

Trait Variations of *Boechera stricta* Along an Elevation Gradient

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Abstract

Plants have been migrating into higher elevations in order to cope with the stresses of the changing climate. However, not all plants are capable of migrating as far or as fast as others. This study examines leaf traits of *Boechera stricta*, a plant with limited migratory capabilities, at different elevations. Stomatal density, water content, and leaf mass per unit area (inverse of specific leaf area) of the collected rosette leaves will be determined in order to understand how these plants are changing their phenotypes to cope with the changing climate. We predict that these physical leaf traits will vary with elevation due to water stress and other biotic and abiotic factors.

Introduction

Global temperatures have been rising rapidly, with earth predicted to become warmer than it's been in the past million years (Thomas et al. 2004) and the most dramatic changes being noted at higher northern latitudes (Nufio 2010). This rapid change in climate could be a major contributing factor to the future endangering and possible extinction of species (Thomas et al. 2004). One of the most significant effects of the changing climate on plants and animals is the alteration of their phenology (Nufio 2010) and habitat range shifts. Overall, species have been observed to be moving up in altitude, or towards, the poles, at a rate of about 6.1km per decade (Parmesan 2003). Seasonal events such flowering time and date of first emergence of insects and animals have been occurring earlier than previously recorded in decades (Nufio 2010). Spring events in the Northern hemisphere have advanced by 1-3 days per decade, most likely due to the fact that timing of snow melt has also advanced in many regions in the Northern hemisphere (Bertin 2008). It seems a key element for plants to maintain biodiversity and ecosystem functioning is their ability to migrate into cooler environments; however, plants are limited by seed dispersal and it has been shown that most plant species will not be able to completely keep up with the projected necessary range shifts brought on by climate change (Cunze 2013). A study showing this limited migratory expansion were done by examining plants that disperse seeds by wind or by mammals, although there are plants that do not use either means to disperse seeds. Plants whose main method of seed dispersal is dropping them directly on the ground around them have small migratory capabilities and are therefore at a greater risk of becoming threatened or extinct (Nielson et al. 2005).

In this study, we surveyed traits of *Boechera stricta*, a plant whose only means of seed dispersal is letting them fall directly to the ground it. We measured the variations in phenotypic leaf traits of *B. stricta* along an elevation gradient, and therefore different climates. The traits we examined are leaf mass per unit area (the inverse of specific leaf area), water content, and stomatal

density. Changes in phenology, such as these, can be good indicators of climate change responses as many biological traits, like flowering time, emergence of species, and migration of species, partly depend on certain weather patterns (Bertin 2008). A positive correlation between elevation and stomatal density is predicted because plants are usually more water stressed in hotter, lower elevations, and thus produce less stomata to prevent desiccation. A previous study done at the Rocky Mountain Biological Laboratory used *Ipomopsis* to show that specific leaf area increases with elevation (Crowell, Unpublished), which reflects the amount of nutrients the plant is taking in (Wilson et al. 1999). The inverse of specific leaf area is leaf mass per unit area, thus, we predict that leaf mass per unit area should decrease with elevation. Another study also shows that phenotypic plasticity did in fact increase with latitude (Molina-Montenegro and Naya, 2012); therefore, we are expecting significant results showing phenotypic leaf trait variations along an elevation gradient. Moreover, we also examined leaves from *Boechera* plants in snow removal plots set up by Zak Gezon at the Rocky Mountain Biological Laboratory. Snow removal in these plots simulates early snow melt caused by rising climate change. Variance in these traits along an elevation gradient will help us understand how plants with short range seed dispersal and migratory capabilities are coping and evolving in response to the changing climate. Furthermore, it will aid in our understanding of how rising temperatures are threatening species and biodiversity and help guide future conservation efforts.

Methods

Study System

Boechera stricta, also known as Drummond's Rockcress, is a short lived perennial, native to the Rocky Mountains. It occupies diverse habitat ranges such as deserts, alpine meadows, montane forests, rocky hillsides, and moist grasslands (Rushworth et al. 2011). Its ability to grow in disturbed areas makes it easy to find along trails and among rocky scree. *Boechera* is a highly self pollinating plant, reproducing primarily asexually about 89% of the time (Song et al. 2006). When its flowers have been pollinated, *B. stricta* produces long and skinny siliques, or seed pods. Once mature, the siliques dry out and eventually break apart, releasing the seeds to the area immediately around them. For this reason, it is unlikely that the seeds will migrate into higher elevations as fast as seeds from other plants. Lastly, the rosette leaves begin to senesce, or dry out and fall off, after the plant has been pollinated and is done producing more flowers for reproduction.

Samples were taken from 28 populations at different elevations ranging from 2700m to 3500m. The populations were named after the description of where they were found and show the variety in their habitat range (Table 1). *Boechera* populations sampled were chosen haphazardly by

searching for rosettes or bolting *Boechera* plants in areas where *Boechera* is commonly found. The minimum number of plants sampled in any given population was four and the maximum number of plants sampled was 35. Such a wide range of samples taken from each population was due to the fact that at the time of this study most plants were done reproducing, therefore, the rosette leaves had already begun to senesce, leaving few plants with leaves viable enough to sample. After finding a population, plants to sample were chosen based on their distances from each other, in order to ensure not just one family was being sampled. Since siliques drop seeds immediately around them, causing very limited seed dispersal, a distance of 5 meters between each plant sampled was ample space. A previous study showed that seeds from siliques travel, at most, about a half meter away from the plant (Bloom 2002). From each plant, we took any viable rosette leaves; the rounder leaves at the base of the plant, not on the bolting part of the plant. Rosette leaves were chosen as opposed to bolt leaves in order to coincide with another study on *Boechera stricta*, being conducted by Jill T. Anderson from the University of South Carolina.

The snow removal plot was at an elevation of 3000m. There were 23 different plots with some randomly chosen to be controls and others as early snow removal manipulations. *Boechera* was found to be naturally occurring in these plots, thus, each plot was surveyed for plants to use in this study. Each sample taken was labeled as being part of the control group or experimental group. The same protocol for leaf trait measurements was taken for these samples.

Trait Measurements

Each set of rosette leaf samples were scanned and processed using ImageJ (NIH freeware) to calculate leaf area. Leaf samples were subsequently oven dried at 50°C over a period of 48 hours and weighed with a balance to the nearest .00001g. Leaf mass per unit area (LMA) was calculated as the ratio of leaf mass over leaf area (leaf mass in grams/ leaf area in cm-squared). Before oven drying the leaf samples, their fresh weight was obtained to calculate the water content of the leaves by subtracting the dry weight from the fresh weight. To help ensure the best measure of fresh weight, the leaf samples were kept in a cooler to slow desiccation and processed as soon as possible. One leaf from about half of each plant population was analyzed for stomatal density. Stomatal density was determined by making slides of nail polish imprints from the underside of the leaves. The slides were sent to the Anderson Lab in South Carolina and analyzed under a high quality microscope to calculate stomatal density, measured as the amount of stomata per unit area. All trait measurements were taken according to techniques previously used in *Boechera* research by Jill T. Anderson.

Statistical Analyses

In order to determine if there was any significant variation along an elevation gradient and in

the snow removal plots, we conducted three separate single variable analyses for stomatal density, leaf mass per unit area, and leaf water content. To account for similarity between individuals in the same population we regressed trait values on elevation and included random effect for population for each analysis (Proc Mixed, SAS ver. 9.3).

Results

For *Boechera* populations along an elevation gradient, there was no statistically significant relationship between stomatal density and elevation ($F_{1, 197}=3.3$, $p=0.07$). However, not all stomatal density slides have been processed and there is a non-significant trend suggesting a positive correlation between stomatal density and elevation. The relationship between leaf water content and elevation also shows no significant pattern between the two ($F_{1, 291}=0.09$, $p=0.76$). Leaf mass per unit area has yielded the most significant results thus far, indicating LMA decreases with elevation ($F_{1, 297}=4.11$, $p=0.0435$).

On the other hand, leaf mass per unit area in the snow removal plots was only marginally significant ($F_{1, 83}=3.68$, $p=0.0586$). Leaf water content yielded the most significant results with leaves from plants in the control treatment group having significantly greater water content than those in the snow removal treatment group ($F_{1,83}=5.45$, $p=0.022$). Stomatal density was also greater in leaves from the control treatment group than the snow removal treatment group ($F_{1, 29}=5.36$, $p=0.0279$).

Discussion

Species have been changing their phenotypes via plasticity in order to respond quickly to the even more rapid change in global temperatures (Anderson et al. 2012). Ecosystems have already been documented to be responding to climate change by moving upwards in range as well as changing their phenology (Kujala et al. 2013). Our results show a clear response to these rising temperatures in certain regions of the Rocky Mountains. As predicted, leaf mass per unit area decreased with elevation in natural *Boechera* populations (Figure 1). In other words, leaves were found to be thicker at lower elevations and thinner at high elevations.

Figures

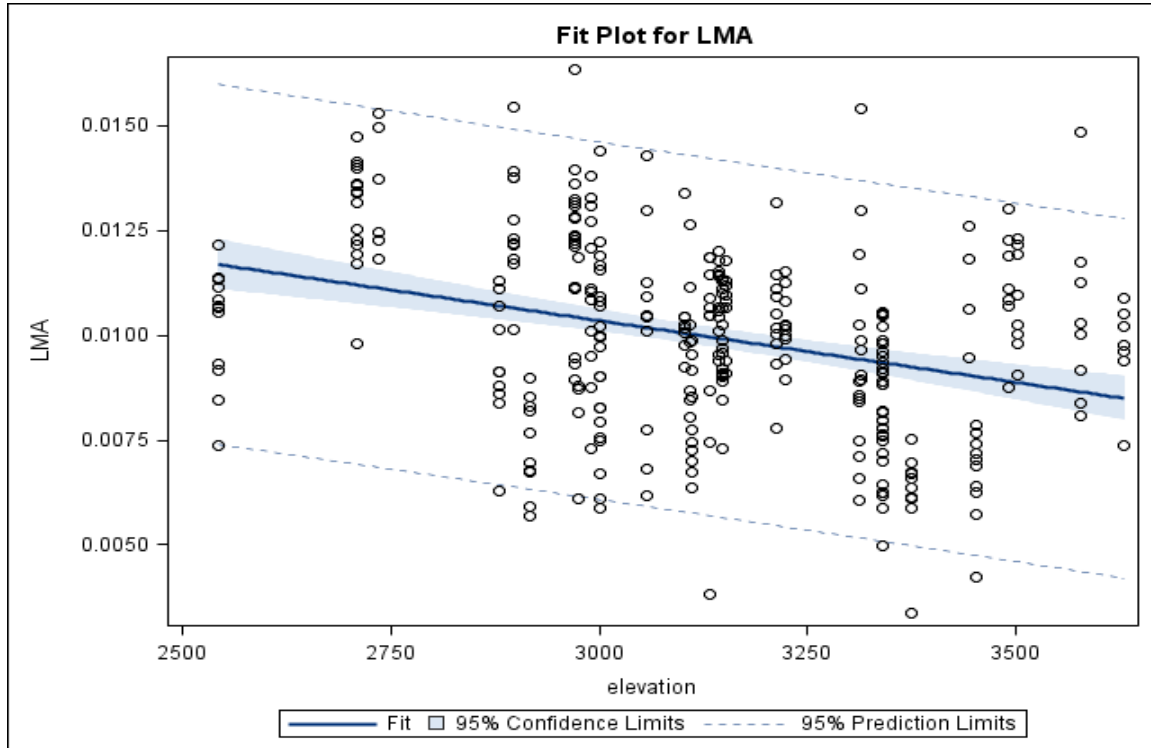


Figure 1. Fit plot for Leaf mass per unit area. A significant trend indicating leaf thickness decreased with elevation.

Figure 2. Comparison of leaf water content in snow removal plots. The control group leaves held a significantly greater amount of water than the snow removal plot leaves.

Figure 3. Comparison of stomatal densities for snow removal plots. Stomatal density was significantly greater for the control group than the snow removal group.

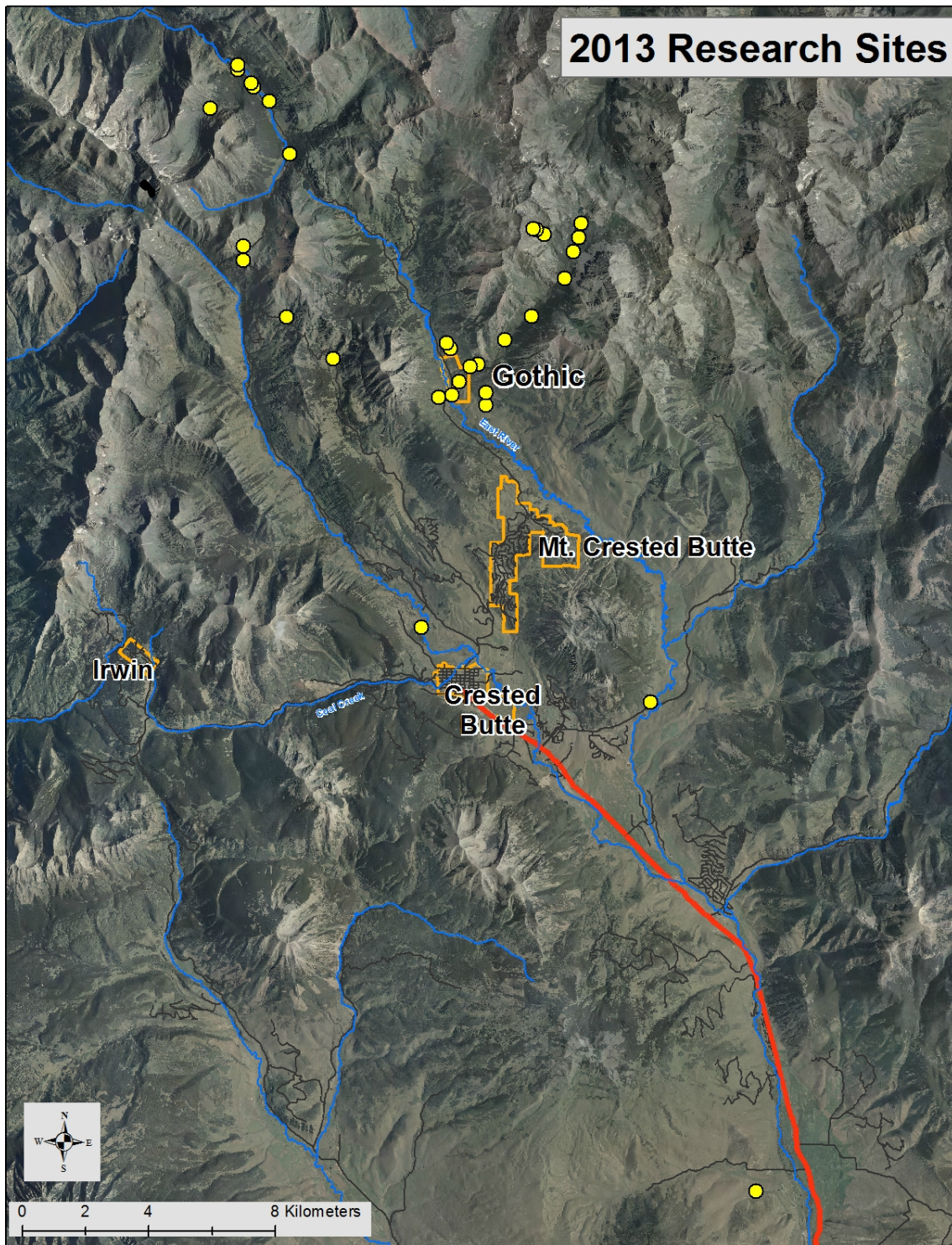
Figure 4. Comparison of LMA for snow removal plots. *Boechera* leaves from the control group were only marginally significantly thicker than leaves from the snow removal group; which is opposite than

predicted.

Tables

Population Identification	Elevation (m)
317 Outcrop	3223
317 Across fall	3103
317 Rock slope	3108
317 Knoll	3143
317 Hillside	3147
Cinnamon Mtn Lake	3443
811 Weather station	3151
Cinnamon GV 254	3490
811 stream	3314
Transect 6	3133
Gothic Valley Natural Population	2881
Brush Creek	2735
North Pole Basin	3340
Estess	2543
Sylvanite Camp	3501
Sylvanite Bluff	3578
Sylvanite Wool	3629
Zak's Meadow	3000
Copper Creek Trail	2991
Judd Falls	2976
Marriage Meadow	3000
Copper Creek Trail Clearing	3212
Copper Creek Trail Triangle Path	3374
Copper Creek Trail Avalanche Meadow	3111
Copper Creek Trail First Willows	3057
Copper Creek Trail Log Path	3453
Copper Creek Trail Climb Right	3311
Peanut Mine	2710
Sunflower Meadow	2897
Above Research Meadow	2916
Sagebrush Slope	2971
Deer Creek Trail First Bend	2884

Table 1. Boechera Populations and their corresponding elevations.



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