

**Plasticity and changes in selection in response to changing
precipitation regimes**

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Abstract

Altered precipitation patterns are a major prediction of climate change models and a recent reality. Changes in climatic variables may affect the evolution of species by affecting the magnitude and direction of natural selection on various traits. Winter precipitation can affect summer-flowering plants by altering the date of snowmelt. By investigating a long-term record of floral and vegetative traits and corresponding fitness in a hybrid zone between two species of subalpine wildflowers, a strong relationship was found between snowmelt date and the strength of selection on corolla length. For other traits, variation in selection could be attributed to pollinator abundances. In addition, a plastic response to winter precipitation was found in specific leaf area, a trait that plants use to balance water usage and carbon acquisition.

Introduction

With the potential for increasingly altered precipitation patterns due to anthropogenic climate change, understanding the sensitivity or insensitivity of selection pressures to precipitation is key to predicting how plants will adapt to this altered abiotic factor (Anderson 2012). Temporal variation in selection has, however, received relatively little attention, with most studies focusing on vertebrates (Siepielski et al. 2009). Vegetative and physiological traits, such as water-use efficiency, are often expected to experience different selection in dry versus wet habitats (Dudley 1996), and so selection on these traits might also be expected to vary between dry and wet years. Selection on floral traits could also depend on precipitation; for example, in an orchid, selection on osmophore area but not nectary depth varied across years with different moisture conditions (Benitez-Vieyra et al. 2012).

The present study examines both the strength of plastic responses in a vegetative plant trait to ambient historical precipitation, and correlations in the magnitude or direction of selection on floral traits with pollinator presence and snow melt date, a proxy for soil moisture during flower development.

The study organisms are the perennial monocarpic herbs *Ipomopsis aggregata*, the closely related *I. tenuituba*, and experimentally generated hybrids, grown alongside natural subalpine populations of each. Based on a previous experiment with the *Ipomopsis* species and hybrids in which precipitation was manipulated, we hypothesize that winter precipitation amount will generate plastic responses across years in the vegetative traits of individuals planted in the field. A plastic trait response is a change in physiology or morphology caused only by environmental, rather than genetic, conditions (Navas and Garnier 2002). For example, many plants have been shown to produce leaves with lower specific leaf area (SLA = leaf area/dry mass) when planted in a drier environment (Knight and Ackerly 2003). This is a physiological response to conserve water (Wright et al. 2001), since more compact leaves have less surface area from which water can transpire. However, lower SLA comes at the cost of reduced photosynthesis, because there is reduced light collection and gas exchange per unit biomass.

Although SLA often shows patterns with changes in natural conditions, the relative roles played by genetics versus plasticity in producing these patterns are little studied. The few studies that have used common gardens to examine genetic and environmental variation in SLA have focused on plasticity across spatial locations (Scheepens et al. 2010). We examine plasticity across years with different environmental conditions and predict that *Ipomopsis* individuals will show a positive response in SLA to winter precipitation amounts.

In subalpine environments, snow melt date affects the amount of time a plant is exposed to damaging environmental events such as frost, desiccation, and herbivory, before it reproduces. Earlier snowmelt, a prediction of many climate models (Anderson 2012) is likely to lower soil moisture during the flowering period of *Ipomopsis*. We hypothesize that increased winter precipitation, measured by snow melt date, will have a positive effect on the strength of natural selection on floral traits (Table 1). For floral traits, directional selection strength is predicted to increase with precipitation since the removal of water limitations can allow the plant to translate increased pollination gained by its attractive traits into the development of fertilized ovules to mature seeds, and hence increased female reproductive fitness.

Both *I. aggregata* and *I. tenuituba* in the region of study are pollinated in most years by the hummingbirds *Selasphorus platycercus* and *S. rufus* (Campbell 2003). Observational studies have demonstrated that hummingbird visitation rate and hence pollination frequency increases with corolla length in some years (Campbell et al. 1991), corolla width, and nectar production (Campbell et al. 1997). Long styles and exerted stigmas (that protrude beyond the petals) are able to capture more pollen (Campbell 1989). If the production of seeds is water-limited, the magnitude of selection through female function is predicted to increase on these traits in wetter years, as plants are released from resource limitation.

Hawkmoths (*Hyles lineata*) are abundant flower visitors in rare years. These moths prefer to visit narrow corollas (Campbell et al. 1997) and do not show the preference for red corollas that hummingbirds do (Meléndez-Ackerman et al. 1997). Thus we predict that selection on corolla width and flower color will also change, depending on whether it is a year with hawkmoths or not.

Selection on SLA is predicted to be positive in wet years because there is a resource benefit to high SLA, and negative in dry years because there is a survival advantage to low SLA in environments with low soil moisture (Volaire 2008). This trend in selection is also expected because plant species growing in areas with higher rainfall tend to have higher SLA (Westoby and Wright 2006).

<i>Trait</i>	<i>Selection with later snow melt date</i>
Corolla length	Stronger for long corollas
Style length	Stronger for long styles
Corolla width	Stronger for wide corollas in years without hawkmoths
Nectar production	Stronger for high nectar production
Flower redness	Stronger for red flowers in years without hawkmoths
Specific leaf area	Positive in wet years, negative in dry years

Table 1. Hypotheses for the effect of precipitation on selection for five floral and one vegetative trait.

Methods

Study System

This study employs two long-running experimental sites in Poverty Gulch, Gunnison County, CO (Map 1). This mountainous valley contains natural populations of *I. aggregata* up to 2,900 m in elevation and *I. tenuituba* at and above 3,200 m, along with a natural hybrid zone between the parent ranges (Campbell et al. 1997). Site 1 is located at 2,900 m on a south-facing slope (Campbell and Waser 2001), and Site 2 is located at 3,050 m on a steeper, drier slope with talus and little vegetation (Waser and Campbell 2004).

In 1997 seeds produced by hand-pollinated crosses of *I. aggregata* and *I. tenuituba* (parental, F_1 reciprocal hybrids, and F_2 hybrids) were planted randomly at 10 cm intervals within 20 1×1 m blocks at Site 1. This was repeated in 1998 at Site 2. A second experiment was started in 2007, using parental, F_1 reciprocal hybrids, and F_2 hybrids at both sites. In 2008, 10 blocks of F_2 hybrids were planted at Site 1 to increase sample size for this second experiment. To avoid encroachment of wild seeds, blocks were placed along strips where there were no flowering *Ipomopsis* individuals from the natural population within 1 m. F_2 hybrids provide a large variation in trait values for selection to act upon. Each June from 2001 through 2006 (for the 1997-98 plantings) and 2008 through 2013 (for the 2007-08 plantings), the surviving experimental plants were censused. Longest leaf length and number of leaves were measured for plants lacking inflorescences at that time. *Ipomopsis* existed as vegetative rosettes for a median of 5 years at Site 1, bloomed during a single season, set seed and usually died (Campbell and Waser 2001).

Trait measurements

Floral traits were measured on every surviving experimental plant that developed inflorescences. About two times per week, up to three open flowers were removed and the following traits were measured: corolla length, corolla width, style length (using calipers), and 48-hr nectar production and sugar concentration (using capillary tubes and a refractometer as described in Campbell et al. 1991).

Two flowers were collected per plant for color analysis. Flower color was measured using an OceanOptics Red Tide USB650 reflectance spectrometer and SpectraSuite software over wavelengths from 400 to 700 nm, averaging 5 scans with an integration time of 100 ms. Flowers of these two species of *Ipomopsis* do not reflect in the UV (Meléndez-Ackerman et al. 1997). Green reflectance (counts integrated from 401-475 nm) was subtracted from red reflectance (626-700 nm) and divided by the total reflectance (401-700 nm) to give flower redness.

Leaf traits were measured on every flowering and vegetative plant. To determine SLA, single leaves were scanned and their area measured in ImageJ (NIH freeware). Leaf samples were oven dried for 2 hours at 60 °C and weighed individually with a balance to the nearest .00001 g. SLA was then calculated as area (cm²)/dry mass (g). For each plant, SLA was based on a single leaf or averaged between two leaves collected two weeks apart.

Seeds were collected from mature fruits and counted up to three times per week just prior to capsule dehiscence.

Data on all of the traits above were recorded for the years 2010-2013 from experiment 2 (seeds planted in 2007 and 2008). An additional data set for 2001-2006 on corolla length, corolla width, style length, and seed number was obtained for experiment 1 (seeds planted in 1997 and 1998; Campbell et al. 2008). In total, flower traits were measured for 256 plants, and SLA for 441 plants. Five years of data are available for examining SLA plasticity, 5 years for examining temporal variation in selection on SLA, and 10 years for examining selection for floral traits (once seed counts from 2013 are completed).

Precipitation data

Precipitation data for the neighboring East River Valley (billy barr, pers. comm.) was obtained for each study year. Winter precipitation was totaled from Oct 1 through May 31 and summer precipitation was totaled from June 1 to July 15, while *Ipomopsis* is developing inflorescences. The total was not extended through August since most of the floral measurements were made in July. As noted by Campbell and Wendlandt (2013), this summer precipitation metric may interact with other climate variables such as temperature. Snow melt date was determined by the Julian calendar day of first bare ground at the weather station in Gothic, CO (2900 m). The sites in Poverty Gulch are 8 km distant and there may be differences in snow, rainfall, and melt timing produced by local geography that cannot be accounted for at present. The likely constant lag in melt time should alter only intercepts of melt date regressions.

Data analysis

SLA was measured in multiple years on the same individual plants, so changes across years must represent plasticity. Since *Ipomopsis* only produces seeds at the end of its life, binary survival to the next year was used as a fitness index for the vegetative traits. Total seeds produced by the plant (seed number) served as the fitness measure for floral traits. Floral traits could usually only be measured in one year, unless the plant was iterocarpic. It is conceivable that plants measured in different years differ genetically, but only if specific genes influence age of reproduction, as all plants in a given experiment originated from the same set of seeds.

To analyze variation in floral trait selection, two approaches were taken. First, a model was constructed with seed number as the response variable and year as a categorical factor, trait, and interaction between year and trait as the independent variables. As the interaction term was entered last in the model, the sum of squares (SS) associated with it took into account the SS due to the main effects. A significant year-by-trait interaction would indicate temporal variation in the strength of selection. If no such interaction effect was found ($p > .05$), the model was rerun without that term, using Type II SS to generate a standard analysis of covariance. A third statistical model nested the slope on the trait within year to estimate separate slopes for each year. The second approach was to examine whether that relationship between seeds and the trait changed systematically with precipitation. For corolla length and width, each year's standardized selection differential (slopes of the fitness regressions divided by average fitness and multiplied by the standard deviation of the trait) was regressed alternately against summer precipitation and snow melt date. Analyses were done in R version 3.0.1, using the *lm* function and *car* package.

To determine the plasticity of the vegetative trait, a repeated-measures ANOVA was performed with SLA as the response variable and year as the independent variable. The parameter estimate for a given year was regressed alternately against summer and winter precipitation. To analyze variation in vegetative trait selection, a logistic ANCOVA was performed with survival to the next year as the binomial response variable and year and SLA as the independent variables using the R function *glm*. This analysis was repeated with flowering state (flowered or vegetative), given survival, as the fitness response variable. In all cases, if no year-by-SLA interaction effect was found based on an analysis of deviance table ($p > .05$), the model was rerun without that term.

Results

To ensure that each floral trait was not merely an indication of plant vigor, the traits were correlated against total flower number, with all $r < 0.22$. Indirect selection could occur if there were phenotypic correlation between traits (Conner and Hartl 2004), but the only strong correlation was that of corolla and style length ($r = 0.62$, $p < 0.0001$).

Year had a strong effect on fitness for all floral trait models (Table 2). For corolla length, style length, and nectar production, there was no detectable trait-by-year interaction on fitness (Table 2). Even though the interaction was not detectable for corolla length, the standardized selection differential for that trait was strongly and positively correlated with snow melt date ($r = 0.89$, $p = .001$, Figure 2). It was not correlated with summer precipitation ($r = 0.50$, $p = .17$).

Selection on corolla width did vary detectably with year (Table 2; Figure 3). Nesting the slope within the year revealed significant selection favoring wider corollas in 2011 ($p = 0.016$) and narrower corollas in 2001 ($p = 0.0011$). Selection differentials on corolla width were not correlated with snow melt date ($r = 0.44$, $p = .24$, Figure 4) or summer precipitation ($r = 0.19$, $p = .63$).

Style length (Figure 5) and nectar production (Figure 6) did not have an overall effect of fitness, nor did selection on either did vary significantly between years (Table 2). There was a significant negative effect of flower redness on fitness, and a detectable year-by-redness interaction (Table 2), indicating that selection on that trait varied across years. Nesting the slope within the year revealed significant selection favoring whiter flowers in 2011 ($p = 0.0008$; Figure 7).

Repeated-measures ANOVA determined that, accounting for individual variation, SLA varied across the years 2011-2013 ($F_{2,90} = 36$, $p < .0001$). SLA from all of the individuals in 2009-2013 was correlated weakly with winter precipitation ($r = 0.47$, Figure 8), but not with summer precipitation ($r = 0.25$).

Logistic ANCOVA of next-year survival found weak year-by-SLA interaction ($p = .10$). Nevertheless, survival to the next year was higher for individuals with low SLA in 2009 and 2011 (both $p < 0.05$), and unrelated to SLA in the other years (both $p > 0.38$). Logistic ANCOVA of next-year flowering state found no year-by-SLA interaction ($p = .42$). With the interaction removed, plants with lower values for SLA were more likely to bloom the next year given that they survived ($p = 0.0007$).

Discussion

Overall selection

Selection was weak or positive on long corollas, consistent with observations that hummingbirds prefer to visit them in some years (Campbell et al. 1991). There was no overall directional selection on corolla width, which could be explained by variation in pollinator abundances (see below). There was also no directional selection on style length, indicating that the benefits of efficient pollen collection on stigmas were small when averaged across years, despite the finding of significant selection in populations of *I. aggregata* by Campbell (1989). This difference between studies could reflect the greater variation in style length seen in hybrid populations compared to those of just *I. aggregata*. Despite hummingbirds' preference for nectar (Campbell et al. 1997), there was no significant effect of nectar production on

fitness. This result is consistent with findings that nectar production in *I. aggregata* had no effect on female reproductive success, only male reproductive success (Mitchell 1993). This could reflect an energy allocation trade-off between the production of nectar to attract pollinators and production of seeds later in the season. Overall selection was negative on flower redness ($p = .04$), which is not supported by observations that hummingbirds prefer redder flowers (Melendez-Ackerman et al. 1997). Since hawkmoths do not demonstrate preference for red flowers, they may be responsible, although they were not sighted in 2011, the year with greatest negative selection. Finally, negative or weak selection on SLA demonstrated that overall, desiccation risk outweighed the gains of broader leaves' resource advantage.. This demonstrates how crucial minimizing water usage is to *Ipomopsis* in this habitat, relative to carbon acquisition.

Variation in selection across years

A clear positive relationship between melt timing and selection strength on corolla length supports the hypothesis that wetter winters allow *Ipomopsis* to convert pollination gains from pollinator attraction into greater seed set. Plausibly, the frost protection and initial moisture provided by later melts gave plants an opportunity to grow bigger at the beginning of the summer, so they could acquire more resources for seeds later in the season. Conversely, in years with early snow melt, increased pollination won by longer flowers did not result in more seeds because either water for seed development was not available or the resource cost of long flowers outweighed increased pollination. Summer precipitation was much less responsible for variation in selection strength, demonstrating that snow is the major factor influencing selection pressure on this trait.

While selection strength on corolla width was not explained by snow melt or summer precipitation, pollinator abundance may have played a role. In 2001 and 2010, hawkmoth pollinators were present in Poverty Gulch (Campbell et al. 2008, Diane Campbell, pers. comm.) Unlike hummingbirds, which prefer to visit wide flowers, hawkmoths actually prefer to visit narrow flowers (Campbell 1997). This could explain the negative selection on width in those years. Hawkmoths were absent in 2011, when there was strong positive selection on width.

Selection on flower color also varied significantly across years. For the three years studied, selection differentials were correlated with snow melt date at the edge of detectability ($r = .99$, $p = .08$). No pollinator preference for white flowers is known at Poverty Gulch, so there may be an unmeasured morphologic or physiologic trait genetically correlated with color that is responsible for this result. Flower color is a chief difference between the parental *Ipomopsis* species, so such a trait is likely split along species lines.

Selection on SLA represented by survival to the next census varied across years. Selection strength is likely correlated with the severity of summer droughts, but there are not enough years with data to test this hypothesis. Mortality could also occur between the next spring's snow melt date and the census.

Vegetative trait plasticity

SLA showed year-to-year plasticity, and some of the variation could be explained by snow melt date. Variation in temperature and soil microenvironment may account for SLA variation within years, since these determine transpiration rates and water availability for individual plants, respectively. Quantitative comparison of this annual individual SLA plasticity to other studies is difficult since many describe plasticity when transplanting along altitude gradients (Scheepens et al. 2010), or compare the mean SLA of populations with naturally or experimentally altered water and nutrient regimes (Wright 2001). The plasticity demonstrated by *Ipomopsis* from the wettest to the driest year in this study (which happened to be consecutive) is equivalent to that shown when moving *Campanula* upwards 1040 m in the Swiss Alps (Scheepens et al. 2010).

Conclusion

This study demonstrated that certain *Ipomopsis* floral traits experience different selection patterns in different years, and that in some cases the variation can be linked to climate variables, most importantly winter precipitation as indicated by snow melt date. For corolla length, selection weakened with earlier snow melt. In the long term, this could lead to altered evolution of corolla length. Selection on width was not correlated with snowmelt, but it did seem to be explained by presence of hawkmoths. *Ipomopsis* also demonstrated functional trait plasticity in response to winter precipitation in the field, as predicted by manipulation experiments (Campbell and Wendlandt 2013). This study reveals important factors that could determine how subalpine plants respond to climate change through a combination of adaptation and acclimatization. In addition, and as argued by Siepielski et al. (2009), the demonstrated existence of annual fluctuations in selection on this population may place limits on the speed at which it can adapt to local conditions by maintaining trait variation.

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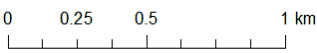
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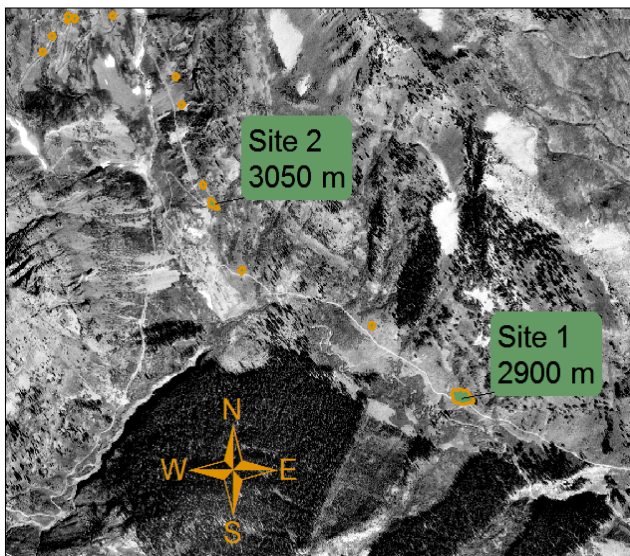
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Tables and Figures

Trait	Effect	With interaction		Without interaction	
		<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value
Corolla length	Year	11.74	<.0001	11.98	<.0001
	Length	2.50	0.12	2.55	0.11
	Year : Length	0.43	0.90		
Corolla width	Year	13.59	<.0001		
	Width	0.71	0.40		
	Year : Width	2.16	0.03		
Style length	Year	11.71	<.0001	11.53	<.0001
	Style	0.20	0.66	0.20	0.66
	Year : Style	1.45	0.18		
Nectar production	Year	9.25	.0003	9.58	.0003
	Nectar	0.02	0.89	0.02	0.89
	Year : Nectar	0.04	0.96		
Redness	Year	14.59	<.0001		
	Redness	4.56	0.04		
	Year : Redness	4.09	0.02		

Table 2. ANCOVA results for 5 floral traits on seed number. Significant effects are in boldface. If the effect of a trait-by-year interaction was not significant, the model was re-run without it.

Map 1. 



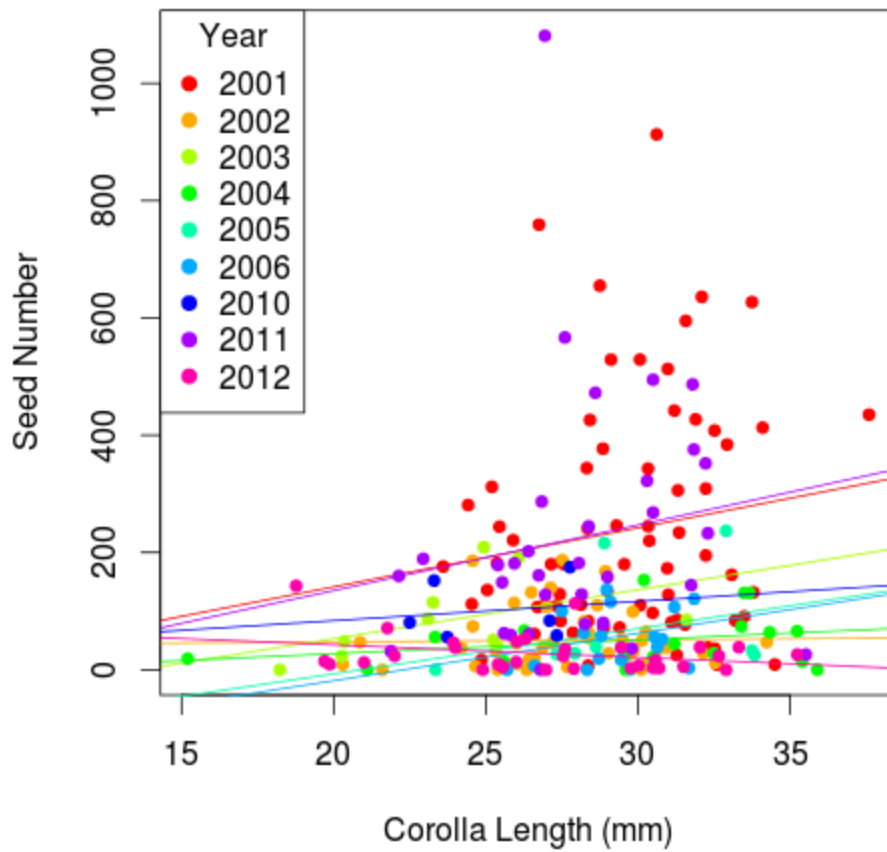


Figure 1. Reproductive fitness as a function of corolla length for all studied plants.

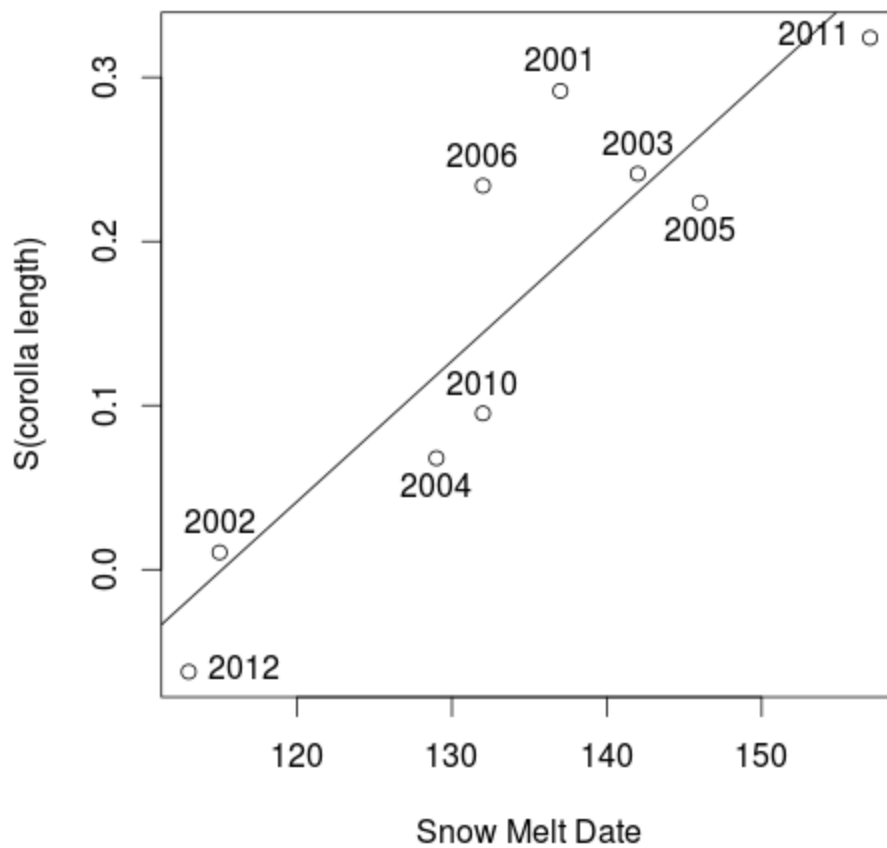


Figure 2. Standardized selection differentials (S) for corolla length against spring snow melt.

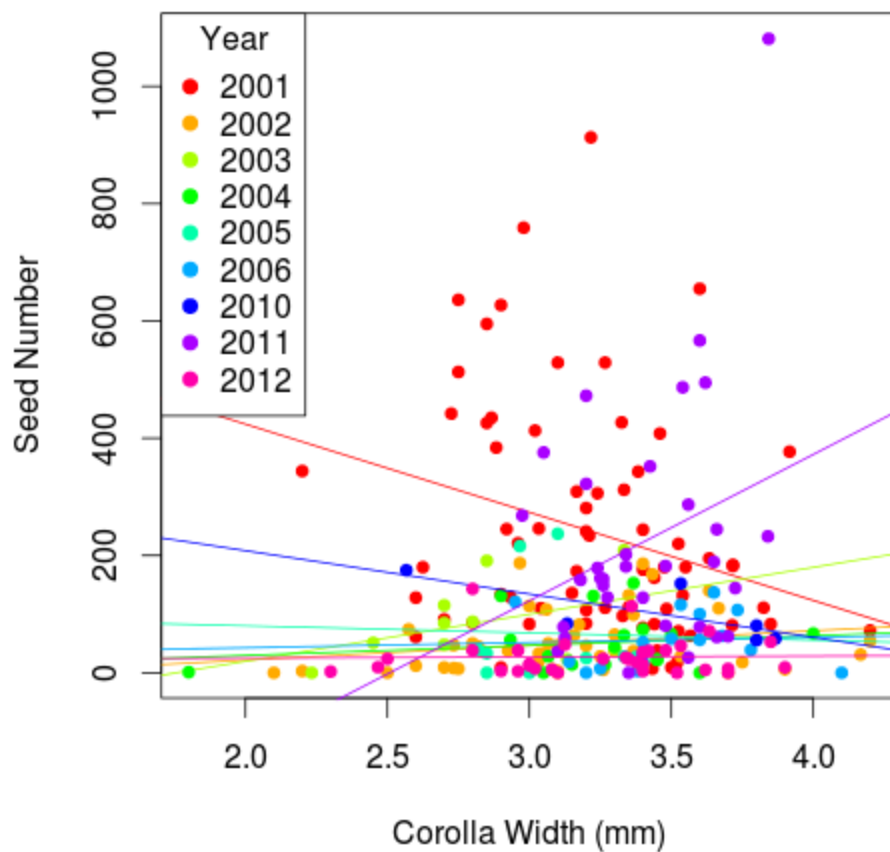


Figure 3. Reproductive fitness as a function of corolla width for all studied plants.

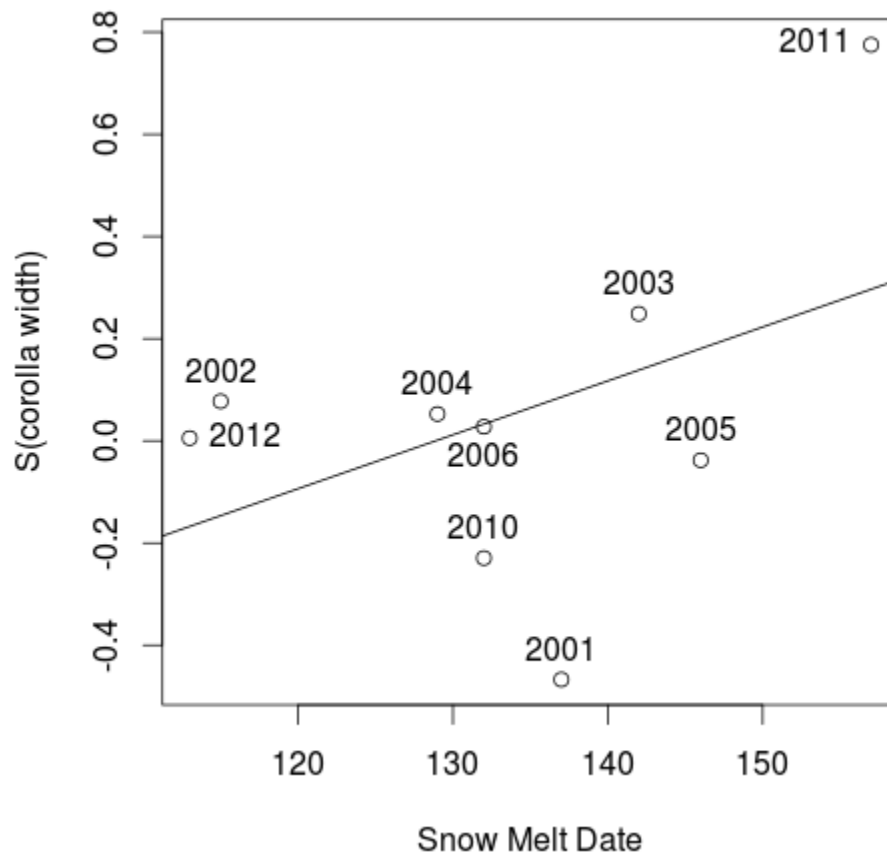


Figure 4. Standardized selection differentials (S) for corolla width against spring snow melt date.

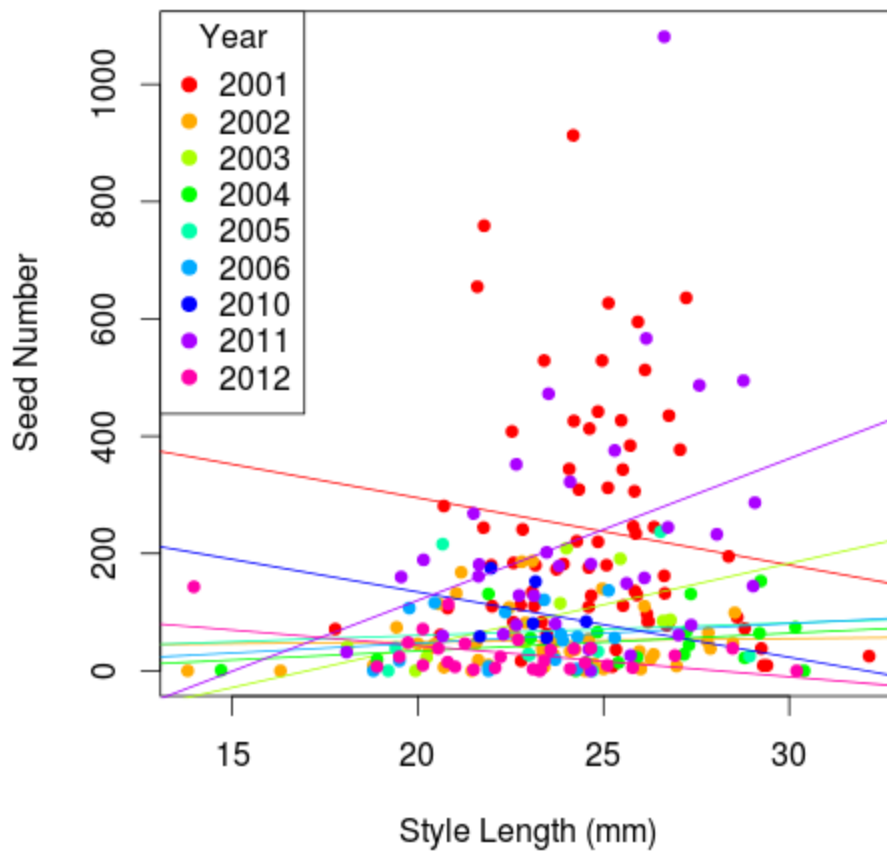


Figure 5. Reproductive fitness as a function of style length for all studied plants.

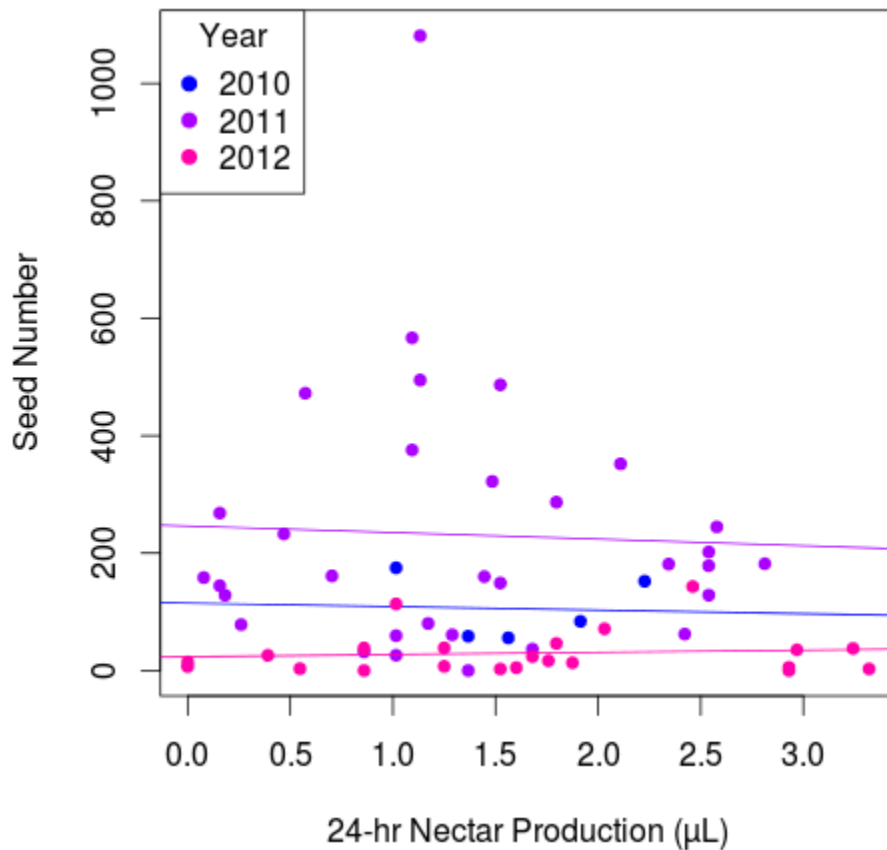


Figure 6. Reproductive fitness as a function of nectar production for flowers from the 2007 and 2008 plantings.

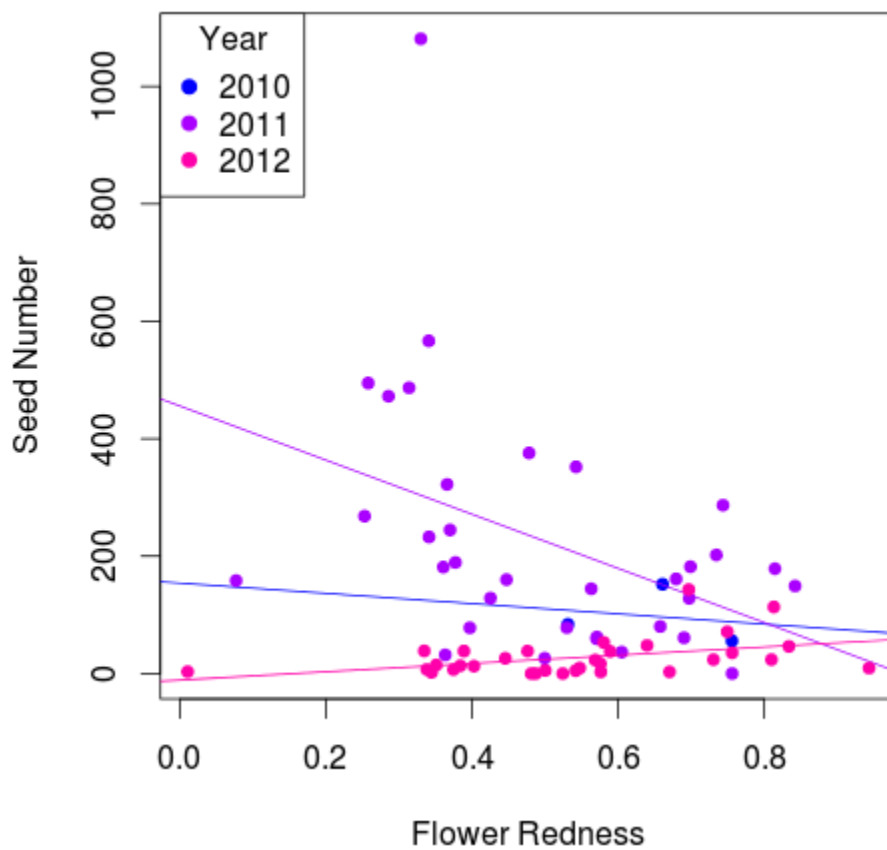


Figure 7. Reproductive fitness as a function of flower redness for individuals from the 2007 and 2008 plantings.

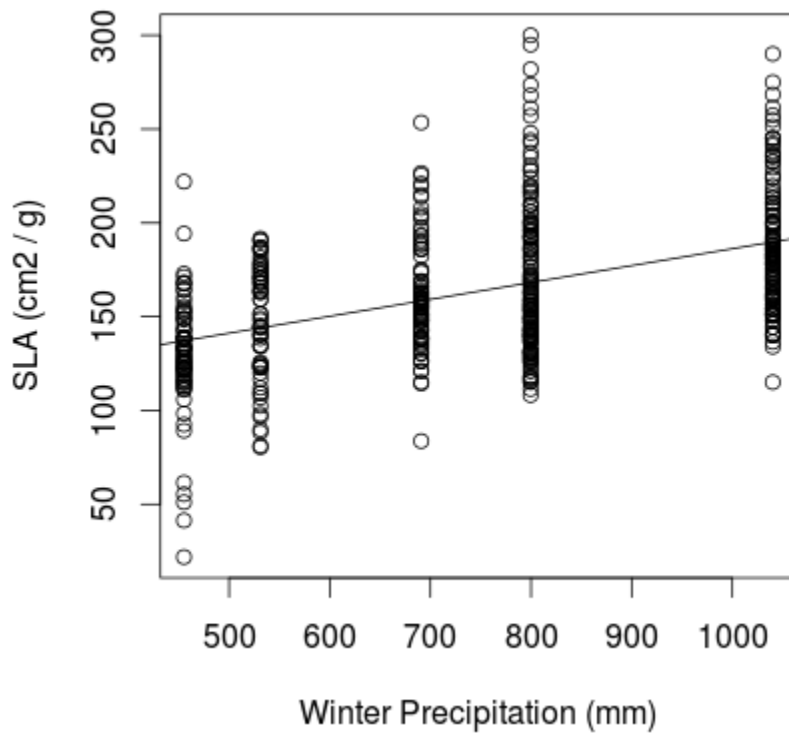


Figure 8. Plastic response of SLA to winter precipitation across 5 years.