EVOLUTION OF INTRASPECIFIC GENETIC DIFFERENCES ACROSS

HETEROGENEOUS ENVIRONMENTS AND THE POTENTIAL IMPACTS ON

ENVIRONMENTAL RESTORATION

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ABSTRACT

Genetic differences evolve between seed sources of widely distributed species that can impact restoration success. Using the herbaceous perennial *Geum triflorum* (Pursh) as a model species, we examine genetic differences that evolve across a species' range in both physiology and fitness. *G. triflorum* occurs across highly differentiated environments consisting of prairie and alvar habitats. Seeds were collected from 22 populations across three eco-geographic regions. Through a common garden experiment in the prairie environment, I first examined differences in physiological traits between source regions and populations. Significant regional differentiation was observed for a majority of traits assessed, with equal or greater trait variation observed at the population scale. Secondly, differences in fitness between source regions were assessed over a period of three years using the ASTER model. Despite being in the home environment, fitness was considerably lower in prairie-sourced plants than alvar sourced plants, both each year and in total.

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LIST OF ABBREVIATIONS

USDA...United Stated Department of Agriculture

CRP..Conservation Reserve Program

AGF...Assisted Gene Flow

DNA..Deoxyribonucleic Acid

GLA ....Great Lake Alvar Region

MBA ....Manitoba Alvar Region

PRA...Prairie Region

MSUM ....Minnesota State University Moorhead

SLA...Specific Leaf Area

LDMC...Leaf Dry Matter Content

WUE ....Water Use Efficiency

SAI..Stomatal Area Index

ANOVA ....Analysis of Variance

EPA...Environmental Protection Agency

ASTER..Aster Statistical Model

ERH...Enemy Release Hypothesis

EICA ....Evolution of Increased Competitive Ability

IPCC..Intergovernmental Panel on Climate Change

LIST OF SYMBOLS

N_e ....Effective population size

GxE ....Genotype by Environment Interaction

V_A...Additive Genetic Variance

V_{pop}..Population Level Genetic Variance

V_{reg} ....Region Level Genetic Variance

V_R...Residual Variance

Q_{ST}..Ratio of additive genetic variance to total genetic variance

1. INTRODUCTION

North American tallgrass prairies are one of the most imperiled habitats in the world.

Approximately 45.8% of land that was historically grassland, savanna or shrubland has been lost

to land conversion globally (Hoekstra et al., 2005). In North America less than four percent of

grasslands remain (Samson and Knopf, 1994; Johnson et al., 2015). Because of this, restoration

efforts have been implemented to conserve and restore this imperiled habitat. However, one of

the major outstanding questions in restoration is how and from where should seeds for

restoration be sourced? This research examines traits important to adaptation and fitness for a

perennial plant species. A half-sibling common garden using seeds sourced from multiple

populations spanning a range of environments was established. Alongside variation in

environment, source populations differ in levels of geographic isolation and include populations

from the core and peripheral distributions of the species' range as well as geographically disjunct

populations. Using tiers of relatedness and quantitative genetic tools genetic variation within and

among populations was estimated for phenotypic trait variation in the common garden. In this

thesis, I examine the proportion of trait variation attributable to regional and population

differences. I also examine fitness variation in individuals sourced from 'home' and 'away'

environments to test hypotheses associated with the fitness expectations of locally adapted

genotypes.

1.1. Ecological Restoration

Restoration involves significant economic investment, where market factors and policy

can drive decision making. Effective justification of seed sourcing decisions and monitoring

post-restoration effectiveness is challenging because policies and practices vary between

practitioners. However, sourcing seed is a common first step in restoration. One factor to

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consider in seed sourcing is the influence different evolutionary processes, such as gene flow,

genetic drift, natural selection, and mutation may have on seed source variation. These forces

influence genetic variation in traits important to adaptation across environments.

Variation in seed sourcing policies often results from varying political pressures. For

example, in the United Kingdom and Australia, spatial proximity is of most importance

(Broadhurst et al., 2008). The U.S Forest Service, however, uses "seed zones", which attempt to

use topographic, edaphic, and climate data when determining seed source (Johnson et al., 2010).

In the United States, another common restoration program is the USDA Conservation Reserve

Program (CRP). CRP emphasizes sourcing native species and has shifted from regional

commercial seed sources to smaller, more local sources as availability increases. Research has

suggested use of local seed sources leads to greater success in establishment and minimizes the

potential consequences of outbreeding depression (Jones and Johnson, 1998; Waters and Shaw,

2003). In their literature review, Johnson et al (2010) indicated most studies show local ecotypes

have the most success in restoration projects. However, these patterns are not universal,

particularly where a species is locally rare, or the target site has been degraded or altered by

change in pathogens, soil, or herbivores. In a drastically altered restoration target site, local

material may no longer be adapted to the changed conditions (Montalvo and Ellstrand, 2000;

Johnson et al., 2010).

1.2. Evolutionary Processes Impacting Population Genetics

1.2.1. Fragmentation

Populations have been subject to fragmentation both from large historical landscape

colonization events (e.g. following glaciation) and rapidly from contemporary anthropogenic

land conversion (row-crop agriculture, expanding cities, etc.). Population demography and

effective population size (N_e) are intimately tied to estimates of genetic variation. In general, populations in the core of a species range have a larger N_e, with reduced potential to lose alleles to genetic drift. However, small populations, disjunct from the core range often have a lower N_e and are susceptible to loss in allelic diversity through genetic drift and inbreeding (Allendorf et al., 2013). The timeline of landscape shifts driving fragmentation and the reduction in genetic variation can vary greatly by species and time since isolation (Young et al., 1993). This can have many different consequences on populations such as driving isolated populations towards speciation or extinction. However, Young et al (1996) pointed out that in their meta-analysis that an initial genetic bottleneck at the time of fragmentation is responsible for reduced genetic variation in disjunct populations, with less impact arising from inbreeding depression or drift as the population evolves in isolation. Thus, considering the influence of historical and contemporary fragmentation and its impact on the distribution of genetic variation across a species' range is important. Considering the factors influencing the distribution of genetic variation across populations may inform which populations are appropriate for restoration across fragmented landscapes and those most likely to persist under changing environmental conditions.

1.2.2. Natural Selection

Natural selection is a process whereby individuals may exhibit differential survival or reproduction in response to changing conditions. Selection may favor individuals with traits adapted to certain conditions, leading to increased reproductive output or survival and concomitant changes to the underlying population genetic structure. The differential reproductive output of heritable traits can change the allele frequency of the population, potentially eliminating maladaptive alleles as unfit individuals die faster than they can replace themselves.

1.2.3. Local Adaptation

A population is considered locally adapted if that population has higher fitness in its 'home' native site than an 'away' foreign site (Kawecki and Ebert, 2004; Leimu and Fischer, 2008). This is because local selection pressures select genetic variance most suitable to the local conditions (Montalvo and Ellstrand, 2000; Savolainen et al., 2013). A meta-analysis examining studies of local adaptation indicated local adaptation was observed 71% of the time, resulting in approximately 45% increase in local population fitness relative to non-local populations (Hereford, 2009).

However, not all populations show local adaptation and the scale of local adaptation may vary widely, leading to the question how local is local? A metanalysis of previous research has shown that the scale of local adaptation is variable, ranging from small habitat patches to wide state/provincial-levels, and is influenced by a multitude of factors. Samis et al. (2016) performed a reciprocal transplant using *Camissoniopsis cheiranthifolia*. Their experiment showed little (if any) evidence of local adaptation, with similar fitness in transplants and local individuals. In a study by Griffith and Watson (2005), transplanting individuals across regions did not affect mortality, but did impact relative fitness as a result of differential phenology in flowering period based on source location. Thus, variation in the effective scale of local adaptation could be the result of the differential contribution of other evolutionary factors that mediate changes in population genetic structure, such as gene flow and drift.

1.2.4. Gene Flow

In restoration, gene flow is an important consideration in the maintenance of evolutionary potential. Gene flow occurs when alleles between populations are exchanged via dispersal by seeds or pollen transfer. Populations that experience high levels of gene flow may have

maladaptive alleles incorporated into the breeding population in proportions that overwhelm allele frequency change from selection and local adaptation (Etterson, 2006; Samis et al., 2016). This can be most pronounced in small populations. However, gene flow can benefit a population by introducing variation if high levels of inbreeding or genetic drift has occurred, or by pre-adapting populations to future conditions, such as those associated with climate change (Aitken and Bemmels, 2016). Based on predictions of the abundant center model, where species abundance is higher in the core of a species range (Soule, 1973; Sagarin and Gaines, 2002), populations at the edge of their species' range tend to have reduced genetic diversity and higher genetic differentiation from other populations (Eckert et al., 2008). Reduced genetic diversity could be exacerbated by reduced seed production at range boundaries, which can further reduce fitness (Jump and Woodward, 2003; Etterson, 2004).

1.2.5. Genetic Drift

The stochastic effect of drift is another evolutionary consideration when comparing the genetics of different populations. Genetic drift, where rare alleles are lost from a population, can impact the distribution of genetic variation across populations. Genetic drift tends to reduce adaptive potential; this is of particular concern for smaller populations, where it is more likely rare alleles will be lost (Shaw and Etterson, 2012).

1.3. Common Garden as a Quantitative Genetic Test

Quantitative genetics is a set of tools that allow for statistical analysis of phenotypic variation attributable to genetic variation within and among populations. Initially used for calculating differential production in domestic livestock and crops, its usage has spread to non-model organisms and wild populations in the past 30 years (Allendorf et al., 2013). Common gardens can be beneficial to restoration practitioners because they can be used to identify

genotypes that may be suitable sources for restoration environments. For example, if a common garden is conducted in an environment mirroring a potential restoration site, the fittest population could be utilized as a seed source. Common garden experiments are conducted by selecting individuals across a species' range and observing phenotypic differences when grown in a shared environment (Reich et al., 2005; De Villemereuil et al., 2016). Phenotypic variation results from multiple factors: genotypic, environmental, and the interaction of the two (GxE). A common garden uses individuals of known descent to calculate additive genetic variance (V_A), or the proportion of variation due to additive effects of the genotype that can be inherited. By looking at differences observed within and among populations and regions we can look at variation attributed to V_{pop} and V_{reg} respectively. Remaining variation between individuals is considered residual variation (V_R) (De Villemereuil et al., 2016). Using these calculations we can estimate the Q_{ST} as the genetic differentiation of populations from total genetic variance.

In relationship to local adaptation, a common garden experiment may show one of three primary outcomes: (1) A 'home-site advantage' may be observed where populations from the local common garden environment exhibit greatest fitness relative to non-local populations. (2) Populations may be maladapted if populations from environments different from the local common garden exhibit greater relative fitness and (3) If no differences are observed across populations this may suggest that there is no evidence for local adaptation.
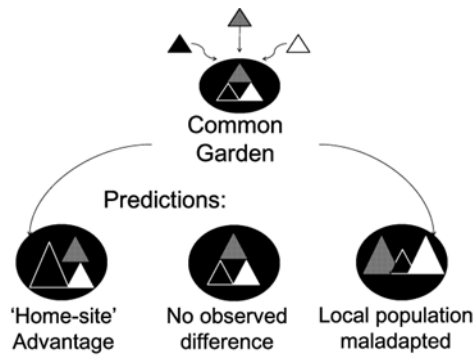
Figure 1.1. Potential common garden outcomes.
Three different scenarios that may be observed in the common garden. Different colored triangles indicate different source habitats. The circles containing the triangles indicate all populations were planted in a common environment, in this case in the black environment.

While local adaptation is not directly tested in common garden experiments, we can compare the relative fitness of local individuals to those from away and provide a geographic scale for relative fitness. If there are high amounts of variation on an individual basis but not on a population level, the measured traits may show stronger effects of plasticity or genotype by environment interaction than adaptive genetic variance. Conversely, if there is very little observed variation at all, there may be low genetic variation in the species. In addition, populations that have been subject to high levels of inbreeding and/or genetic drift may have low genetic variation for traits observed.

Gould et al. (2014) used a series of common garden experiments to show the heritability of traits in *Clarkia xantiana*. They determined that for focal traits such as flowering time, seed mass, height and branchiness, all had significant heritability. By using a family-structured common garden with knowledge of source region and population, they were able to estimate genetic variation within and among regions, populations, and families. Their results indicated a highly heterogeneous genetic structure across the species' range, with the majority of phenotypic variance observed within populations. Though populations were genetically differentiated, their

results indicated low levels of local adaptation. Local adaptation may not have been detected because of the environment in which *C. xantiana* grows. *C. xantiana* inhabits a highly heterogeneous environment, with temperature, photoperiod, and precipitation all varying across different axes across the range. As a result, few traits were determined to be an adaptive response to a specific environmental condition.

Samis et al. (2016) used a set of common gardens in a reciprocal transplant experiment to evaluate fitness of edge populations. Their study was a test of the 'abundant center model' (Soule, 1973; Sagarin and Gaines, 2002) where it is thought that genetic diversity is highest towards the center of a species range. Counterintuitively, they found limited effects of local adaptation, with similar fitness estimates occurring in local and foreign-sourced individuals, and fitness increasing at the range edge. These findings conclude that the abundant center model is not universal, and that for this species, the range edge is based on limited dispersal ability rather than reduced fitness.

Miller et al. (2011) established common gardens of five plants species to determine if genetic variation occurs among populations as a function of associated geography, climate, or habitat. The primary objective of their study was to examine if the scales set for seed transfer (such as U.S. EPA 'ecoregions') were appropriate indicators of suitability of seed sources. They found evidence of local adaptation to climate for four of the five species studied. However, three of the species demonstrating adaptation did not have suitable geographic differentiation to establish appropriate seed transfer guidelines (Miller et al., 2011). The authors proceeded to recommend using Omernik level III ecoregions as guidelines for seed transfer as effective due to both availability and suitability of seed at this level, with the caveat that genetic data is largely

unknown and there are still risks associated with transferring species with unknown genetic composition.

Anderson and Gezon (2015) examined the ability of populations to adapt to future climate change conditions through two common gardens at two separate elevations. These gardens would represent difference in conditions that are expected to occur with climate change. By planting offspring from the same family in two separate places they were able to examine whether trait variation was genetically heritable (if plants from the same family had the same responses within and across gardens) and if traits responded to different conditions plastically (i.e. if plants from the same family differed by garden, but were the same within a garden). They used experimentally-advanced snowmelt timing to simulate climate change. They found across ten traits measured, significant heritability and plasticity in several of the traits (Anderson and Gezon, 2015). Furthermore, the authors determined the heritability of plasticity in traits by looking at genotype by environment interaction compared to total genetic variation. The authors found that there is a genetically-encoded ability for populations to response to climate change through plastic changes, particularly in two traits, specific leaf area and height at flowering. Genetic difference in plasticity for these traits suggests that variation in plasticity may evolve as a response to fluctuating environmental conditions.

1.4. Climate Variation and Anticipated Change

The current rate of climate change far surpasses that of earlier eras (Shaw and Etterson, 2012). Climate warming has varying effects on the distribution of plants and pollinators, including altering their phenology and fitness (Etterson, 2006; Hegland et al., 2009). It is predicted that variation of 2°C above optimal temperature could lead to wheat yield reductions of up to 50 % via perturbations in physiological, biological and biochemical processes (Liu et al.,

2015). While wheat isn't directly pertinent to ecological restorations, a reduction of yield in a

crop may correlate with reduction in fitness in natural populations, or shift in priority from

restoration to increasing agricultural land. Rapid climate change should affect seed sourcing

decisions: the changing environment will drive evolutionary change by altering selection

pressures impacting local adaptation and speciation.

Etterson (2004) conducted a reciprocal transplant experiment by planting *C. fasciculata*

in three prairie environments to measure differences in response to a climate gradient. The three

prairies were similar, except in latitude. Planting along latitudinal and elevational gradients

provide the opportunity to evaluate response to climatic variation. However, photoperiod and

duration of growing season is also intertwined and lies along the same gradients. Their results

showed evidence of local adaptation, with fitness being significantly lower outside of seeds'

native distribution. This was observed despite plastic responses in phenology at vegetative

stages. Populations with high levels of genetic diversity have more adaptive potential and

theoretically increased evolutionary potential in the face of rapid climate change (Aitken and

Bemmels, 2016). Despite having high genetic diversity in northern populations, Etterson

suggested northern populations would still be negatively impacted by climate change due to

reduced fitness in warmer environments, and that adaptive evolution would occur slower than

anticipated climate changes.

The current rate of climate change may exceed the ability of a species to disperse and

shift its distribution to match suitable climatic conditions. Aitken and Bemmels (2016) suggested

that 'Assisted gene flow' (AGF) may be important to provide restoration populations' genetic

variation that might not needed to persist under changed conditions. In most cases, AGF will not

incorporate new alleles, but alter the allele frequencies to increase alleles pre-adapted to a

changing climate. For example, transplanting individuals from a warmer climate to one that was historically cooler may shift the allelic frequencies of the native population introducing warm-adapted alleles, pre-adapting the population to increased temperatures predicted under climate change.

1.5. *Geum triflorum* (Pursh) as a Model Species

Geum triflorum or Prairie Smoke is a mesic herbaceous plant, a member of the Rosaceae family, and an allohexaploid with a phylogeny strongly affected by reticulate evolution and genome duplication (Gajewski, 1959; Smedmark and Eriksson, 2002). It is one of the earliest plants to flower in the spring, with flowering occurring from March until June. Flowers consist of one or more inflorescences containing three florets that are pale-pink to purple, with closed corolla. *G. triflorum* die back annually, but some leaves overwinter green from year to year (pers. obs.). Distribution varies from abundant to locally rare across the range. The core range of *G. triflorum* is in the prairies, with peripheral populations occurring on alvar habitats in Manitoba around the Interlake Region. In addition to peripheral populations in Manitoba, there are geographically disjunct populations found on the alvar habitats surrounding the Great Lakes.

Hamilton and Eckert (2007) used microsatellite DNA markers to assess genetic diversity across the range of this species. They sampled populations from the core of the species range in the Northern tallgrass prairies, as well as range edge and disjunct populations on alvars near the North American Great lakes. Their findings showed that the central prairie populations possessed the most allelic diversity, and contained alleles private to the region, while the alvar populations contained reduced allelic diversity overall (Hamilton and Eckert, 2007). Interestingly, the alvars also maintained higher genetic distinction between populations than populations in the prairie

region. This could be driven by a number of factors largely categorized as landscape level and habitat differences.

1.5.1. Habitat Conditions *G. triflorum* Inhabits

1.5.1.1. Prairies

Prairie is the colloquial name for the expansive grasslands of North and Central America. Grasslands by definition are dominated by species of the Poaceae family, and often include a diversity of herbaceous forbs—predominately from the Asteraceae and Fabaceae families (Anderson, 2006). Prairies are the historically dominant habitat type of the North Central U.S and Central Canada, spanning approximately 162 million ha prior to agricultural conversion (Samson and Knopf, 1994). Prairies consist of landscape with typically thick, nutrient-dense soil with strong seasonal climates. The prairie community of North America is divided largely in to tallgrass, mixed-grass, and shortgrass communities. These regions are differentiated by vegetation height corresponding to precipitation amounts (Anderson, 2006).

1.5.1.2. Alvars

Alvars are a unique ecological community. Abiotic conditions consist of shallow soils over dolomitic limestone bedrock, and are prone to extreme seasonal flooding and drought (Catling and Brownell, 1995; Stark et al., 2004). Alvars occur in the Great Lakes Region of North America, Sweden, Baltic Europe, and Western Ireland (Reschke et al., 1999). The North American alvars were likely established during the Lake Huron draw down between 10,000 B.P and 4500 B.P. based on elevation. Alvars harbor a unique assemblage of plant species more commonly observed in prairies, boreal, cordilleran regions, largely disjunct from the core of their range. They also harbor a number of endemic species found only on alvar habitat, including *Hymenoxys herbacea* and *iris lacustris* (Catling and Brownell, 1995; Stark et al., 2004).

Previous research suggests that *G. triflorum* likely colonized alvar habitats via range expansion during the warming Hypsithermal period ~5000BP following the expansion of prairie communities, but were later isolated from the core population when the species' range contracted (Hamilton and Eckert, 2007).

1.6. Research Objectives

The objectives of this thesis were to examine how different evolutionary processes influence the distribution of genetic variation underlying traits important to adaptation across the range of a species important to restoration. Specifically, I evaluated differences in phenotypic traits of seeds sourced across the range of the perennial plant, *Geum triflorum*. Seeds were collected from a range of environments, including a number of populations within alvar and prairie regions, each representing a distinct environment. Physiological traits were assessed to test what proportion of physiological trait variance was attributed to regional and population-level differences. Multi-year fitness data was used to test the fitness consequences of seed transfer, evaluating cumulative fitness differences between alvar and prairie populations sampled from across the range of *G. triflorum* in the prairie environment. Reproductive output, measured as seed mass produced per individual, was compared within the common garden to test for a 'home site advantage' for individuals from the prairie region relative to individuals from the 'away' alvar environment.

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2. EVOLUTION OF QUANTITATIVE TRAIT DIFFERENTIATION ACROSS SCALES OF LANDSCAPE HETEROGENEITY[1]

2.1. Introduction

Understanding the scale over which phenotypic traits evolve is crucial to successful habitat restoration. Both macro and micro-evolutionary processes contribute to the evolution of broad- and fine-scale genetic variation for traits important to adaptation (McKay et al., 2001; Hamilton et al., 2019). However, if quantitative trait differences evolve over varying eco-geographic scales, seed transfer across those scales could impact restoration success. Reduced fitness may result where genotypes are maladapted to a site of introduction following restoration (Langlet, 1971; Aitken and Bemmels, 2016). While extensive research has established seed transfer guidelines in forested ecosystems based primarily on latitude, elevation, and climate (Knapp and Dyer, 1998; Broadhurst et al., 2008; Johnson et al., 2010; O'Neill et al., 2017), similar guidance is limited in grassland ecosystems. Seed transfer guidelines are often defined by separate federal, state/provincial, or local jurisdictions, and recommendations may be region- or species-specific (Miller et al., 2011; Potter and Hargrove, 2012; Crow et al., 2018). Consequently, quantifying the eco-geographic scale over which trait differences vary across grassland ecosystems may provide important guidance in establishing seed transfer recommendations for future restoration.

The evolution of complex traits results from a combination of genetic and environmental variation, as well as their interaction, and can lead to the evolution of locally adapted genotypes

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[1] The material in this chapter was co-authored by Zebadiah Yoko, Kate Volk Ned Dochtermann, and Jill Hamilton. JH designed and established the experiment, ZY and KV collected data, ZY and ND contributed to data analysis, ZY and JH wrote the manuscript. This work is in review for publication with Annals of Botany Plants

(Escudero et al., 2003; Wright et al., 2004; Martin et al., 2007). Locally adapted genotypes exhibit trait values that may be advantageous in their 'home' environment, but maladaptive in an 'away' environment (Hereford, 2009; Johnson et al., 2010; Bucharova, Durka, et al., 2017). Thus, varying selection pressures across environments may contribute to substantial variation in quantitative traits across a species' range. In addition to variation in the direction of selection, the strength of selection may also vary across eco-geographic scales, contributing to regional and population-level differences in traits important to adaptation (Manel et al., 2003; Messier et al., 2010; Hovick et al., 2018). Indeed, not all traits are differentiated across region- or population-level scales. This may be due, in part, to a lack of trait variation on which selection may act, or if traits are subject to stabilizing rather than directional selection across environments (Levin, 1992; Ackerly, 2003). Identifying the degree to which genetic differences have evolved across a species' range, and quantifying the proportion of trait variation attributable to regional and population-level effects within and across functional trait classes will be valuable in establishing seed transfer recommendations in the future.

In this study, we use *Geum triflorum* (Pursh) or Prairie Smoke, to quantify the scale(s) over which trait differences have evolved across its range. We compare regional and population trait differentiation for a range of individual quantitative traits and functional trait classes. *G. triflorum* is an herbaceous perennial native to North America with a wide distribution spanning the Great Plains of the U.S and Central Canada (hereafter referred to as 'Prairie'), as well as alvar habitats surrounding the Great Lakes Region and into Manitoba (Fig. 2.1). Prairie and alvar habitats exhibit contrasting environments. Prairie habitats are typically characterized by cold, dry winters and hot, humid summers and experience unpredictable variation in water availability that can be partially mitigated by the presence of thick, nutrient-rich soil (Risser et al., 1981;

Anderson, 2006). In contrast, alvar habitats, which exhibit shallow soils over dolomitic limestone, are prone to predictable extremes in seasonal water availability with annual transitions between complete flooding in the spring to total desiccation by early summer (Catling and Brownell, 1995; Stark et al., 2004). Variation in water availability during the growing season likely contributes to the evolution of genetic differences in quantitative traits between alvar and prairie regions. Thus, understanding the eco-geographic scale of trait differentiation across the landscape may influence development of recommendations for seed transfer between regions, or between populations within regions, for restoration. Here we evaluate fifteen quantitative traits organized broadly by functional trait class; including resource allocation, stomatal characteristics, and leaf morphological traits. Trait variation within these classes is often associated with environmental differences (Ackerly et al., 2002; Hulshof et al., 2013; Martin et al., 2017), as all plants balance carbon gain with water loss. To quantify genetic differences in traits for seeds sourced from across the range of *G. triflorum*, we quantified the proportion of trait variances explained by regional- and population-effects for a number of traits; including those related to resource allocation, stomatal, or morphological differences. In addition, we compare the proportion of trait differentiation explained by region- and population-scales across these three functional trait classes. We predict that the scale over which trait classes differ will vary depending on how selection at the regional or population-scale impacts the distribution of trait variation across contrasting environments. Identifying the scale over which functional trait classes differ across eco-geographic scales will be informative for developing seed transfer guidelines for restoration for the future.
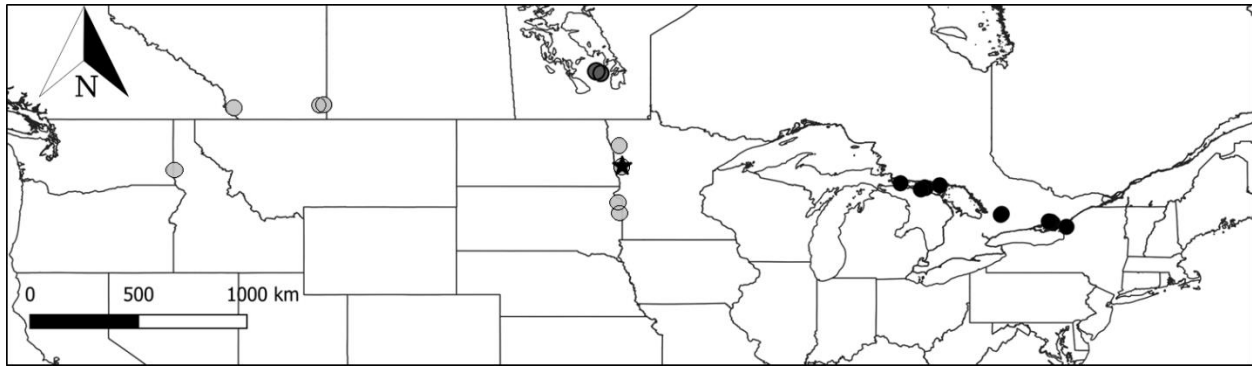
Figure 2.1. Map of collection sites of *Geum triflorum* (Pursh).
Black dots represent Great Lake Alvar populations, two populations in intermediate gray dots near Lake Manitoba represent Manitoban Alvars, and the lightest gray dots represent Prairie collection sites. Common garden location depicted as a star.

2.2. Methods

2.2.1. Field Sampling of *G. triflorum*

In the spring of 2015, seeds from 22 populations of *G. triflorum* were collected across much of the species' range. Nineteen populations were sampled spanning three distinct eco-regions; including eleven from the Great Lake alvars (GLA) region, two from the Manitoba alvars (MBA) region, and six from the Prairie (PRA) region (Fig. 2.1, Table 1). Forty open-pollinated maternal seed families were collected along a 100m transect within each population (see details in Hamilton and Eckert 2007). In addition to field collections, three bulk seed collections were provided by commercial growers (SD-PMG, MN-PMG) and the United States Department of Agriculture (WA-BLK) from the prairie region (Fig. 2.1, Table 1).

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Table 2.1. Names of source populations of *G. triflorum* separated by region.

Populations:	Latitude	Longitude	Elevation (m)	No. Indv. seeded	No. Indv. in Garden	Percent Survival (%)	Dist. from garden (km)
Great Lake Alvars (GLA)						—	
CAR-NBA	44.68502	-79.0515	268	120	97	81	1368.191
CAR-PSR	44.64526	-79.0946	250	120	75	63	1366.181
MAN-FOX	45.89713	-82.5789	186	120	98	82	1068.226
MAN-KIP	45.87036	-82.5394	183	120	100	83	1071.794
MAN-LCI	45.99426	-81.8944	182	117	77	66	1118.294
MAN-MIS	45.80825	-82.7591	193	119	96	76	1056.428
MI-DRI	46.08578	-83.692	188	120	103	86	979.8749
NAP-ASS	44.26533	-76.7119	126	120	46	38	1559.351
NAP-CE	44.33003	-76.7897	166	120	70	58	1551.28
NAP-SCH	44.34399	-76.8934	154	120	92	77	1542.945
WNY-CB	44.09764	-76.0829	93	120	92	77	1612.813
Manitoba Alvars (MBA)						—	
MB-CRN	51.07094	-97.4613	231	120	91	76	472.9779
MB-MR	51.18429	-97.6268	231	120	87	73	487.3889
Prairie (PRA)						—	
AB-HSC	49.63639	-110.33	721	120	5	4	1070.778
AB-LL	49.54361	114.2472	929	120	108	90	1348.389
AB-RL	49.66528	-110.108	721	120	0	0	1055.739
AB-RO	49.67194	110.1472	721	120	6	5	1058.689
MN-PMG	47.7742	-96.6081	267	24	8	33	101.3225
ND-BSP	46.85845	-96.4717	274	120	39	33	1.896
SD-MUD	44.76309	-96.5879	531	120	99	83	234.4168
SD-PMG	45.2186	-96.6336	351	24	11	46	184.0634
WA-BLK	46.68551	116.9719	786	24	12	5	1558.248
Common Garden	46.86913	-96.4522	259	2348	1412	58	

No. of indv. seeded represents the number of individual seeds from the respective population included in the initial experimental design setup, while No. indv. in garden represents individuals that germinated and were transplanted to the permanent field site. Percentage survival represents difference seeded and germinated by population, along with the garden total. Latitude and longitude and elevation (m) of source populations listed. Distance from garden calculated as greater circle distance (Pearson, 2013) between source location and common garden location established at Minnesota State University Regional Science Center.

2.2.2. Common Garden Experiment

A common garden experiment was established on November 7, 2015 at North Dakota State University. Twenty-two populations were planted across twelve randomized complete blocks. For field-collected populations, ten maternal seed families were planted per population, including 12 individuals per maternal seed family. For bulk-seed collections, 24 seeds were planted including two replicates per block for each source (Table 1). Seeds were treated with a .02% PPM^{TM} fungicide treatment and grown in conetainers (Stuewe & Sons, 158mL) filled with Sungro horticulture mix soil for approximately two months, following which surviving germinants were re-potted into mini-treepot containers (Stuewe & Sons, 1014 mL). Seedlings were grown for 27 weeks under controlled greenhouse conditions, maintained at a 15h:9h daylight to darkness photoperiod with supplemental light from halide lighting at a measured flux density of 0.3383 mmol m^2 s^{-1} and temperatures between 18.3°C and 23.9°C. Plants were watered bi-weekly, and provided a slow release fertilizer mix (Osmocote 14N-14P-14K) intermittently throughout the course of the experiment. In May 2016, surviving germinants were transferred to a permanent outdoor research facility at the Minnesota State University Moorhead (MSUM) Regional Science Center (Table 1, 46.86913N, -96.4522W). The randomized complete block design was maintained in the field planting. Seedlings were planted directly into soil through cutouts in a weed barrier to limit competition. Percent survival was calculated following transplant to the outdoor garden as the number of individuals successfully established per population versus the number planted in the initial design (Table 1). The number of individuals established ranged from 5 to 108 per population, with the exception of AB-RL, which exhibited zero percent emergence in the greenhouse (Table 1).

2.2.3. Measurement of Quantitative Traits

2.2.3.1. Morphological measurements

Leaf morphological trait variation was quantified for all surviving individuals in the greenhouse prior to transfer to the permanent outdoor research facility. For each individual (n=1396), one leaf was randomly sampled, photographed and measured for variation in midvein length, sinus depth and mini leaflet presence and shape on a $1cm^2$ grid. Midvein length was measured as the total length of the primary vein per sampled leaf. Sinus depth refers to the depth of the margin between the apex lobe and the next nearest lobe. Mini-leaflets, defined as small leaflets along the midvein between lobes, were assessed as present or absent and the shape of leaflets was assessed as lobed or non-lobed (Fig. 2.2). All measurements were quantified using ImageJ software (Schneider et al., 2012).
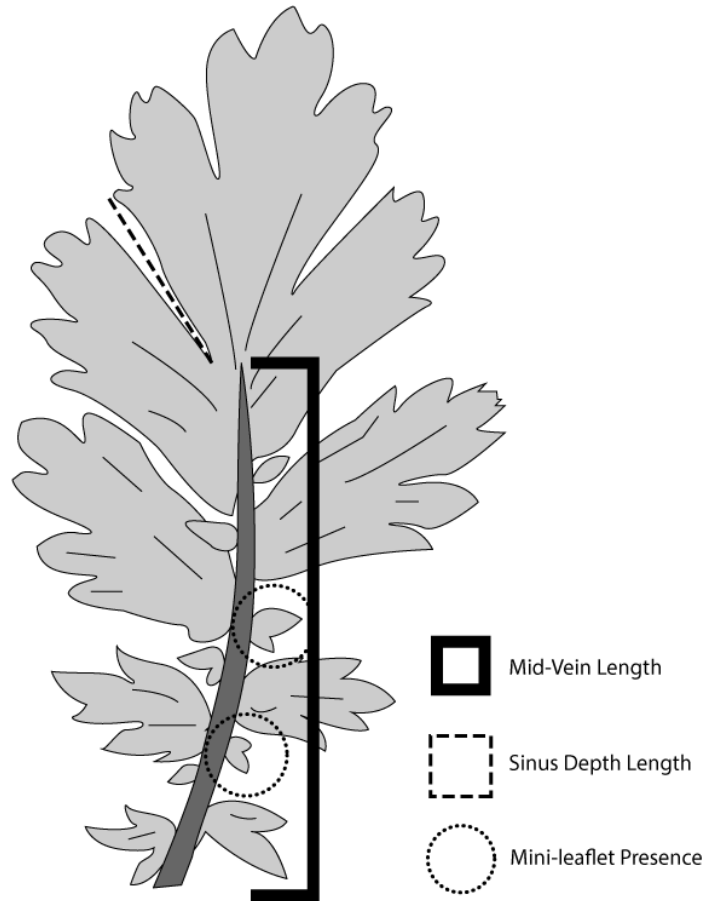
Figure 2.2. Morphological measurements of leaves of *G. triflorum*.
The bracket outlines the length of the midvein, the dashed line represents the length of the major sinus depth, the lightly dashed circles illustrate the mini-leaflets, which were assessed as presence/absence, and if present, if leaflets were lobed or not.

2.2.3.2. Resource allocation measurements

To evaluate genetic differentiation for traits associated with resource allocation we assessed specific leaf area, chlorophyll fluorescence, leaf dry matter content, and water use efficiency for a subset of individuals during the summer of 2018. These traits encompass a physiological tradeoff between efficient resource acquisition, specifically carbon sequestration while minimizing the consequences of water loss via transpiration (Reich et al., 1997; Messier et al., 2010). One to five individuals per population were evaluated. Due to the unbalanced number

of populations per region, between 9 and 56 individual measurements were taken per trait per region (Table 2).

Specific leaf area (SLA), calculated as a ratio of leaf area to dry mass, was measured over one day using a LI-3000C (*Li-Cor Biosciences*) portable area sensor for 99 individuals (approximately 5 individuals per population for a total of 33 PRA, 56 GLA, and 10 MBA individuals). The surface area of one randomly selected mature leaf was estimated alongside fresh and dry mass. Leaves were dried for 68 hours at 50□C, following which dry mass was taken. SLA is calculated as dry mass per unit leaf area. Leaf dry matter content (LDMC) was also calculated with these data, as LDMC is calculated as the ratio of fresh to dry mass.

Chlorophyll fluorescence was quantified over a single 90-minute period in the field common garden using a CCM-300 (Opti-Sciences) on 98 individuals, approximately 5 individuals per population for a total of 33 PRA, 56 GLA, and 9 MBA individuals. The CCM-300 records emission ratios of 700 and 735 nm (red and far red wavelengths) as a proxy for chlorophyll content (Gitelson et al., 1999). Here, we use chlorophyll fluorescence as a proxy for individual leaf chlorophyll content.

To quantify integrated water-use efficiency (WUE), we used carbon isotope composition as measured by $\delta^{13}C$ (Farquhar et al., 1989b). Leaf samples from approximately five individuals per population (53 GLA, 9 MBA, and 31 PRA individuals) were sampled from the field common garden and oven-dried at 55°C over a twenty-four hour period. Following this, leaf samples were homogenized into a fine powder using a TissueLyser II (Qiagen, Hilden, Germany) and 4-5 mg of each sample were weighed and placed into a tin capsule (Costech, Valencia, CA, USA) for ^{13}C isotope analysis using a continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope Facility (Davis, CA, USA). To assess the repeatability

of isotope measurements across samples, 23 technical replicates were evaluated. A correlation of r=0.793 across technical replicates provides confidence in the repeatability and precision of the assay. Reported $\delta^{13}C$ values are expressed as relative to the Vienna Pee Dee Belemnite.

2.2.3.3. Stomatal measurements

Stomatal density and size were measured for adaxial and abaxial leaf surface impressions for individuals within the common garden experiment. Newskin 'liquid bandage' was applied to either adaxial or abaxial surfaces for two leaves per individual within the common garden for a total of 650 leaf surface impressions (417 GLA, 91 MBA, and 142 PRA individuals, Table 2). Surface impressions were mounted onto slides and photographed using a Zeiss Stereo Discovery (V8) digital microscope with a Canon Rebel T3 E0S 1100D digital camera. Stomatal density was calculated as the number of stomata from either the abaxial or adaxial surface divided by the area of the impression image (0.32 x 0.42mm)**.** Guard cell length was measured using ImageJ software (Schneider et al., 2012) and calculated based on an average of three stomata per surface impression. Stomatal area index (SAI), which is the total amount of area covered by stomata on a leaf surface, was calculated as the product of guard cell length and stomatal density (Bertel et al., 2017).

Stomatal conductance was measured using a Decagon SC-100 Porometer (METER Group) between 09:00am and 11:30am over the course of five days (August 8, 2018 – August 12, 2018) for 99 individuals in the common garden experiment (approximately 5 individuals per population for a total of 33 PRA, 56 GLA, and 10 MBA individuals, Table 2). Stomatal conductance estimates transpiration, calculating water transpiration via stomata over 30 second intervals. The rate of conductance is intrinsically linked to transpiration as well as gas exchange (CO_2 acquisition), reflecting an important tradeoff in plant growth and water-use. Five

individuals were sampled per population (Table 2), with one individual within each population measured per day to minimize effects of temporal environmental variation on population-level estimates. A subset of ten individuals were measured repeatedly over the course of sampling to assess repeatability within individuals, while accounting for changes in daily temperature and humidity. We used a linear model to assess repeatability, with individual as a fixed effect, and temperature and humidity as random effects. Temperature and humidity had little effect on stomatal conductance measurements (R^2=0.151, and R^2=0.0, respectively), and repeatability within individuals was fairly low ($R^2 = 0.372$)

Table 2.2. List of traits assessed and number of individual *G. triflorum* plants assessed for each trait.

Quantitative Trait	Quantitative Trait category	Number Prairie individuals sampled	Number Great Lake alvar individuals sampled	Number Manitoba alvar individuals sampled	Total Number of individuals per trait
Sinus Depth	Morphological	277	940	174	**1391**
Midvein Length	Morphological	277	940	174	**1391**
Presence of mini leaflets	Morphological	277	939	174	**1390**
Presence of Lobed minileaflets	Morphological	222	837	137	**1196**
Chlorophyll Content	Physiological	33	56	9	**98**
Specific Leaf Area	Physiological	33	56	10	**99**
Leaf Dry Matter Content	Physiological	33	56	10	**99**
Carbon Isotope Discrimination)	Physiological	31	54	10	**95**
Stomatal Conductance	Stomatal	32	56	10	**98**
Stomatal Density (abaxial)	Stomatal	123	356	85	**564**
Stomatal Density (adaxial)	Stomatal	123	349	83	**555**
Stomatal Size (abaxial)	Stomatal	122	353	83	**558**
Stomatal Size (adaxial)	Stomatal	122	346	82	**550**
Stomatal Area Index (abaxial)	Stomatal	122	353	83	**558**
Stomatal Area Index (adaxial)	Stomatal	122	346	82	**550**

Number of individual *G. triflorum* plants assessed for each morphological, physiological, and stomatal trait within the common garden experiment separated by region. Total number of individuals sampled per trait in bold

2.2.4. Statistical Analysis

2.2.4.1. Assessing the scale of genetic differentiation across complex landscapes: regional and population effects

In a common garden experiment, trait differences observed can be associated with genetic differences as the shared environment controls for much of the environmental variation that might otherwise contribute to the expression of trait differences (De Kort et al., 2014). Phenotypic traits were assessed for normality and homogeneity of variance using the Shapiro-

Wilk test and Bartlett test, respectively. Of the 15 traits evaluated, specific leaf area (SLA) was log-transformed to meet assumptions of normality. In addition, a square root transformation was used for stomatal density, stomatal conductance, adaxial and abaxial stomatal area indices (SAI), and sinus depth. Midvein length failed both the Shapiro-Wilk test and Bartlett test, but was visually assessed as normal.

To estimate the proportion of variance explained by region and population, we fit a linear mixed effect model using the lme4 package in R (Bates et al., 2015; R Core Team, 2018) for each trait. The full model for each trait was:

$$y_{ijk} = R_i + p_j + e_{ijk}$$

Where y_{ijk} is the predicted trait value for individual from region i and population j; with R being the effect of region i, p is the effect of population j, and e as the residual variance for individual k. Within the mixed model, region (R_i: PRA, GLA, or MBA) was classified as a fixed effect and population as a random effect, as populations represent a random selection of the total number of populations (p_j) within each region. Interaction terms were not included in the model because no population occurred in more than one region. Normality of residuals was visually assessed for all traits. All statistical tests were conducted in R.

To determine the impact of eco-geographic scale on trait differentiation, we estimated the proportion of variance explained by fixed (region) or random (population, residual) effects for each trait using the rptGaussian function in the rptR package (Stoffel et al., 2017; R Core Team, 2018). The rptR package estimates the proportion of variance explained by a given effect, which can be considered equivalent to a goodness of fit, or R^2 (Nakagawa and Schielzeth, 2013). We estimated the R^2 value using region as a fixed effect. As estimates of regional difference could have an impact on random (population) effects, regional differences were accounted for by

including fixed effect estimates in the denominator of the R^2 (i.e. marginal R^2's (Nakagawa and Schielzeth, 2013), or unadjusted repeatabilities (Nakagawa and Schielzeth, 2010)) for population effects. These estimates of R^2 can be biologically interpreted as the proportion of variation in traits that is attributed to genetic differentiation at the corresponding eco-geographic scale (region or population), with residual variation representing differences in trait values from effects not evaluated in this study.

2.2.4.2. Comparing the proportion of variance explained by region and population on quantitative traits and across trait classes

To determine the significance of fixed (region) effects estimates, an Analysis of Variance (ANOVA) was conducted on the linear mixed effect model (Table 3). The proportions of variance were then bootstrapped (n=1000) to provide a 95 percent confidence interval for variance explained for each trait dependent on the predictor variable. P-values for random effects (population) were obtained from likelihood ratio-tests.

To assess differences in trait classes at different scales, proportions of variance for each trait were averaged within each trait class. Due to a significant difference in homogeneity of variance across trait classes, Kruskal-Wallis tests were used to compare trait class variance attributed to region and population. Quantitative traits were grouped into 'morphological', 'resource allocation' or 'stomatal' trait classes based on primary function within the leaf (Table 2). A Dunn test post-hoc evaluation was conducted using the Dunn.test (Dinno, 2017) package to compare variance explained across trait classes. All tests were run using R (R Core Team, 2018, version 3.5.0).

2.3. Results

2.3.1. Quantitative Traits Differentiation Across Complex Landscapes: Regional and Population Effects

Regional and population-scale differences explained a considerable amount of variation for quantitative traits. The proportion of phenotypic variance explained by region varied between $R^2=0.0125$ to 0.201 for all traits (Table 2.3, Fig. 2.3), and population from zero to 0.292 (Table 2.4). Ten of the fifteen traits sampled had a significant proportion of variance explained at the regional scale (Table 2.3). Thirteen of the fifteen traits had a significant proportion of variance explained at the population scale (Table 2.4). This indicates that region and population differences explain a substantial proportion of variation for some traits, but not all. Below we elaborate on the variation explained by region and population organized by trait class.
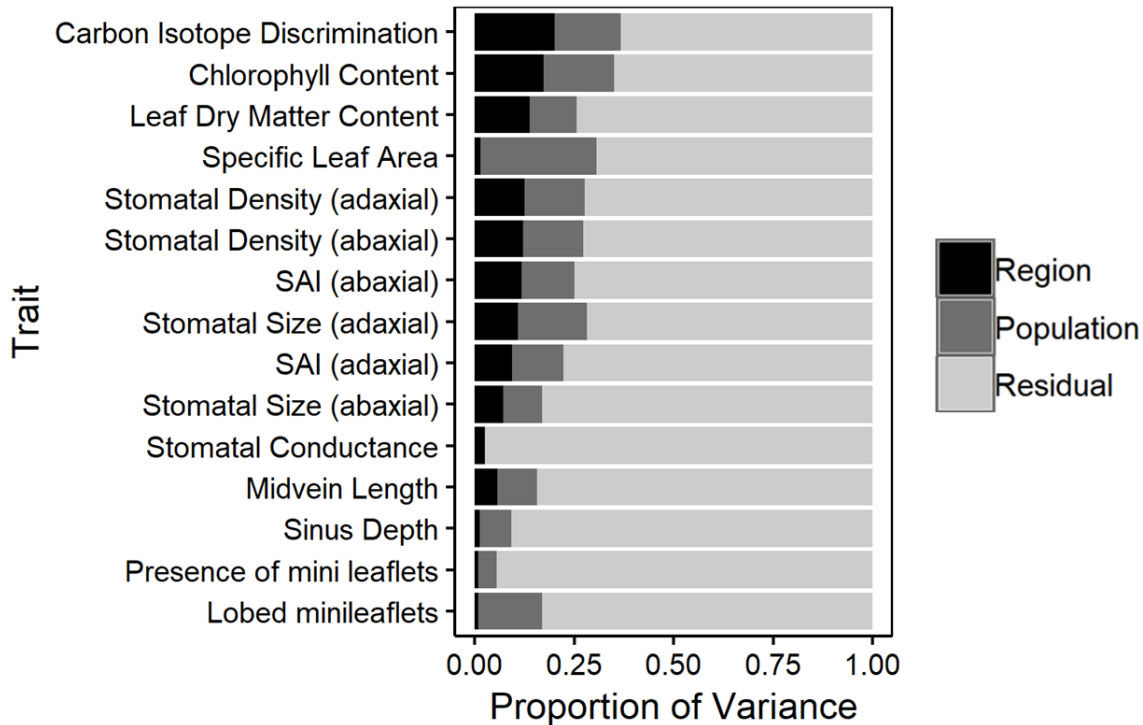
Figure 2.3. Proportion of variance explained by each scale factor for each trait. Proportion of variance explained by region (black), population (dark gray), and residual individual (light gray) variance for quantitative traits measured in a common garden experiment. Linear mixed models were used to calculate the proportion of variance explained with region as a fixed effect and population as a random effect, with all other variation considered individual residual variance.

2.3.1.1. Morphological traits

Collectively, regional differences explained a limited amount of morphological trait variability. The proportion of variance explained by region was significant for midvein length (R^2=0.056, p=0.02). However, regional differences did not have a significant effect on variability observed in leaf sinus depth (R^2=0.0125, p = 0.245), presence of mini leaflets (R^2=0.009) and the presence of lobed or unlobed leaflets (R^2=0.008) (Table 2.3, Fig. 2.3).

As was the case for region, population also explained little variation in morphological traits—albeit marginally more. The proportion of variance explained by population-level differences for sinus depth and midvein length was R^2= 0.079 (p = <0.001) and R^2= 0.1 (p =

<0.001), respectively (Table 2.4). Population-scale effects explained more variation than region for presence of mini leaflets and whether mini leaflets were lobed or not R^2=0.047 (p = <0.001) and R^2=0.161 (p = <0.001), respectively.

2.3.1.2. Resource allocation traits

Resource allocation traits exhibited a large range in the proportion of trait variance explained by region. While a small proportion of variation was explained by region for specific leaf area (SLA) (R^2=0.014, p = 0.7179), a significant proportion of variability was explained for carbon isotope discrimination (R^2 = 0.201, p = 0.0275). In addition, the proportion of trait variability explained by region for leaf dry matter content and chlorophyll content was significant (R^2= 0.138, p = 0.017 and R^2= 0.173, p = 0.012) (Table 2.3, Fig. 2.3).

While regional effects explained a significant proportion of trait variability for resource allocation traits, population-scale effects also contributed substantially to trait variances (Table 2.4). Interestingly, while region explained limited variability in specific leaf area, a substantial proportion of variability was explained by population-level variation (R^2=0.292, p = 0.00135). Region and population explained the same proportion of variance for chlorophyll fluorescence (region and population R^2= 0.177, Tables 2.3, 2.4) and a similar proportion of variability was explained by both region and population for leaf dry matter content (Tables 2.3, 2.4, region R^2= 0.138, population R^2= 0.117). A greater proportion of variation in carbon isotope discrimination was explained by regional differences relative to population-level differences, though both were significant (region R^2= 0.201 p = 0.007, population R^2= 0.166, p= 0.0275, Tables 2.3, 2.4).

2.3.1.3. Stomatal characteristics

Both regional and population-level differentiation explained a considerable proportion of variation across stomatal traits. Similar proportions of trait variation were explained by region

for stomatal density and SAI (density: $R^2 = 0.122$, p = 0.006 abaxial; $R^2 = 0.125$, p = <0.001 adaxial; SAI: $R^2 = 0.118$, p= 0.0055 abaxial; $R^2 = 0.094$, p = 0.011 adaxial; Table 2.3, Fig. 2.3). However, the proportion of variance explained by region for stomatal size differed substantially across leaf surfaces ($R^2 = 0.072$, p = 0.016 abaxial; $R^2 = 0.108$, p = 0.015 adaxial; Table 2.3). Finally, while a substantial proportion of variability was explained by region across all stomatal traits, population-level differences explained a greater proportion of variability (Table 2.4). This excludes stomatal conductance, where variability was not explained by either regional or population-level differences ($R^2 = 0.026$, p = 0.2465; $R^2 = 0$, p = 0.5).

Table 2.3. Proportion of variance explained by each region per each trait model.

Trait	Quantitative Trait Category	Effect Size of Region	p-value of Region
Leaf Sinus Depth	Morphological	0.013	0.245
Midvein Length	Morphological	0.056	*0.02*
Presence of Mini Leaflets	Morphological	0.008	NA
Presence of Lobed Mini Leaflets	Morphological	0.008	NA
Chlorophyll Fluorescence	Resource Allocation	0.173	*0.01195*
Specific Leaf Area (SLA)	Resource Allocation	0.014	0.7179
Leaf Dry Matter Content (LDMC)	Resource Allocation	0.138	*0.01723*
Carbon Isotope Discrimination	Resource Allocation	0.201	*0.00679*
Stomatal Conductance	Stomatal	0.026	0.2465
Stomata Density (abaxial)	Stomatal	0.122	*0.006*
Stomata Density (adaxial)	Stomatal	0.125	*0.006*
Stomata Size (abaxial)	Stomatal	0.072	*0.016*
Stomata Size (adaxial)	Stomatal	0.108	*0.015*
Area Index (abaxial)	Stomatal	0.118	*0.0055*
Area Index (adaxial)	Stomatal	0.094	*0.011*

Quantitative trait category represents which trait class the trait is categorized as. P values of effect sizes are listed for region, with significant values italicized and in bold. P-values for fixed effects of binary measurements (presence of mini-leaflets and presence of lobed minileaflets) are not obtainable and are listed as NA.

Table 2.4. Proportion of variance explained by population and residual effect per each trait model.

Trait	Quantitative Trait Category	Effect Size of Population	p-value of Population	Residual Variance
Leaf Sinus Depth	Morphological	0.079	***<0.001***	0.908
Midvein Length	Morphological	0.1	***<0.001***	0.844
Presence of Mini Leaflets	Morphological	0.047	***<0.001***	0.944
Presence of Lobed Mini Leaflets	Morphological	0.161	***<0.001***	0.831
Chlorophyll Fluorescence	Resource Allocation	0.177	***0.0149***	0.649
Specific Leaf Area (SLA)	Resource Allocation	0.292	***0.00135***	0.693
Leaf Dry Matter Content (LDMC)	Resource Allocation	0.117	0.118	0.745
Carbon Isotope Discrimination	Resource Allocation	0.166	***0.0275***	0.633
Stomatal Conductance	Stomatal	0	0.5	0.974
Stomata Density (abaxial)	Stomatal	0.15	***<0.001***	0.728
Stomata Density (adaxial)	Stomatal	0.151	***<0.001***	0.724
Stomata Size (abaxial)	Stomatal	0.096	***<0.001***	0.832
Stomata Size (adaxial)	Stomatal	0.174	***<0.001***	0.718
Area Index (abaxial)	Stomatal	0.134	***<0.001***	0.749
Area Index (adaxial)	Stomatal	0.129	***<0.001***	0.777

Quantitative trait category represents which trait class the trait is categorized as. P values of effect sizes are listed for population, with significant values italicized and in bold.

2.3.2. Comparison Across Trait Classes

We compared the proportion of trait variances explained by regional and population-scale differences for traits grouped into three distinct trait classes: morphological, resource-allocation, and stomatal trait classes. Stomatal conductance was removed from this comparison as no variation was explained by regional or population-scales and the trait exhibited limited repeatability. From the Kruskal-Wallis test, the proportion of variance explained by region significantly varied across trait classes ($X^2 = 7.9143$, $p = 0.02$). Resource allocation and stomatal

trait classes explained a statistically greater proportion of regional trait variance relative to

morphological traits, (Fig. 2.4). Resource allocation traits exhibited the greatest proportion of

regional trait variance, with the stomatal trait class only slightly lower. Despite regional

differences in the proportion of variance explained across trait classes, there was no significant

difference in the proportion of phenotypic variance explained across trait classes at the

population-scale ($X^2 = 4.8286$, p-value = 0.089, Fig. 2.4). This reflects the increased variability

around the mean at the population scale.
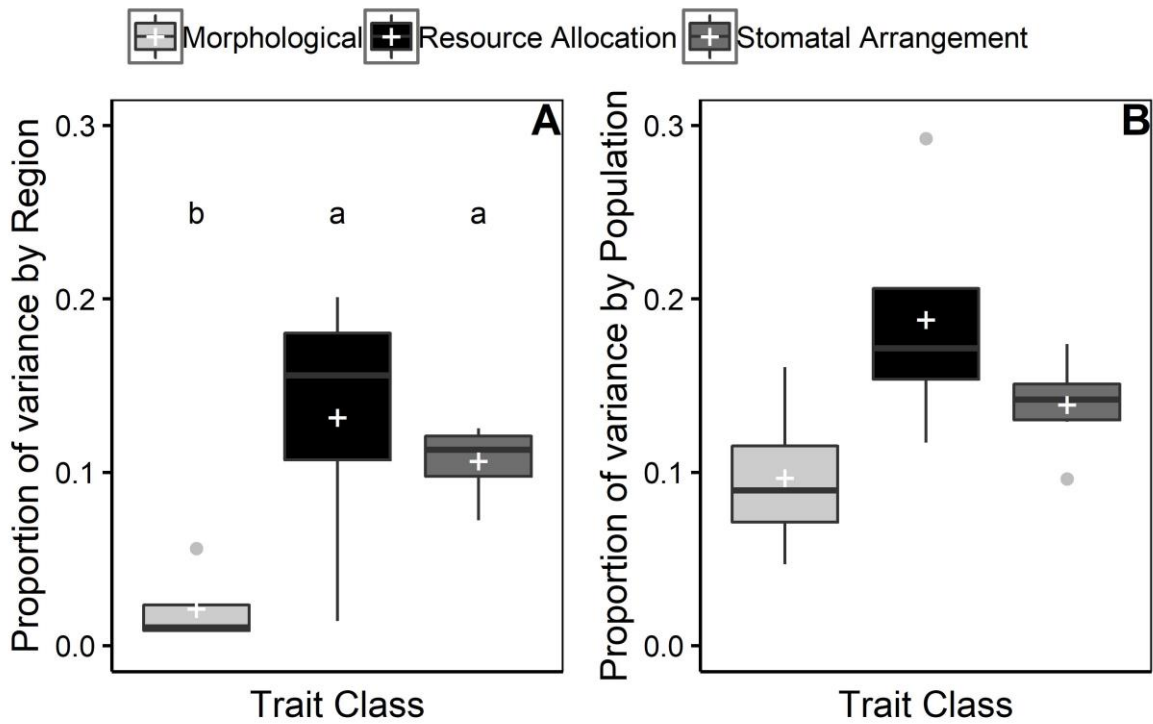
Figure 2.4. Proportion of variance explained across functional trait classes.
Proportion of variance explained across functional trait classes for regional (A) and population
(B) effects. Significant differences were observed across trait classes, including morphological
(light gray), resource allocation (black) and stomatal traits (medium gray) for regional effects,
whereas no significant differences were observed for population effects. Statistical differences
between trait classes are indicated by a and b. Significant differences are denoted by a letter
change (p<0.05) and same letters indicate no significant difference. White crosses indicate mean
variance explained for each trait class.

2.4. Discussion

Understanding the scale over which trait differences and functional trait classes evolve will impact development of seed transfer guidelines for restoration. We examined quantitative trait differentiation in a common garden experiment for individuals sourced from much of the range of *G. triflorum*. We found a large proportion of phenotypic variance was explained by regional and population-scale differences. Consistent with previous literature, genetic variation is partitioned at broad regional- and fine population-scales across complex landscapes (Messier et al., 2010; Hovick et al., 2018; Baughman et al., 2019). Our results suggests that while genetic differences in traits important to adaptation have evolved between ecologically distinct regions (Etterson, 2004; Durka et al., 2017), fine-scale evolutionary processes likely also contribute to the distribution of trait variation (Messier et al., 2010; Hovick et al., 2018). Additionally, the distribution of trait variation differed by functional trait class at the regional level. Stomatal trait and resource allocation trait classes had greater variance explained by regional differentiation relative to morphological traits. The tighter association of stomatal and resource allocation traits to fitness may reflect directional selection over varying eco-geographic scales. Thus, selection across eco-geographic scales likely contributes to the distribution of trait variability in complex landscapes.

2.4.1. Trait Differentiation Across Scales in Complex Landscapes

The distribution of trait variances suggests that landscape-level processes likely play an important role in shaping quantitative trait differences at the regional scale (Manel et al., 2003; De Kort et al., 2014; Maia et al., 2017). Across fifteen quantitative traits observed in the common garden, region explained between nearly zero and twenty percent of the observed variation (Table 3). In this system, the contrasting environmental extremes of alvar and prairie

habitats likely contribute to the evolution of regional genetic differences. Alvar habitats, with very thin layers of soil over limestone experience seasonal extremes in water availability: from spring flooding, to summer drought (Hamilton and Eckert, 2007). This contrasts with prairie habitats which experience infrequent flood and drought, and have a substantial layer of soil to mediate fluctuations in water availability (Anderson, 2006). Given this, selection associated with regional differentiation in water availability has likely influenced the distribution of trait variation.

While a substantial proportion of quantitative trait variation was explained by regional differences, equal or greater variance was explained by population-scale effects. This suggests alvar and prairie regions are not only differentiated from each other, but populations within regions are also highly differentiated from each other. While the direction and magnitude of selection likely vary across regions and populations, gene flow and drift may additionally influence the distribution of genetic variation (Manel et al., 2003). Stochastic changes associated with reduced connectivity and demography can contribute to differences observed. Previous research from Hamilton and Eckert (2007) indicated within the same geographic distance alvar populations were more genetically different from each other at neutral genetic loci than prairie populations. They attributed population-scale differentiation within regions to the combined influence of reduced gene flow and fine-scale environmental selection. For geographically disjunct alvar populations the combined influence of reduced gene flow and selection may lead to greater variance explained for population-scale variation relative to more continuous prairie environments.

2.4.2. Variance Across Trait Classes for Regional and Population Level Differences

To broadly compare the proportion of variance explained by region and population for functional trait classes we grouped fifteen individual quantitative traits into resource allocation, stomatal, and morphological trait classes. Our results suggest that differences have evolved across functional trait classes, but that those differences do not manifest equally across all trait classes. Resource allocation and stomatal trait classes exhibited significantly greater regional differentiation relative to the morphological trait class (Fig. 4). Resource allocation and stomatal traits are likely important to adaptation, and may be under strong divergent selection across regional environments. Carbon isotope discrimination is typically viewed as a proxy measure of water use efficiency (Farquhar et al., 1989a) and differentiation in this ratio suggests genetic differences have evolved likely as an adaptive response to extremes in water availability in the alvar ecosystem. Resource allocation traits measured here also represent part of the 'leaf economic spectrum' (Grime et al., 1997; Wright et al., 2004), where tradeoffs exist between resource investment and leaf lifespan. We expect that differential investment in leaf traits associated with alvar and prairie environments likely contribute to substantial regional differences for this trait class.

In addition to resource allocation, traits related to stomatal characteristics, specifically size and number of stomata may be under differential selection at the regional scale. Alvar plants experience seasonal flood to drought cycles, which likely selects for increased efficiency in managing extremely variable water availability. The most efficient arrangement of stomata is to rapidly respond to environmental change is smaller size and greater number of stomata (Drake et al., 2013; Carlson et al., 2016). Therefore, we expect divergence in the direction of selection between alvar and prairie habitats. Alvar populations exhibit smaller size and greater number of

stomata while prairie populations exhibit larger, but fewer stomata (pers. obs.). Larger stomata may facilitate greater gas exchange capabilities, increasing productivity when plants are not under water stress in prairie environments (Lawson and Blatt, 2014; Dittberner et al., 2018). The large amount of variation explained by region for these traits appears to be driven by these contrasts.

Little quantitative trait variance was explained by regional effects within the morphological trait class. These data suggest that morphological traits, while important to plant form and function, are not under strong diversifying selection across regional or population scales for *G. triflorum* or do not exhibit enough genetic variation for which natural selection to act upon. This contrasts with the stomatal characteristic and resource allocation trait classes, suggesting that differentiation within morphological traits may more adequately reflect differentiation due to stochastic processes. Differentiation in morphological traits at the regional scale may be a product of drift or demographic processes, particularly if differences in these traits do not affect relative fitness of individuals (Hereford, 2009).

Local adaptation can occur on multiple scales (McKay et al., 2005), and genetic differences can evolve between populations as a result of fine-scale response to selection. While a greater proportion of variance is explained at the population scale, no significant differences were observed in that proportion across trait classes (Fig. 4). While populations have evolved differences, the evolutionary processes contributing at the population-scale likely represent a combination of stochastic and deterministic processes. Reduced connectivity among alvar populations and prairie fragments, alongside site-specific selection not directly studied here could have substantial influence on the distribution of population genetic variation for quantitative traits (Hamilton and Eckert 2007).

2.4.3. Evolutionary Factors Impacting Trait Variance Across Scales

Our data support the evolution of genetic differences across heterogeneous landscapes in response to varying selective pressures, but we cannot rule out the possibility that other evolutionary processes have shaped the distribution of quantitative trait variation. Alvar habitats were likely colonized by *G. triflorum* during a range expansion from prairie environments during the warming Hypsithermal period (Hamilton and Eckert, 2007). Stochastic demographic processes during colonization, including founder events and population bottlenecks, likely contributed to observed differentiation in contemporary quantitative traits. In addition, the alvar populations of *G. triflorum* are disjunct not only from prairie environments, but also from each other. If effective population size is small, and barriers to gene flow exist between regions, genetic drift may have contributed to accumulation of genetic differences (Lande, 1992; Knapp and Rice, 1996; Young et al., 1996).

While our findings support regional and population-scale differentiation for traits important to adaptation across the range of *G. triflorum*, the relationship with fitness, including reproductive success or number of reproductive events undertaken, for the individual plants studied has not been estimated. The traits we examined have frequently been related to fitness through life history tradeoffs and provisioning resources to reproduction (Dudley, 1996; Wright et al., 2004; Martin et al., 2007; Muir, 2015), but do not directly capture reproductive life history variation in *G. triflorum.* As *G. triflorum* is a perennial species, quantifying lifetime fitness variation would provide a means to relate trait variation to fitness consequences in a given environment. Additionally, to truly test whether populations are locally adapted, reciprocal transplant experiments would be necessary (Griffith and Watson, 2005; Ackerly et al., 2006; Hamilton and Eckert, 2007).

2.5. Conclusions

Sourcing seeds for restoration frequently aims to identify seed with the appropriate genetic variation for the restoration environment (Lesica and Allendorf, 1999; Gallagher and Wagenius, 2016). Understanding how genetic variance for quantitative traits is distributed will aid in establishing guidelines for seed transfer during restoration. For *G. triflorum*, individuals exhibited genetic differences for functional traits across regions and populations. If seed transfer is proposed, consideration of the impact functional trait differences have on success in the restoration environment is necessary (Bucharova, Michalski, et al., 2017). Furthermore, different traits or trait classes may be more or less appropriate for the development of seed transfer guidelines and may be context-specific depending on environmental selection pressures.

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3. EVALUATING CUMULATIVE DIFFERENCES IN FITNESS ACROSS THE RANGE OF GEUM TRIFLORUM[2]

3.1. Introduction

Sourcing seed for ecological restoration can be challenging if species used in restoration exhibit differential success depending on source provenance (Anderson et al., 2014; Nagel et al., 2018). Therefore, research on the evolutionary causes of genetic differentiation is undertaken to inform suitability of seed sources and optimize success of restorations. Grassland species are a particular focus of restoration efforts, as this ecosystem is one of the most at-risk biomes (Hoekstra et al., 2005). However, the genetic consequences of seed transfer and extent of genetic differentiation between populations of herbaceous species is largely unknown.

Current seed sourcing practices implemented by agencies such as the U.S. Forest Service, the B.C Ministry of Forests, and the U.S EPA use geographic, environmental and biotic factors to determine seed transfer zones (Johnson et al., 2010; Potter and Hargrove, 2012; O'Neill et al., 2017). Sourcing seeds from geographically or environmentally similar populations attempts to preserve local adaptation, where individuals have evolved genetic differences for phenotypic traits best suited to their local environment (Montalvo and Ellstrand, 2000; Joshi et al., 2001; Kawecki and Ebert, 2004; Leimu and Fischer, 2008; Savolainen et al., 2013). Individuals with traits locally adapted have greater fitness than foreign individuals in their home environment (Ackerly et al., 2006).

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[2] The material in this chapter was co-authored by Zebadiah Yoko, Mason Kulbaba, and Jill Hamilton. JH designed and established the experiment, ZY collected data, MK contributed to data analysis, MK, ZY, and JH wrote the manuscript. This work is in preparation for submission to Heredity

Preserving local adaptation is of particular interest for species that occur across heterogeneous environments. Highly differentiated environments may support genetically divergent populations of a species as a result of varying selection. Common garden experiments are often used to observe if there are genetic differences occurring across genotypes that span a species' range that impact fitness. By growing seeds from different sources across a species range in a common environment, differences observed can be attributed to genetic differences between the sources (Reich et al., 2005; De Villemereuil et al., 2016)

ASTER is a useful model to assess fitness in perennial plants as it jointly analyzes life history traits and their impact on reproductive output (Geyer et al., 2007) by accounting for differences in multiple traits with different statistical probabilities associated with distributions including survival, flowering, and an estimate of fitness. Previous studies have used ASTER to evaluate the impact of genetic differences between populations on fitness, ranging from determining diverging adaptation between populations (Sheth et al., 2018), to identifying additive genetic variance for lifetime fitness itself (Kulbaba et al., 2019). Relative to previous standard Lande-Arnold fitness landscape estimates, Aster provides an analytical method that addresses assumptions of distributions when conducting statistical tests that estimate fitness (Shaw and Geyer, 2010).

Here we use *G. triflorum* (Pursh), which is a perennial herbaceous forb endemic to three distinct eco-geographic regions that present varying environmental conditions. Across its distribution, *G. triflorum* ranges from abundant to locally rare. One eco-geographic region is the North American grasslands, known colloquially and hereafter referred to as 'Prairies.' Prairie habitats are typically characterized by cold, dry winters and hot, humid summers and experience unpredictable variation in water availability that is partially mediated by the presence of thick,

nutrient-rich soil (Risser et al., 1981; Anderson, 2006). In addition to prairie habitats, *G. triflorum* also occurs within the unique alvar habitat. Alvars occur in two distinct geographic regions within North America: one surrounding the Great Lakes of Eastern North America and the other adjacent to Lake Winnipeg in Manitoba, Canada. Alvar habitats have shallow soils over dolomitic limestone and are experience predictable extremes in seasonal water availability. (Catling and Brownell, 1995; Stark et al., 2004). Winters are very wet with lake effect snowfall, but during the growing season conditions transition from complete flooding in the spring (due to snowmelt), to total desiccation in early summer (Reschke et al., 1999).

We hypothesize that variation in water availability during the growing season contributes to the evolution of genetic differences between populations of *G. triflorum* in alvar and prairie regions. A common garden experiment was designed, with the objective of determining if there is a genetically-driven fitness difference between eco-geographic regions. Specifically, we assess if there are differences in reproductive effort between regions, and whether reproductive differences between regions differ across years, to evaluate variation in cumulative reproductive success across three years in a perennial plant species.

3.2. Methods

3.2.1. Field Collections

Seeds of *G. triflorum* were collected from 22 populations spanning much of the species' range in the spring of 2015. Nineteen populations were sampled spanning three distinct eco-regions; including eleven from the Great Lake alvars (GLA) region, two from the Manitoba alvars (MBA) region, and six from the Prairie (PRA) region (Fig. 3.1). Seeds were collected from forty open-pollinated maternal families along a 100m transect within each population (as in Hamilton and Eckert 2007). In addition to field collections, three bulk seed collections were

provided by commercial growers (SD-PMG, MN-PMG) and the United States Department of

Agriculture (WA-BLK) from the prairie region.
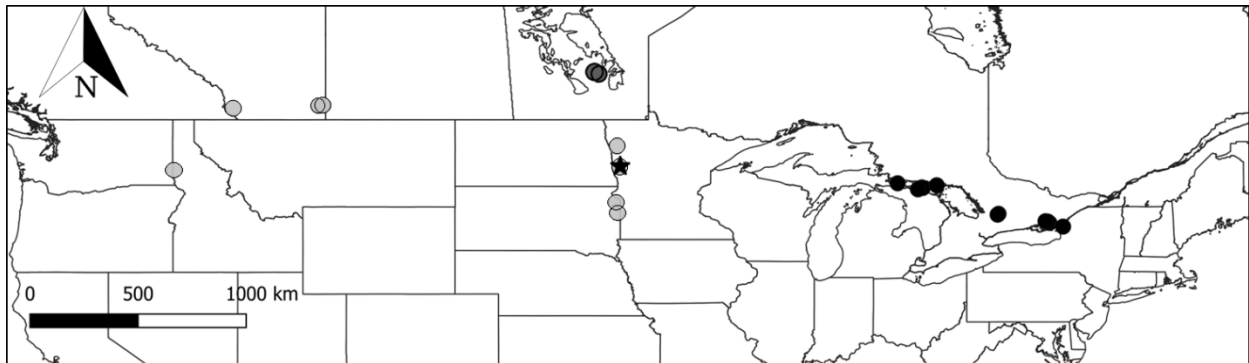
Figure 3.1. Map of collection sites of *Geum triflorum* (Pursh).
Black dots represent Great Lake Alvar populations, two populations in intermediate gray dots
near Lake Manitoba represent Manitoban Alvars, and the lightest gray dots represent Prairie
collection sites. Common garden location depicted as a star.

3.2.2. Common Garden Experiment

A common garden was established on November 7, 2015 at North Dakota State

University. Twenty-two populations were planted in twelve randomized complete blocks

including ten maternal seed families per population and 12 individuals per maternal seed family.

For bulk-seed collections, 24 seeds were planted including two replicates per block for each

source. 2348 individuals were initially seeded for the experiment (Table 1). Seeds with a .02%

PPM™ fungicide treatment were planted in Stuewe & Sons 158mL 'cone-tainers' filled with

Sungro horticulture mix (1N:45P:12K) soil. Plants were re-potted into 1014mL mini-treepot

containers (Stuewe & Sons) following approximately two months of seedling development.

Greenhouse conditions were maintained at a 15h:9h daylight to darkness photoperiod with

supplemental light from halide lighting at a measured flux density of .3383 mmol m^2 s^{-1} and

temperatures between 18.3°C and 23.9°C throughout the course of the experiment. Plants were

watered bi-weekly and were provided a slow release fertilizer mix (Osmocote 14N-14P-14K)

intermittently throughout the course of the experiment. In May 2016, surviving germinants were

transferred to a permanent outdoor research facility at the Minnesota State University Moorhead Regional Science Center (Table 1, 46.86913N, -96.4522W, Fig. 3.1). The randomized blocks were kept in the same arrangement. One population, AB-RL did not have any individuals germinate. The number of individuals in the final garden for all other populations ranged from 5 to 108 (Table 1), excluding AB-RL, which exhibited 0% emergence in the greenhouse, and were planted directly into the ground through cutouts in a weed barrier to limit competition.

Table 3.1. Names of source populations of *G. triflorum* separated by region

Populations:	Latitude	Longitude	Elevation (m)	No. Indv. seeded	No. Indv. in Garden	Percent Survival (%)	Dist. from garden (km)
Great Lake Alvars (GLA)						—	
CAR-NBA	44.68502	-79.0515	268	120	97	81	1368.191
CAR-PSR	44.64526	-79.0946	250	120	75	63	1366.181
MAN-FOX	45.89713	-82.5789	186	120	98	82	1068.226
MAN-KIP	45.87036	-82.5394	183	120	100	83	1071.794
MAN-LCI	45.99426	-81.8944	182	117	77	66	1118.294
MAN-MIS	45.80825	-82.7591	193	119	96	76	1056.428
MI-DRI	46.08578	-83.692	188	120	103	86	979.8749
NAP-ASS	44.26533	-76.7119	126	120	46	38	1559.351
NAP-CE	44.33003	-76.7897	166	120	70	58	1551.28
NAP-SCH	44.34399	-76.8934	154	120	92	77	1542.945
WNY-CB	44.09764	-76.0829	93	120	92	77	1612.813
Manitoba Alvars (MBA)						—	
MB-CRN	51.07094	-97.4613	231	120	91	76	472.9779
MB-MR	51.18429	-97.6268	231	120	87	73	487.3889
Prairie (PRA)						—	
AB-HSC	49.63639	-110.33	721	120	5	4	1070.778
AB-LL	49.54361	114.2472	929	120	108	90	1348.389
AB-RL	49.66528	-110.108	721	120	0	0	1055.739
AB-RO	49.67194	110.1472	721	120	6	5	1058.689
MN-PMG	47.7742	-96.6081	267	24	8	33	101.3225
ND-BSP	46.85845	-96.4717	274	120	39	33	1.896
SD-MUD	44.76309	-96.5879	531	120	99	83	234.4168
SD-PMG	45.2186	-96.6336	351	24	11	46	184.0634
WA-BLK	46.68551	116.9719	786	24	12	5	1558.248
Common Garden	46.86913	-96.4522	259	2348	1412	58	

No. of indv. seeded represents the number of individual seeds from the respective population included in the initial experimental design setup, while No. indv. in garden represents individuals that germinated and were transplanted to the permanent field site. Percentage survival represents difference seeded and germinated by population, along with the garden total. Latitude and longitude and elevation (m) of source populations listed. Distance from garden calculated as greater circle distance (Pearson, 2013) between source location and common garden location established at Minnesota State University Regional Science Center.

3.2.3. Life History Measurements in a Common Garden

Emergence from the soil and formation of true leaves were recorded for juvenile plants in 2015. Plants were flagged and recorded for whether they initiated the reproductive stages of flowering as well as whether they proceeded to seed set each year. To capture annual reproductive output per plant, mesh bags were tied around inflorescences at the initiation of seed set, but before development was complete and seeds dispersed. At the end of the growing season, bags were collected from the garden and processed in lab facilities at North Dakota State University, where the number of inflorescences that successfully set seed (deemed 'fruit') were quantified. A correlation was established between the number of seeds and mass of seeds in 2016 (Fig. 3.2). Due to a strong correlation between the number of seeds and seed mass ($R^2 = 0.528$), seed mass was used as a proxy for number of seeds and cumulative seed mass across all fruits represents the fitness product of an individual. After harvesting mesh bags, flowers that were still standing in the field that had senesced prior to setting seed were recorded as "non-reproductive flowers". All inflorescences that were collected in the mesh bags that also did not develop seeds were added to the 'non-reproductive flowers' count of the respective individual. Non-reproductive flowers and total number of fruit were combined to quantify the total number of flowers produced per individual per season. Lastly, survival of plants was assessed at the end of each growing season each year.
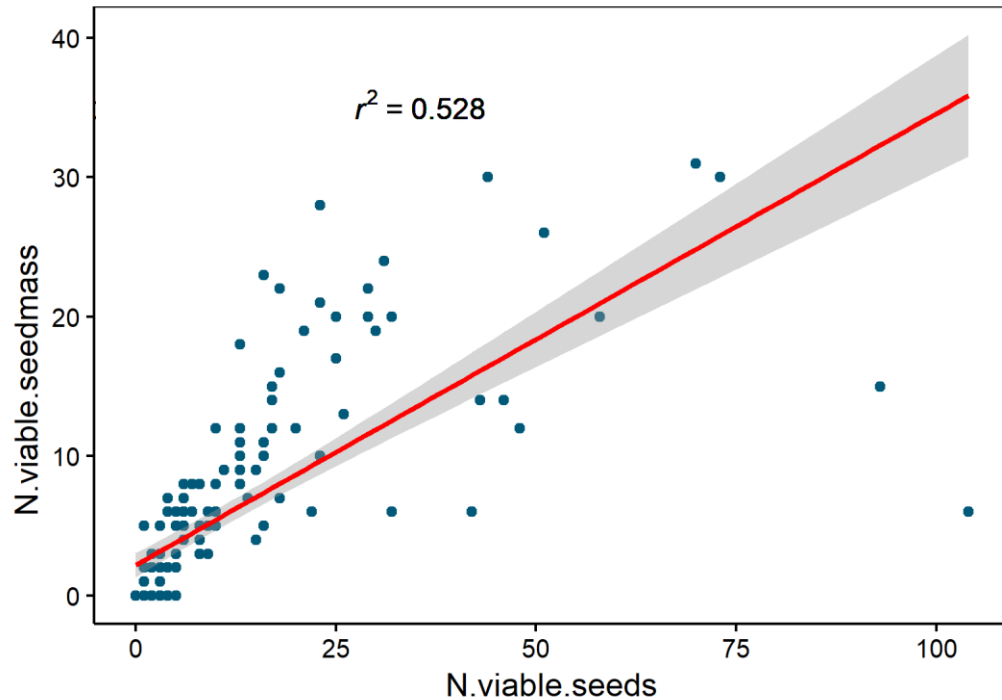
Figure 3.2. Correlation of number of viable seeds to seedmass.
The number of seeds an individual produced was correlated to the seedmass in milligrams (r2) = 0.528. Measurements were taken in 2016.

3.2.4. Estimating Cumulative Fitness

To estimate cumulative mean fitness, the ASTER package in R was used (Geyer et al.,

2007; R Core Team, 2018). ASTER uses multiple stepwise life-history nodes for each individual

to calculate fitness estimates for each region, cumulating fitness effort per year, to estimate total

overall fitness. Life history stages assessed are presented in a graphical model (Fig. 3.3) and

include emergence and survival, which were assessed in the first year (2015) and modeled as a

Bernoulli-distributed variable. Survival, flowering, and fruit set each year, from 2016 through

2018, were assessed annually as Bernoulli-distributed variables. Nodes for number of flowers,

number of fruit, and seedmass for each year (2016 through 2018) were analyzed as negative

binomial distributions, due to variance dispersion exceeding the Poisson distribution (Bliss and

Fisher, 1953). The three years of data were combined in the full graphical model presented

below (Fig. 3.3). For the ASTER analysis, Region, Population, and Family were considered

fixed effects. Due to a significant difference between randomized blocks, a mean block value

was used for fitness estimates. Seedmass was the terminal fitness node of the ASTER model

each year (Fig. 3.3) and estimates of cumulative fitness per region were presented for each year

(Fig. 3.4).

Greenhouse	Year 1 (2016)	Year 2 (2017)	Year 3 (2018)
0 (root) $\xrightarrow{Ber.}$ Germ $\xrightarrow{Ber.}$	$\xrightarrow{Ber.}$ Survival.Y.N $\downarrow$ Ber.	$\xrightarrow{Ber.}$ Survival.2017 $\downarrow$ Ber.	$\xrightarrow{Ber.}$ Survival.2018 $\downarrow$ Ber.
Distributions:	Flw.Y.N.2016 $\downarrow$ Poi.	Flw.Y.N.2017 $\downarrow$ Poi.	Flw.Y.N.2018 $\downarrow$ Poi.
Ber. –Bernoulli	Flw.No.2016 $\downarrow$ Ber.	Flw.No.2017 $\downarrow$ Ber.	Flw.No.2018 $\downarrow$ Ber.
Poi. –Poisson, 0-inflated Poisson, or negative binomial	Frt.2016 $\downarrow$ Poi.	Frt.2017 $\downarrow$ Poi.	Frt.2018 $\downarrow$ Poi.
	Frt.No.2016 $\downarrow$ Poi.	Frt.No.2017 $\downarrow$ Poi.	Frt.No.2018 $\downarrow$ Poi.
	Seed.mass.2016	Sum (Seed.mass.2016 + 2017)	Sum (Seed.mass.2016 + 2017 + 2018)

Figure 3.3. Graphical model of ASTER model with predictors used and distributions analyzed
for each node.
Survival.Y.N is a measure of whether a plant survived to that year. Flower.Y.N represents
whether the plant flowered in the year labeled. Flw.No. is the number of flowers produced by an
individual in the year labeled. Frt.YEAR represents wither a plants' flowers set fruit in the
respective year. Frt.No. represents the number of infloresence that set fruit on a plant in the
labeled year. Seed.mass is the mass of seeds in milligrams that was produced by a plant in the
given year, and is cumulated across years to have an estimate of total seedmass produced per
individual in 2018.

3.3. Results

Estimates of fitness varied substantially by region, and by year (Table 3.2, Fig. 3.4). Fitness in 2016 was essentially 0mg for the MBA and PRA in 2016, with GLA having a slightly greater fitness of 3.027mg (standard error ± 1.246). In 2017, fitness increased considerably in all regions, but the increase was not even across all regions. GLA had a much greater expected seedmass than both MBA and PRA regions. The GLA region had a fitness estimate of 544.625mg (standard error ±163.027). The MBA region had a fitness estimate of 93.6mg (standard error ±81.898) in 2017. PRA had the lowest fitness estimate in 2017 with 70.379mg (standard error ± 75.872). Regional differences in fitness increased further in 2018, the final year of the study. GLA had much greater cumulative fitness, increasing in divergence from the other two regions of study with an estimate of 926.324 (± 268.496) (Table 3.2). The MBA region had an increase in fitness, but was still less than the other alvar region (GLA) with an estimate of 248.6 (± 194.65). The fitness of the PRA region was considerably lower than GLA as well, but there was less fitness differentiation between PRA and MBA, as the PRA cumulative fitness estimate was 94.445 (± 100.454)

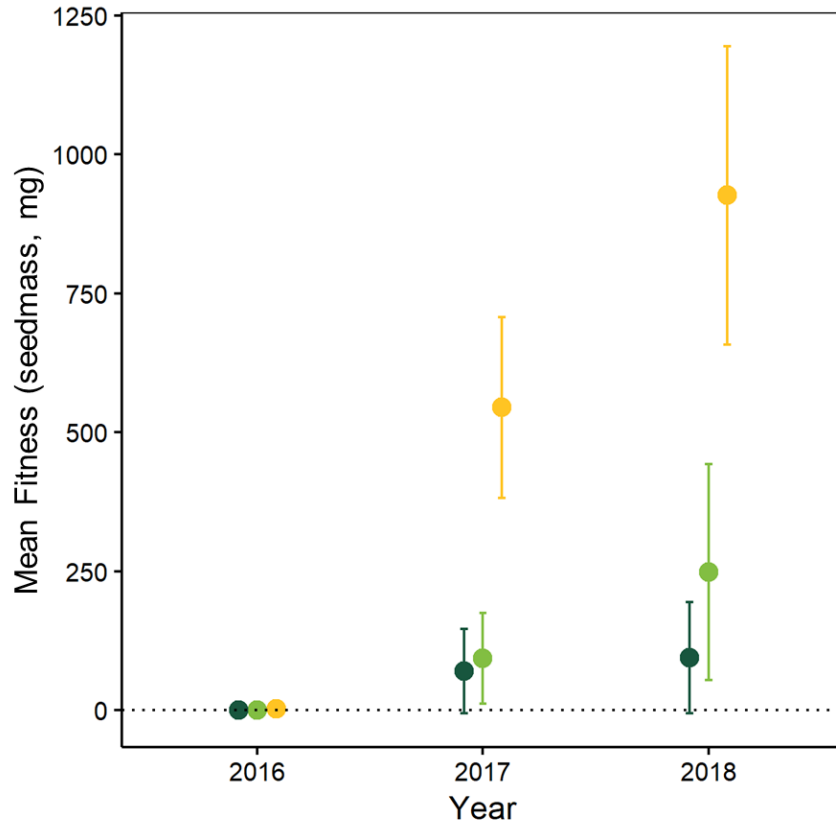Figure 3.4. Expected fitness values for *G. triflorum* per source region per year.
Expected fitness values for plants sourced from three distinct eco-regions. Dashed line at zero. During 2016 expected fitness was essentially zero for all populations. 2017 and 2018 fitness outputs represent cumulative outputs of fitness, suggesting that while there were differences in 2017 they were further exacerbated in 2018.

Table 3.2. Estimates of fitness per year obtained from ASTER model.

Year	Region	Mean Fitness seedmass (mg)	Standard error
2016	GLA	3.027	1.246
2016	MBA	2.50E-10	n/a
2016	PRA	8.06E-12	n/a
2017	GLA	544.651	163.027
2017	MBA	93.6	81.898
2017	PRA	70.379	75.872
2018	GLA	926.324	268.496
2018	MBA	248.6	194.65
2018	PRA	94.445	100.454

GLA represents the Great Lake alvar region, MBA represents Manitoba alvar region, and PRA represents the Prairie source region. Seedmass in milligrams was used as Fisher's estimate of mean fitness per region.

3.4. Discussion

Our comparison of mean fitness for seeds of *G. triflorum* sourced from prairie and alvar habitats grown within a prairie environment indicates that geographically and environmentally distant alvar seed exhibited greater mean cumulative fitness relative to seed sourced within the home prairie environments (Fig. 3.4). If prairie plants were locally adapted to the prairie environment, we would predict that PRA plants would exhibit greater relative fitness in the prairie environment when compared with foreign genotypes. Below, we elaborate on several possible reasons why we observe greater reproductive output for seeds sourced from the GLA environment relative to the PRA environment. Our findings suggest that there are genetic differences between the seed sources for reproductive output that could impact restoration success.

3.4.1. Greater Fitness in Alvars

In our study, the nonlocal alvar populations exhibited increased cumulative fitness relative to the local prairie populations (Fig. 4). Establishment of the disjunct alvar populations was hypothesized to occur during an overall range expansion of the species and then contraction (Hamilton and Eckert, 2007). Founder effects during establishment of alvar populations and bottlenecks during range contraction could have reduced allelic diversity through evolutionary processes of drift and inbreeding (Ellstrand and Elam, 1993; Young et al., 1996). It is possible greater fitness in alvar populations than prairie populations may be a result of purging of genetic load. Genetic purging could have occurred if maladaptive alleles were lost disproportionately during the process of establishment and then isolation on the alvars (Ellstrand and Elam, 1993; Lynch and Walsh, 1998), though this is not a common consequence of isolation (Young et al., 1996).

Additionally, given the extreme nature of the alvar environment, selection has likely favored those individuals that exhibit increased reproductive output early in life. Annual transitions between complete flooding in the spring, to total desiccation by early summer occur in the alvar environment (Catling and Brownell, 1995; Stark et al., 2004). Frequent disturbances and stresses such as these can favor plants that invest in reproduction and flower early as this increases the likelihood of lineage persistence through re-establishment of progeny (Grime, 1988). Extreme environmental conditions may result in selection for specific life history strategies, such as a live fast, die young strategy (Geber and Dawson, 1990; Donovan et al., 2009). While prairies also have varying water availability, the thicker soil and detritus layers mediate extremes in water availability by retaining moisture (Samson and Knopf, 1994; Knapp et al., 2001). The prairie may not have the same selective pressure for early reproduction, providing

potential for a contrast of slow, steady reproductive output over a longer lifespan in the prairie plants. Further study of the generation time of plants in the common garden may aid in identifying if the relative fitness of prairie populations increases beyond the three-year term of this experiment over their lifespan.

However, if we were to see disparate investment in reproduction early in alvars, we would expect to see a decrease in subsequent years. While slow, steady reproduction was observed in PRA plants, (nearly an order of magnitude less than GLA plants each year), there was no apparent reduction in alvar fitness across the three years observed in the experiment. An important note to consider with our findings is that, though multiple years of fitness were investigated, it is still a snapshot of reproduction. The lifespan of *G triflorum* is unknown, and continued observation over the lifespan of these plants may lead to reproductive effort changing over time. For example, the alvar populations could senesce earlier or reduce reproductive output, while the prairies continue to be reproductively active for more seasons. Additionally, due to the varied nature of the prairie habitat, extreme environmental effects (such as flood or drought) that did not manifest during the years studied could differentially impact fitness in future years.

3.4.2. Local is not Best?

Local adaptation has been found in many studies (Joshi et al., 2001; Leimu and Fischer, 2008; Savolainen et al., 2013; Wilson et al., 2016), however there are also studies that suggest that local individuals may not be the best suited to their home environment. Bucharova et al. (2017) conducted a reciprocal transplant experiment consisting of six perennial species reciprocally transplanted across three to four environments for a total of 22 species and location contrasts. They found that most transplants did not show a local advantage or disadvantage.

Interestingly, a non-local ecotype was best in three of the 22 contrasts, whereas local was found best in only two of the 22 contrasts, and both of those were within the same species. Our findings along with these studies suggest that while local adaptation is common, it is not necessarily present in every species. Local adaptation may not be detected in species with high levels of gene flow throughout the range, where beneficial alleles spread rapidly across populations (Hereford, 2009).

Gallagher and Wagenius (2016) found establishment and fitness in seeds sourced across a range of populations for three native grass species varied widely, and not in accordance with local adaptation. They suggested that differences in their experiment could have been a result of genetic issues not related to adaptation (particularly stochastic effects impacting some of the smaller populations used in their experiment). Additional non-genetic effects resulting from different maternal environments may have impacted their results, as some populations were wild-collected and others were nursery grown. It is possible observed differences in fitness in *G. triflorum* could be subject to the same confounding effects, as maternal effects were not controlled and source population sizes varied. However, due to the perennial nature of the species, it would be expected that maternal effects would not affect the results across multiple study years.

Hereford (2009) in his metanalysis noted that locally adaptive differentiation occurs through tradeoffs, where populations perform worse in foreign environments (deemed the magnitude of local adaptation) than home environments they presumably would be locally adapted to. Hereford found that fitness differences across environments were, on average, only 45 percent greater fitness in the home environment than foreign environment (2009). Therefore, when testing for local adaptation, if the environments tested across do not differ substantially,

local adaptation may not be evident if fitness differences do not emerge. In our study, it may be that the prairie environment is not substantially different enough from the alvar environment to show reduced performance of alvar populations (which could suggest adaptive differences between prairie and alvar plants) in the prairie environment, though this is not expected to be the case in our study. This is because climate and edaphic conditions between prairie and alvar environments are quite different. Alvars consist of very shallow soil prone to frequent flood and drought (Reschke et al., 1999), while prairies consist of deep soil that can mediate water availability (Anderson, 2006). Due to the large habitat differences between prairie and alvar, we considered even those geographically distant PRA populations such as AB-RL and AB-RO as local, though future research may be useful in determining if these populations are 'local'. It is possible the inclusion of these populations skews the prairie fitness estimates lower, but this alone likely does not cause the near order of magnitude difference in fitness between regions.

3.4.3. Enemy Release and Increased Competitive Ability

Increased fitness in alvar populations relative to prairies could suggest alvars are benefiting from the absence of natural enemies that are found in their home environment (the 'enemy release hypothesis') (Keane and Crawley, 2002; Joshi and Vrieling, 2005). ERH has been suggested when there is significantly more mortality in native individuals than foreign individuals (Vilà et al., 2005), particularly from pathogen load. This does not seem to be the case in our study. While there was mortality, it appeared to be from random herbivory or destruction. Furthermore, enemy release is often found in exotic species, where success is disproportional due to a lack of specialist predators (Keane and Crawley, 2002). This is unlikely here since *G. triflorum* does not have any documented specialist pathogens or parasites known in its range. A survey of fungal colonization of *G. triflorum* in natural prairie and alvar soils could determine if

there are pathogens affecting *G. triflorum,* and furthermore if there are pathogens specific to either environment.

Alvar population success could be attributed to a similar hypothesis, the evolution of increased competitive ability (EICA). The EICA hypothesis suggests that foreign populations may be more competitive because they shift allocation of resources from defense tissue to growth if there is a reduction in herbivores in the foreign environment (Blossey and Notzold, 1995). However, there have been mixed results in past common garden experiments testing EICA (Bossdorf et al., 2005; Colautti et al., 2009). Blumenthal and Hufbauer (2007) found effects of EICA only when plant interspecific competition was excluded in their study, and not when competition was limited or uncontrolled. EICA is an unlikely explanation for our results; competition was limited in the common garden, but it was not completely excluded, nor were generalist herbivores. While not directly studied, there did not appear to be preferential herbivory or obvious pathogen impact on any specific source population or region in the garden. Moreover, EICA suggests an evolutionary response to the novel environment (Colautti et al., 2009), and since our seeds were sourced directly from their home populations, evolution to the novel environment could not have occurred.

3.4.4. Maladaptation of Prairie Populations

Our findings of reduced fitness in the local populations in comparison to foreign-sourced seeds suggest the possibility that prairie populations may be maladapted to the common garden conditions. Changing climate conditions could drive genetically correlated traits in different directions, exacerbating potential maladaptation (Etterson and Shaw, 2001; Anderson, 2016). For example, with the frequency of extremes in temperature and precipitation that is expected to change as climate change progresses (Parmesan and Yohe, 2003; IPCC, 2013), in *G. triflorum*,

selection for advancing earlier phenology as the climate warms could lead to earlier leaf growth and flowering that may be damaged by extreme weather events.

In addition, plasticity can vary between populations, and can be important for persistence of populations in changing conditions (Franks et al., 2014; Espeland et al., 2018), such as the aforementioned seasonal water extremes or different conditions presented by restoration sites. Friedman et al stated in their review that plants have been found to adjust their reproductive output based on differing conditions such as drought stress (Friedman et al., 2019). It may be that alvar populations have greater plasticity and were better able to respond to the experimental conditions present.

3.4.5. Caveats

Early growth conditions in the greenhouse may have impacted populations differently, as the greenhouse is not the 'home' environment for any regions. Indeed, fitness was lowest in plants from all sources in the first year, possibly due to greenhouse effects, or more likely transplant shock. Shock very likely disrupted flowering in the first year, as plants were transferred during the species' typical flowering time in May. Flowering onset occurred throughout the growing season, but with little reproductive success (roughly six percent of the garden produced fruit in the first year). It is possible plants did not have sufficient resources to invest in seeds the first year as a result of the transplant. Another possibility is a mismatch with timing of flowering and availability of local pollinators occurred, as *G. triflorum* is insect pollinated. However, effects from the greenhouse (other than mortality) would not be expected to manifest in subsequent years of the study. This is supported by flowering phenology returning in subsequent years to what is the typical flowering time for *G. triflorum*, and reproductive success increased dramatically in 2017 and was stable in 2018.

3.4.6. Future Directions

A reciprocal transplant experiment should be conducted to test if prairie populations are maladapted (Kawecki and Ebert, 2004; Hereford, 2009), or if alvar populations have greater fitness across environments. Local adaptation is detected 65-71 percent of the time based on a metanalysis of reciprocal transplant experiments by Hereford (2009), though our results do not suggest a reciprocal transplant would find local adaptation in *G triflorum,* it may still be worth examining, particularly if seeds are to be sourced across eco-geographic regions for restoration. Additionally, germination tests should be conducted to determine if estimates of seed mass, currently viewed as proxies for fitness, correlate with individual fitness. A germination test would assess if there are differences in viability of the seeds by region, and support or refute the assumption that greater seed mass (indicating greater number of seeds) is a valid proxy for reproductive output. Lastly, the heritability of differences in fitness, and traits that are thought to impact fitness, should be investigated. Heritability of a trait can inform adaptive capacity, and whether results observed in our study could be repeated in other experiments and persist across generations.

3.5. Conclusions

In our study, differences in fitness across years corresponding to the eco-geographic region seeds were sourced from were found. Contrary to much of the literature, the local populations had reduced fitness relative to foreign populations. When observing in a single common garden experiment, differences in fitness could be a result of many different evolutionary processes. While locally sourcing seeds is thought to preserve local adaptation (Lesica and Allendorf, 1999), but due to the significantly lower fitness in PRA plants, our findings suggest when sourcing *G. triflorum* seeds for restoration, local sources of seeds may not

be important. Due to the greater fitness in the Great Lake alvar region, alvar populations may be considered for seed sourcing regardless of geographic distance. Future work should emphasize conducting a reciprocal transplant to further test impact of genetic differences in seed sources and their potential impact on restoration in grassland environments.

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4. RESEARCH SUMMARY AND FUTURE DIRECTIONS

The objectives for this study were to evaluate genetic differences in seeds sourced across the range of *Geum triflorum* from a variety of populations and habitat regions. Understanding how genetic variance is distributed and the impact it has on trait differentiation aides in establishing guidelines for seed transfer in ecological restorations (Broadhurst et al., 2008; Potter and Hargrove, 2012). To assess genetic differences between populations spanning multiple eco-geographic regions, quantitative physiological traits were examined during a single growing season, and a three year assessment of reproductive output was measured for individuals grown in a common environment.

4.1. Research Summary

We found regional- and population-scale differences explained a considerable amount of variation in the observed quantitative traits. This is consistent with other literature that genetic variation is partitioned at both broad and fine scales throughout a species' range (Messier et al., 2010; Hovick et al., 2018; Baughman et al., 2019). The physiological traits measured in *G. triflorum* represented classes of traits that balance adaptive tradeoffs related to water usage via stomatal arrangement (Lawson and Blatt, 2014; Dittberner et al., 2018) and a 'resource use axis' of leaf economics (Reich et al., 1997; Wright et al., 2004). These traits were expected to be under divergent selection pressures between the contrasting environments of prairies and alvars. Variation was explained by regional differences as expected, but as much or more variation was explained by population level differences for each trait. This suggests that broad (regional) and fine (population) scale selection on trait variation may be equally important in the context of seed transfer for restoration.

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Differences in trait variation across scales and, particularly, differences in classes of traits suggests certain traits or trait classes may be more informative for the development of seed transfer guidelines. Resource allocation and stomatal trait classes explained a statistically greater proportion of regional trait variance relative to morphological traits. For *G. triflorum,* we would expect differences in water usage traits due to the difference in water availability across both environments studied (Samson and Knopf, 1994; Catling and Brownell, 1995; Knapp et al., 2001). Regions may experience the substantial divergent selection pressure and therefore be the most informative of adaptive genetic differences between sources. The impact functional trait differences can have on success is an important consideration when sourcing seeds for restoration (Bucharova, Michalski, et al., 2017). To better understand the impact physiological trait differentiation may have on restoration, differences in fitness between regions was evaluated.

We detected differences in individuals corresponding to the three eco-geographic region seeds were sourced from. Using ASTER, we determined these differences persisted across years. GLA individuals had an order of magnitude greater expected fitness each year in comparison to PRA individuals. This was in contrast to expectations from much of the literature (Montalvo and Ellstrand, 2000; Joshi et al., 2001; Kawecki and Ebert, 2004; Leimu and Fischer, 2008; Savolainen et al., 2013) and current practices when sourcing seeds for restoration (Hereford, 2009; Johnson et al., 2010; Miller et al., 2011), as local populations had reduced fitness relative to foreign populations. There are several possible explanations for greater fitness in foreign populations. Our findings suggest when sourcing *G. triflorum* seeds for restoration, alvar populations may be considered for seed sourcing in prairie environments, although further study

is required to evaluate long-term fitness consequences of seed transfer as we have only monitored fitness across three years and differences in longevity and fitness could yet emerge.

Two prominent hypotheses, the enemy release hypothesis (ERH) and evolution of increased competitive ability (EICA), suggest that foreign populations can better compete in a novel environment than natives. However, the lack of known predators or pathogens specialized to *G. triflorum* refutes ERH (Keane and Crawley, 2002). Additionally, since seeds were sourced directly from their respective home environment, there was no ability for an evolved response such as EICA to occur (Colautti et al., 2009).

Another possibility is that GLA populations have evolved genetic variation capable of plastic responses to environmental conditions (Pigliucci, 2005; Anderson et al., 2014). Plasticity may have evolved in response to strongly predictable seasonal cues associated with the extreme heterogeneity variation in water-availability (Callahan and Pigliucci, 2002; Lázaro-Nogal et al., 2015; Acasuso-Rivero et al., 2019). If the GLA populations exhibit 'generalist' genotypes and are more plastic to changing environmental conditions, this could be desirable for restorations. Lastly, the short term of our experiment did not capture the full lifespan of the observed individuals, if alvar populations have adapted a 'live fast, die young strategy' (Geber and Dawson, 1990; Donovan et al., 2009), the fitness differences could diminish or reverse with the PRA population in subsequent years over the course of the remaining lifespan of the studied plants.

4.2. Future Directions

Future work should emphasize conducting a reciprocal transplant to fully test the influence genetic differences in seed sources have on restoration success in grassland and alvar environments. A reciprocal transplant experiment could test relative fitness across environments

providing a test for 'home vs. away' hypothesis of local adaptation (Kawecki and Ebert, 2004; Hereford, 2009), as this will be an important consideration for seed transfer for this species. It is possible the alvar populations would show greater fitness across environments, further suggesting a 'generalist' genotype that would be valuable for ecological restoration.

Additionally, the heritability of differences in fitness, and traits that are thought to impact fitness, should be investigated. Heritability of traits can inform adaptive capacity. Preserving adaptive capacity is another reason seed transfer guidelines are being extensively researched (Hamilton et al., 2019; Kulbaba et al., 2019); the ability for populations to adapt to future climate change is expected to be crucial (Prober et al., 2015; Aitken and Bemmels, 2016). Estimating heritability of observed traits will help determine whether results observed in our study could be repeated in other experiments, such as a reciprocal transplant, or be expected to persist across generations. Lastly, conducting a germination test on collected seeds would provide important support to the findings that there is greater fitness in nonlocal populations. A germination test would evaluate the assumption that greater seed mass or number of seeds is an accurate representation of fitness, and that seed mass correlates to reproductive success of future generations.

4.3. References

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