

Genetic variation in plant functional traits as drivers in arthropod community structure

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Abstract

Genetic variation and environmental influences are important drivers of variation in individual traits. Environment is an important selective force in shifting genetic variation as individuals respond to climate change. Climate change has a very strong effect on precipitation which affect many organisms especially plants, which leads to genetic variation among plant species as they respond to the effects of climate change. Genetic variation in plants has been shown to influence not only phenotypes of the plant itself, but also the community of organisms the plant interacts with. The objective of this study is to determine how traditional and extended phenotypes differ among source populations and maternal families and what plant traits drive differences among arthropod community interactions. We studied *Valeriana edulis* Nutt. ex Torr. and A. Gray subsp. *edulis*, a long lived dioecious perennial herb native to western North America and its associated arthropod community. A common-garden was planted containing 424 replicates of *Valeriana edulis* from five source populations across an elevation gradient. On these plants, we measured traits known to influence arthropod abundances and sampled the arthropod community. The results showed a significant difference in the variation of traits across the source populations as expected, however there was no significant effect of arthropod communities being associated to any specific traits on the plants. This can lead to further analysis looking at the development of the plant to determine at what point the plant traits are important for arthropod communities. Telling us more about the variation in community genetics depending on the maturity of the host plants.

Introduction

Genetic variation is an important driver of variation in individuals traits and, coupled with environmental factors, influences fitness (Hartl and Clark 1988). We know that phenotypes are shaped by genotypes and the environmental interactions (Johnson and Agrawal 2005). Abiotic factors such as climate influence selection on genotypes across environmental gradients through local adaptations. Climate is especially influential in sessile organism like plants which must adapt to constant changes in the environment that is beyond their control. Climatic variation influences the phenotype of plants through variations in temperature and precipitation (Lynch and Lande 1993). Plants are completely dependent on their environment for all of their resources. They have been known to alter their phenotype in order to allocate their resources in a way that ultimately maximizes reproduction (Chapin 1991). One of the resources essential for survival, especially in plants, is water.

Water is one of the main environmental limiting factors for plants (Teramura and Iwanzik 1983). Due to changes in climate plants are moving to higher elevations where they are better adapted to precipitation levels despite all other factors that might be altered like soil nutrients (Jump and Penuelas 2005). Previous studies have shown that when plants are water stressed they tend to grow slower and produce smaller leaves (Stone and Bacon 1994). Plant metabolism is also affected and to counteract osmotic pressures due to water stress they produce nitrogen-containing osmoprotectants which herbivores prefer because nitrogen is a limiting resource for insect herbivores (White 1984). Thus, when plants are experiencing water stress due to their environment they are more vulnerable to being attacked by herbivores (White 1984, Huberty and Denno 2004). As the climate continues to change plants are moving higher in elevation (Jump and Penuelas 2005) and adapting to cope with water stress by a mixture of stress avoidance and tolerance strategies that vary with genotype. Some adaptations include producing smaller leaves, developing storage reserves in the roots or stem, closing stomata, or even changing photosynthetic

processes for better water conservation like C4 and CAM plants (Chaves et al. 2002). However, how these shifts will affect plant defense strategies against herbivores as they experience higher water stress with climate change is unclear.

Plant genetic variation has been shown to influence not only phenotypes of the plant itself, but also the community of organisms that associate with the plant ("community genetics": reviewed in Whitham et al. 2006). This is important in understanding the diversity and complexity of a community. Before we are able to understand the complexity of the interactions that occur within diverse taxa it is essential to consider the role that genetic interactions play in shaping these diverse and complex communities (Whitham et al. 2006). Genetically controlled plant traits have been seen to be very influential in diversity of arthropod communities (Johnson and Agrawal 2005). In a study by Wimp and Whitham (2001) the diversity of the arthropod communities on host plant was affected by the genetic differences among host plants, which contributed to the success of aphid populations. Aphids also attracted tending ants to the plant. The study found that when ant-aphid mutualism was present it would have a negative impact on the arthropod biodiversity. This study demonstrates the strong effects that plant genotypes have on arthropod diversity at multiple trophic levels. Yet there has been little research done on the actual mechanism behind this plant trait and arthropod community interaction (but see Whitham et al. 2003, Petry et al. 2013). Most previous studies in this area involved a common garden, however, the source populations used to represent the genetic variation across spatial scales were taken too far away from the common garden and did not properly represent a variation that might be observed in nature (Tack et al. 2012). Future studies need to be done to look at the relationship between plant traits and arthropod community interaction at realistic spatial scales (i.e. within the dispersal distance of plant pollen/seeds and arthropods).

The objective of this study is to determine how traditional and extended phenotypes differ among source populations and maternal families and what plant traits drive differences among arthropod community interactions. The questions that we will be investigating are: **1) What effect does climatic variations over an elevation gradient have on genetic variation in plant traits?** **2) Is genetic variation in plants associated with variation in arthropod community structure?** **3) Which genetically variable plant traits drive variation in arthropod communities?**

Methods

Study site and organisms

The study was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado. The study site was set at Vera Falls (approximately 38.962068 N, 106.992824 W) at an elevation of about 2900m. See Langenheim (1962) for a description of the vegetation.

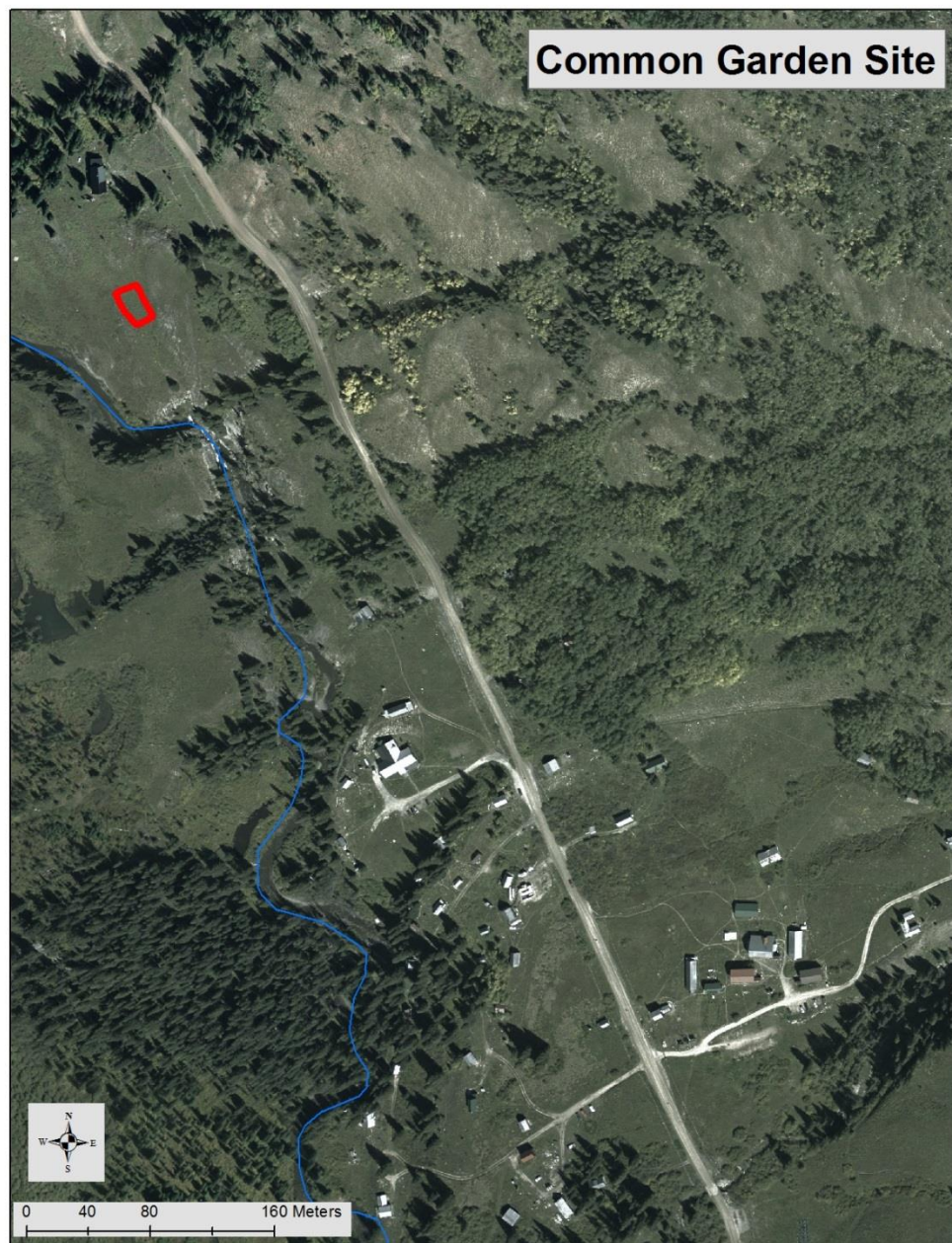


Fig.1 The location of the common garden in Vera Falls meadow north of Gothic, Colorado.

The plant used in this study was *Valeriana edulis* Nutt. ex Torr. and A. Gray subsp. *edulis*, is a long lived dioecious perennial herb native to western North America (Meyer 1951). It is commonly found in moist pastures, creek bottoms, sagebrush plains, limestone cliffs, and subalpine

parks. The plant grows for several years as a basal rosette and finally during the months of May to September begins flowering with one to many inflorescences of white flowers about (3-5mm in diameter). The plant inflorescences consist of a stalk supporting one to several compound cymes (Soule 1981). Once the flower bolts after snow melt, it is fed upon by ant-tended aphids *Aphis valerianae*. These aphids are tended by ants which provide the aphid with protection from predators and in turn the ant consumes the honeydew that the aphid secretes. The predators that have been known to attack the aphids include Araneae, Coccinellidae (Coleoptera), Syrphidae (Diptera), Miridae and Reduviidae (Heteroptera), Braconidae, Ichneumonidae and Vespidae (Hymenoptera), and Chrysopidae (Neuroptera) (Petty et al. 2013). In early June *V. edulis* is fed upon by lepidopteran larvae (Tortricidae). The caterpillars initially feed on the basal rosette and after bolting they move up to feed on the cymes (Mooney et al. 2012). *Valeriana edulis* is also fed upon by grasshoppers (Orthoptera) and vertebrate herbivores (e.g. deer and elk).

Common-garden experiment

A common-garden was planted in Vera Falls (2900m) in the summer of 2012 containing 424 replicates of *Valeriana edulis* from five source populations across an elevation gradient. The source populations include Brush Creek Range (2780m), Judd Falls (2918m), North Rustlers Gulch (2989m), Gothic/Baldy Saddle (3270m), and Bellview II (3440m), all within 11km of the common garden. From each source population there were between three to five seed mothers to account for any variation that might be present within the population. There are five to fifteen individuals that come from each seed mother to be able to look at the genetic variation within individuals of the species, maternal families, and populations.

Q1- Testing for genetic variation in plant functional traits

The plant traits (Table 1) that were measured were those that have been shown in previous studies to influence arthropod abundances. Leaf number was assessed by counting all the leaves within each basal rosette including leaves that had been damaged by herbivores or had desiccated but were still visible. Leaf size was measured as length of the longest leaf and width of the widest leaf (Mooney et al. 2012, Johnson and Agrawal 2005). Drought stress tolerance was measured by recording the xylem water pressure by using a leaf cut from the base of the plant and placing it into a Scholander pressure chamber and increasing the pressure on the leaf until water could be seen coming from the stem of the leaf (Ritchie and Hinckley 1975). Architecture was measured by how accessible it was for predators to find herbivores on the plant the basal circumference was measured and the diameters were also measured for the widest part of the plant and perpendicular to the widest diameter. Both values will be used to determine openness of the plant (Marquis et al. 1996).

Each plant trait being measured had an expected relationship with local climatic variation over elevation based on previous studies (Table 1). There is also an expected effect that this relationship will have on the arthropods depending on their category: sap feeding herbivores, chewing herbivores, and predators/parasitoids. An ANOVA was used to analyze these data with plant traits as a dependent variable, and maternal family nested in source population as the independent variables.

Table 1. The predicted direction of relationship of plant trait being studied with increased elevation and effect on arthropod.

Trait	Expected relationship as elevation increases	Effect on arthropod	Sources
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Leaf number	+	Sap feeders: + Chewers: +	Mooney et al. 2012 Johnson and Agrawal 2005
Leaf toughness	-	Sap feeders: - Chewers: -	Sands and Brancatini 1991 Yao 2004
Leaf size	+	Sap feeders: + Chewers: +	Mooney et al. 2012 Johnson and Agrawal 2005
Specific leaf area (SLA)	+	Sap feeders: + Chewers: +	Wilson et al. 1999
Drought stress tolerance	-	Sap feeders: + Chewers: -	Stone and Bacon 1994 Hubert and Denno 2004
Architecture (canopy openness)	-	Predators/Parasitoids: + Sap feeders: - Chewers: -	Marguis 1996 Grevstad and Klepetka 1992

Q2- Testing for genetic variation in arthropod communities

The arthropod abundance was sampled using a butterfly net over a period of 13 days from the 8-20 of July between 9:00 and 18:00 during peak arthropod activity. At least two plants were skipped between each plant that was sampled for arthropod to avoid disturbing arthropods present on the plant before they were caught with the net. Herbivory was measured by comparing the total number of living leaves to the total number of damaged leaves on each plant and each plant was rated on herbivory based on total above ground biomass on a scale of 0-100%. Where 0% is no visible herbivory damage and 100% is if only a small stub in the ground of the plant is left. The arthropod community composition within each plant genotype was scored two dimensionally using nonmetric multidimensional scaling (NMDS) ordination techniques. The scored form the NMDS were used as the dependent variable in a permutational MANOVA. Maternal families were nested in source population as the independent variables and 999 permutations to generate p-values.

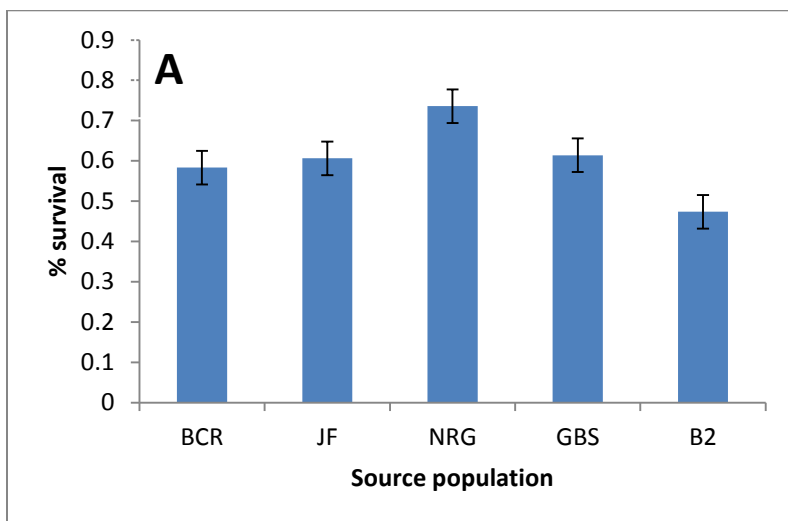
Q3- Identifying plant trait drivers of genetic variation in arthropod communities

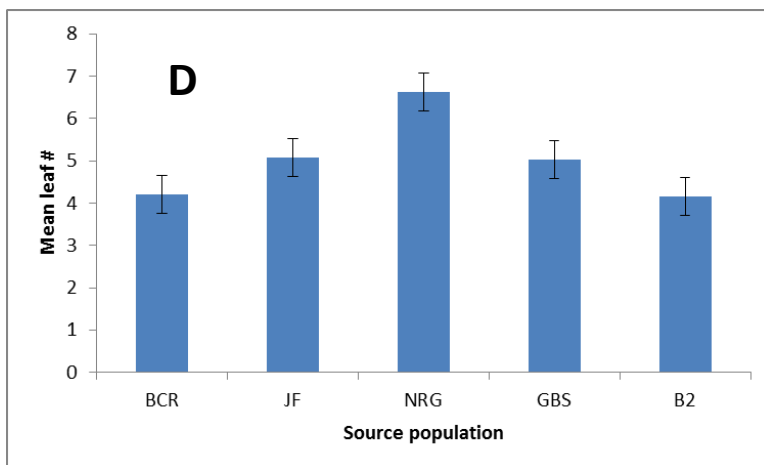
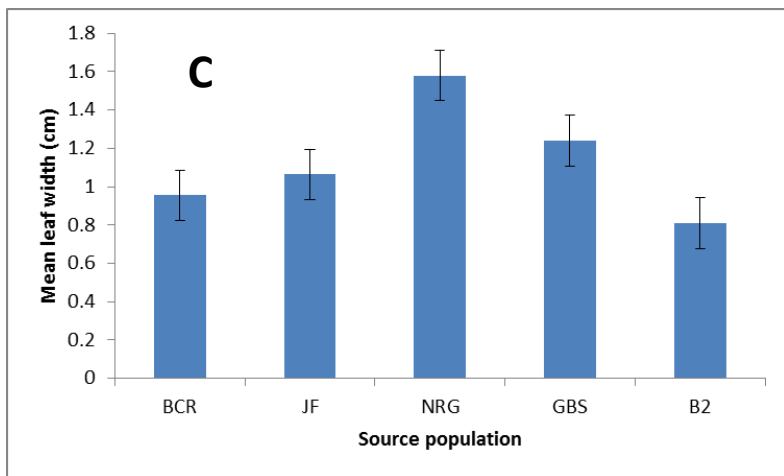
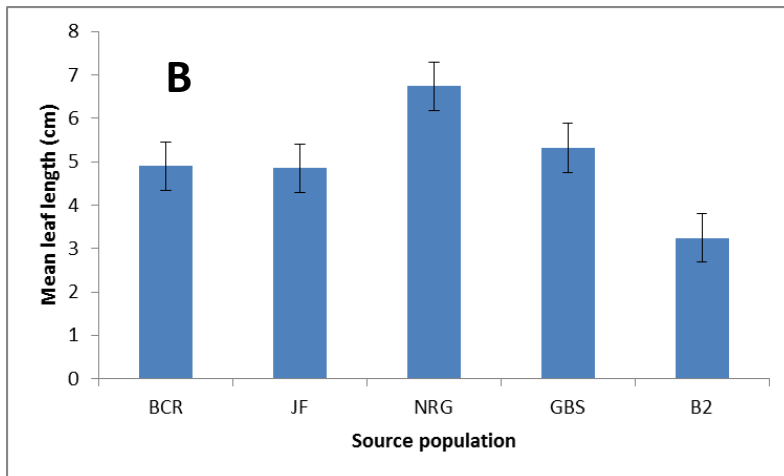
This was done by looking at the arthropod data and trait data that was collected from the first two questions. The data was analyzed looking at the arthropod communities that were found on each plant and correlating that to the specific traits that associated to the source plant. This was done using a regression and NMDS.

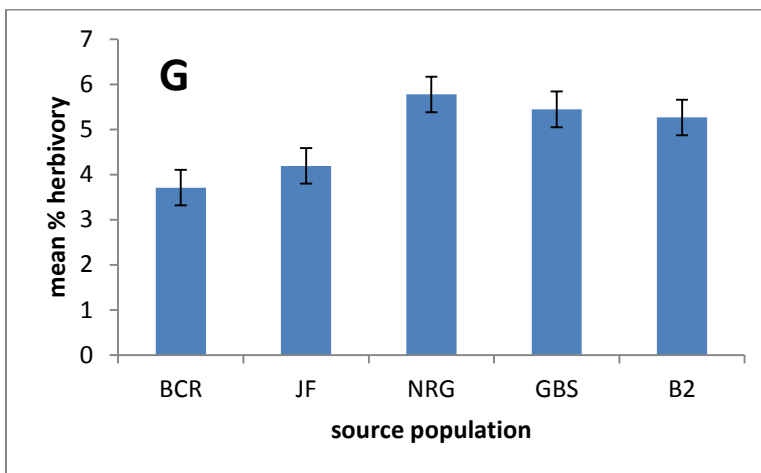
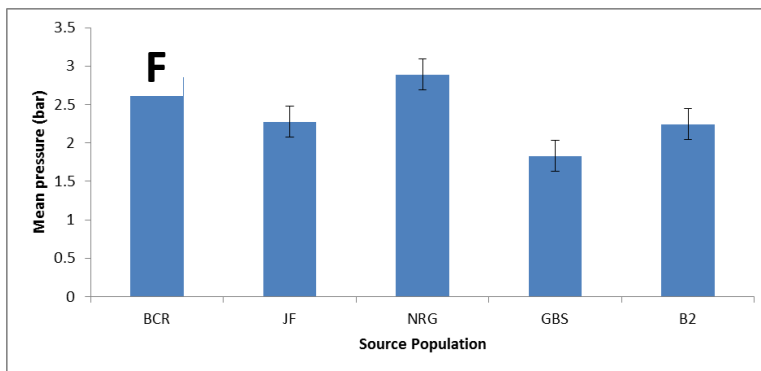
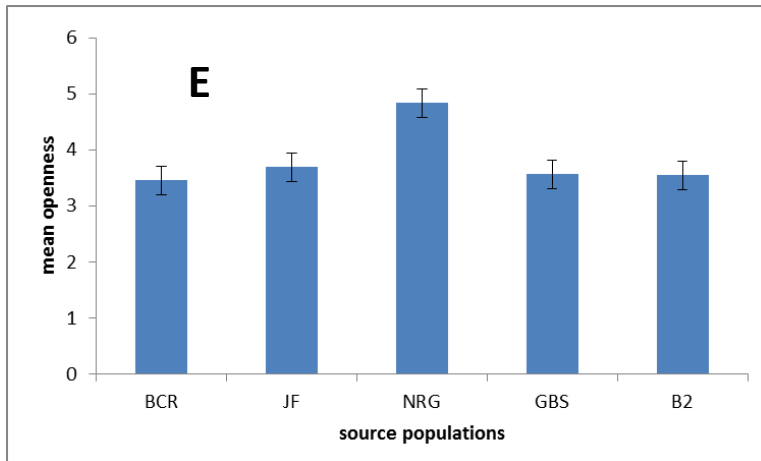
Results

Question one: genetic variation in plant functional traits

When the data was analyzed looking at the survival of the individual plants within the source populations there was a significant difference present ($\chi^2 = 15.09$, $df = 4$, $P = 0.0045$) (fig.2a). Most of the traits that were measured showed significance *at* the population level and at the maternal level. For the leaf length there was a significant difference among source populations ($F = 5.391$, $DF = 4, 265$, $P = 0.000346$) (fig.2b), however it showed no significance among maternal families ($F = 1.468$, $DF = 26$, $P = 0.070963$). Leaf width was also significant within source populations ($F = 4.378$, $DF = 4$, $P = 0.00191$) (fig.2c), but not within maternal families ($F = 1.763$, $DF = 26$, $P = 0.01459$). Leaf number was one of the traits that showed no significance among source populations ($F = 1.023$, $DF = 4$, $P = 0.396$) (fig.2d) or maternal families ($F = 1.065$, $DF = 26$, $P = 0.383$). Architecture was significant at the population level ($F = 5.106$, $DF = 4$, $P = 0.000561$) (fig.2e), but was only marginally significant at the maternal level ($F = 1.525$, $DF = 26$, $P = 0.053375$). When analyzing the leaf water potentials there was no significance found in source populations ($F = 1.765$, $DF = 4$, $P = 0.137$) (fig.2f) or in maternal families ($F = 0.761$, $DF = 26$, $P = 0.794$).







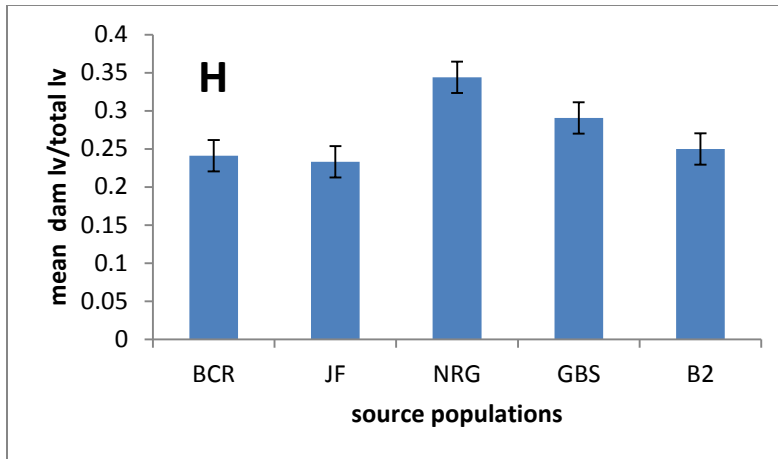


Fig. 2 **a** Percent survival of the individuals within each source population, **b** Mean $\pm$ 1SE leaf length of the individuals within each source population, **c** Mean $\pm$ 1SE leaf width of the individuals within each source population, **d** Mean $\pm$ 1SE number of leaves on each individual from each source population, **e** Mean $\pm$ 1SE measure of openness for each individual within the source population, **f** mean water pressure in leaf of each individual within the source population, **g** Mean $\pm$ 1SE value of percent herbivore damage, **h** Mean $\pm$ 1SE ratio of total damaged leaves over total leaves on the individual plants from each source population.

Question two: genetic variation in arthropod communities

There was no significance found between arthropods and traits among source populations or maternal families (STATS; fig.3)

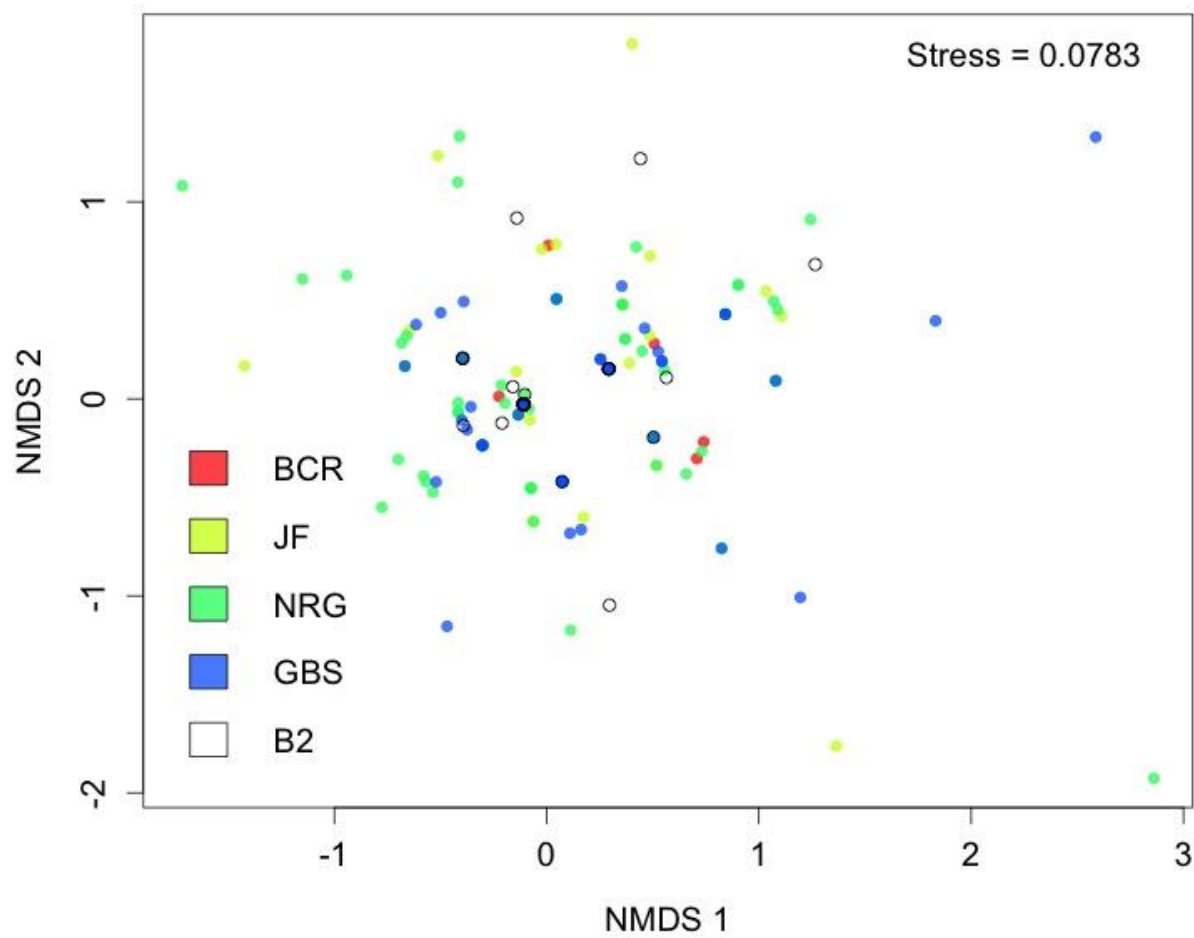


Fig.3. Two-dimensional nonmetric multidimensional scaling ordination of arthropod communities by plant source population.

Question three: Identifying plant trait drivers of genetic variation in arthropod communities

The results for this question could not be done due to there being no variation among arthropod communities in question two.

Discussion

We found significant results that showed genetic variation among source populations and maternal families. There was variation in survival rate and in most of the traits that were measured. This is consistent with what was expected because the plants came from different sources that have different environments. Because the plants were grown in a common garden and all experienced a similar environment these results variations in traits can suggest that there is genetic variation among maternal families and source populations. However, despite there being genetic variation in plant phenotypes there was no significant variation in arthropod communities among the plant genotypes. This results was not as expected from what has been shown in previous studies on plant trait variation influencing arthropod diversity. Because of these result we could not identify the plant traits that were significant to influencing arthropod diversity.

The results from the survival analysis show us that there was variation in the probability of the population being successful in the new environment. This shows that there is significant genetic variation within each of the source populations in terms of survival. The data also show a pattern survival across the elevation gradient. Populations who are naturally found farther away from the location of the common garden had a significantly lower percent of survival than the populations who are naturally found closer to the location of the common garden. This could de due to the plants from either extreme of the elevation gradient not having developed enough plasticity to survive in such a different environment than where they were genetically adapted to. A population cannot become established beyond its range because it will experience negative growth rates in a different habitat it is not adapted to (Bridle and Vines 2007).

The results from the trait data show significant variation among source populations and among maternal families, consistent with our expectations base on variation in elevation and thus environmental conditions among the source populations. Such heterogeneity has been shown to influence patterns of genetic variation through adaptation to local environmental conditions (leimu

and fischer 2008). We also expected there to be variation among the maternal families because there also needs to be significant amount of genetic variation in communities within an environment because of variations in microclimates that each mother could have been adapted to. We could have also not seen any genetic variation within each source population at all which would have demonstrated the phenotypic plasticity. Genetic variation within and among populations is something that has been found to occur when looking at the same or related species (Sandquist and Ehleringer 2003)

The results from the arthropod community data showed no significant variation among source populations. Although this contrasts with previous studies of arthropod communities among plant genotypes (CITES), a study done by Coley (1983) looking at herbivory and defense characteristics in the leaves of tropical trees suggests that leaf age is strongly correlated with susceptibility to herbivores. The younger the leaf is the less defend it is against predators and the more nutritious it is, so it is much harder to see significant variation in arthropod communities if the plants are not mature enough for the arthropods to detect any trait variation. Thus, because this study focused on juvenile plants of a slow-growing, long-lived perennial, statistically detectable variation among plant traits may not have been apparent to arthropods against a background of palatable young tissue. Furthermore, attractive traits associated with reproduction (e.g. floral nectar) that may be particularly important to higher trophic levels (Petty et al. 2013) simply are not expressed in juveniles.

Understanding the genetic interactions that occur between a community of species and there abiotic environment is important because it tells us more about the mechanisms that play a role in shaping the community genetics. We need to understand the mechanisms of community genetic effects that can be translated out into the field. This will give us a better understanding of how to fully understand the complexity of the interactions that occur among organism. The results from

this study on the interactions among the arthropods and plant demonstrate that there is still much more we need to know about the development of the plant before we can begin to see variation in arthropod communities. The plants had not fully expressed the traits that varied among the genotypes and thus we saw no relationship between plant traits and arthropod communities. This is something that can be taken into consideration when studying other interactions among other organisms.

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