale and the direction of plasticity.

Experimental study

In 2012, Jill Anderson planted N=1200 juveniles from 24 genotypes into two gardens (elevations: 2884 m and 3130 m). The 24 genotypes range in elevation of origin (provenance elevation) from 2800 m to 3650 m. In each garden, genotypes were randomized into blocks of 48 plants, with 2 replicates of each genotype in each block (Anderson and Mitchell-Olds, in preparation).

We measured a series of foliar and life history traits, including specific leaf area (SLA), stomatal density, leaf water content, date of first flowering, and height at first flowering. We monitored the gardens for phenology 2-3 times per week during the growing season. Future studies will examine patterns of local adaptation using data on lifetime fitness (number of viable seeds; Anderson and Mitchell-Olds, in prep).

In order to analyze microenvironment, we also evaluated fine-grain variation in soil moisture, plant species diversity (Shannon-Weiner index) and plant cover. To quantify one facet of the abiotic environment in both gardens, we measured soil moisture in each block using a Hydrosense (Campbell Scientific). We took soil moisture measurements in each garden on two separate days, measuring on both the north and south sides of each block, to calculate an average per block. We estimated the biotic environment by conducting a census of the plant assemblage and percent cover in each block with a 10cm buffer zone included to assess the role of diversity and evenness on foliar traits.

Statistical Analyses

To examine the role of climate on plasticity, we conducted a series of generalized linear models, analyzing plant traits as a function of the elevation of origin for each genotype (Genotype), transplant garden (Environment), and provenance elevation by garden interactions (Genotype x Environment). We included random effects for genotype, block and genotype nested within block crossed with transplant in these generalized linear models (Proc Mixed, SAS ver. 9.3). To investigate microenvironmental plasticity, we analyzed foliar and life history traits as a function of elevation of origin and three environmental metrics: soil moisture, plant species diversity (Shannon-Weiner index) and plant cover.

For both spatial scales, we conducted separate analyses for the following traits: leaf water content, stomatal density, specific leaf area, flowering phenology, and plant size at first flowering. In all cases the residuals were normally distributed and homoscedastic.

Results

Macroplasticity

At a broad spatial scale, we found plasticity in specific leaf area and timing of first flowering, indicated by the effect of environment on different genotypes grown in the same location. Specific leaf area shows evidence for both genetic control and plasticity. The genotype by environment interaction for SLA indicates that genotypes vary in the level of plasticity. *B. stricta* individuals in the high elevation garden have significantly higher SLA than those grown in the low elevation garden (Table 1; Fig 1). Each genotype responded quite differently to the reciprocal transplant, indicating genetic variability in the plasticity of SLA (Fig 2). Additionally, we found a nonsignificant trend suggestive of plasticity in stomatal density (Table 1). Plants at the high elevation site appear to have higher stomatal density, on average, than at lower elevations (Figure 3).

Date of first flowering was influenced by genotype and environment, independently (Table 1). Plants in the low elevation garden flowered, on average, 24 days before plants in the high elevation garden (Fig. 4), and plants from high elevation source populations flowered earlier than those from low elevations (Fig. 5a, b). High elevation genotypes flower at smaller sizes, consistent with the short growing seasons of high elevation locations (plant height at flowering: Table 1). Leaf water content was not suggestive of genetic or environmental influence (Table 1).

Microplasticity

We found evidence of microplasticity in both gardens; however, the patterns varied for various traits at different garden sites. At the low elevation garden, we found a significant plastic response to all three environmental factors for specific leaf area (SLA), as well as elevation of origin (genotype) (Table 2a). We also found a trend suggestive of plasticity for leaf water content based on soil moisture in the low elevation garden (Table 2a). In contrast, at the high elevation garden, soil moisture and plot diversity had a significant effect on height at flowering (Table 2b). Stomatal density, non-significant for all environmental factors at the low elevation garden, was significantly correlated with both plot diversity and percent cover on each plot at the high elevation garden (Table 2b). SLA at the high elevation garden was influenced by percent cover and the genotype by percent cover interaction (E_3 : Table 2b, $G \times E_3$: Num DF=1, Den=126, $F=6.27$, $p=0.0136$).

Discussion

In this study, we asked if (1) scale significantly affected plant response to genetic and environmental factors. We found different patterns of foliar traits and phenology at the macro and microenvironmental scales, supporting the hypothesis that the scale at which environment is defined has an effect on the outcomes of genotype by environment effects on plasticity. Second, we hypothesized that (2) plants alter both phenological timing and foliar traits to cope with different environmental factors. The data suggest that plants are plastic both in physiological qualities as well as the timing of life history events. Lastly, we hypothesized that (3) plants respond to both abiotic and biotic factors. Our results were based on only three environmental factors, but we found significant effects of these abiotic and biotic factors on *B. stricta*. We found that while elevation is a proxy for environment, additional ecological and environmental factors can significantly affect plasticity.

Macroplasticity

Between gardens, we expected to see a plastic trend for decreased SLA in the low elevation garden because plants at lower elevation experience greater drought stress. Thicker leaves dry out more slowly than thin leaves. Another mechanism for drought resistance is to decrease stomatal density to reduce transpirational water loss (Ackerly 2000, Dudley 1996b). In our study, there was a slight trend for increased stomatal density in the higher vs. lower elevation garden. Whereas Heschel et al (2005) found a similar pattern of increased stomatal density for long-lived annual plants, this data suggests that the same may be true even in the plasticity of small perennials. At high elevation, plants with high stomatal density could have a photosynthetic advantage, because the growing season is shorter.

We found that plants originally from high elevations flower earlier than those of low provenance elevation. This reproductive strategy has been found in many habitat types, as plants at high elevation must complete the reproductive cycle more quickly during their shorter growing season (Stinson 2004). Selection for earlier flowering has been shown as a mechanism for drought resistance in response to the reduction in soil moisture seen in years with diminished snowpack (Giménez-Benavides 2007).

We found a genetic influence on SLA, date at first flowering, and height at first flowering by which high elevation plants flower earlier in the season. The significant effects of provenance elevation indicate that genetic population differentiation occurs along an elevational gradient. Our future studies will examine whether or not this genetic divergence is adaptive. Because the growing season is shorter at high elevation, *B. stricta* must flower earlier, and cannot afford to grow taller before initiating reproduction. In contrast, at low elevations where the growing season is longer, it is advantageous to grow taller for better dispersal (Stinson 2004).

Microplasticity

Some of the trends at the microenvironmental scale mirror those at the macroenvironmental scale. We found a significant effect of soil moisture on SLA at the low elevation garden, and suggestive trends of soil moisture with date and height of first flowering at the high elevation garden. Additionally, we found variable significant effects for plant diversity and percent vegetative cover on SLA at the low elevation garden. The significance of, and discrepancies between, these effects suggests plasticity to microenvironment based on the abiotic and biotic state of the ecosystem. Not only does elevation of reciprocal transplant matter, but also factors that are heterogeneous at fine scales can have an impact on the ability of a plant to adapt to its local climate.

Because we see different patterns of plasticity at the macro and microenvironmental scales, these results suggest that scale is an important factor to consider in the study of plasticity. While many studies have been conducted on plasticity using the genotype by environmental analysis scheme, our results stress the importance of defining the spatial scale of the environment through both biotic and abiotic effects. In much of the work in reciprocal transplant experiments, the environment term has been defined simply as 'garden.' Our research suggests that environment has a complex role in determining adaptation in plant populations through both ecological and evolutionary processes. These effects can be seen in both spatial scale and abiotic and biotic factors that may be heterogeneous across a landscape. By defining the environment

through a variety of metrics, we can better understand what facets of environment drive the plasticity observed across so many ecosystems.

Although it could be true that plasticity is advantageous for populations undergoing rapid climatic change, high plasticity can lead to developmental instability, and jeopardize heritability of traits advantageous in certain environments (Tonsor et al. 2013). Tonsor suggests that there exist evolutionary tradeoffs for plasticity that will limit the level of plasticity seen in populations pressured by changing climate and other selective stressors. Because plasticity can lead to reduced heritability and local adaptation, plastic traits will become more important players in adaptive mechanisms.

Our future studies will focus on investigating the role of plasticity in plant response to anthropogenic climate change. Not only will plant populations experience a singular increase in annual temperature, but must also endure an increasingly unpredictable abiotic and biotic environment through time and space. The advent of novel climates will increase selective pressures on plants, and plasticity may allow plants and other organisms to phenotypically keep pace with climate change and variation. Through further experiments we hope to better understand the role of selection on plasticity in these transformed landscapes. The future of research on plant plasticity must include analyses of defined spatial scale and evolutionary tradeoffs in order to explore the evolutionary capacity for plasticity.

Macroplasticity	<i>SLA</i>		<i>Stomatal Density</i>		<i>Leaf water content</i>		<i>Date of first flower</i>		<i>Height at first flower</i>	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>Genotype (G)</i>	6.67	0.0105	1.94	0.1731	0.02	0.89	16.39	<0.0001	14.74	0.0001
Num DF Den DF	1	217	1	32	1	217	1	355	1	360
<i>Environment (E)</i>	4.43	0.0412	3.32	0.0758	0.30	0.5852	10.55	0.0022	1.17	0.2852
Num DF Den DF	1	44	1	40	1	44	1	45	1	45
<i>G x E</i>	15.84	<0.0001	1.58	0.2174	0.13	0.7234	3.82	0.0515	1.04	0.3084
Num DF Den DF	1	217	1	32	1	217	1	355	1	360

Table 1. Role of genotype and environment on foliar traits on the macroenvironmental scale.

Microplasticity GV Garden	<i>SLA</i>		<i>Stomatal Density</i>		<i>Leaf water content</i>		<i>Date of first flower</i>		<i>Height at first flower</i>	
Degrees of freedom	Num DF=1 Den DF=89		Num DF=1 Den DF=6		Num DF=1 Den DF=87		Num DF=1 Den DF=13		Num DF=1 Den DF=12	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>Genotype (G)</i>	7.4	0.0078	0.16	0.7028	.98	.3254	1.12	0.3089	0.14	0.7115
<i>Soil Moisture (E₁)</i>	4.58	0.035	0.36	0.5693	3.19	0.0774	0.04	0.8379	0.6	0.4529
<i>Diversity (E₂)</i>	7.98	0.0058	0.58	0.4766	.59	.4433	1.87	0.1942	0.34	0.5686
<i>Percent cover (E₃)</i>	7.17	0.0088	0.09	0.776	1.24	0.2694	0.13	0.7278	0.01	0.9448

Table 2a. Role of genotype and three environmental descriptors on foliar traits on the microenvironmental scale at the low elevation garden (2884m).

Microplasticity Schofield Garden	<i>SLA</i>		<i>Stomatal Density</i>		<i>Leaf water content</i>		<i>Date of first flower</i>		<i>Height at first flower</i>	
Degrees of freedom	Num DF=1 Den DF=126		Num DF=1 Den DF=24		Num DF=1 Den DF=127		Num DF=1 Den DF=341		Num DF=1 Den DF=347	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>Genotype (G)</i>	0.29	0.5928	3.38	0.0784	0.01	0.9113	5.91	0.0156	1.43	0.2322
<i>Soil Moisture (E₁)</i>	0.13	0.7168	0.18	0.6758	0.02	0.8931	3.2	0.0746	3.34	0.0684
<i>Diversity (E₂)</i>	2.82	0.0954	11.74	0.0022	0.06	0.8136	0.02	0.8775	2.95	0.0866
<i>Percent cover (E₃)</i>	4.79	0.0305	9.24	0.0056	0.44	0.5101	0.17	0.6799	0.7	0.4037

Table 2b. Role of genotype and three environmental descriptors on foliar traits on the microenvironmental scale at the high elevation garden (3130m).

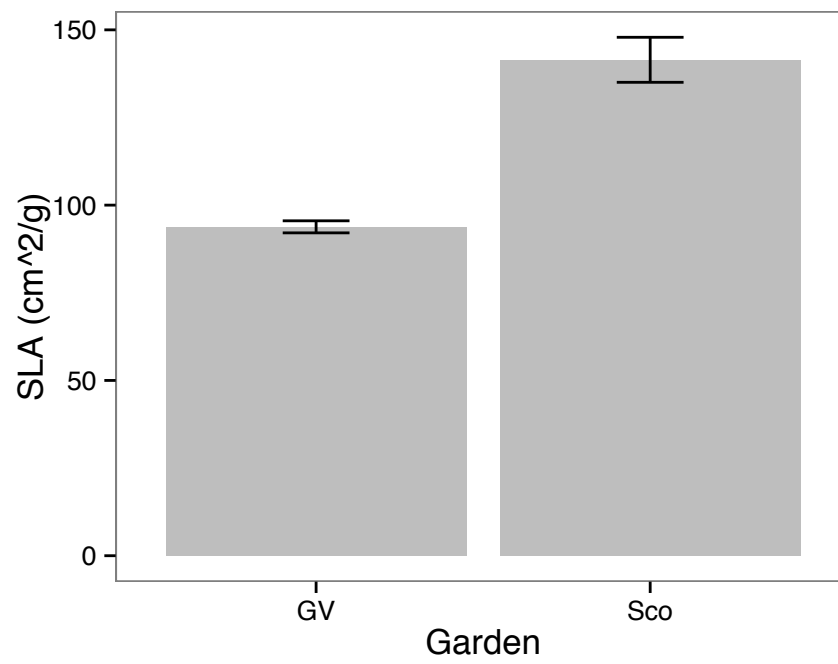
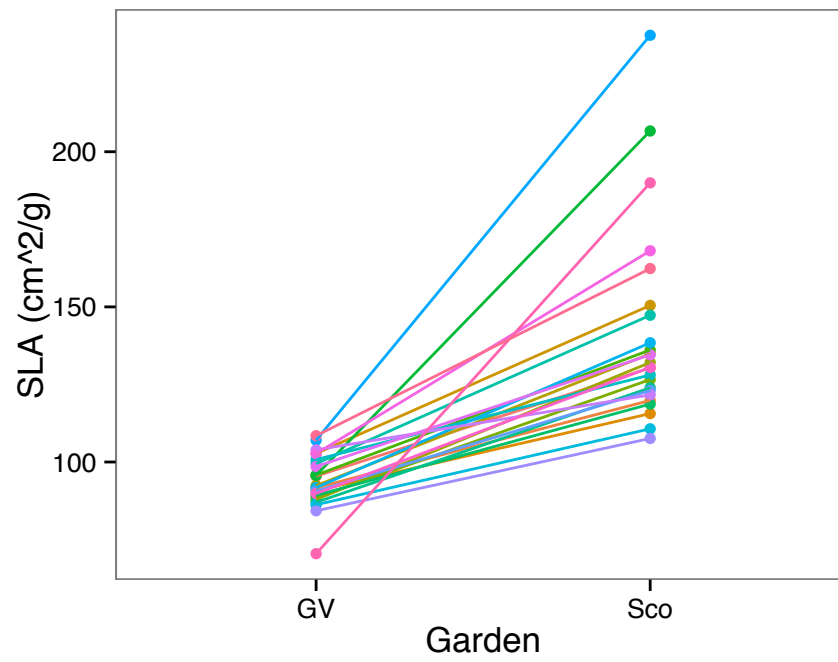


Figure 1. Means of Specific Leaf Area by garden.



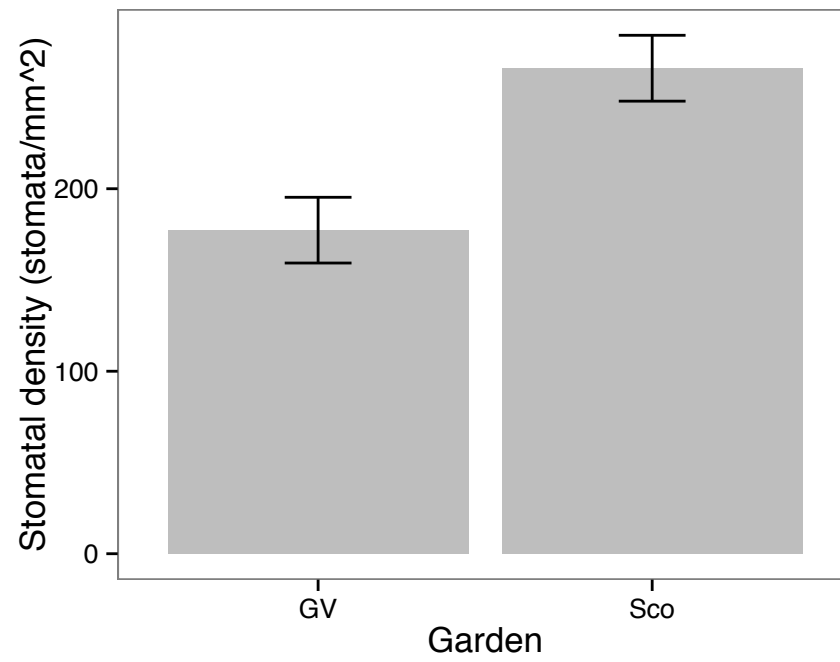


Figure 3. Mean stomatal density by garden.

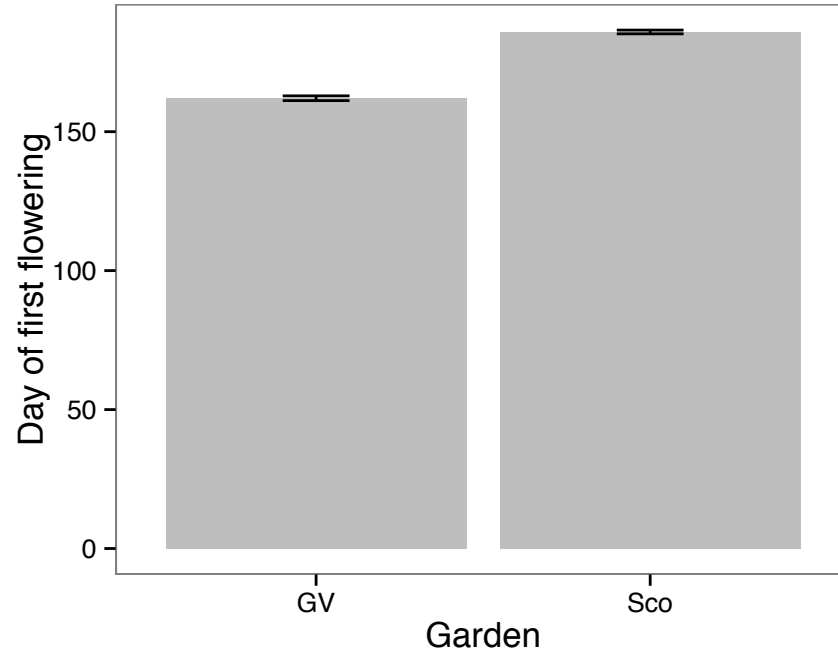
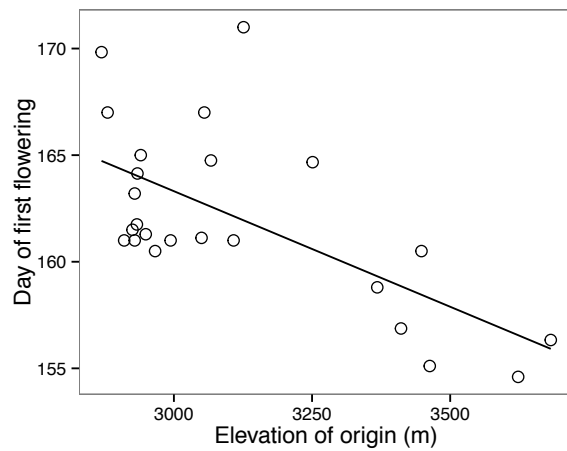


Figure 4. Mean date of first flowering by garden.

GV Garden



Schofield Garden

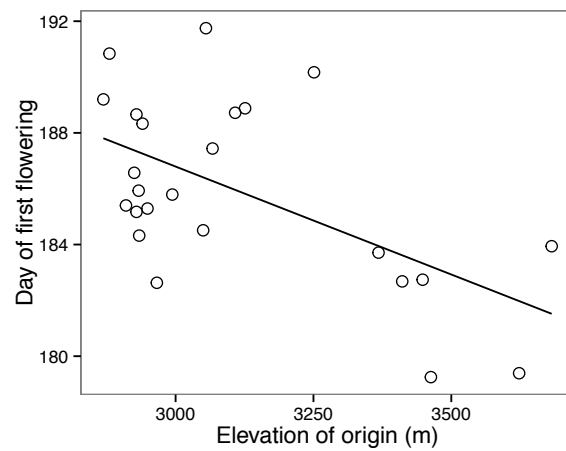


Figure 5 a and b. Day of first flowering by provenance elevation for both the GV garden and Schofield garden. Each dot represents one source population.

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