

Analyzing the effect of climate change on *Boechera stricta* seed germination and fitness along an elevational gradient

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Abstract

Climate change alters many biotic and abiotic factors in environments around the world. At higher elevations in particular, climate change brings warmer mean temperatures, reduced snowpack, earlier snowmelt, and more extreme drought. In this study, we analyzed the effect of early snow removal on *Boechea stricta* seed germination and establishment along an elevational gradient. We predicted that climate change would alter long-standing patterns of local adaptation by favoring seeds from hot and dry low elevation populations (whose current climate reflects the future climate in higher elevation locations). In 2014, the Anderson lab planted *B. stricta* seeds with 200 distinct genotypes into five common gardens at differing elevations (2530m to 3340m) in Gothic Valley in Colorado and subjected half of the seeds to an early snow removal treatment. We found the lowest germination and establishment success at the most stressful site (2530m) where hot and dry conditions severely restricted seed germination. Local adaptation was more apparent in the mid-elevation gardens (2710m, 2890m) where seeds from lower elevation families had a higher probability of germination than those from higher elevations. At one of the mid-elevation sites (2890m), the snow removal treatment eliminated evidence for local adaptation. Local adaptation was also evident in a higher elevation garden (3133m) such that germination success actually increased with source elevation under control treatments. However, as predicted, snow removal completely disrupted local adaptation there, favoring lower elevation genotypes. In the current experiment, we found no evidence for local adaptation in the highest garden (3340m). From these data we can see that germination success and fitness are influenced in different ways and to differing degrees based on their environment.

Introduction

Climate change has widespread effects on the ecosystems and organisms of the world. In alpine ecosystems in particular, climate change has profound effects on the biota (Walther et al. 2002, Parmesan 2006). We have already witnessed increases in surface air temperatures and they are projected to continue to increase as the century progresses (Rangwala et al. 2012). Warmer winters cause precipitation to fall more as rain instead of snow, contributing to a reduced snowpack at high elevations and latitudes (Knowles, Dettinger, and Cayan 2006). Climate change is also advancing the timing of annual snowmelt, in turn changing the volume and timing of stream runoff and local water availability (Stewart, Cayan, and Dettinger 2004, Knowles, Dettinger, and Cayan 2006). These changes in water supply, along with increased evapotranspiration associated with warming temperatures during the growing season, result in more severe and widespread droughts throughout the region (Rangwala et al. 2012).

In response to changing climates, plants can migrate to higher latitudes or elevations, alter their phenotypic plasticity, or possibly adapt to novel selection (Byars, Papst, and Hoffman 2007). A number of studies have provided evidence that organisms can alter their geographic ranges to remain within their tolerance zones (Pauli, Gottfried, and Grabherr 1996). For example, in a recent meta-analysis, species distributions shifted at a median rate of 16.9 km per decade to higher latitudes and 11.0 meters per decade to higher elevations (Chen et al. 2011). While range shifts are fairly well understood, very few studies to-date have demonstrated adaptive responses to climate change, but a recent study showed that *Brassica rapa* has the ability to rapidly adapt to its environment, altering its flowering phenology in response to a period of extreme drought (Franks, Sim, and Weis

2007). It is important to understand plants' ability to adapt to their environment so that we can predict how their survival and reproduction will be affected by climate change or other disturbances.

This study aimed to analyze the effect of climate change on local adaptation in montane ecosystems, specifically through seed germination success and subsequent establishment. I studied *Boechnera stricta*, a subalpine perennial forb of the Brassicaceae family with a wide natural geographic range that spans the Rocky Mountains and covers elevations from less than 1500m to 3700m (Anderson et al. 2015). *Boechnera stricta* primarily self-fertilizes, and has the ability to adapt to environmental differences across short geographic areas (Anderson et al 2015). Since the 1970s, the timing of flower of *B. stricta* has advanced approximately 13 days, likely due to increasing temperatures and earlier snowmelt (Anderson et al. 2012). This current study manipulated the timing of snowmelt in an evolutionary framework in gardens at varying elevations. We analyzed critical early life history components of fitness (seed germination success and establishment) as a function of source elevation, treatment, and their interactions to test: (1) whether natural populations are adapted to local environments across a complex climatic gradient, (2) whether climate change could reduce fitness in the short term, and (3) whether climate change might disrupt local adaptation, instead favoring families from hot and dry low elevation locations.

Methods

Study Location

Over the course of the past three years, the Anderson lab established five common gardens in close proximity to the Rocky Mountain Biological Laboratory (RMBL) in Gothic,

Colorado. The gardens span a range of elevations (2530m, 2710m, 2890m, 3133m, 3340m) and vary in temperature, soil moisture, growing season length, and timing of snowmelt (Fig. 1). All gardens are within the geographic and elevational range of *B. stricta* and there are robust natural populations of *B. stricta* at each garden site.

Manipulation

In the fall of 2014, the Anderson lab planted 19,752 seeds from approximately 200 maternal families into the gardens. The families came from a variety of *B. stricta* populations of differing elevations located near RMBL.

Each seed was planted in a 1cm x 1cm x 1cm cell within a seed grid filled with Colorado compost potting soil. Seeds from different maternal families were randomized in the seed grid to control for microclimate effects from soil, precipitation, and competitors. Seeds were buried approximated 5mm deep. Within each seed grid, some cells were left unplanted to measure possible natural seed bank contamination.

Each year, in the late winter (March-May) the Anderson lab removed snow from half of the plots at each garden to mimic the effects of early snowmelt induced by climate change. When the snowpack was 1 m high, the snow was shoveled to a height of 10 cm. There was a 0.5 m buffer around each plot.

Measurements

For each of the five gardens, the Anderson lab recorded the date of snow shoveling of experimental plots and the date of complete snowmelt. In 2016, they installed two all-weather cameras at each garden that take photos every four hours to document the progression of snowmelt throughout the season.

Once the snow completely melted from the gardens, Anderson and students censused the seed grids at each garden for germination (first year only), growth, and reproduction, collecting data from each seed grid approximately 2 times a week.

Analysis

We analyzed the data using the statistical program, SAS (ver. 9.2), to determine if there are significant differences in the germination success and establishment of seeds based on treatment (control vs. snow removal) and elevation of the original seed family population, with all 2- and 3-way interactions among treatment, source elevation, and garden. The dependent variables we analyzed are germination success and seed establishment. We analyzed germination success and establishment at the family level, compiling data about seeds from the same treatment and family within a garden. We determined probability of germination as the number of germinants divided by the number of seeds planted for each family and each treatment at each garden. Probability of establishment is the number of germinants that survived the growing season divided by the number of seed planted for each family and treatment at each garden.

Results

At Estess (2530m), our lowest elevation site, we found overall low germination success for all seeds, regardless of elevation of origin or treatment (Fig. 2). Neither elevation, nor treatment had a significant effect on probability of germination (Table 2). Seed establishment was also uniformly low at Estess (Fig. 3).

In our Peanut Mine garden (2710m) we saw that, regardless of treatment, seeds from low elevation families had significantly higher germination success than those from

higher elevations ($p=0.0003$) (Fig. 4, Table 3). Treatment was not significant (Table 3). In terms of seed establishment, establishment was generally low, with slight negative linear slopes (Fig. 5).

In our mid-elevation Gothic garden (2890m), there was a significant difference in germination success based on the treatment ($p<0.0001$), source elevation ($p=0.0423$), and their interaction ($p=0.0008$) (Table 4). In the control plots, seeds from low elevation families had higher germination success than those from high elevations, but in snow removal plots family elevation had no effect on germination (Fig. 6). Seed establishment in the Gothic garden had similar trends to germination success (Fig. 7).

In our Schofield garden (3133m), seed germination was not significantly affected by elevation or treatment (Table 5, Fig. 8). However, seed establishment was significantly affected by both treatment ($p=0.0037$) and elevation by treatment ($p=0.0099$) (Table 6). Elevation alone had no significant affect on establishment (Table 6, Fig. 9).

At our highest elevation site, North Pole garden (3340m), we found that neither seed germination nor establishment was significantly affected by elevation or treatment (Table 7, Table 8).

Discussion

We found evidence at multiple gardens that natural populations are locally adapted to contemporary conditions at the seed to seedling transition. In addition, two mid elevation gardens (2890m and 3133m) showed strong evidence for local maladaptation in the context of climate change, as snow removal either eliminated or completely altered patterns of adaptation. In the lowest elevation garden (2530m), the low germination and establishment across all seeds, regardless of family or treatment, suggests that harsh

abiotic conditions may restrict germination success (Fig. 2, Fig. 3). Warm and dry climatic conditions resulted in high mortality, which could have eliminated evidence for any trends. It might also be a sign that climate change is already altering the Estess garden environment such that seeds originally from similar elevations to Estess have not had time to adapt to the warmer, drier climate.

At Peanut Mine (2710m), the increased germination and establishment in seeds from mid and low elevation families provides evidence for both local adaptation and local maladaptation (Fig. 4, Fig. 5). Seeds from the same elevation as Peanut Mine do well at the garden in both the experiment and control plots, suggesting that local adaptation is at play. Seeds from elevations even lower than Peanut Mine do even better than local seeds, providing evidence that there is local maladaptation where seeds from lower, drier environments do better than local seeds due to climate change.

At our middle elevation garden (2890m), we again saw evidence for local adaptation and local maladaptation in the control plots – low elevation families tended to have higher germination success than seeds from high elevation families. Once again, the negative linear trend seen in seed germination with source elevation might be an indicator that climate change is already warming the garden so that its current environment resembles a climate similar to that of historically lower elevations. The snow removal plots in the Gothic garden had a uniformly low level of germination success across seeds from all source elevations, suggesting that reduced snowpack could have resulted in higher water stress and high mortality, eliminating any evidence of local adaptation.

At our Schofield garden (3133m), neither of our variables affected germination success, while treatment significantly influenced seed establishment and we found

significant elevation by treatment interactions (Fig. 8). Under control conditions, establishment success increased with source elevation, whereas the opposite pattern emerged in the snow removal treatment (Fig. 9). The trends in establishment we saw provide evidence for local adaptation since seeds from lower elevations that have adapted to longer growing seasons and drier conditions did better in snow removal plots, while seeds from higher source elevations did better in control plots.

Seed germination and establishment at the highest elevation site (3340m) was not affected by treatment or source elevation (Fig. 10, Fig. 11). Despite the lack of statistical significance, seeds in snow removal plots generally had higher germination and higher establishment than seeds in control plots, likely due to the increased growing season and conditions that resembled lower-elevation environments.

In conclusion, the germination and establishment success of *B. stricta* seedlings from varying source elevations under differing treatments provides insight into the effects of climate change on local adaptation. At a number of gardens we saw higher germination and establishment among local seeds in control plots, offering evidence for local adaptation under contemporary conditions. In other cases, the snow removal treatment disrupted the local adaptation that was seen in control plots, instead favoring seeds from lower source elevations or entirely removing any effect of source elevation.

As climate change continues to progress, it is important to continue researching its impact on plant fitness, particularly in early life stages. Further analysis of the data collected in this study, including growth rate, herbivory, reproductive success, and soil temperature and moisture data, will contribute to a broader understanding of local adaptation and fitness in this study system.

Literature Cited

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Tables and Figures

Table 1: P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	1969	5.34	0.0209
Treatment	1	1969	0.17	0.6772
Elevation*Treatment	1	1969	0.02	0.8996
Garden	4	1969	5.79	0.0001
Elevation*Garden	4	1969	7.20	<0.0001
Treatment*Garden	4	1969	5.79	0.0001
Elevation*Treatment*Garden	4	1969	3.91	0.0036

Table 2: Estess Garden P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	201	0.19	0.6639
Treatment	1	201	0.13	0.7180
Elevation*Treatment	1	201	0.05	0.8284

Table 3: Peanut Mine Garden P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	347	13.56	0.0003
Treatment	1	347	3.25	0.0723
Elevation*Treatment	1	347	2.09	0.1492

Table 4: Gothic Garden P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	201	4.18	0.0423
Treatment	1	201	17.15	<0.0001
Elevation*Treatment	1	201	11.63	0.0008

Table 5: Schofield Garden P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	351	1.44	0.2311
Treatment	1	351	3.41	0.0658
Elevation*Treatment	1	351	2.68	0.1027

Table 6: Schofield Garden P values for family level seed establishment ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	351	0.17	0.6807
Treatment	1	351	8.54	0.0037
Elevation*Treatment	1	351	6.73	0.0099

Table 7: North Pole Garden P values for family level seed germination success ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	352	1.72	0.1907
Treatment	1	352	0.11	0.7348
Elevation*Treatment	1	352	0.14	0.7040

Table 8: North Pole Garden P values for family level seed establishment ANOVA using linear model

Effect	DF	DF	F Value	Pr > F
Elevation	1	352	2.13	0.1455
Treatment	1	352	0.00	0.9874
Elevation*Treatment	1	352	0.57	0.4509

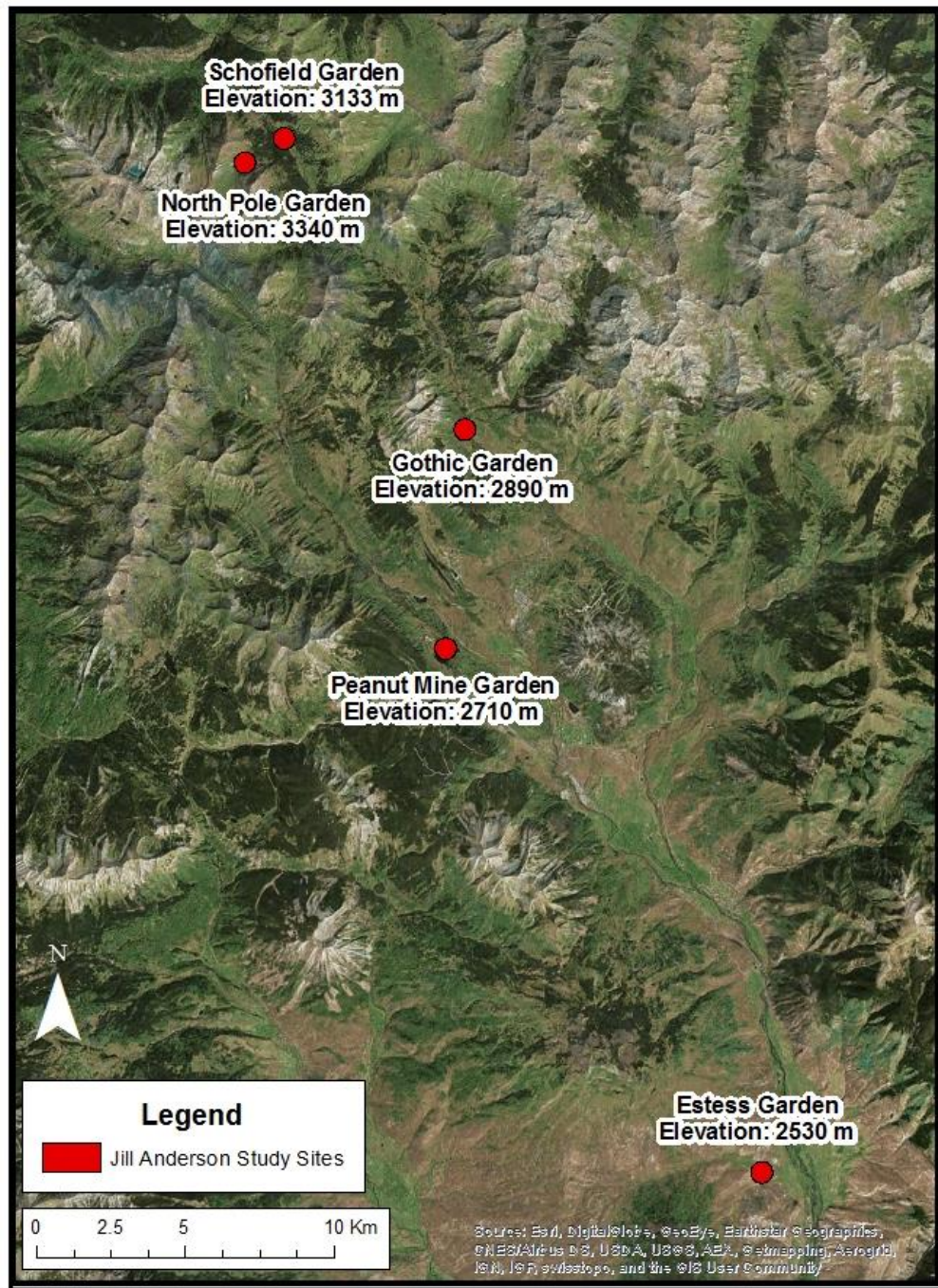


Figure 1: Jill Anderson study sites near Gothic, Colorado. At each elevational site (2530m, 2710m, 2890m, 3133m, 3340m) there is a common garden with experimental and control seed grids.

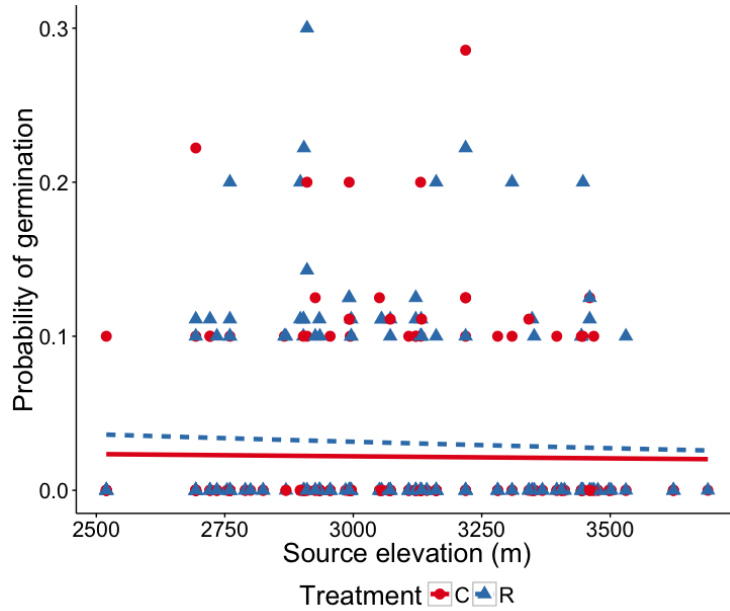


Figure 2: Estess garden (2530m) seed germination success by source elevation using family-level data. Probability of germination is calculated by $\text{\#germinants}/\text{\#seeds}$ planted for each genotypic family. Neither elevation ($p=0.6639$) nor treatment ($p=0.7180$) significantly affected probability of germination.

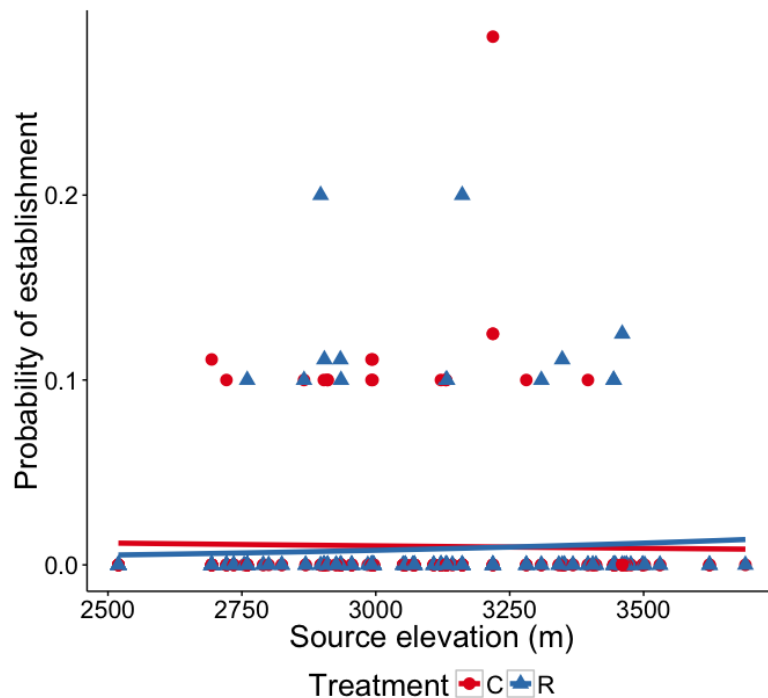


Figure 3: Estess garden (2530m) seed establishment by source elevation using family-level data. Probability of establishment is calculated by $\text{\#survived germinants at end of the season}/\text{\#seeds}$ planted for each genotypic family.

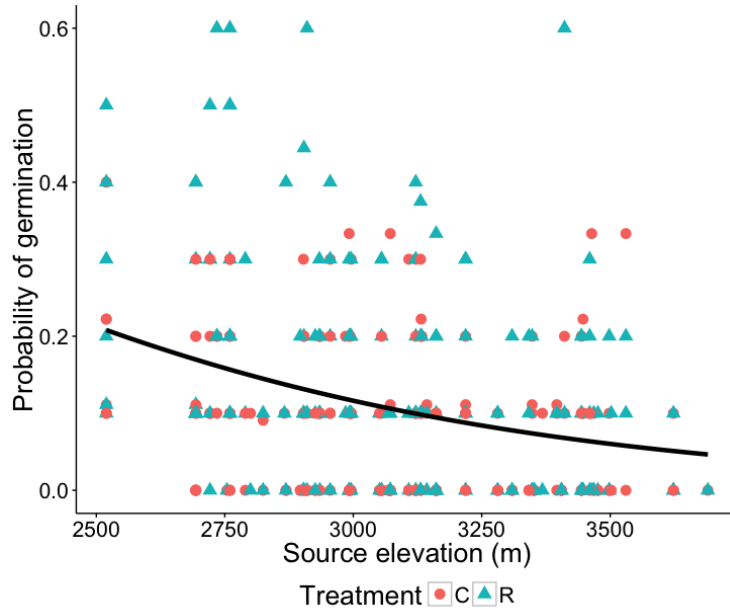


Figure 4: Peanut Mine garden (2710m) seed germination success by source elevation using family-level data. Probability of germination is calculated by #germinants/#seeds planted for each genotypic family. Elevation significantly affected probability of germination ($p=0.0003$). Treatment did not significantly affect probability of germination ($p=0.0723$).

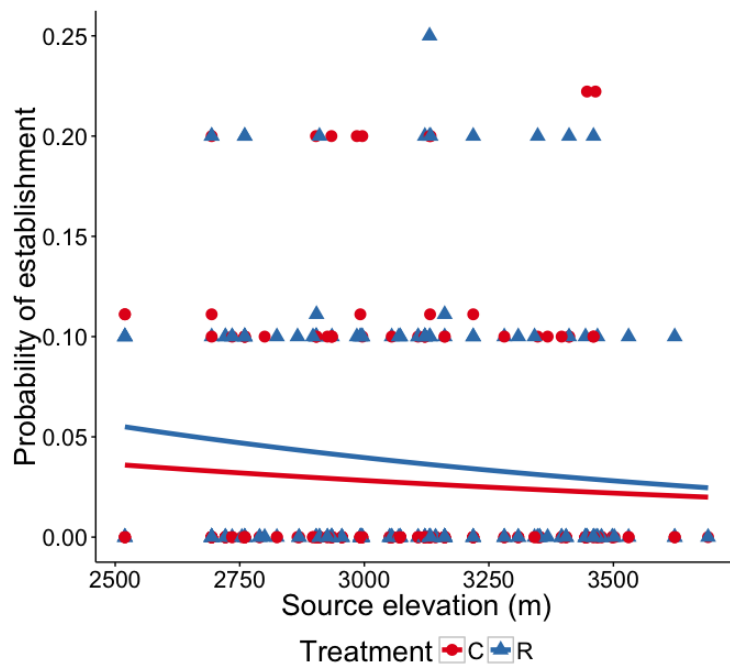


Figure 5: Peanut Mine garden (2710m) seed establishment by source elevation using family-level data. Probability of establishment is calculated by #survived germinants at end of the season/#seeds planted for each genotypic family.

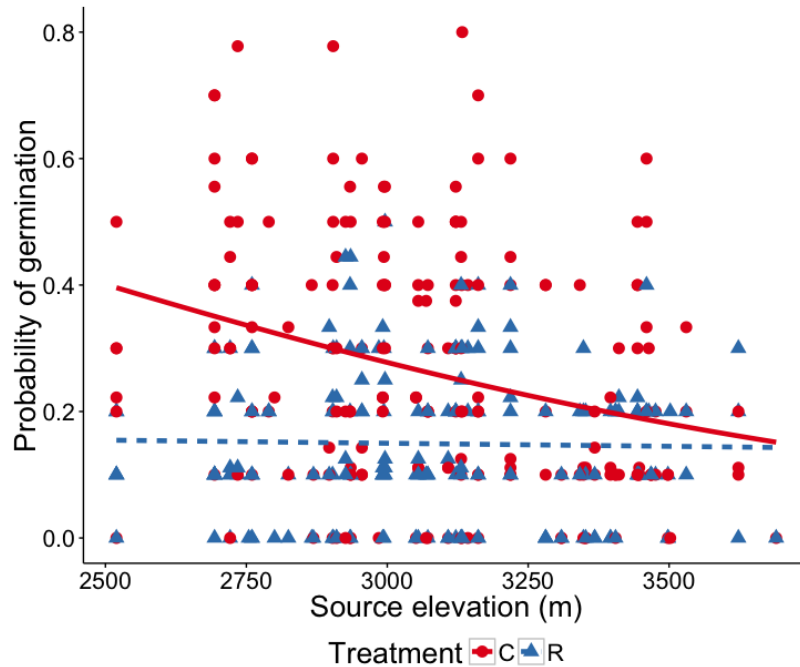


Figure 6: Gothic garden (2890m) seed germination success by source elevation using family-level data. Probability of germination is calculated by $\# \text{germinants} / \# \text{seeds planted}$ for each genotypic family. Elevation significantly affected probability of germination ($p=0.0423$). Treatment significantly affected probability of germination ($p<0.0001$).

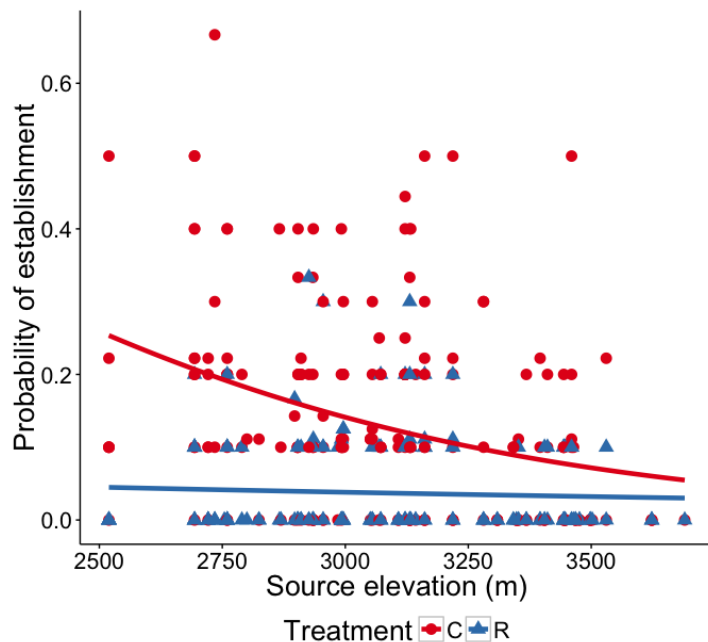


Figure 7: Gothic garden (2890m) seed establishment by source elevation using family-level data. Probability of establishment is calculated by $\# \text{survived germinants at end of the season} / \# \text{seeds planted}$ for each genotypic family.

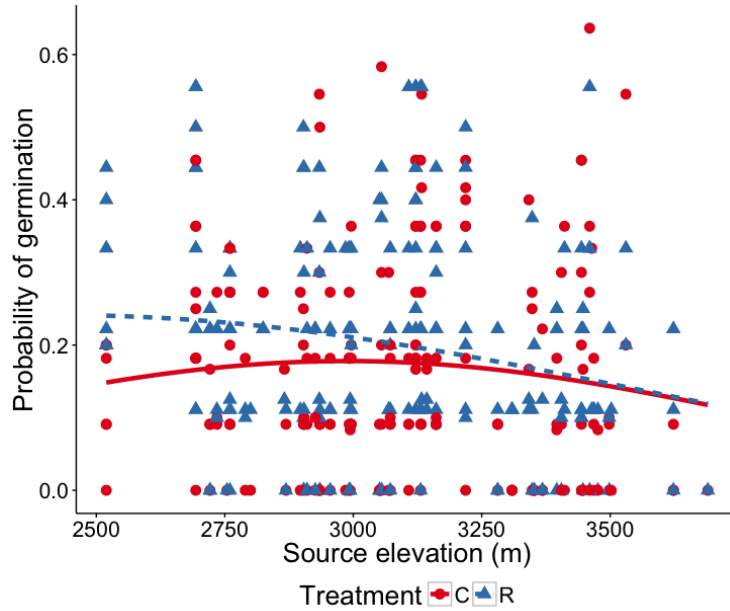


Figure 8: Schofield garden (3133m) seed germination success by source elevation using family-level data. Probability of germination is calculated by $\#germinants/\#seeds$ planted for each genotypic family. Neither elevation ($p=0.2311$) nor treatment ($p=0.0658$) significantly affected probability of germination.

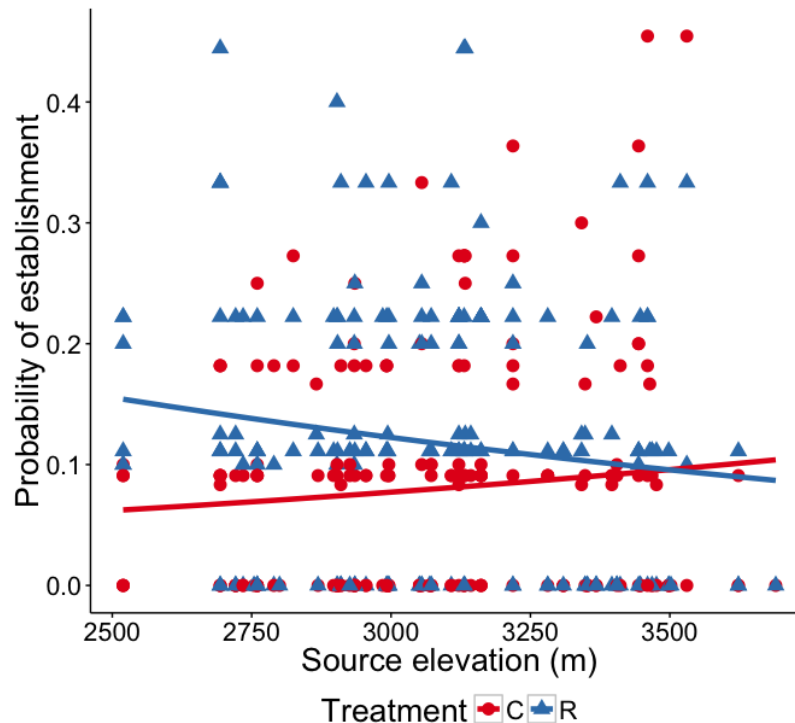


Figure 9: Schofield garden (3133m) seed establishment by source elevation using family-level data. Probability of establishment is calculated by $\#survived\ germinants$ at end of the season/ $\#seeds$ planted for each genotypic family. Elevation did not significantly affect probability of establishment ($p=0.6807$), but treatment was significant ($p=0.0037$).

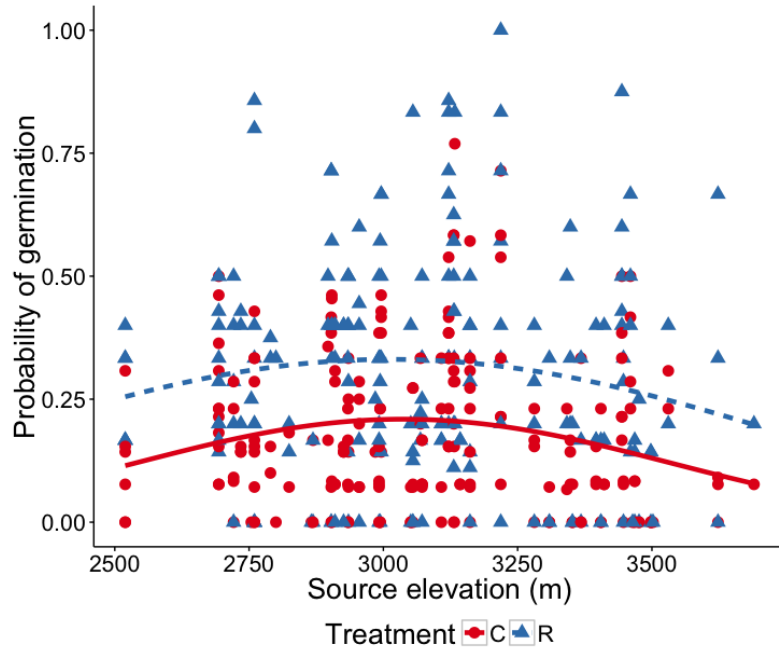


Figure 10: North Pole garden (3340m) seed germination success by source elevation using family-level data. Probability of germination is calculated by $\#germinants/\#seeds$ planted for each genotypic family. Neither elevation ($p=0.1907$) nor treatment ($p=0.7348$) significantly affected probability of germination.

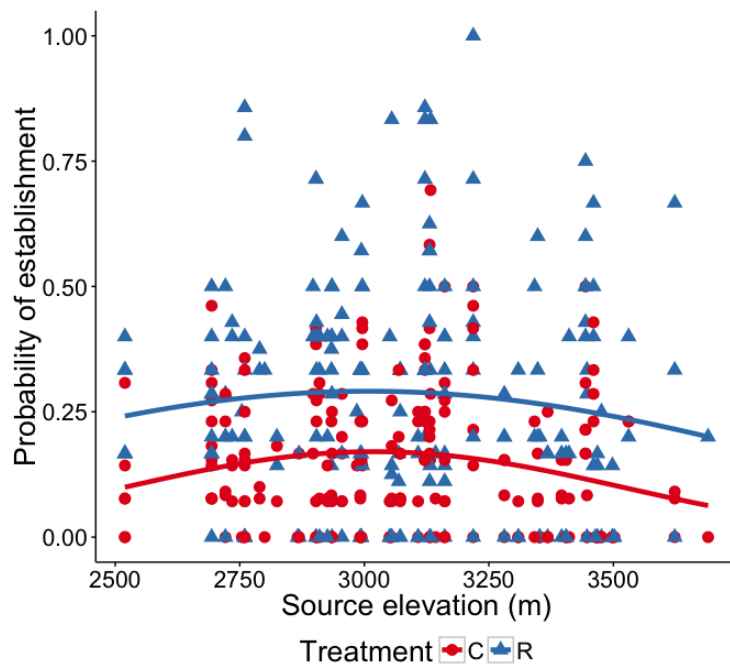


Figure 11: North Pole garden (3340m) seed establishment by source elevation using family-level data. Probability of establishment is calculated by $\#survived\ germinants$ at end of the season/ $\#seeds$ planted for each genotypic family. Neither elevation ($p=0.1455$) nor treatment ($p=0.9874$) significantly affected probability of establishment.