Trichome density differences in *Boechera stricta* across an elevational gradient

Student: Katherine Soto
Mentor: Jill T. Anderson

NSF REU- Full Time Independent Research
Summer 2014

Abstract

Insect herbivores consume plant tissue, reducing plant fitness in the process. In response, plants have evolved a variety of mechanical and chemical defenses to protect themselves. This paper focuses on trichomes, which are hair-like appendages that extend from leaf tissues, and plants use them as a defense mechanism against herbivores as well as a way to reduce evapotranspiration. Over the course of the study, we collected rosette leaves from experimental *Boechera stricta* plants located in five common gardens. These gardens are located across an elevational gradient of 2530 m - 3430m, and contain a total of 8,200 plants with 120 genotypes. In each garden, half of the siblings of each family experienced early snow removal, whereas the other half experienced natural snowmelt. We found significant genetic variation in trichome density in the lower elevation gardens. Similarly, trichome density varied with garden, suggesting that environment influences trait expression. Finally, we found that across all gardens, low elevation genotypes had much higher trichome density than high elevation genotypes.

Introduction

Insect herbivores consume various plant tissues, in the process severely reducing plant fitness and imposing strong selection on plant populations (Prasad *et al.*, 2012). Plants protect themselves against herbivores via morphological structures such as trichomes (Agren *et al*., 1993) as well as a suite of secondary compounds (Prasad *et al.*, 2012). The focus of this study was on the genetic and environmental variation of trichome density in *Boechera stricta* across an elevational gradient. Trichomes are hair-like appendages which extend from the epidermis of aerial tissues and occur in a variety of types (Levin, 1973). Leaf trichomes reduce damage done

by insect herbivores, but not all insect herbivores are deterred by leaf trichomes (Løe et al., 2007). Herbivore damage and trichome-producing plants are both shown to be negatively related to altitude in some systems (Løe et al., 2007) yet we do not know whether this variation is due to environmental differences across elevations, genetic variation in trichome density, or both. Trait variation along an elevational gradient can be due to genetics, the environment, or both (Byars, 2007).

Climate change models predict increased mean annual temperatures for the next hundred years (IPCC, 2007). This change in mean annual temperature over a short period of time may radically amplify existing pressures, such as herbivore damage (Rassman *et al.* 2014). Insects exhibit some of the strongest responses to climate change because they are ectothermic, thermal changes therefore affect their development, reproduction and survival (Bale *et al*. 2002). Potential responses of insects due to climate change include range expansions and contractions, and altitudinal shifts in their distribution (Hodkinson, 2005; Rassman *et al.,* 2014). Studies have shown a general trend that herbivory rates decrease with elevation (Rassman *et al.,* 2014), but this pattern is variable, in some cases increasing responses were observed (Hodkinson 2005). In regards to plants, individuals of most species experience different environmental conditions across their ranges, resulting in substantial phenotypic variation (Weis *et al.*1990).

Rapidly changing climatic conditions will expose natural populations to novel selection and could amplify existing stresses, such as drought and herbivory. A major effect of rising temperatures is that snowpack levels are expected to decrease in Western North American Mountains which in turn results in reduced soil moisture (Hall et al. 2008). In a low water environment trichome densities have been shown to increase because of their ability to reduce evapotranspiration (Ehleringer and Mooney, 1978). Trichome densities in leaf tissue traditionally

vary depending on altitude, soil moisture, and herbivory risk. The specific objectives of this study were to test whether: (1) low elevation populations have higher trichome densities, as they are likely exposed to greater herbivory; (2) snow removal induces increased trichome density; and (3) trichome density is a heritable trait.

Methods

Boechera stricta is a short-lived perennial species native to the Rocky Mountains (Anderson *et al.* 2011). This species grows along an elevational gradient and has been present in remote locations in this area for ~3000 years (Anderson *et al*. 2011). *B. stricta* was chosen for this study because it is it is an ecologically and genetically tractable system, because natural populations occur in sites that differ substantially in climate, and because phyloecological studies suggest that this species has expanded and contracted its range in response to previous bouts of climate change (Song et al. 2006; Kiefer et al. 2009; Rushworth et al. 2011).

In the fall of 2013, the Anderson lab planted ~8200 *B. stricta* juveniles into five 20m x 20m experimental gardens located around the Rocky Mountain Biological Laboratory in Gothic, Colorado. These five gardens range in elevation from 2530 m to 3430 m (Table 1and Figure 1). In each garden there are 120 genotypes of *B. stricta* that originate from populations spread across a broad elevational gradient of 2690 m -3690 m. All genotypes from varying elevational origins were transplanted into each garden and four replicas of each genotype were randomly planted into a snow removal treatment and control plot. In April-May 2014, Anderson lab personnel conducted a snow removal manipulation in half of the experimental blocks at each site, allowing the other half of the blocks to experience natural snow melt conditions. Members of the lab

shoveled snow down to 10 cm, which both advanced the timing of snowmelt and reduced snow pack.

Garden	Latitude	Longitude	Elevation (meters)
Estess	38˚43'40.62 " N	106˚52' 20.00" W	2530
Peanut Mine	38˚53'07.85 " N	106˚59' 52.18" W	2710
Gothic Valley (Maxfield Meadow)	38˚57'05.18 " N	106˚59' 27.87" W	2890
Schofield garden	39°02.346 " N	107˚03.818 " W	3133
North Pole Basin	39˚01'53.328 " N	107˚04' 42.770" W	3430

Table 1: Experimental garden locations

Trichome Density

Trichome density was quantified by collecting 1-2 rosette leaves from B. *stricta* transplants located in each of the five gardens. Five images of each leaf were taken using a Keyence Digital Microscope System and a VH-Z100W wide-range zoom lens, at a magnification of 200x (area = 1647µm x 1235µm). The trichomes were then counted from the images. Trichome density was calculated by taking average counts of the trichomes on the images then dividing it by the area of each image (1.647mm X 1.235mm).

We examined how trichome density varied with treatment, garden, source elevation, and 2 and 3-way interactions among these fixed effects. We incorporated random effects for source population, genotype nested within source population, and block nested within treatment by garden. Those random effects account for non-independence of plants from the same population, siblings from the same maternal family, and plants in the same block.

Results

In all gardens, trichome density declined significantly with source elevation, indicating that low elevation genotypes have significantly higher trichome density than high elevation

genotypes in all test environments (Figs. 3-7; Table 2). We also found a significant effect of environment on trichome density (Fig. 2; Table 2). Overall, there is a trend for declining trichome density with garden elevation, except our garden at 2710 m (Peanut Mine) is somewhat of an outlier, with trichome densities that resemble high elevation gardens.

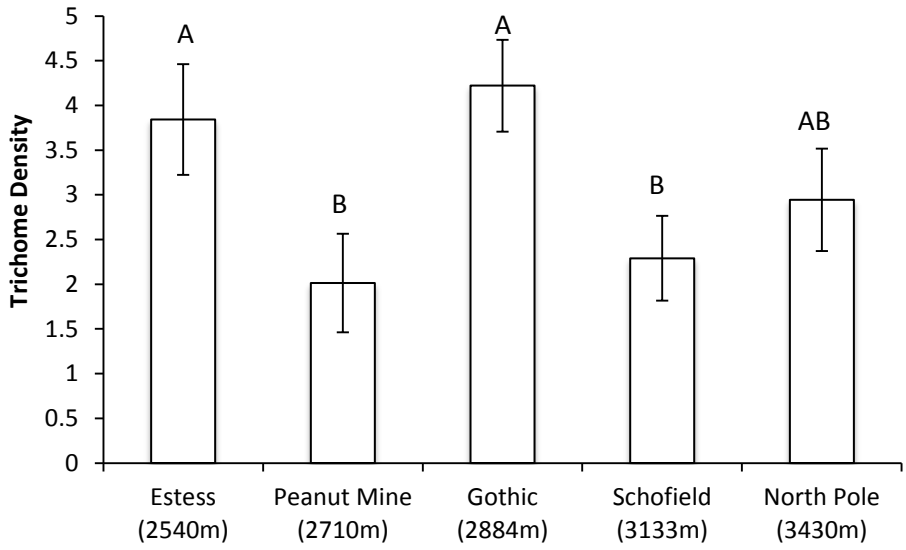

Figure 2: Graph for garden means ± SE. The different letters indicate which gardens are significantly different from each other. Estess and Gothic have similar trichome densities, as do Peanut Mine and Schofield. Estess does not differ from North Pole, but Gothic does. North Pole does not differ from

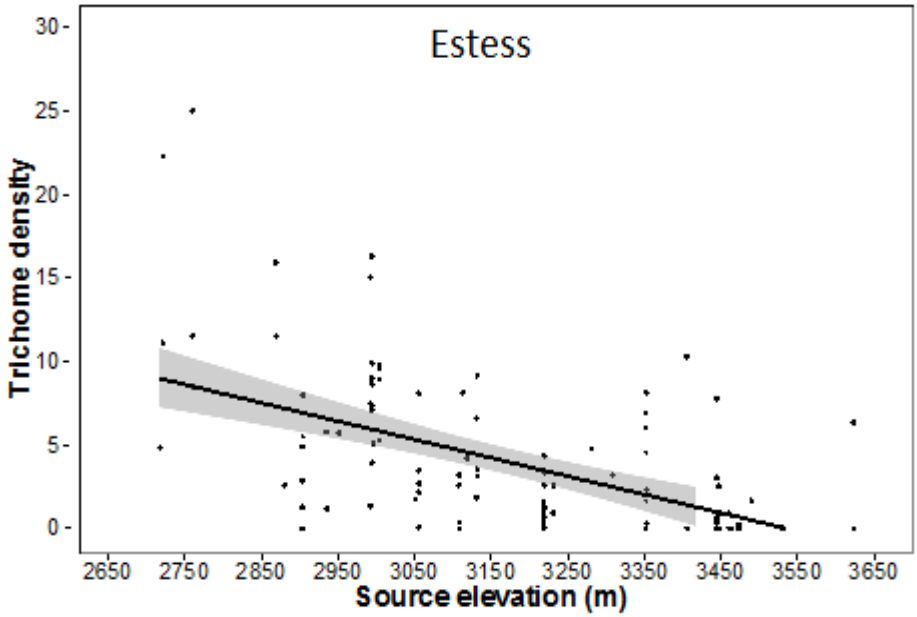

Figure 3: Relationship between trichome density (trichomes/mm^2) and source elevation of all plants from Estess garden with 95% CI (Gray).

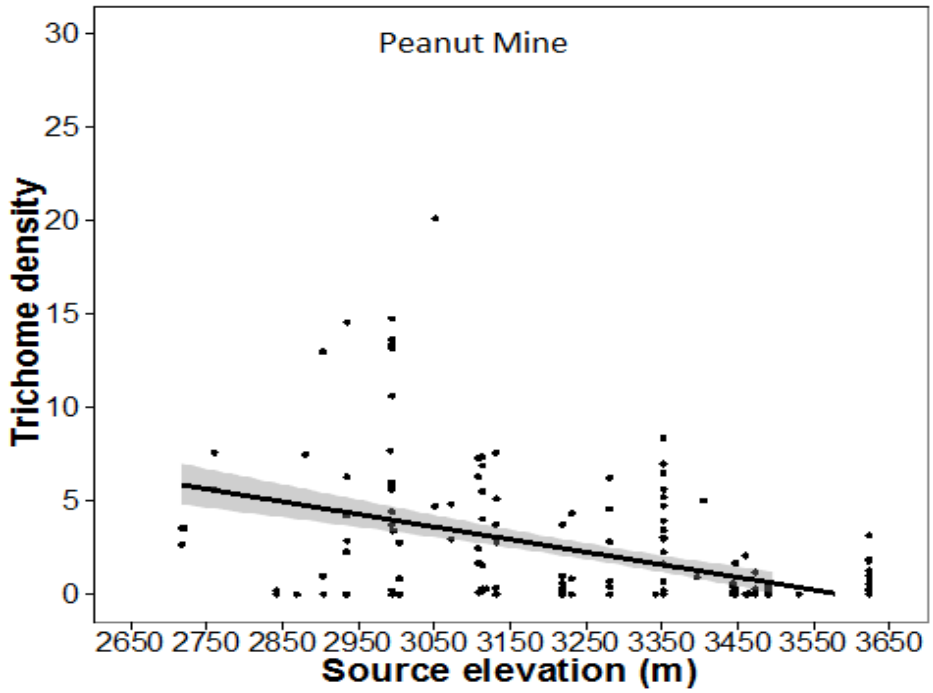

Figure 4: Relationship between trichome density (trichomes/mm^2) and source elevation of all plants from Peanut Mine garden with 95% CI (Gray).

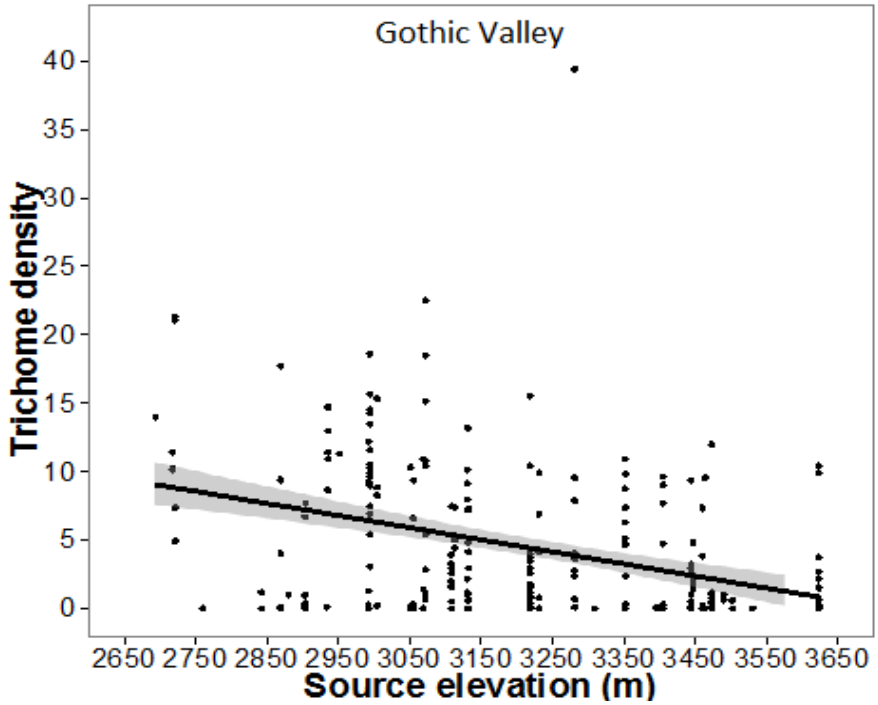

Figure 5: Relationship between trichome density (trichomes/mm^2) and source elevation of all plants from Gothic Valley garden with 95% CI (Gray).

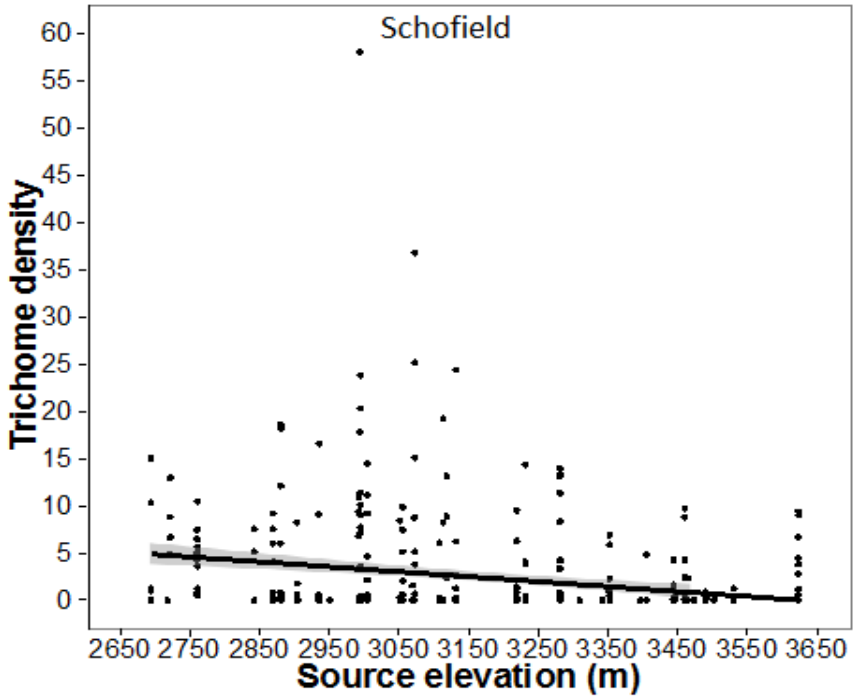

Figure 6: Relationship between trichome density (trichomes/mm^2) and source elevation of all plants from Schofield garden with 95% CI (Gray).

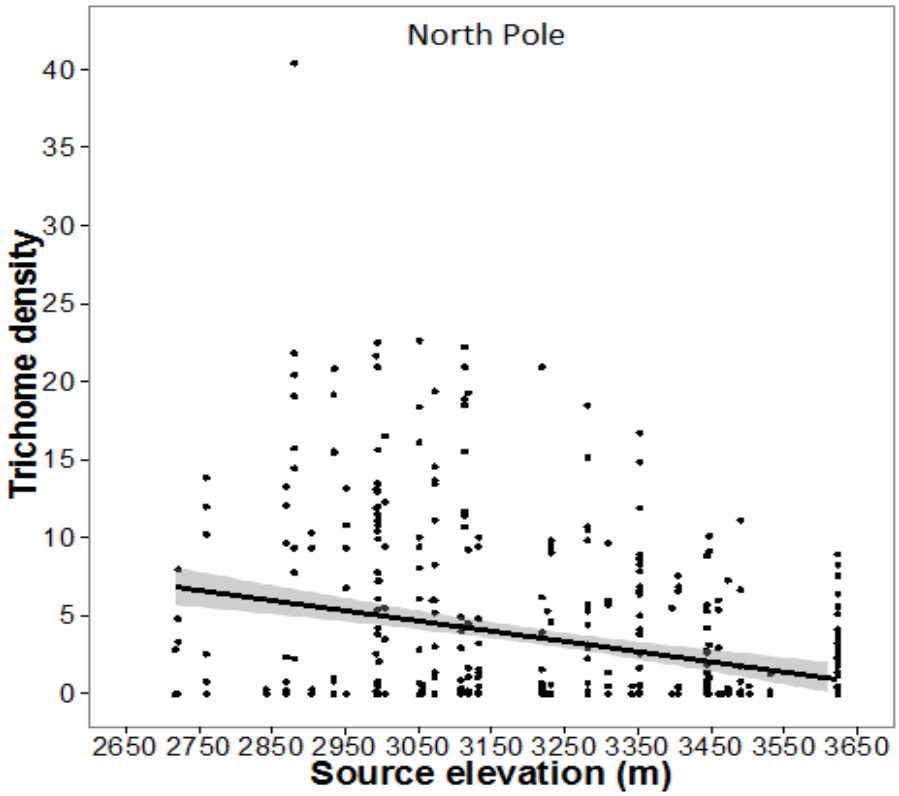

Figure 7: Relationship between trichome density (trichomes/mm^2) and source elevation of all plants from North Pole garden with 95% CI (Gray).

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F-value	p-value
Treatment	1	58	3.81	0.0559
Source elevation	1	1113	28.81	**<0.0001**
Treatment × Source elevation	1	1113	3.49	0.062
Garden	4	58	4.34	**0.0039**
Garden × Treatment	4	58	1.63	0.18
Garden × Source elevation	4	1113	3.59	**0.0064**
Garden × Source elevation × Treatment	4	1113	1.59	0.1737
Source population			χ^2=36.8	
Genotype (population)			χ^2=2.6	
Block crossed with treatment nested within garden			χ^2=10.8	

Table 2: Examined how trichome density varied with treatment, garden, source elevation, and 2 and 3-way interactions among these fixed effects

Heritability depends on the environment and in Table 3; we assessed heritability separately for each garden. We did not have sufficient sample sizes of trichome density data to estimate heritability for both the control and early snow removal treatment plots. We found significant heritability in trichome density in the lower elevation gardens.

Garden (Elevation)	H^2	χ^2	p-value
Estess (2540m)	0.331 ± 0.16	5.9	*0.015*
Peanut Mine (2710m)	0.24 ± 0.123	9.0	**0.0027**
Gothic (2884m)	0.305 ± 0.105	15.4	**<0.0001**
Schofield (3133m)	0.002 ± 0.017	0	1
North Pole (3430m)	0.007 ± 0.024	0.2	1

Table 3: Broad sense heritability (± SE) of trichome density in five common gardens. We estimated heritabilities from variance components in REML (Proc Mixed) using individual level data, and present uncorrected p-values (based on a likelihood ratio tests; χ^2, degrees of freedom = 1). Significant heritabilities after Bonferroni correction for 1 trait × 5 gardens ($\alpha = 0.05/5=0.01$) are in bold.

Discussion

We found significant genetic variation in trichome density in the lower elevation gardens. Trichome densities varied with garden, suggesting that environment influences trait expression. Finally, we found that across all gardens, low elevation genotypes had a much higher trichome density than high elevation genotypes.

In previous studies done in Norway, the frequency of trichome-producing plants was negatively related to altitude among the Norwegian populations (Løe et al., 2007). In our study, we found a significant decline in trichome density as a function of source elevation in all gardens.The garden by source elevation interaction occurs because the slope of the relationship between trichome density at source elevation is different for each of the gardens.

Our data suggest that trichome density depends both on the environment and the genetic background. Indeed, trichome density is heritable in other systems (Van Dam *et. al* 1990).

Climate conditions are rapidly changing and natural populations of many plants will be exposed to greater drought and herbivory. Plants possess trichomes which are a morphological structures that through this study suggest is a characteristic of being influenced both

environmentally and genetically. Ultimately, trichomes could serve as an adaptive way to deal with increased herbivory risk and drought.

Soil moisture and temperature are additional factors that we will examine. Soil moisture has been shown as a factor which contributes to trichome density, because trichomes enhance the plants ability to retain water (Ehleringer and Mooney, 1978). Trichomes have been shown to deter some insect herbivores suggesting that trichomes are an effective defense mechanism against insect herbivory (Agren *et al*., 1993). We are currently collecting fitness data to test whether trichomes confer a fitness advantage in the presence of herbivores, and follow-up analyses to understand whether plants with greater trichome density have lower leaf damage from insect herbivores.

Acknowledgements

I am very grateful to my mentor Jill T. Anderson for her guidance and valuable comments during the duration of this project. Bashira Chowdhury for her valuable technical support in the lab and dedication in the field. Cassidy Way, Sarah C. Daws, and Robert Burns for their immense help in the field collecting data. A huge thank you to the Rocky Mountain Biological Laboratory for their wonderful hospitality and working environment; and the National Science Foundation for the REU Award which provided me with this amazing opportunity.

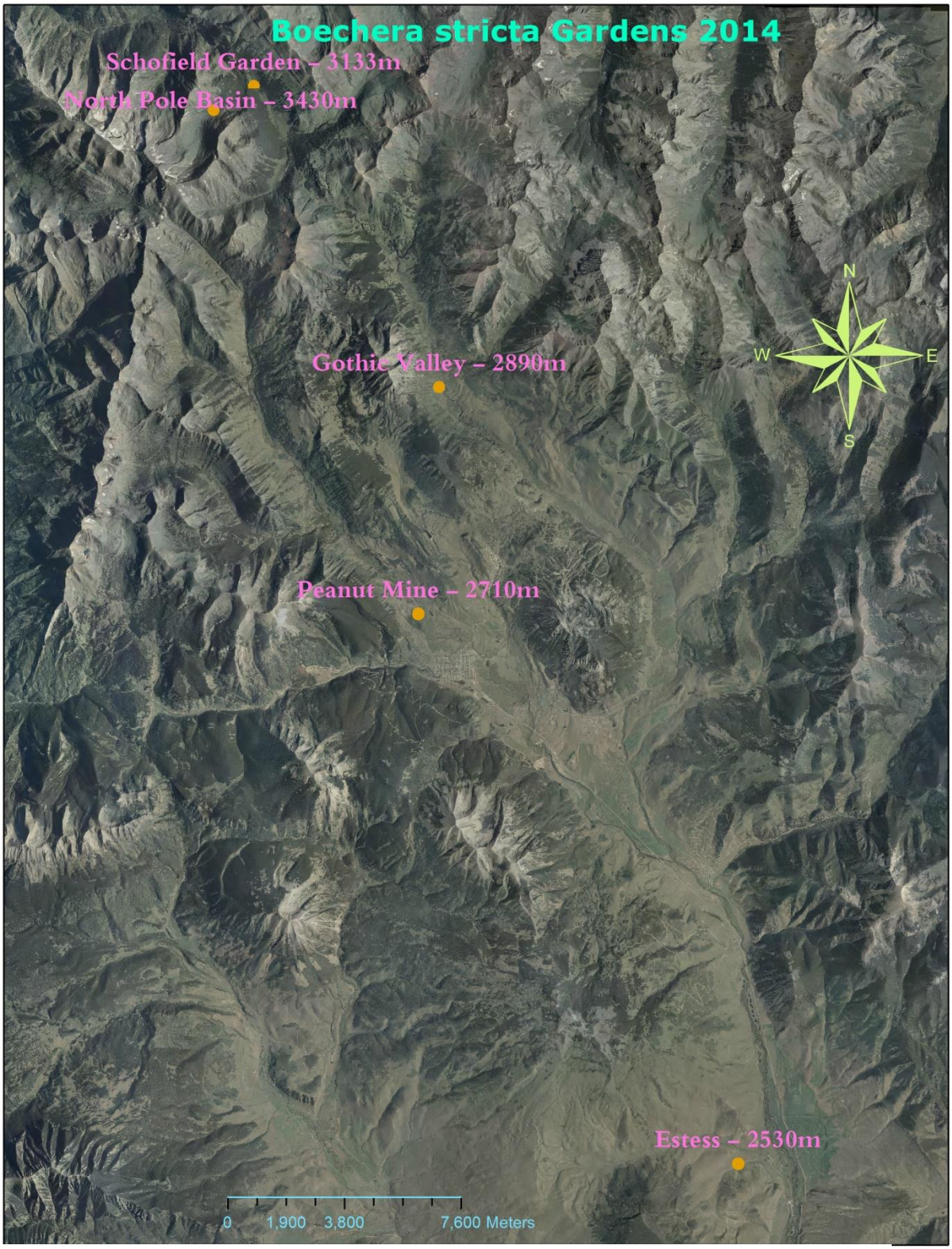

Figure 1: Map of *Boechera stricta* experimental garden sites with elevation

Literature Cited

Agren, Jon, and Douglas W. Schemske. (1993)The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. *American Naturalist*. 338-350.

Anderson, Jill T., Cheng-Ruei Lee, and Thomas Mitchell-Olds. (2011) Life-history QTLs and natural selection on flowering time *Boechera sricta*, a perennial relative of *Arabidopsis*. *Evolution* 65(3),771-787.

Bale, Jeffery S., Gregory J. Masters, Ian D. Hodkinson, Caroline Awmack, T. Martijn Bezemer, Valerie K. Brown, Jennifer Butterfield et al. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* 8, 1-16.

Byars, Sean G., Warwick Papst, and Ary A. Hoffmann. (2007). Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. *Evolution* 61(12), 2925-2941.

Ehleringer, J. R., and H. A. Mooney. (1978) Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. *Oecologia* 37(2),183-200.

Hall, Noah D., Bret B. Stuntz, and Robert H. Abrams. (2008) Climate change and freshwater resources. *Natural Resources & Environment*. 30-35.

Hodkinson, Ian D. (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* 80(3),489-513.

IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kiefer C, Dobes C, Sharbel TF, Koch MA (2009) Phylogeographic structure of the chloroplast DNA gene pool in North American*Boechera* - A genus and continental-wide perspective. *Molecular Phylogenetics and Evolution*, **52**, 303–311.

Levin, Donald A. (1973) The role of trichomes in plant defense. *Quarterly Review of Biology*. 3-15.

Løe, Geir, Per Toräng, Myriam Gaudeul, and Jon Ågren. (2007).Trichome production and spatiotemporal variation in herbivory in the perennial herb *Arabidopsis lyrata*. *Oikos* 116(1), 134-142.

Rasmann, Sergio, Loïc Pellissier, Emmanuel Defossez, Herve Jactel, and Georges Kunstler. (2014) Climate-driven change in plant–insect interactions along elevation gradients. *Functional Ecology* 28, 46-54.

Rushworth, M. F. S., Noonan, M. P., Boorman, E. D., Walton, M. E., and Behrens, T. E. (2011). Frontal cortex and reward-guided learning and decision-making. *Neuron* 70, 1054–1069.

Song B-H, Clauss M, Pepper A, Mitchell-Olds T (2006) Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of *Arabidopsis*. *Molecular Ecology*, **15**, 357–369.

Prasad K, Song B-H, Olson-Manning C *et al.* (2012) A gain of function polymorphism controlling complex traits and fitness in nature. *Science,* 336, 1081-1084.

Van Dam, N. M., J. D. Hare, and E. Elle. (1990) Inheritance and distribution of trichome phenotypes in *Datura wrightii*. *Journal of Heredity* 90(1),220-227.

Weis, Arthur E., and Wendy L. Gorman.(1990) Measuring selection on reaction norms: an exploration of the Eurosta-Solidago system. *Evolution*, 820-831.